Medicinal properties of some dietary herbs and spices

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Submitted by

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DECLARATION

Ihereby declare that the thesis entitled "***Medicinal properties of some dietary herbs and spices"*** has been prepared by meunder the supervision of Professor Usha Chakraborty of Department of Botany, University of North Bengal**.** No part of this thesis has formed the basis for the award of any degree orfellowship previously.

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CERTIFICATE

October 24, 2017

Certified that Mr. Sandip Dev Chaudhuri has prepared his thesis entitled "***Medicinal properties of some dietary herbs and spices***", for the award of PhD degree of the University of North Bengal, under my supervision. He has carried out the work at Plant Biochemistry Laboratory of Department of Botany in the University of North Bengal. To the best of my knowledge and belief the contents of the thesis, in full or in parts, have not been submitted earlier to any other Institute or University for the award of any degree or diploma.

Further certified that Mr. Dev Chaudhuri has followed all the rules and regulations formulated by the University for fulfilment of requirements for the degree of Doctor of Philosophy (PhD) in science.

Usha Chakraborty

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ABSTRACT

A plant-based dietary health food is a rich source of multiple phytochemicals along with a high-class of natural antioxidant molecules. Consumption of natural antioxidants through foods and drinks prepared particularly with lofty herbs and spices not only promotes the quality of health and life, but also protects against chronic oxidative stress-related (OSR) diseases or disorders.Herbs and spices are included among the common food adjuncts which have been used as flavouring, seasoning and colouring agents, and sometimes as preservatives throughout the world for thousands of years. Use of herbs and spices in cooking is the oldest form of aromatherapy that stimulates gastric secretion and creates appetites, creates positive moods, stimulates the body, relieves cold symptoms and respiratory problems, and eases muscle pains. The active components in herbs and spices are considered as powerful means to create a state of wellness.

In the present study, twelve commonly used herbs and spices namely *Mentha piperita*, *Trigonella foenum-graecum*, *Coriandrum sativum*, *Murraya koenigii*, *Glinus oppositifolius*, *Foeniculum vulgare*, *Illicium verum*, *Myristica fragrans*, *Ceiba pentandra*, *Capsicum annuum*, *Parmelia perlata* and *Dregea volubilis* were selected and evaluated their biological activities. Plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively.

Preliminary phytochemical screening to detect the presence or absence of some significant phytochemicals viz. phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard protocols. Phytochemical analysis revealed that phenol, flavonoid, reducing sugar, free amino acids, tannins were detected in all plants tested; anthraquinone was absent in *I. verum*,*C. pentandra* and *P. perlata*; triterpenoid was not detected in *C. sativum*, *T. foenum-graecum* and *M. fragrans*; cardiac glycosides were detected in all samples except *T. foenum-graecum*, *I. verum* and *C. pentandra*; alkaloid was detected in all the test plants; saponin was absent in *C. sativum*; steriod was absent in *C. annuum*; phlobatannin was present only in *P. perlata* and *D. volubilis* and cardenolide was presen only in *D. volubilis*.

Samples were evaluated for the total soluble sugar content, reducing sugar content, soluble protein content and total lipid content, total phenol, flavonoid and flavonol content along with vitamin C and E. *G. oppositifolius* and *I. verum* had highest amount of total phenol, total flavonoid, total flavonol, total sugar and vitamin C. Highest amount of reducing sugar and total and vitamin E was present in *G. oppositifolius* and *I. verum*. Protein content was found to be highest in *M. koenigii*, lowest in *G. oppositifolius*.

Quantity of different plant pigments like total chlorophyll content and total carotenoid content was estimated and found highest in *G. oppositifolius* for both the pigments. Carotenoid content was lowest in *M. piperita*.

Extraction of the samples with methanol revealed higher yield than hot aqueous which may be due to the higher solubility potential of phytochemicals in methanol. Amongst the samples, *P. perlata* showed the highest yield.

Analysis of *in vitro* antioxidant activities of the extracts, DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, superoxide anion scavenging activity and nitric oxide radical scavenging activity were performed. It was found that, all herb and spice samples showed antioxidant activity at different levels. Among samples, *I. verum* and *G. oppositifolius* showed the highest antioxidant activity as well as phytochemical components. In all the cases, gradual rise in the activity with the increase in the concentration was observed but it was insignifcantly different to each other in case of majority of herb extracts.

Antimicrobial activity of methanolic extracts of different herbs and spices against both Gram positive bacteria (*Bacillus cereus* MTCC 10665 and *Bacillus pumilus* MTCC 1684) and Gram negative bacteria (*Serratia marcescens* NCBI GENBANK no.JN020963 and *Pseudomonas aeruginosa* MTCC 2453 by disc-agar diffusion method.*B. cereus* and *B. pumilus* were inhibited only by the *M. piperita* and *T. foenum-graecum* extracts at higher doses. No inhibition was observed by the other herbal extracts and against other test organisms. The MID value of *M. piperita* extract against *B. cereus* and *B. pumilus* was found to be 8.5 and 5.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID value of *T. foenum-graecum* extracts against *B. cereus* and *B. pumilus* was determined as 3.5 and 7.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID values of other herb extracts against respective organisms were found to be >10 mg lyophilized methanolic extract $disc^{-1}$. Among the spice extracts, extract of *I. verum* was found to be most potent showing

highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. *P. perlata* also found to be active against both Gram positive and Gram negative bacterial strains. The MID value of *P. perlata* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 2.0, 2.0, 3.0 and 2.0 mg lyophilized methanolic extract $disc^{-1}$ respectively. *M. fragrans* and *C. annuum* were also effective against all microorganisms at comparatively higher doses.

Anti-quorum sensing activities of different herbs and spices evaluated through preliminary screening for inhibition of violacein synthesis by whole plant parts. Among the plant tested *I. verum* was found to be most potent in inhibiting the violacein production in *C. violaceum*, followed by *P. perlata*. Methanolic extract of *I. verum* was also able in reducing the virulance phenotypes such as pyocyanin synthesis, ptotease production, swarming motility and biofilm formation in *Ps. aeruginosa*.

Evaluation of *in vivo* anti-diabetic activity was performed in Streptozotocin-induced rats using *I. verum* and *G. oppositifolius* methanolic extracts as they were traditionally reported as antidiabetics. The methanolic extracts were reconstituted in sterile distilled water and used to determine the toxicity and pharmacological effects on rats. Before performing *in vivo* assay, the crude extracts were tested for their acute toxicity at a concentration of 2000 mg kg^{-1} BW and analyzed the lethal and safer doses of extracts. For anti-diabetic assay 500 mg kg^{-1} BW and 250 mg kg^{-1} BW doses were selected as safer and non-toxic. Streptozotocin-induced diabetic rats treated orally with both the sample extracts and Metformin were able to reverse the diabetic conditions to near normal. Various biological markers such as fasting blood sugar level, cholesterol, triglycerides liver enzymes (SGPT and SGOT), serum urea and creatinine were reduced to nearly normal level while significant increase in body weight and HDL-cholesterol level was observed in compared to the diabetic controls. Among the plant extracts,*I. verum* extract (*Iv*ME)showed comparatively better *in vivo* antidiabetic activity than *G. oppositifolius* extract(*Go*ME). This may due to various antidiabetic compounds present in the extract.

Further, characterization of bioactive compounds present in the fractions of *I. verum* (*Iv*ME) and *G. oppositifolius* (*Go*ME) were performed by GC-MS analysis. GC-

MS profiling revealed the presence of myriad of chemical compounds including volatile compounds, phenolics, terpenoids, fatty acids, phytosterol etc. Many of them are reported to have antidiabetic and antimicrobial properties. Some of the compounds identified through GC-MS analysis of methanolic fraction of *I. verum* were Linalool, Estragole, n-Hexadecanoic acid; Benzaldehyde, 4-methoxy-; Benzene, 1-methoxy-4-(1-propenyl)-; cis-Vaccenic acid; 2-Propanone, 1-(4-methoxyphenyl)-; Benzhydrazide, 4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; Octadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-. The chemical compounds identified through GC-MS analysis of hexane fraction of *I. verum* were Benzaldehyde, 4-methoxy-; Anethole; Anisaldehyde dimethyl acetal; 2-Propanone, 1-(4-methoxyphenyl)-; 4-(p-Methoxyphenyl)-1-butanol; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; cis-Vaccenic acid; (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-;4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-. Some of the compounds identified through GC-MS analysis of ethyl acetate fraction of *I. verum* were Linalool; Estragole; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; n-Hexadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and cis-Vaccenic acid.GC-MS analysis of different fractions of *G. oppositifolius* also revealed different types of chemical compounds. Compounds identified through GC-MS analysis of methanolic fraction of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; 1-Deutero-2,2,5,5-tetramethylcyclopentanol; n-Hexadecanoic acid; Phytol; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d]-naphthalene;(4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-exahydronaphthalen-2-yl and Retinol, acetate. Compounds identified through GC-MS analysis of hexane fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; Mome inositol; Hexadecanoic acid, methyl ester, n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; Linoelaidic acid; Octadecanoic acid; Squalene and Chondrillasterol. Chemical compounds identified through GC-MS analysis oethyl acetate fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; DL-Proline, 5-oxo-, methyl ester; Mome inositol; n-Hexadecanoic acid; Phytol; Lanosterol; Lup-20(29)-en-28-ol; Beta.-copaen-

4 .alpha.-ol and Retinol, acetate. Many of the identified compounds are reported to have versatile bioactivities. Compounds such as cis-1,2-Dihydrocatechol, trans linalool oxide, estragole, benzaldeyde, 3-methoxy and several others have antimicrobial, antioxidant and antidiabetic activities.

Hence, all the herb and spice samples tested showed nutritional and antioxidant potential confirming their nutraceutical and medicinal properties. But among them *I.verum* and *G. oppositifolius* were found to possess highest scavenging activities, good amount of phytochemicals, thus should be explored for nutraceutical and pharmacological applications.

PREFACE

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Date:..... ……..….....

Place:..... (Sandip Dev Chaudhuri)

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CHAPTER 1

INTRODUCTION

1. Introduction

Association and dependence of man with or on plants have originated at the very beginning of life as man couldn't think his life without the plants or plant products that provide 'life saving' oxygen, 'energy giving' food, 'life protecting' shelter and 'disease curing' medicine - the basic human necessities for sustaining, maintaining and continuing the life forms on earth. Ever since the birth of mankind there has been a close affinity among man, medicinal plants and medication systems. Over the time span man has acquired knowledge to recognize and categorize the available plants for multifarious uses. The use of medicinal plants can be traced to the earliest of myths, traditions and documentations used to codify these plant materials that could relieve pains and cure diseases. These medicinally valued plants form the core structure of the traditional medicine systems that have been in existence for an age-long and continue to serve humankind with newer remedies and possibilities. The evolution of plant-based system of medication, primarily originated within a local area, and then flourished as popular indigenous systems of medicine such as the Ayurveda, Siddha and Unani of the Indian sub-continent, the Chinese and Tibetan in other parts of Asia, the Native American of North America, the Amazonian of South America, and several unorganized systems of folk-medicine within Africa. Throughout the world, the use of conventional medicine is common but nearly 70-80 % of the primary health care remains based on plants (Mamedov and Craker, 2012).

Food, the most important among the basic ones of life's prerequisite helps to nurture the life in a healthy style. Many of the foods, usually we take in our everyday life as normal diet have been prepared with lots of herbs and spices. With the increasing interest in "functional foods" among researchers and health professionals, herbs and spices have been receiving a thought-provoking attention for their power to confer health benefits beyond basic nutritional assistance. In the modern era of science and technology, the foods or food components, which are a part of an everyday diet and is demonstrated to offer disease-preventive, health-protecting and physiological benefits beyond the widely accepted nutritional effects, are known as "functional foods" (Hasler, 1998; FFC, 2012). Functional foods can prevent or delay the onset of chronic diseases as well as provide basic nutritional requirements (Medoua *et al*., 2009). Functional foods which contain variety of phytochemical constituents can have long-term health promoting or medicinal qualities. Herbs and spices have a long

traditional history of medicinal usages, with significant roles in cultural heritage, and in the appreciation of foodstuff and its link to good health (Krishnaswamy, 2008).

Foods in combination with herbs and spices have pharmacological agents in plenty; they act as drugs in the body system, and depending on the food one eats, effects at the cellular level can be observed. This inspires to investigate a Food-Health-Disease (FHD) connection which has led to the realization of amazing potentials of phytochemicals as because plants are the richest and widest source of bioactive phytoconstituents and antioxidant nutrients (Elless *et al*., 2000). It is now broadly accepted that certain classes of plant-based compounds such as dietary fibers, phenolic acids, flavonoids, vitamins and neuro-pharmacological agents, and antimicrobial compounds play preventive role against the incidence of some common deadly diseases or disorders like diabetes mellitus, cancer, cardiovascular and neurodegenerative disorders, and many infectious microbial diseases of human beings (Fan *et al*., 2007; Siddhuraju *et al*., 2007; Liu *et al*., 2008). A majority of these present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body system. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the present day life, or due to the poor scavenging in the body caused by depletion of the dietary antioxidants (Schulz *et al.*, 2000; Dringen, 2000). In diet-based health benefits, early investigations confirmed the positive effects of various dietary spices including garlic, ginger, onion and many more.

India possesses a diversity of medicinally important herbs and spice plants and many of them hold good health promoting natural components. The natural components viz. antioxidants, antidiabetics and antimicrobials have the potential ability to decrease blood cholesterol levels and protect against osteoporosis and cancer development, and many emerging infectious diseases of humans. These aforementioned properties of plants, herbs and spices have awarded them to be as nutraceuticals (Wildman, 2001). The culinary application of herbs and spices is the oldest form of aromatherapy used to stimulate gastric secretion and to enhance appetites, create positive moods, perk up the body and organs, relieve from cold symptoms and muscular pains. The active components in herbs and spices are considered as powerful agents to create a state of wellness including production of enzymes that inhibit

cholesterol synthesis, detoxification of carcinogens, lowering the blood pressure, blocking the estrogens and preventing the blood clotting (Uhl, 2000).

In most of the countries of the world, herbs and spices are popular as common food adjuncts which have been used as flavouring, seasoning and colouring agents, and sometimes as food preservatives for thousands of years (Srinivasan, 2005). Beyond their popular house-hold use they are widely admired as traditional medicines (Gao *et al*., 2000; Srinivasan, 2005). Traditional medicines of late have been re-evaluated by the scientists to search a source of "qualified leads" from the bioactive agents for use in the production of next generation drugs. Many of the herbs and spices have been well recognized in traditional medication and possess medicinable bioactive components that conduce beneficial effects on human health through the antioxidant activity, digestive improvement activity, anti-inflammatory, anti-microbial, hypolipidemic activity, antidiabetic activity, anti-carcinogenic activity, etc. (Dorman and Deans, 2000; Pizzale *et al*., 2002; Sokovic *et al*., 2002; Lampe, 2003; Srinivasan, 2005). All these biological activities of herb and spice plants consecutively characterises their medicinal properties.

The increasing demand for herbs and spices that played an important role in world history stimulated by the exploration of the globe and the initiation of trade and cultural interaction between the countries over the world. Focus on herb or spice phytochemicals continues to increase because of their presumably safe nature. Moreover, despite the potentiality of herbs and spices to contribute more than just taste-and-flavour to our food, in many cases dietary recommendations do not yet set for their specific consumption. In recent times the work is only beginning to come forth, providing validation of traditional medicinal practices, and revealing more detailed investigation that must be pursued such as the effective amount of a particular herb or spice that need to be consumed in order to see the long-term health-promoting and health-protective benefits.

Natural antioxidants and antimicrobials present in herb-spice-based foods have gained considerable interest because of their huge commercial applications and therapeutic effects. As most of the synthetic antioxidants do not fall under the status of generally regarded as safe (GRAS), hence they are eliminated from many food applications. The increasing interest in the search for natural replacements for synthetic antioxidants has also led to the evaluation of antioxidant activity of a number of new

plant sources. Nowadays significance of natural antioxidants is increasing with the changes in the pattern of life style, increased intake of artificial additives through processed foods and drinks, stress and strain, air pollution etc. Intake of foods of plant origin with high amount of antioxidants helps to inhibit damages to the cell's macro molecular cascades, and to reduce the risk factor of deadly diseases caused by the free radicals. A variety of antioxidant molecules are present in different parts and tissues of plant. Vitamin A, C and E, and the phenolic compounds are common natural antioxidants present in plant based foods (Javanmardi *et al*., 2003).

Universally herbs and spices are valued as condiments in the national and international cuisine. Beyond that use they have been honoured as basic ingredients of incense, embalming preservatives, perfumes, ointments and cosmetics. Although these are abundantly used in making delicious foods and drinks, the traditional system of medicines to cure both infectious and degenerative diseases is still based solely on some of them. Lack of earlier reports on the diverse medicinal use of dietary herbs and spice plants showing antioxidant, antimicrobial, anti-quorum sensing and antidiabetic activities in both aqueous and solvent systems offered plenty of scope to explore them as new source of pharmacologically active compounds. To evaluate the biological activities of medicinal plant extracts, both *in vitro* and *in vivo* studies are undertaken. Hence the present investigation was carried out based on the following objectives:

- To carry out a survey work, collect and authenticate the commonly consumed dietary herbs and spices and to enlist the medicinally used herbs and spices
- To evaluate qualitative and quantitative phytochemical analysis of the secondary metabolites present in them
- To evaluate the antioxidant activity of selected herbs and spices
- To evaluate the antimicrobial and anti-quorum sensing activities of selected herbs and spices
- To evaluate the antidiabetic activity of selected herb and spice
- To isolate, purify and characterize the bioactive phytocomponent(s) showing biological activities.

CHAPTER 2

REVIEW OF LITERATURE

2. Review of literature

The Plant Kingdom represents a 'chemical treasure trove' of structurally and functionally diverse classes of bioactive phytochemicals. Plants, specially the medicinal and aromatic plants (MAPs) synthesize enormous numbers and amounts of secondary metabolites including reducing sugars, polyphenols, flavonoids, carotenoids, terpenoids, alkaloids, saponins and the aroma compounds. The influential effect of MAP extracts on health originally resulted from these biologically active compounds present in them. Recently, there is a great attention and concern involved both in food and pharmaceutical industries for the plant produced compounds because of their multi-functional properties and multi-biological activities. Studies on bioactivities of different MAPs including the medicinal herbs and spices have been conducted in recent decades, but further investigation is still needed to clarify the specific chemical based activity of plant extracts. Modern research on different bioactivities of plant includes antioxidant activity, antimicrobial activity, anti-quorum sensing activity, antidiabetic activity, anti-inflammatory activity, anti-tumour activity, and so on. Further, the relationship among bioactivity (antioxidant and antimicrobial activity) and phytochemical content (total phenolic and total flavonoid contents) of a large number of medicinal herbs and spices has not yet been systematically and thoroughly investigated. In addition, much of the early research focused only on the antimicrobial activity of some dietary herbs and spices, but there are reduced or scanty reports on anti-QS potentiality exhibited by them. Furthermore, search for potent antidiabetic principles from common herb and spice matter is still an immense amount of work to be done. So, an expanded research on biological activities by specific plant extracts is necessary to fully understand the protective roles of dietary herbs and spices in biological systems through the evaluation of bioactivities and phytochemical analyses.

In the present context, elaborately reviewed information of 'landmark' studies on the medicinal properties of dietary herbs and spices along with their bioactivities like antioxidant, antimicrobial, anti-quorum sensing and antidiabetic or anti-hyperglycaemic activities of commonly used dietary herbs and spices has been compiled with available data and information from the published literature of various sources. However, relevant earlier works from other plant species have also been incorporated for critical evaluation and logical conclusion of the outcomes.

2.1. Dietary herbs and spices

The food and beverage that influence a person's health are collectively termed as diet. Basically diet is recognized as the corner stone of a healthy life. Dietary components may include carbohydrates, proteins, lipids, vitamins, minerals, herbs, spices or other botanicals as a source of various phytometabolites. Thus herbs and spices that are exclusively used in regular diets are known as dietary herbs and spices. But the definition and distinction of dietary herbs and dietary spices are not consistent.

Spices are often referred to as the non-leafy parts of plants which are used for seasoning and flavouring food, while herbs are generally defined as the leafy parts of low-growing shrubs or herbaceous plants (Kumar *et al*., 1997). Fresh or dried, they are used in small amounts for flavouring, aroma or colour in cooking. Herbs are generally considered a sub-set of spices. Examples of some popular culinary herbs include basil, caraway, chives, dill, marjoram, parsley, oregano, sage, rosemary, savory,celery and thyme leaves. These can be used as fresh or dried. Dried forms may be of whole, crushed, or ground type (Rathore and Shekhawat, 2008). Contrarily spices are defined by the US Food and Drug Administration (FDA) as "aromatic vegetable substances, in the form of whole, broken, or ground, whose significant function in food is seasoning rather than nutrition". The Cambridge Dictionary defines spice as "a substance made from a plant that is used to give a special flavour to food". Other dictionaries mention spices as "various pungent, aromatic plant substances, such as cinnamon or nutmeg, used to flavour foods or beverages" or "something that adds zest or flavour". Spices are used as medicines, cosmetics, perfumes, food preservatives and even in sacred rituals. Spices usually come from different plant parts such as the bark (cinnamon), bulb (garlic, onion), root (ginger), buds (cloves), seeds (black mustard, yellow mustard, poppy and sesame), berry (black pepper), or the fruit (allspice, paprika, star anise), aromatic seeds (cumin), and even the stigma of a flower (saffron) of tropical plants and trees. Many of the aromatic seeds known as spices are actually gathered from plants when they have finished flowering. It is difficult to differentiate the herbs from spices on the basis of whether the plants are herbaceous or woody, or whether the utilized parts are leaves or other plant organs. For example, both basil and rosemary are generally considered as herbs, but basil is herbaceous and rosemary is woody in nature. Seeds of coriander and dill are often considered as spices, but leaves of such plants are usually counted as herbs. When referring to the stem and roots of coriander, which are

used in cooking, and to onions, garlic and the bulb of fennel, these parts of these plants tend to be classified along with herbs, as they are often used fresh and applied in a similar way to cooking.

Thousands of chemical compounds have been identified in plant based foods. Herbs and spices are the rich source of diverse chemical compounds. Herbs and spices are generally used for nutrition, seasoning, beverages, cosmetics, dye and smoke, medicinal and industrial purpose. Herbs and spices can have complementary and overlapping actions, including antioxidant effects, modulation of metabolic detoxification, stimulation of the immune system, reduction of inflammation and antibacterial, antifungal and antiviral effects in human (Lampe, 2003).The aroma or smell of the original herb or spice is dependent on its chemical composition mainly essential oils or volatile oils. In many cases no single component has the characteristic aroma, but a complex mixture influences the overall odour quality. In addition to their aroma and pungency factors, spices contain variable amounts of protein, fat, carbohydrate, small quantities of vitamins (e.g., carotene, thiamine, riboflavin, and niacin) and inorganic elements (calcium, magnesium, manganese, phosphorus, potassium, chlorine, copper, iron, sodium, and zinc). Some spices also contain fatty acid, starch, sugars, cholesterol, and fibre. Some spices like paprika, turmeric and saffron have the advantage of not only giving a flavour but also giving attractive colours to foods.

So far numerous studies on antioxidant properties of many spices have been evaluated using different assay methods (Dorman *et al*., 2000; Exarchou *et al*., 2002; Dorman *et al*., 2003; Ninfali *et al.*, 2005; Albayrak *et al*., 2011). However, the wide variety of oxidation systems and ways to measure activity used in antioxidant assessment make it difficult to directly compare the results from different studies. Even though intensive studies on the bioactive components and their total content in many spices have been carried out, the phenolic identification data are insufficient and incomplete. In particular, quantitative data on the individual phenolics in the spices is currently lacking. Also, there are few comparisons of phenolic constituents identified in various spices from different spice families. The structure-activity relationships of phenolic compounds in the spices have not been thoroughly discussed and revealed. Moreover, the relationship between total antioxidant activity and total phenol content of a large number of spices was not systematically investigated before. Many

researchers claimed that the phenolic compounds in spices were responsible for their antioxidant activity, but few could establish real correlative relationships and provide convincing statistical data to reveal the relationship between the activity and phenolics on the basis of large numbers of herbs and spice samples.

2.2. Dietary herbs and spices as rich sources of medicinal phytochemicals

Phytochemicals are the chemical constituents of organic nature which are formed in plants through the activity of their individual cells by enzymatic process called biosynthesis. Phytochemicals are generally categorized under plant secondary metabolites (PSMs). The medicinal properties of plant materials are typically resulted from the combinations of secondary metabolites present in the plant tissues. Although PSMs have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the eco-physiology and defence strategy of plant species. However, recent research has indicated the therapeutic roles of PSMs as medicinable or pharmacological agents. The therapeutic effect of plants can be attributed to particular "active principle" or "chemical entity". Pharmaceutical industries all over the world are in search of new drug leads from plant resources. The most important of the bioactive phytochemicals of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al*., 2005). The advent of high-throughput, activity-based *in vitro* and *in vivo* bioassays coupled with candidate plant species from painstaking ethno-pharmacological research has resulted in the discovery of new pharmaceutical drugs. According to Harborne (1973) phytochemical evaluation for pharmacologically potent drugs from plant species involves the following steps:

- Taxonomic authentication of plant sample
- Extraction of the plant material
- Separation and isolation of the plant constituents
- Characterization of the isolated and purified compounds
- Investigation of the biosynthetic pathways to particular compounds
- Quantitative and quantitative analyses
- Evaluation of biological activities.

Among the bioactive phytochemicals, phenolic compounds are one of the most diverse groups of plant secondary metabolites reported from a wide variety of fruits, nuts, herbs, spices, vegetables,legumes, seeds, stems and flowers as well as tea, wine, honey and propolis, representing a common food ingredient of the human diet (Cuevas-Rodríguez*et al*., 2010; Yao *et al*., 2011). Major classes of plant polyphenolics are phenolic acids, flavonoids, tannins, and lignins. Each class of polyphenols possesses particular chemical configuration that characterize them with specific functional attributes. Flavonoids are the most widely occurring polyphenol and are present in almost every vegetable-based food items consumed by human. Dietary flavonoids have gained much interest because of their many fold beneficial biological properties, which may play an important role in the maintenance of good health. Flavonoids act as potent antioxidants, free radical scavengers and metal chelators as well as strong inhibitor of lipid peroxidation. They exhibit different physiological activities including anti-allergic, anti-carcinogenic, anti-inflammatory, anti-arthritic, anti-hypertensive, and antimicrobial activities. Consumption of polyphenol-rich dietary plants including fruit, vegetables, herbs and spices has commonly been associated to a reducing of the risk of cardiovascular diseases in human population. Amongst the 9000 phytochemical compounds identified so far from plant groups, the largest group of polyphenolic compounds is the flavonoid family (Whiting, 2001). Flavonoids have been found to be the most abundant polyphenols in our diets. Family of flavonoids can be further divided into six subclasses according to the degree of oxidation of the oxygen heterocycle: flavones, isoflavones, flavanones, flavonols, flavonols, and anthocyanins (Puupponen-Pimia *et al*., 2001).

2.3. Medicinal attributes of some dietary herbs and spices

Throughout the centuries, plants have been a valuable source of natural products for maintaining human health and well-beings, especially in last few decades, with more intensive studies devoted to natural therapies. Till now, folk wisdom and traditional knowledge of herbal practices persist in influencing the modern culture over the synthetic medicines. Herbs and spices in food are regarded as biomedicine with a designation of new “magic bullet” or “fountain of youth”(Mandal *et al*., 2009). Regular intake of antioxidant compounds through foodstuffs can be recommended as a part of health promoting way of life. In India, the use of plant compounds for pharmaceutical purposes has gradually increased with time. India is one of the 17 mega diversity

countries in the world and has been recognized for its medicinal herbs and spices which exhibit a wide range of physiological and pharmacological properties (Arora *et al*., 2003). Current pharmacological researches are focused on their scientific merits, to provide evidence-based studies for their traditional uses and to develop either functional foods or nutraceuticals (Krishnaswamy, 2008). Plant based foods contain variety of phytochemicals such as flavonoids, phenolic acids, that show a remarkable pharmacological activity.

Members of the *Allium* family (garlic, onions, and chives); members of the Lamiaceae family (basil, mint, oregano, rosemary, sage, and thyme); members of the Zingiberaceae family (turmeric and ginger); licorice root; green tea; flax; members of the Umbelliferae family (anise, caraway, celery, chervil, cilantro or coriander, cumin, dill, fennel, and parsley); and tarragon have been reported to have immense medicinal uses (Caragay, 1992). Furthermore, these herbs and spices contain a variety of medicinable phytochemicals such as phytosterols, triterpenes, flavonoids, alkaloids, saponins, and carotenoids etc, which have been shown to be cancer protective. These beneficial substances act as antioxidants and electrophile scavengers, modulate the immune system, inhibit nitrosation and the creation of DNA adducts with carcinogens, control hormonal imbalance and metabolic pathways associated with the development of cancer, and persuade phase I or II detoxification enzymes (Haraguchi *et al*., 1995).

2.4. Biological activities of medicinal herbs, spices and other plants

Biological activity or pharmacological activity is described as the evaluation of beneficial or adverse effects of a bioactive extract or pure compound through *in vitro*, *ex vivo* and *in vivo* tests. Such activity is exerted by the plant's active phytochemical or pharmacophore. Generally, biological activity is dependent on the dosage of crude extracts or isolated pure compounds. Biological activity determines the medicinal effectiveness of a plant extract. For the preparation of herbal drugs, quantification of different phytochemicals, isolation and identification of the active principle from authenticated plant samples must be followed. In recent times the claims of therapeutic efficiency and lack of toxicity of many medicinal plants have been scientifically established. However, there are thousands of plant species with questionable value among the enormous repertory of indigenous herbal drugs. It would be praise worthy step if one tries to select the best out of them. There are large numbers of plants, which have to be screened thoroughly for their useful activities.

2.5. Overview of antioxidant concept

The concept of antioxidant emerged in the 19th century when engineers discovered a particular chemical substance which was able to prevent the "metal corrosion" by shutting off the oxidation process, is known as the “antioxidant”. From the mid-20th century to till date the innovative mind of human renders these “chemicals” from metal to food to cells to extend life expectancy and quality (Matill, 1947; Resveratrol, 2011). Early research is associated only with the role of antioxidantsin preventing the oxidation process of unsaturated fats (German, 1999). However, it was the identification of antioxidant micronutrients such as vitamin A, vitamin C, and vitamin E that revolutionized the field of science and led to the realization of the fact that antioxidant molecules have immense importance for the cellular biochemistry of living organisms. The possible mechanism ofantioxidant action was explored fot the first time when it was recognized that a substance with antioxidant activity is likely to be one that is itself readily oxidized (Moreau, 1922). Recent studies on how antioxidant molecules prevent the lipid-peroxidation process led to the identification of such molecules as reducing substances that terminate oxidation process by scavenging free radicals before they can harm the living system (Wolf, 2005; Lobo *et al.*, 2010).

2.5.1. Cellular metabolism and generation of life-threatening free radicals

Life on earth of aerobic organisms is solely dependent on di-oxygen (O_2) molecule that in its ground state is relatively un-reactive; but partial reduction of it gives rise to life-threatening reactive oxygen species (ROS) which includes singlet oxygen ($^1O_2$), superoxide anion ($\cdot O_2^-$), hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2) etc. The production of oxygen-based free radicals (OFRs) is baneful to all the aerobic organisms. This variety of molecules is generated endogenously as by-products during the mitochondrial electron transport of aerobic respiration or by oxido-reductase enzymes or metal-catalyzed oxidation and exogenously by environmental factors such as UV light, ozone, tobacco smoke, xenobiotics, ionizing radiation, herbicides, pesticides (Cadenas *et al.,* 1997; Halliwell and Gutteridge, 1999) etc.Oxidative stress, a result of imbalance between the antioxidant defence system and the formation of ROS, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases (Gulcin *et al*., 2006; Katalinic *et al*., 2006; Prakash *et al.,* 2007). Despite the presence of strong antioxidants, defense mechanism to

counteract the ROS and to minimize plausible oxidative damage, ROS-mediated damage to cellular macromolecules accumulates during the lifetime of organisms. Consequently, ROS have been implicated in many deleterious diseases and disorders presented below (Halliwell and Gutteridge, 1984; Maxwell, 1995; Halliwell, 2000; Young and Woodside, 2001; Moskovitz *et al*., 2002; Heinecke, 2003). These diseases can be prevented or cured by regular intake of dietary antioxidants (Atoui *et al*., 2005; Alasalvar *et al*., 2005).

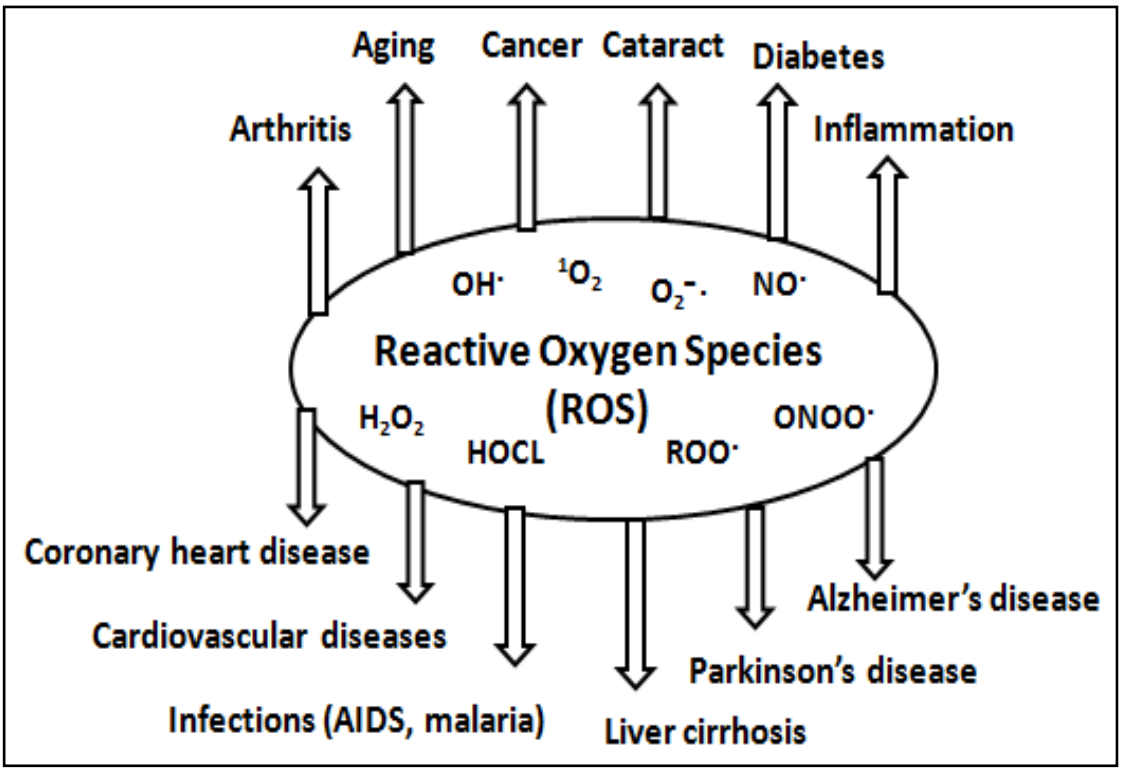

Figure 1. Diseases and disorders caused by reactive oxygen species, ROS. Here, hydroxyl free radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxyl radicals ($ROO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$), andnitric oxide radical ($NO^{\bullet}$). Over production of such chemicals generate oxidative stress and modification of cellular macromolecules of biological importance leading to cause different health issues (Modified after Parekh, 2007).

2.5.2. Definition and classification of antioxidants

A now old definition attempts to define an "antioxidant" as ''any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate'' (Halliwell and Gutteridge, 1999). Over the years this definition has come to be recognized as ''clearly imperfect''

(Halliwell, 2007) because it excluded proteins and other macromolecules to be considered strictly as antioxidants. A new, much more general concept defined "antioxidant" as ''any substance that delays, prevents or removes oxidative damage to a target molecule'' (Halliwell and Gutteridge, 2007).

Classifying antioxidants generally done according to their action, function or nature. Classically, antioxidants are of three types depending on their mechanism of action: (a) chain breaking antioxidants (vitamin E, polyphenols), (b) preventive antioxidants (intracellular enzymes, such as CAT, SOD etc.), and (c) complementary antioxidants (vitamin C, β-carotene, flavonoids) (Williams and Elliott, 1997). Based on physiological function, another commonly used classification antioxidants is to divide them into three distinct groups: primary, oxygen scavenging antioxidants; secondary, enzymatic and chelating/sequestering antioxidants; and tertiary antioxidants (Dapkevicius, 2002, Butnariu and Grozea, 2012). During the past two decades, several naturally occurring phytocompounds have been included into the panel of antioxidant category that prove their antioxidant efficacy against oxidation of unsaturated fats and oils and most of them fall into the multifunctional category. Classification of antioxidants according to the mode of activity as primary and secondary is preferred in the present discussion. Primary antioxidants interrupt the free radical chain formation in oxidative reactions by transfering the hydrogen atom from the phenolic hydroxyl groups, thus forming stable free radicals(examples: carotenoids, catalase, glutathione peroxidase, ferritin, lactoferrin, selenoprotein, transferrin, etc.). On the other hand, secondary antioxidants trap free radicals, scavenge ROS, chelate metal ions, regenerate primary antioxidant molecules, or act as peroxide destructors (Haworth, 2003). Tertiary antioxidants repair the oxidized molecules (some proteolytic enzymes, enzymes of DNA, etc.) through sources like dietary or consecutive antioxidants.

Depending on the origin and synthesis, antioxidants may be of natural and synthetic. Most common synthetic antioxiants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT),propyl gallate (PG) and tertiary-butylhydroquinone (TBHQ), etc. and natural antioxidants such as ascorbic acid and tocopherol, are extensively used in cosmetics and food industries because of their protecting ability against oxidation-reduction reactions (Roberto *et al.,* 2000). It is known that BHT and BHA retard lipid oxidation, however, due to increasing consumer awareness of health aspect; their use is slowly replaced by alternative antioxidants,

which are devoid of toxic effect. Recently, there is growing interest in the use of natural antioxidant in food products. Naturally occuring antioxidants are perceived as safe, non-toxic and beneficial for human health; however due to their expensiveness, generally they are notcommercialized in a wider aspect. Sources of natural antioxidants are plants such as herbs and spices, and such materials have been used throughout history for flavouring and preservative agent. Besides playing an important role in physiological system, antioxidants have been used in food industry to prolong the shelf life of foods, especially those rich in poly-unsaturated fatty acids (PUFAs). These components in foods are readily oxidized by molecular oxygen and are a major cause of qualities deterioration, nutritional loses, off-flavor development and discoloration. The greatest advantage of synthetic antioxidants is related to their availability and low cost. Other advantages are their well studied chemical and technological properties, which in most cases meet the demands of producers. That makes synthetic antioxidants dominating in the world market. Among natural antioxidants, however, only a small percentage has been thoroughly analyzed and even fewer are actually being used. Up to now only tocopherols, carotenoids, ascorbic acid and its derivatives, as well as extracts from rosemary and sage have been industrially applied in foods (Dapkevicius, 2002).

There are a wide variety of naturally occurring antioxidant molecules based on their composition, physical and chemical properties, and mechanisms of action. Some of the major classes of antioxidants are mentioned in Table 1.

2.5.3. Antioxidant defence in human body system

The human body employs three general categories of antioxidants to safeguard against free radicals namely endogenous antioxidants, dietary antioxidants, and metal-binding proteins. Humans have relied on plants to supplement their bodies with natural defence strategies through consumption of dietary antioxidants. There is considerable interest in dietary antioxidants as bioactive components of food. Many plants (fruits, vegetables, spice, medicinal herbs, etc.) contain a wide variety of free radical scavenging molecules and provide a rich source of natural antioxidants. The antioxidant activities in these plants range from very slight to extremely great.

Table 1: Major classes of antioxidant with their mechanism of action

Antioxidant classes	Examples	Mechanism of action	Reference
Enzymatic antioxidants	Superoxide dismutase (SOD), Catalase (CAT), Glutathion peroxidise (GPx), etc.	Transform reactive oxygen species and reactive nitrogen species into the stable compounds	Mates *et al*., 1999
High molecular weight antioxidants	Albumin, transferin, ceruplasmin, etc.	Restrict production of metal catalysed free radicals	Khanam, 2004
Low molecular weight antioxidants	Tocopherol, quinines, bilirubin (lipid soluble); ascorbic acid, uric acid (water soluble) etc. and some polyphenols.	Effective inhibitors of free radical processes	Blois, 1958; Skorokhod and Kurdysh, 2014
Antioxidant minerals	Selenium (Se), Copper (Cu), Manganese (Mn), Zinc (Zn), Chromium (Cr) etc.	Forms metalloenzymes that have critical role in protecting the intracellular constituents from oxidative damage	Gupta and Sharma, 2006; McDowell *et al*., 2007
Antioxidant vitamins	Vitamin A, C, E, K	Prevent peroxidation damage in the biological system	Mantena, 2003
Hydrophilic antioxidants	Ascorbic acid, glutathione, uric acid	Free radical scavengers	Kapoor Mehta and Gowder, 2015
Hydrophobic antioxidants	Ubiquinol, carotenes, α-tocopherol	Protect cell membranes from lipid peroxidation	Halliwell, 2000

These include compounds such as carotenoids, phenolics, terpenoids, nitrogen compounds, vitamins, and some other endogenous metabolites, which are rich in antioxidant activity. The best known natural antioxidants that have proven important in the food industry and in human health are carotenoids, tocopherols and vitamin C (Maizura *et al.,* 2011).Dietary antioxidants may act as free radical scavengers, hydrogen donors, electron donors, singlet oxygen quenchers, peroxide decomposers,

enzyme inhibitors, synergists, and metal chelators. In both of the intracellular and extracellular environment, enzymatic and non-enzymatic antioxidant defence systems exist to detoxify free radicals. According to Niki (1993) and Lobo *et al*. (2010) defence performance exerted by the antioxidant molecules occurs at four line of defence such as preventive, radical scavenging, repair and *de novo*, and the adaptation. Here is a brief outline of antioxidant defence:

- **The first line of defence** is shown by the preventive antioxidants, which retrict the formation of free radicals. Some antioxidant enzymes reduce hydroperoxides and hydrogen peroxide beforehand to alcohols and water, respectively, without generation of free radicals. Glutathione peroxidase, glutathione S-transferase, peroxidise and phospholipid hydroperoxide glutathione peroxidise are able to decompose lipid hydroperoxides to alcohols. Glutathione peroxidase and catalase reduce hydrogen peroxide to form water.
- **The second line of defence** is the radical-scavenging antioxidants that help to terminate chain initiation and/or break the chain propagation reactions. Various endogenous radical-scavenging antioxidants are included in this category: vitamin C, uric acid, albumin, bilirubin, thiols, vitamin E and ubiquinol. Vitamin E is accepted as the most potent radical-scavenging antioxidant.
- **The third line of defence** is the repair and *de novo* antioxidants. The proteolytic enzymes, proteases, proteinases and peptidases, present in the cytosol and mitochondria of mammalian cells, identify, make target, degrade, and remove oxidatively modified proteins and prevent the accumulation of oxidized proteins in the cells. The DNA repair systems also play an important role in the total defense system against oxidative damage. Glycosylases and nucleases, which repair the damaged DNA, are well known examples.
- **The forth line of defence** is called the adaptation where the signalling for the production and reactions of free radicals influences the formation and transport of the specific antioxidant to the right site for action.

2.5.4. Dietary herbs and spices as functional foods and nutraceuticals

Recent research has pointed out that nutrition plays a vital role in the prevention and reduction of chronic diseases of human beings, as most of them can be associated with the diet. Functional foods employ the concept of considering foods not only necessary

for living but also for the maintenance of mental and physical well-being, reducing the of risk factors of life-style related diseases or enhancing certain physiological functions for health benefits (López-Varela *et al*., 2002). A food is considered as functional if it is satisfactorily demonstrated to affect beneficially one or more physiological functions in the body system, beyond its nutritional aspects, in a way which is relevant to either promotion of a state of health and wellness, and/or reduction of the risk of a disease or a pathologic condition. Simplest example of functional food is the whole foods. Herbs and spices including the green leafy vegetables like mustard, turmeric, coriander etc. extensively used in Indian cuisine, also fall under this category. "Nutraceutical" is a term coined by Stephen DeFelice in 1979 (DeFelice, 1992) and defined as "a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease". In other words, a nutraceutical is any non-toxic food extract supplement that has scientifically proven health benefits (Dillard, 2000). Examples of nutraceuticals may account from isolated nutritional components, dietary supplements, and "designer" food, herbal products, and processed products such as cereals, soups, and beverages. The major active nutraceutical ingredients from plant origin are polyphenols and flavonoids. These typical phytochemicals can act as potent antioxidants and metal chelators, and have long been recognized to possess different biological activities including antidiabetic, anti-inflammatory, anticarcinogenic, hepatoprotective, antithrombotic, antibacterial, antifungal, and antiviral activities (Tapas, 2008).

Phytochemicals contributing the functional properties to foods are vitamins, minerals, dietary fibers, antioxidants, polyphenolics, oligosaccharides, essential fatty acids (ω-3), and lignin. To some extents these chemical constituents are present in every herb and spice. Indian systems of traditional medicine have the belief that complex diseases of human beings can be treated with a combination of botanical extracts unlike in west, with single drug (Lobo *et al*., 2010). Hence whole foods are used as functional foods rather than supplements. Dietary constituents having functional attributes of herbs and spice include onion, garlic, mustard, turmeric, ginger, chilies, cinnamon, clove, saffron, curry leaf, fenugreek etc.(Vidya and Devasagayam, 2007).

2.5.5. Antioxidant activity of herbs and spices

Medicinal herbs, spices and other botanicals are known to possess a variety of biological activities and antioxidant properties (Zheng and Wang, 2001). For at least 50 years, herbs and spices have been a target of investigation because of their excellent antioxidant properties. As early as 1952 many herbs and spices were examined and found to retard the oxidation (Srinivasan, 2005). Many studies indicated that rosemary, sage, oregano and thyme in the family Lamiaceae, exhibited high antioxidant activity (Zheng and Wang, 2001; Pizzale *et al*., 2002). Researches also showed that black pepper, clove, cinnamon, and coriander had antioxidant properties (Gulcin *et al*., 2004; Melo *et al*., 2005). Phenolic compounds in these plant species are closely associated with their antioxidant activities (Pridham, 1995). The antioxidant effect of phenolic compounds is mainly due to their redox properties, and is the result of various possible mechanisms of action: free radical scavenging activity, transition metal-chelating activity and/or singlet oxygen-quenching capacity (Rice-Evans *et al*., 1995; Rice-Evans *et al*., 1997; Chen and Ahn, 1998; Luiz *et al*., 2002). Extracts of different herbs and spices are also known to play a crucial role in stabilizing lipid-peroxidation and inhibition of various oxidizing enzymes. These multiple potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for health-beneficial effects, and also offer a possibility to use phenolic compounds or plant extracts rich in them to extend shelf-life of lipid-rich foods (Yanishlieva and Marinova, 2001).

Shobana and Naidu (2000) exploredthe potential antioxidant activities of garlic, ginger, onion, mint, cloves, cinnamon and pepper extracts using enzymatic lipid peroxidation method. Hydroalcohol extract (1:1) of these spices inhibited oxidation of fatty acid, linoleic acid in presence of soybean lipoxygenase in dose-dependency. Among the spices tested, cloves exhibited highest while onion showed least antioxidant activity. The relative antioxidant activities decreased in the order of cloves > cinnamon > pepper > ginger > garlic > mint > onion. Synergistic antioxidant activity was exhibited by spice mix namely ginger, onion and garlic; onion and ginger; ginger and garlic on anti-lipid peroxidation model. The antioxidant activities of spice extracts were found to to be retained even after boiling for 30 min at 100°C, indicating that the presence of thermo-tolarent chemical constituents. According to them the antioxidant activity of these dietary spices possessed potential health benefits.

Albayrak *et al*. (2011) investigated methanol extracts, infusions and decoctions of Cassia angustifolia (Senna tea), Foeniculum vulgare (fennel), Pimpinella anisum (anise), Laurus nobilis (laurel), Tilia vulgaris (linden tea), Urtica dioica (nettle), Petroselinum crispum (parsley) and Anethum graveolens (dill) for their antioxidant activity using phosphomolybdenum and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays: The results showed that the highest antioxidant activity was found in methanolic extract of linden tea. Linden methanolic extract also contained the highest amount of phenolic compounds. Kim *et al.* (2011) evaluated the radical scavenging-linked antioxidant activities of hot water extracts from commonly used herbs and spices in Korea. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical and superoxide anion scavenging activities of bay extract were 39.5% and 22.1%, respectively. The hydroxyl radical scavenging activity was in order of dill (50.0%) > bay (31.3%) > garlic (27.9%) > white pepper and black pepper (15.1–15.3%) > onion (10.1%) extracts. They reported that bay extract had the highest total phenolic content (17.86 μg CE/g). High correlation coefficients were found between the total phenol content and DPPH radical scavenging activity (R = 0.9162). The results indicated that herbs and spices have high antioxidant activity that may be partly due to the phenolic compounds.Antioxidant activity of the methanol extract of Zingiber officinale was determined by Amir *et al.* (2011) using Reducing power assay, Superoxide anion scavenging activity assay, Hydroxyl radical scavenging activity assay, Nitric oxide scavenging activity assay, DPPH free radical scavenging assay, and hydrogen peroxide method. Preliminary phytochemical screening revealed that the extract of Z. officinale possesses flavonoids, phenolic compounds and volatile oils. In the present investigation, quantitative estimation of phenols and flavonoids was carried out by colorimetric methods, using Folin-Ciocalteu reagent and aluminium chloride respectively. They reported that Z. officinale extract showed significant activities in all antioxidant models compared to the standard antioxidant reference in a dose dependent manner and remarkable activities to scavenge free radicals may be attributed to the presence of high amount of hydrophilic phenolic compounds. The results of this study indicated that Z. officinale extract is a potential source of natural antioxidant. Further, Nahak and Sahu (2011) studied the antioxidant activity and phytochemical compounds analysis of *Piper nigrum* and *Piper cubeba* in different solvent system. In preliminary screening and confirmatory test, presence of alkaloid, flavonoids, tannins and saponins was confirmed. High antioxidant activity via DPPH radical scavenging assay was reported in ethanol extract of *Piper cubebai.e.*

77.61±0.02% in comparison to *Piper nigrum* extracts with 74.61±0.02% with IC_{50} values 10.54±0.12 μg mg^{-1} and 14.15±0.02 μg mg^{-1} respectively.

Patel and Jasrai (2012) made an attempt to determinethe antioxidant capacity of hexane extracts of *Anethum sowa* seeds, *Cinnamomum zeylanicum* bark, *Cinnamomum tamala* leaves, *Citrus sinensis* fruit peel, *Coriandrum sativum* seeds, *Cuminum cyminum* seeds, *Cymbopogon caesius* leaves, *Elettaria cardamomum* fruits, *Foeniculum vulgare* seeds, *Illicium verum* fruits, *Mentha piperita* leaves, *Myristica fragrans* fruit, *Ocimum sanctum* leaves, *Santalum album* wood, and *Trachyspermum ammi* seeds. Antioxidant activity was screened through the DPPH radical scavenging assay. All the plant extracts had shown an excellent antioxidant activity where maximun activity was recorded in *Ocimum sanctum* extract with 76.608 ± 0.063 % for DPPH free radical scavenging.

Kouřimská *et al.* (2013) studied the antioxidant activity of oregano, Greek oregano, marjoram, summer savory, rosemary and two varieties of leafy. The activity of tested dry herbs was significant (protection factors for fat oxidation ranging from 1.7 to 11.4) and linearly increased at concentrations from 10-100 g kg^{-1}. Prooxidant effect did not occur under the Schaal test conditions. The antioxidant activity of plants decreased in the following order: marjoram > Greek oregano > flat parsley > rosemary > summer savory > curly parsley > oregano, which did not correspond with their total phenol content (TPC). Panpatil *et al*. (2013) evaluated the antioxidant activity of spice extracts from ginger, turmeric and garlic by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The antioxidant activity of these spices was found to be in the order of ginger > turmeric ≥ dry garlic > fresh garlic. The study confirmed spices like ginger, garlic and turmeric have significant antioxidant property.

Popova*et al*. (2014) investigated *in vitro* antioxidant activity and total phenolic compounds content in 70% ethanol extracts of *Chrysanthemum balsamita* L., *Melissa officinalis* L. and *Allium bulgaricum* L. growing in Bulgaria. They determined the antioxidant activity of the extracts through ABTS radical scavenging activity, DPPH radical cation decolorization activity, ferric reducing antioxidant power (FRAP) assay and copper reduction (CUPRAC) assay. Total polyphenolic content was estimated to be in the range from 0.41 ± 0.08 to 2.71 ± 0.15 mg GAE g^{-1} of fresh weight. *M. officinalis* fresh leaves showed the strongest antioxidant activity.

Tchokouaha *et al*. (2015) studied the antioxidant capacities of vegetable and spices used in the preparation of traditional soups in different regions of Nigeria. The antioxidant capacity of the soups was evaluated by measuring the total phenolic content (TPC), total flavonoids content (TFC), Ferric reducing antioxidant power (FRAP) and radical scavenging activity (DPPH). From the results obtained it was observed that based on the individual spices antioxidant, *Adansonia digitata* and *Hibiscus sabdariffa* had the highest TPC and FRAP while *Monodora myristica* had the highest TFC. On the other hand, *Corchurus olitorious* from the Southern Region of Nigeria had the least TPC followed by *Gongronnema latifolium*, while *Pterocarpus soyauxil* had the least TFC followed by *Corchurus olitorious*.

Different extracts from different spices and herbs *viz*. cumin, cinnamon, clove, ginger and thyme by using cold and hot extract methods were tested by Abdelfadel *et al*. (2016). They investigated the effect of extraction method on antioxidant activity, chemical compositions and total phenolic compounds for each plant. Results showed that the main phenolic compounds in thyme and cumin were found to be caffeic, cinnamic, pyrogall, vanillic and salicylic acids; ginger and cinnamon contained pyrogall, caffeic, vanillic and cinnamic acids, and clove with caffeic, catechol, cinnamic, gallic, pyrogall and vanillic acids. The main flavonoid compounds in thyme and cumin were naringin, hisperiden, hespertin, rosmarinic and rutin; whereas ginger and cinnamon was with rutin; and clove with apegenin, hisperiden, rosmarinic, rutin and naringin. Hot water xtract led to increase the amount of total phenolic compounds of thyme, cumin and cinnamon from 302.0-340.6, from 270.3-299.0 and from 270.0-282.0 mg GAE/100 mL of extract, respectively. Antioxidant activity evaluated through DPPH scavenging activity was also found to be increased for thyme, cumin and cinnamon extracts from 82.35-91.93%, from 16.47-48.91% and from 24.37-53.28%, respectively. They also reported that the total phenolic content of clove and ginger extracts were decreasing from 268.6-241.3 and from 376.0-348.0 mg GAE/100 mL of extract, respectively. While, DPPH scavenging activity was found to decrease from 15.97-12.10% and from 93.60-89.58% for clove and ginger extracts, respectively.

2.6. Overview of antimicrobial activity

Microbial species affect human life more than any other life forms with which we share the blue planet, but our understanding about these invisible inhabitants has developed in a staggering pattern. Usually when we are threatened by infectious microorganisms,

we visualized these potentially lethal organisms from which we must remain isolated by sanitation and which we have to kill by immunization and chemical antimicrobial compounds. Infectious diseases are defined by the World Health Organization as diseases caused by microbes; these microbes may include bacteria, fungi, protozoa, and viruses (WHO, 2010). These organisms may be found in either the environment or participate in normal commensal flora for humans, plants, or other animals. When in their natural habitat, these microbes are typically kept in balance by the surrounding flora. Thus, many of these organisms can be beneficial to their environment by helping nutrient turnover; but when these organisms are introduced into a foreign niche within the human body, they may cause diseased symptoms. Diseases caused by bacterial species can range from severe to mild and may include wound infections, endocarditis, septicaemia, pneumonia, colds, and eye and ear infections (Todar, 2008). Infectious diseases continue to be the leading causal factor of death and illness worldwide (Livermore, 2004; Morens *et al*., 2004). The discovery of Penicillin by Alexander Fleming in 1929 introduced the era of antimicrobial chemotherapy, which has saved millions of lives by controlling many serious bacterial infections (Fleming, 1929; Drews, 2000). Over the years, antimicrobial or antipathogenic drugs had saved the lives of millions of people successfully by easing the sufferings. From 1940s to 1980s many classes of antibiotics had discovered and for many years, conventional antibiotics were thought to be the "end-all" curative agent for several microbial infections and were considered to be the wonder drug in treating infections caused by Staphylococci, Streptococciand other Gram-positive organisms (OTA, 1995). But extensive use, misuse and abuse of antibiotics, an ever increasing frequency of bacterial mutations has resulted with the incidence of antibiotic resistance and the horizontal transfer of resistance genes to other bacteria of the same or different species shown to create bacterial populations with

- Enzymatic modification or alteration of active sites for target drugs;
- an increased ability to degrade antibacterial drugs directly;
- decreased cellular permeability;
- decreased affinity for the antibiotic;
- increased efflux of different antibiotics; or, finally,

- development of biofilm communities through quorum sensing system (Lewis, 2001; Sheldon, 2005).

Of late drug-resistance to human and animal microbial pathogens is one of the best-documented cases of biological evolution and a serious problem in developing as well as developed countries. More than one ton of antimicrobial drugs per day are consumed in some European countries, which has resulted in the emergence and spread of a vast amount of antibiotic resistance determinants or "Superbugs" among bacterial and other microbial populations, thus creating a critical public health problem. World Health Organisation officially deemed antibiotic resistance is the number three public health concern of the 21st century (Levy, 2002). In 2010, the Infectious Disease Society of America (IDSA) launched the "10 x' 20" initiative to assist the development of 10 new antibiotic drugs by the end of 2020. The main focus of this drug discovery is to target "ESKAPE" pathogens namely *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumamnii, Pseudomonas aeruginosa,* and *Enterobacter* species. Literally, "ESKAPE" signifies also the adaptive ability of these microorganisms to "escape" present-day antimicrobial treatments (IDSA, 2010). Clearly, in present conditions exploration of new and alternative antibiotic treatments for infectious diseases is vital and in urgent need.

2.6.1. Plants as biological source of novel antimicrobials

Natural antimicrobial agents derived from plant sources have been recognized and used in medical cares for centuries. Worldwide there is almost 250,000 to 500,000 species of plants known to man, of which more than 10% are used for medicinal purposes (Lewis, 2006; Pliego, 2007). The curative effects of medicinal plants have been extensively documented throughout history. Early civilizations, such as the Indian, Chinese or others in the Middle East have documents describing the usages of plants as medicinal remedies five thousand years ago. The recent trend of the general public to reconsider the alternative herbal medicine has attracted the attention of the drug industry and the scientific community to generate reliable information regarding the claimed therapeuticeffects of medicinal plants (Li, 2003). Plants, flowers, herbs and spices are widely recognized to have antimicrobial properties and were used by ancient people to treat the pathogenic diseases. Advanced studies in recent times have been conducted to analyze the effectiveness of natural antimicrobials from botanical origin, and to isolate and purify specific phytochemicals responsible for their antibacterial or bactericidal,

and antifungal or fungicidal effects. The majority of studies curried out on phytochemicals have attempted to correlate antimicrobial activity with phenolics, specifically phenolic acids and flavanoids (Ahn *et al.,* 2004, Ozkan *et al.,* 2004, Cushnie and Lamb 2005). Dietary phytochemicals from medicinal herbs and spices are the rich sources of carotenoids, phenolics, flavonoids, alkaloids, terpenoids, lectins, and polypeptides, nitrogen-containing compounds, and organosulfer compounds and are suggested that their commercial use as natural antimicrobials could be expanded. Due to their contribution as antibacterial and antifungal activity, classes of phenolic compounds and antioxidant biomolecules were the hot topic of anti-infective research for many years (Cushnie and Lamb 2005; Fattouch *et al*., 2007; Szabo *et al*., 2010). The anti-pathogenic activities suggested that phenolics and flavonoids can be used as natural chemotherapeutic drugs, food preserving agents and disinfectants (Dorman and Deans 2000). These classes of phytochemicals can significantly affect the growth and cellular metabolism of microorganisms depending on their constitution and concentration (Alberto *et al.,* 2006; Nazzaro *et al*., 2009).

Chemical compounds from medicinal plant species showing antimicrobial activities have the potential for fulfilling the present demand to replace the conventional antibiotic therapy as because their structural configurations are different from those of the much studied microbial sources, and therefore their mode of actions are very likely to differ. There is tremendous interest in correlating the phytochemical constituents of medicinal plants with their biological and pharmacological activities. Screening the active compounds from plants has lead to the discovery of new pharmaceutical drugs which have efficiencies to cure various diseases with less or no side effects. Presently, medicinally important plants are the key source of antibiotic leads for pharmaceuticals currently used around the world. These pharmaceutical drugs either contain plant-derived chemical components, or compounds derivatized from plant based principles. Plant-derived antimicrobials are believed to be risk-free, safe and superior to chemically synthesized antibiotics used in chemotherapy for improvement of human health. The human body recognizes components that derived from plants and has sophisticated means for metabolizing such compounds. The naturally occurring bioactive compounds in plants may sometimes have less potency than synthesized drugs; however, as these are consumed in significant amounts through

diet, they may provide long term physiological and therapeutic benefits without detrimental side effects (Espin *et al*, 2007).

2.6.2. Mechanism of action of plant-derived antimicrobials

The medicinal effects of plant-derived antimicrobials typically result from synergistic actions of secondary metabolites present in the plants or in the plant products. Plant secondary metabolites have defensive roles against pathogen attack, facilitating pollination by attracting pollinators, protective actions against abiotic and biotic stresses and their roles as plant growth regulators, modulators of gene expression, and in signal transduction at cellular level have also been shown (Kaufman *et al*., 1999; Wink, 1999). In comparison to synthetic antimicrobials based upon single chemical compound, phytochemicals from medicinal plants exert their pharmacological effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites (Tyler, 1999). Regarding the role of plant secondary metabolites as defensive chemical weapons, medicinal plant extracts represent a mixture of multiple chemical compounds having additive or synergistic actions at several target sites would not only ensure the medicinal effectiveness against a vast array of microbial pathogens but also decrease the chances of these harmful organisms for developing drug-resistance traits (Kaufman *et al*., 1999; Wink, 1999).

The antimicrobial activity of phytochemicals, especially the phenolic compounds and flavonoids are well established facts in recent literatures (Milovanović *et al*., 2007). According to Fattouch *et al*. (2007) and Xia *et al*. (2011), the mechanisms of action responsible for phytochemical toxicity to microorganisms include adsorption and disruption of cytoplasmic membrane structure and function, interaction with enzymes and metal ion deprivation, interruption of DNA/RNA synthesis and function, interference with intermediary metabolism,induction of coagulation of cytoplasmic constituents and interruption of normal cell communication or quorum sensing.

The antimicrobial action usually includes the following sequence of events: phytochemicals or phenolic compounds interact with the cell membrane, diffuse through the membrane *i.e.,* penetrate into the interior of the cell, and interact with intracellular constituents or cellular processes. It is reported that antimicrobial activity and mechanism of action of different plant-based antimicrobials are highly influenced by some factors namely type of the target cells (bacterial or fungal cell, Gram-positive or Gram-negative bacteria), and also by some environmental factors such as

hydrophilicity, concentration, temperature and *p*H (Denyer and Stewart, 1998). The effect of botanical compounds is expected to be very similar in action for both the microbial groups, Gram-positive bacteria and fungal organisms, where the main target is the cell envelope, whose disintegration and changes in permeability are followed by an efflux of the intracellular biomolecules and coagulation of cytoplasm (Kalemba and Kunicka, 2003).

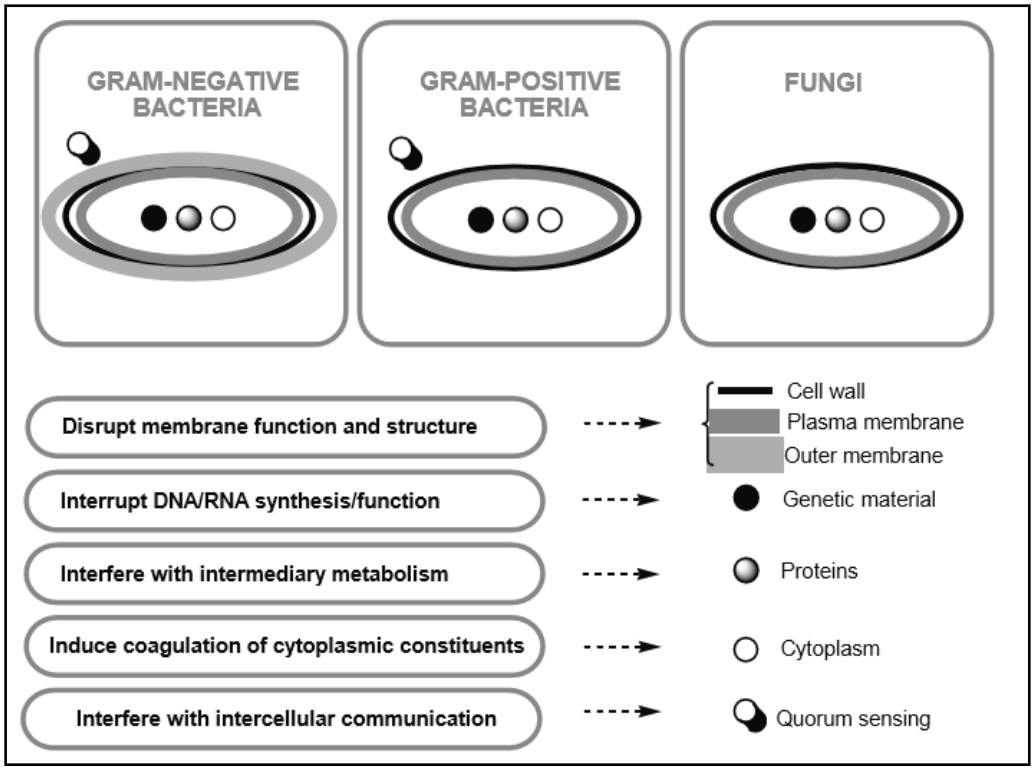

Figure 2. Mechanism of action of plant-derived antimicrobials on possible targets (Adapted from Radulović *et al*., 2013).

2.6.3. Antimicrobial activity of herbs and spices

Investigations have assigned to search the potent antimicrobials with a broad spectrum of activity against numerous pathogenic bacteria and fungi are always in highest priority of concern. Early researches reported that clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymusvulgaris*), oregano (*Origanum vulgare*) and vanilla (*Vanilla planifola, V. pompona, V. tahitensis*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), cilantro (*Coriandrum sativum*), tea tree oil (*Melaleuca alternifolia*) and finger-root extract (*Boesenbergia pandurata*) have antimicrobial activities (Casterton *et al.*, 2005; Davidson, 2005). In recent years many works have been executed to explore the antimicrobial potentiality of medicinal herbs

and spices and hence an attempt is made to review some of the most recent investigations.

Venugopal *et al.* (2009) studied antimicrobial effect of aqueous extracts of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), tulsi (*Ocimum tenuiflorum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*) and asafoetida (*Ferula asafoetida*) against *E. coli* using paper disc method, agar ditch method, turbidometry method. They reported that all the herb and spice extracts were able to inhibit the growth of *E. coli*, but thyme (herb) and turmeric (spice) were found to be most effective. Keskin *et al*. (2010) performed agar well diffusion method to evaluate the antimicrobial activity of some Turkish medicinal plant spices which are used in the traditional system of medicine. Extracts of *Alchemilla vulgaris, Laurus nobilis, Melissa officinalis, Silybum marianum, Camellia sinensis, Rosmarinus officinalis, Hibiscus* sp. and *Foeniculum vulgare* showed broad-spectrum antimicrobial activity against 10 pathogenic bacterial species and yeast, *Candida albicans* with zone of inhibition ranging from 4-32 mm, except *Erica vulgaris*. The most resistant organisms were *Escherichia coli* and *Salmonella typhimurium* and most susceptible organisms were *Candida albicans* and *Kocuria rhizophila.* MICs of crude extracts were ranging from 2.92 to 10 $\leq$ mg mL^{-1} and determined for the three highly active plant species that inhibit growth of *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Kocuria rhizophila, Enterococcus faecalis* and *Candida albicans.*

Albayrak *et al*. (2011) investigated methanol extracts, infusions and decoctions of Anethum graveolens (dill), Cassia angustifolia (Senna tea), Foeniculum vulgare (fennel), Laurus nobilis (laurel), Petroselinum crispum (parsley), Pimpinella anisum (anise), Tilia vulgaris (linden tea)and Urtica dioica (nettle) for their antimicrobial activity against bacteria and yeasts using the agar diffusion method. Ch and Smitha (2011) reported that *Ferula assafoetida* (resin), *Zingiber officinale* (rhizome), and *Glycyrrhiza glabra* (root) were used together in traditional medicine as 'Chitrakadivati' for the treatment of flatulence, gut microflora, and indigestion. Their study focused on determining the antimicrobial efficacies of methanolic extracts of these plant parts independently and in combination by measuring the zone of inhibition and minimum inhibitory concentration (MIC). Antimicrobial activity was tested against bacterial and fungal species.The zones of inhibition of individual extracts were lower (0-15 mm)

against microbes than in combination (9-23 mm). Preliminary phytochemical analysis confirmed the presence of reducing sugars, phenolics, flavonoids, cardiac glycosides, terpenoids, carbonyls and tannins. They explored the evidence of synergism among the phytochemcials when used in combination. Antimicrobial activity of natural spice extracts from *Allium cepa*, *Allium sativum*, *Coriandrum sativum*, *Citrus aurantifoliaPiper nigrum* and *Zingiber officinale* on multi-drug resistant *Escherichia coli* isolates was investigated by Rahman *et al*. (2011). All the bacterial isolates were found susceptible to undiluted lime-juice and none of them were found to be susceptible against the aqueous extracts of garlic, onion, coriander, pepper and ginger alone. However, all the isolates were susceptible when applied in combination with a ratio of 1:1:1 aqueous extract of lime, garlic and ginger. Gupta *et al*. (2011) studied *in vitro* antimicrobial effects of alcoholic and aqueous extracts from *Carum carvi* (carway), a medicinal herb from the family Apiaceae. Antibacterial activity was investigated by disc diffusion method against *E.coli* while antifungal activity was evaluated by poisoned food technique against *Aspergillus niger*. Phytochemicals such as carvone, germacrene D, limonene and transdihydrocarvone isolated from *C. carvi* have inhibitory effects. Usha *et al*. (2012) evaluated *in vitro* antibacterial activities of ethanol and acetone extracts of cinnamon bark (*Cinnamonum zeylanicum*) and ajowan fruits (*Trachyspermum ammi*) against *Pseudomonas* sp. and*Escherichia coli*, and *Bacillus subtilis* and *Staphylococcus aureus* through disc diffusion assay. Results revealed that ethanol extract of cinnamon and ajowan had significant antibacterial activity against *Pseudomonas* sp., while acetone extract exhibited highest activity against *Escherichia coli*. Acetone extract of cinnamon and ajowan showed no activity against *Bacillus subtilis* and *Staphylococcus aureus*. The results obtained from the study suggested that the ethanol extract of *Cinnamonum zeylanicum* and *Trachyspermum ammi* have a significant scope to develop a novel broad spectrum of antibacterial herbal formulation and can be used for food preservation.

Mukhtar and Ghori (2012) reported the antibacterial activity of water and ethanol extracts of garlic, cinnamon and turmeric against *Bacillus subtilus* (DSM 3256) and *E.coli* (ATCC 25922) at different concentration by disc diffusion method. Among the selected spices garlic had the best inhibitory activity showing maximum zone of inhibition against both *Bacillus subtilis* and *E.coli*. The aqueous extracts of garlic were appeared to be more effective than ethanolic extract. In the case of cinnamon and

turmeric, the ethanolic extracts were more effective exhibiting inhibition zones of 16 mm against *B. subtilis* and that of 17 mm against *E.coli.* The ethanolic extract of cinnamon was equally effective against both Gram negative and Gram positive bacterial strains. Das *et al*. (2012) further screened Indian herbs and spices against some entero-pathogenic, probiotic or food-spoiler microbes using disc diffusion and MIC bioassays. Results showed widest inhibition zones (12-14 mm, diameter of zone of inhibition) were seen in cases of aqueous extracts of fenugreek, mustard and henna. Gram positive bacteria were more susceptible to these spices or herbal extracts than Gram negative bacteria and fungus. *Klebsiella pneumonie* and *Aspergillus niger*were the most resistant microbes while *Staphylococcus aureus* and *E. coli* were most susceptible strains. Combinations of the herb and spice extracts exhibited synergistic or additive effect where cumin plus fenugreek and black cumin plus mustard combinations demonstrated higher synergistic antimicrobial effects.

Panpatil *et al*. (2013) evaluated antimicrobial activity of spice extracts such as ginger, turmeric and garlic by Slant method. The antimicrobial activity of these spice extracts was found to be in the order of turmeric > ginger > garlic**.** The study indicated that the spices like garlic, ginger and turmeric have significant antimicrobial activity. In another study, Sethi *et al*. (2013) evaluated antimicrobial activity of spices with methanolic extract of *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Syzygium aromaticum* (clove), *Cuminum cyminum* (cumin), *Brassica* j*uncea* (musturd), *Emblica officinalis* (amla), *Aloe vera* and *Crocus sativus* (saffron) against food borne pathogens such as *E.coli, Bacillus subtilis, Citrobacter frendii, Pseudomonas florescens, Serratia marscens, Kleibsiella pneumonia, Staphylococcus aureus* and *Proteus vulgaris* by agar dilution method and MIC determination. They found extracts of *Syzygium aromaticum* and *Cuminum cyminum* had excellent antimicrobial activity against all the target organisms. *Syzygium aromaticum* showed the highest inhibition zone against all except *Serratia marscens* and *Proteus vulgaris.* Clove extract had the highest antibacterial activity followed by cumin, whereas extracts of ginger, garlic, mustard, amla, aloe, and saffron showed weak activity against the tested strains. They reported that the most sensitive strain to different spice extracts was *Citrobacter frendii and* the most resistant species was *Proteus vulgaris.*

Shete and Chitanand (2014) investigated the antimicrobial activity of eight Indian spices (cumin seeds or *Cuminum cyminum*, star anise or *Iilicium verum*,

cardamom or *Elettaria cardamomum*, malabar leaves or *Cinnamomum tamala*, cloves or *Syzygiumarromaticum*, black pepper or *Piper nigrum*, cinnamon or *Cinnamomum zeylanicum* andstone floweror *Parmelia perlata*) against both Gram positive and Gram negative bacterial pathogens *viz., S. aureus, B. subtilis, B. cereus, E.coli, S. typhi,* and *P. aeruginosa* using aqueous, ethanolic and methanolic extracts. It was found that alcoholic extract of *Ilicium verum* and *Piper nigrum* had maximum antimicrobial activity against Gram negative bacteria while alcoholic extract of *Syzygium arromaticum* and *Piper nigrum* exihibited maximum action against Gram positive bacteria. Ibrahim and Abu-Salem (2014) studied antibacterial activity of methanol and aqueous extracts of jatropha, jojoba, clove and ginger against *Bacillus cereus* (Gram positive), *Staphylococcus aureus* (Gram positive) and *Salmonella typhimurium* (Gram negative) by agar diffusion and disc diffusion method. Screening results showed potential antibacterial activity of the tested plant extracts against the screened bacterial strains. Methanol extracts exhibited higher antibacterial activity than aqueous extracts. Methanol extract of *Jatropha* produced highest zone of inhibition against *Staphylococcus aureus* with 24 mm in diameter, compared to the other plant extracts followed by clove. Meanwhile, the zone of inhibition by methanol extracts of jojoba and ginger were found to be the same (12 mm).They concluded that Gram positive bacteria were more sensitive to aqueous and methanol extracts than Gram negative bacteria.

Ranganathan(2015) determined the antimicrobial activity of some important naturally grown spices against Gram positive bacteria (*Bacillus pumilus*, *Bacillus cereus* and *Staphylococcus aureus*) as well as Gram negative pathogenic bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) using aqueous, ethanolic, methanolic and liquid nutrient extracts. Among the extracts tested alcoholic extracts of cardamom (*Elettaria cardamom*), clove (*Eugenia caryophyllus*) and lemon grass (*Cymbopogon citratus*) showed maximum antimicrobial activity against Gram negative bacteria while alcoholic extract of cardamom (*Elettaria cardamom*) and lemongrass (*Cymbopogon citratus*) showed maximum activity against Gram positive bacteria.

Antibacterial activity of spices against *Vibrio cholerae, Vibrio parahaemolyticus,* and *Vibrio vulnificus* using agar well diffusion method were studied (Srivastava *et al*. 2016). Extracts obtained from *Cuminum cyminum*, *Elettaria*

cardamomum, *Corianderum sativum Piper nigrum* and *Cinnamomum verum* showed anti-Vibrio effect. Black pepper (*Piper nigrum*) showed maximun activity zone at 100% ethanol and methanol extracts and minimum at 70% ethanol extract. Coriander (*Coriandrum sativum*) exhibited maximun antibacterial activity zone at 85% ethanol extract and minimum at 70% ethanol extract. Cinnamon (*Cinnamomum verum*) showed maximum antibacterial zone at 85% and 100% acetone extracts and minimum at 70% ethanol. Green cardamom (*Elettaria cardamomum***)** showed a very significant result with maximun inhibitory effect at 100% ethanol extract and minimum at 70% methanol as well as acetone extracts. Dhiman*et al*. (2016) investigated the antimicrobial activities of different spices, *Curcuma longa*, *Mentha arvensis* and *Zingiber officinale*, and medicinal herbs, such as *Centella asiatica*, *Emblica officinalis*, *Rauvolfia serpentina*, *Terminalia arjuna* and *Withania somnifera*against *Bacillus cereus*, *Serratia* sp., *Rhodotorula mucilaginosa*, *Aspergillus flavus*, and *Penicillium citrinum* using different solvent systems like water, acetone, ethanol and methanol. Extracts from the medicinal herbs and spices showed significant antibacterial activity revealing *B. cereus* was the most sensitive while *R. mucilaginosa* was the most resistant among the test microorganisms. Ethanolic and methanolic extracts of *C. asiatica* showed maximum inhibition zone against bacteria and yeast, and inhibition of mycelial growth against the mould species.

Akinnibosun and Ogu (2017) studied antibacterial activity of aqueous and ethanolic leaf extracts of *Murraya koenigii* and *Telfairia occidentalis* synergy against *Escherichia coli*, *Bacillus subtillis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Shigella dysenteriae* by the Kirby-Bauer disc diffusion method. Synergistic antibacterial action of the aqueous extract ranged from 0.0 to 20.0 mm while synergistic activity of the ethanolic extract ranged from 5.0 to 25.0 mm. Diameter of zones of inhibition were observed larger in ethanolic extract than the aqueous extract. The MIC and MBC values were found to be ranged from 31.25 to 250.00 mg mL^{-1} and 250.00 to 500 mg mL^{-1} respectively for the aqueous extract, while the ethanolic extract showed MIC and MBC values ranging from 31.25 to 62.50 mg mL^{-1} and 125.00 to 500.00 mg mL^{-1} respectively. The ethanolic extract was found to have lower MIC and MBC values than the aqueous extract. The phytochemical screening of the extract revealed the presence of alkaloids, reducing sugars, flavonoids, glycosides, tannins, terpenoids, anthraquinones, saponins and steroids, which conferred the antibacterial

property of the plants. The presence of such phytochemicals was more prominent in the ethanolic extract than in the aqueous extract. Bankova and Popova (2017)investigated antimicrobial effect of hot and cold water extracts and infusions of herbs oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Pasteurella multocida*, *Enterococcus faecalis* and *Candida albicans* using classical agar-gel diffusion method. The highest antibacterial and antimycotic effect *in vitro* exhibited the infusions of both herbs. The cold water extracts of both herbs showed less pronounced antimicrobial effect, which was slightly higher in case of thyme. The cold extract of oregano showed significant antibacterial effect against *K. pneumoniae* and*P. aeruginosa*,but did not affect the growth of *P. multocida*. The hot aqueous extracts of the herbs manifested weakest antimicrobial activity *in vitro* showing the inhibitory effect of oregano was slightly higher than that of thyme. In another study, Mostafa *et al*. (2017) investigated antimicrobial activity of five plant extracts against *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using agar disc diffusion assay. Ethanolic extracts of *Punica granatum*, *Syzygium aromaticum*, *Thymus vulgaris*and *Zingiber officinales*were potentially effective with variable efficiency against the target bacterial strains at a concentration of 10 mg mL^{-1} while extract of *Cuminum cyminum* was effective only against *S. aureus*. Ethanolic extracts of *P. granatum* and *S. aromaticum* were found to be the most effective against the highly susceptible strains of *S. aureus* and *P. aeruginosa* with MICs ranged from 2.5 to 5.0 mg mL^{-1}.

2.7. Overview of anti-quorum sensing activity

The use of antimicrobial medicines against crude infectious diseases is a general strategy for first-line-treatment designed to reduce morbidity and mortality of human population. A frequent and indiscriminate use and reuse of antimicrobial drugs for controlling or killing bacterial pathogens is so often helped to adapt them some built-in-abilities to antimicrobial-resistance. The increasing rise of antimicrobial-resistant (AMR) bacterial strains becomes an enigma for human population worldwide. World Health Organization recently released a panel list of "priority pathogens" which have the power to pose catastrophic public health threat due to their resistance towards major classes of antibiotics (WHO, 2017). Every year in India, the number of people who acquire severe infectious diseases from AMR bacteria is approximately twice than

United States resulting large proportion of population died for untreatability of conditions complicated by AMR-infections (Laxminarayan and Chaudhury, 2016).

In view of the fact that quorum sensing (QS) is involved with development of infectious diseases by pathogenic microorganisms, research efforts have recently targeted on cell-to-cell communication and developing anti-pathogenic agents of plant origin to control bacterial diseases (Adonizio, 2008; Al-Hussaini, 2009). Anti-quorum sensing or anti-QS phytochemicals would offer a new way of controlling microbial infections with the advantage of reducing risks of microbial resistance (Adonizio, 2006). The continuing search for newer and novel antimicrobials and anti-pathogenic drugs has highlighted on the fact that plants surviving in an environment with high bacterial density have been seen to possess protective mechanisms against infections (Cos, 2006). Following this concept, researchers are increasingly looking at botanical products in quest for new therapeutic and anti-pathogenic agents which might be nontoxic inhibitors of quorum sensing, thus controlling infections without encouraging the appearance of resistant bacterial population (Hentzer, 2003). It has been suggested that targeting pathogenesis instead of killing the causal organism may provide less selective pressure and therefore decreasing the emergence of resistant strains (Whitehead *et al.*, 2001). Hence, interest is growing more and more in practical applications of anti-QS especially when faced with increased incidence of drug failure due to the large number of pathogenic bacteria developing resistance to available antibiotics.

2.7.1. Quorum sensing: a phenomenon of signal-for-intercellular communication

The term ‘quorum sensing (QS)’, first introduced in an article by Fuqua *et al*., 1994, has been employed to describe a density-dependent signal induced phenomenon. Quorum sensing essentially reflects the minimum threshold level of individual cell mass required to initiate a concerted population response. The signal molecule used for communication was represented as ‘autoinducer’, owing to its origin inside the bacterial cell and the desired response can be attained by of the autoinducer in a process called as ‘autoinduction’. In other words, the whole circuit relies on the intracellular production and export of a low-molecular mass signalling molecule, the extracellular concentration of which grows with the population density of the producing bacterial organism. The signalling molecule can be sensed and reimported into these cells, thus allowing the whole population to respond to changing environment or requirement once

a critical concentration (corresponding to a particular cell density) has been achieved. Till date several classes of microbially-derived signalling molecules have been identified. Broadly, such molecules can be grouped into two main categories (i) amino acids and short peptide derivatives, commonly utilized by Gram-positive bacteria (Shapiro, 1998) and (ii) fatty acid derivatives, called homoserine lactones (HSLs) or N-acyl homoserine lactones (AHLs) frequently utilized by Gram-negative bacterial species (Dunny and Winans, 1999; Whitehead *et al.*, 2001). Whatever may be the chemical nature of the signaling molecule, the whole circuit functions by its reentry into the cell either via diffusion or an active transport (Whitehead *et al.*, 2001). The signalling mechanism involves subsequent interaction of the signal molecule with an intracellular effector that induces the expression of the concerned phenotype.

Quenching or inhibiting microbial QS with new antimicrobial or antipathogenic or QS inhibiting (QSI) agents or anti-QS agents from plants have become a very pressing priority for control of microbial infections (Coates, 2002). This strategy results from the realization that many single-celled microbial organisms, including bacterial and fungal pathogens, can communicate with each other and act collectively in the regulation of infection-related traits, including expression of virulence genes and production of biofilms. The pathogens produce, detect and respond in a population density-dependent manner to specific small signal molecules, ranging from fatty acid derivatives to oligopeptides and furanones, thus synchronizing the expression of virulence genes among family members (Waters and Bassler, 2005). Based on this novel mechanism of action, QS has been suggested as an opportunity to fight bacterial infection/virulence by means other than growth inhibition, overcoming the problem linked to antibiotic resistance (Lynch and Wiener-Kronish, 2008). Over a short period of time, numerous anti-quorum sensing phenomena have been observed with promising results. The discovery of anti-quorum sensing agents from plants will provide us with yet another type of “antimicrobial” or “antipathogenic” agents to cope with the serious problem of antibiotic resistance and biofilm infections.

2.7.2. Quorum sensing: a new target for the treatment of biofilm infections

Quorum sensing is a population-dependent expression of genes that influences biofilm formation and disease development. Although infectious diseases are not exclusively a consequence of biofilm formation, up to 60% of all human infections are caused by biofilms (Spoering and Lewis, 2001). Among the microorganisms that cause serious

infections due to their ability to form biofilms are *Aeromonas hydrophilia*, *Burkholderia cepacia*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, etc (Aparna and Yadav, 2008). Microbial infections associated with biofilms have over the years become difficult to treat and cure with the use of conventional antimicrobials. For biofilm disease treatment, it requires an antibiotic dosage of hundred to thousand times greater concentration than with non-biofilm infections (Costerton, 1999). The excessive and frequent use of conventional antimicrobials has resulted in an increase in microbial resistance to available drugs, making it difficult to eradicate most of the common microbial infections. Among the various factors contributing to microbial resistance, is the ability of the microbial species to exist in biofilm forms that allow them to withstand against antimicrobial action. The increased prevalence of drug resistance among microbial species has led to the introduction of alternate therapy with high treatment efficacy. The reduction of biofilm biomass by plant extracts shows potential in the development of new herbal antimicrobials that prevent microbial adhesion and biofilm formation thus reducing the incidence of microbial infections.

By definition, a biofilm is a complex community of microbial cells attached to either a biotic or abiotic surface enclosed in an exopolysaccharide matrix (Yerly *et al*., 2007). Biofilms have been reported to show increased resistance to antimicrobial drugs due to:

- One, the exopolysaccharide matrix creates a physical barrier by which diffusion of antibiotic into the microbial community is minimized.

- Two, due the negative charge on the exopolysaccharide matrix, penetration of antibiotic may also be restricted by the charge attraction and thus become adsorbed onto the matrix.

- Three, even if antibiotic infiltrates the exopolysaccharide barrier, the antibiotic may still have difficulty in reaching the internal cells of the mushroom-like structures.

- Four, during biofilm development, specialized virulence gene expression may occur ensuring the expression of antibiotic denaturing enzymes, efflux pump, increased plasmid exchange and synthesis of several signalling molecules. The

development and expression of many of these virulence factors is typically under QS-control (Lewis, 2001; Camara *et al*., 2002).

Although a lot of research works on medicinal plants and their active constituents is currently ongoing, the main focus is largely on the antimicrobial properties against planktonic microorganisms. However, the biofilm structure that is more resistant to antimicrobial drugs and hence more difficult to control, remains largely unexplored (Bupesh *et al.*, 2007). Higher plants produce a large numbers of chemical compounds some of which may interfere with the QS-regulated gene expression in the invading organism (Rasmussen *et al*., 2005). These compounds are identified as quorum sensing inhibitors (QSIs). QSIs may reduce microbial virulence by interrupting intercellular communication thus preventing microbes to attack the host as a unified army, by inhibiting the expression of pathogenic and virulence gene expression, and by preventing biofilm formation. Several QS inhibiting compounds have been recognised from medicinal plants that have the ability to interfere with QS-mediated gene expression (Manefield *et al*., 1999) through competitive inhibition, thus decreasing biofilm thickness (Hentzer and Givskov, 2003).

2.7.3. Anti-quorum sensing activity of herbs and spices

Anti-QS agents were first characterized in the red marine alga, *Delisea pulchura* (Manefield *et al.*, 1999). This alga was investigated for its anti-fouling properties, and was found to contain halogenated furanones, compounds which act as anti-QS agents.Since the discovery of AHL inhibitors in *D. pulchura*, anti-QS activity has been found in a south Florida *Caulerpa* species (Willsie, 2000) and a number of higher plants including various fruits and vegetables (Rasmussen *et al.*, 2005; Adonizio *et al.*, 2006). *Pisum sativum* (pea) seedlings and root exudates produced an inhibition of pigment production, exochitinase activity, and protease activity in *C. violaceum* (Teplitski *et al.*, 2000). Some fractionation of the crude extract was attempted; however, no active compounds were purified. Garlic (*Allium sativum*), carrot (*Daucus carota*), chamomile (*Matricaria* sp.), water lily (*Nymphaea* sp.) and various peppers (*Capsicum* spp.) were found to possess anti-QS activity (Rasmussen *et al.*, 2005). Garlic was also found to inhibit biofilm formation in *P. aeruginosa,* and prevented nematode death in a limited analysis (Rasmussen *et al.*, 2005). Garlic extract was partially fractionated in this study, but again, no purified compounds were elucidated.

Because of its extensive antifungal reputation in medicinal folklore, *Allium sativum* L., commonly known as garlic, has been examined for this type of activity. Persson *et al.* (2005) reported that toluene extracts of garlic contained several compounds with varying levels of quorum sensing inhibition against Gram-negative bacteria.

Various fruits and herbs were recently shown to possess anti-QS activity in a *Chromobacterium violaceum* biomonitor strain and on the swarming motility of *E. coli* and *Pseudomonas aeruginosa* (Vattem *et al.*, 2007). Fruits including raspberry, blueberry, blackberry, cranberry, and grape, and herbs such as thyme, ginger, basil, kale, oregano and turmeric exhibited moderate inhibition of these QS-controlled processes. Other than signal mimics such as the furanones and synthetic derivatives, the compounds ellagic acid, tannic acid, and epigallocatechin gallate have been shown to inhibit QS in both an *E. coli* and a *Pseudomonas aeruginosa*biomonitor strain (Huber *et al.*, 2004). These and related polyphenolics are widespread throughout the plant kingdom and should be further explored as anti-QS compounds.

Aparna *et al*. (2010) evaluated the effect of aqueous extracts of six Indian spices *Allium sativum, Brassica junceae, Piper nigrum,Syzygium aromaticum, Trigonella foenum-graecum* and *Trachyspermum amoni* for their anti-quorum sensing activity against *Pseudomonas* sps. Among the six spices tested *Allium sativum*, *Piper nigrum* and *Syzygium aromaticum* showed significant inhibition in quorum sensing activity by reducing the virulence factors *i.e.* protease production and pyocyanin production. Their findings suggested that the quorum quenching mechanisms are not related to bacteriostatic or bacteriocidal effects exerted by plant extracts. Ding *et al*. (2011) screened traditional Chinese medicines (TCMs) comprising different herbs (*Rheum palmatum* L., *Rheum officinale* Baill., *Peucedanum decursivum* (Miq.) Maxim., *Lithospermum erythrorhizon* Sieb., *Rheum palmatum* L., *Fraxinus chinensis* Roxb.) for novel quorum-sensing inhibitors (QSIs) that inhibit bacterial biofilm formation. Six out of 46 active compounds from these Chinese herbs were recognised as putative QSIs based on molecular docking studies. Among them, three compounds (Rhein, Chrysophanol and Fraxin) inhibited biofilm formation by *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* at 200 mM concentration. Emodin, a fourth compound found in *Rheum palmatum* L. significantly inhibited biofilm formation at 20 mM and induced proteolysis of the quorum-sensing signal receptor TraR in *Escherichia coli* at a concentration of 3 to 30 mM. They reported emodin increased the activity of ampicillin

against *P. aeruginosa*. Krishnan*et al*. (2012) studied anti-quorum sensing activity of hexane, chloroform and methanol extracts of an Ayurveda spice, namely clove (*Syzygium aromaticum*). It was found that both hexane and methanol extracts of clove inhibited the response of *C. violaceum* CV026 to exogenously supplied N-hexanoylhomoserine lactone, by preventing synthesis of violacein; whereas chloroform and methanol extracts of clove significantly reduced bioluminescence production by *E. coli* [pSB1075] grown in the presence of auto inducer N-(3-oxododecanoyl)-L-homoserine lactone. They demonstrated that clove extract inhibited QS-regulated phenotypes in *Pseudomonas aeruginosa* PA01, including gene expression of lecA::lux by hexane extract, pyocyanin synthesis by hexane extract and swarming motility by methanol extract.

In 2013, Kalia reported that turmeric (*Curcuma longa* L.) produces curcumin, which significantly inhibits the expression of virulence genes of *Ps. aeruginosa* PA01 in *A. thaliana*/*Caenorhabditis elegans* pathogenicity models. He further investigated that Cinnamaldehyde, an organic compound, isolated from cinnamon (*Cinnamomum verum* J. Presl) and its derivatives affect QS-regulated biofilm formation in *Ps. aeruginosa* and AI-2-mediated QS in different *Vibrio* spp. by decreasing the DNA binding ability of LuxR, resulting in several phenotypic changes, including reduced virulence and increased susceptibility to stress. The ethanolic extracts of leaves, flowers, fruits, and bark from *Laurus nobilis* L. were found to possess anti-QS activities against *C. violaceum* (Kalia, 2013). Quorum quenching activity has also been reported from garlic(*Allium sativum* L.), and has been found that extract of garlic has a preference for the genes belonging to the group (toxins, enzymes, and alginate), targeting 11 genes (22 % of the total functional genes of *Ps. aeruginosa*) like elastase and protease coding genes LasA, LasB; rhamnolipid coding gene rhlAB ; chitinase coding genes chiC, as well as aprA, phzA1B, phzS, phzC2D2E2F2G2 and PA1L associated with the virulence and pathogenesis of *Ps. aeruginosa* (Kalia, 2013). Kumutha *et al*. (2013) evaluated crude hexane, chloroform and methanol extracts of *Phyllanthus amarus*, a traditional Chinese herb for its anti-quorum sensing properties through different bioassays. Among the extracts of *P. amarus*, the methanolic extract only exhibited anti-quorum sensing activity against *Chromobacterium violaceum* CVO26 and reduced bioluminescence in *E. coli* [pSB401] and *E. coli* [pSB1075]. The methanolic extract also significantly inhibited QS-regulated virulence determinants of

Pseudomonas aeruginosa PA01. According to them, increasing concentrations of methanolic extracts had inhibitory effects on pyocyanin synthesis, swarming motility and *P. aeruginosa* PA01 lecA::lux gene expression.

Tolmacheva *et al*. (2014) studied twenty medicinal plants including some culinary herbs (*Achillea millefolium, Arctostaphylos uva-ursi, Betula verrucosa, Bidens tripartite, Calendula officinalis, Chelidonium majus, Comarum palustre, Eucalyptus viminalis, Inula helenium, Juniperus communis, Ledum palustre, Matricaria chamomilla, Plantago major, Quercus robur, Rosa majalis, Salvia officinalis, Taraxacum officinale, Tussilago farfara, Vaccinium vitis-idaea* and *Viola tricolor*), using wild-type and reporter *Chromobacterium violaceum* bioassays, for novel compounds that target bacterial cells and their communication systems. Among them, seven plant extracts showing direct growth-inhibition, the strongest effect was shown by *Arctostaphylos uva-ursi* leaves. Many plants stimulated the violacein production in *C. violaceum* ATCC 31532 in a non-specific manner, and only the herb *Bidens tripartita* contained compounds that mimic acyl-homoserine lactone and operated as a QS agonist. They reported that anti-QS activity was found in eleven plants including *Quercus robur* cortex extract, *Betula verrucosa* bud extract and *Eucalyptus viminalis* leaf extract.

Aparna *et al*. (2014) attempted to check the efficacy of food spice *Syzygium aromaticum* for its quorum quenching activity against *Serratia* sp. Aqueous extract of *S.aromaticum* was tested for growth inhibition and reduction in virulence factors like protease secretion, prodigiosin production, DNase production and swarming motility in *Serratia* sp. The spice extract was found to inhibit all the virulence determinants except for DNase. Anti-QS activity of the spice extract was further confirmed on bio indicator strain *Chromobacterium violaceum* 12472 in which inhibition of violacein production was evaluated.

Mutungwa *et al*. (2015) investigated the anti-quorum sensing activity and total phenolic and flavonoids contents and of some Indian spices. The methanolic extracts of (*Brassica juncea, Cariandrum sativum, Capsicum annuum, Papaver somniferum, Pipper nigrum, Syzygium aromaticum, Trigonella foenum-graecum* and *Nigella sativa* were studied for their phytochemicals and quorum sensing inhibition. anti-quorum sensing activity was determined by qualitative and quantitative violacein inhibition using *Chromobacterium violaceum* 12472. Anti-biofilm formation ability and

inhibition of virulence factors *viz*. pyocyanin, exopolysaccharide production (EPS), proteolytic enzyme production and swimming motility were assessed using *Pseudomonas aeruginosa*. Results revealed that methanolic extracts contained carbohydrates, phenolic compounds, flavonoids, tannins, terpenoids, cardiac glycosides and alkaloids. Among the spices, *Sygygium aromaticum* contained highest phenol (35±0.53 mg GAE g^{-1} dry weight) and flavonoid (18±0.22 mg QE g^{-1} dry weight) contents. *S. aromaticum* exhibited highest quorum sensing activity by reducing violacein synthesis (57.63±04%) and anti-biofilm activity by 49.36±1.5% inhibition at 200 mg mL^{-1}. *S. aromaticum* also exhibited dose dependent inhibition of virulence factors such as pyocyanin pigment, EPS production, proteolytic enzyme and swimming motility in*Pseudomonas aeruginosa*. ATR-IR (Attenuated total reflectance infrared) analysis of S. *aromaticum* extract revealed the presence of phytochemicals with aromatics, alkene, anhydrites, alkynes, esters, hydroxyl, nitro compounds, sulfoxide and halogen functional groups.

Namasivayam and Vivek (2016) evaluated anti-quorum sensing activity of ethanolic extract of *Aegle marmelos*, *Azadirachta indica*, *Curcuma longa*, *Cynodon dactylon*, *Eucalyptus globules* and *Ocium tenuiflorum*against QS-mediated virulence factors of human pathogenic bacteria *Proteus vulgaris* and *Salmonella paratyphi*. Among the plants *Eucalyptus globules* revealed maximum inhibition of QS-mediated virulence factors in*P. vulgaris*. In the case of *Salmonella paratyphi*, *Aegle marmelos*, *Eucalyptus globules* and*Ocimum tenuiflorum* brought about maximum effect on QS-mediated virulence phenotype. They suggested that these plants have immense potential for treating microbial infections through antibacterial activity or anti-quorum sensing activity.

2.8. Overview of antidiabetic activity

One in three or 33 per cent of Indian people over the ages of 30 years is suffering from one or more lifestyle diseases like diabetes, high cholesterol, cardiovascular complications, high blood pressure, thyroidism and cancer (IDF, 2017). According to International Diabetes Federation, China, India and USA are among the top three countries with a high number of diabetic populations. India currently faces an uncertain future in relation to the potential threat that may impose upon the country. World Health Organisation estimated every 26 per 100,000 persons die due to diabetes in India (WHO, 2014).

Diabetes mellitus (DM) or simply the diabetes is a chronic non-communicable disease (NCD) generally symptomized by elevated blood glucose levels more than normal range, a condition biomedically known as hyperglycemia, with presently more than 415 million diabetic people worldwide and without intervention the number is predicted to be 642 million by the year of 2040 due to the expected increase in new cases (IDF, 2017). The disease has been recognized as an important public health problem in developing countries and is associated with oxidative stress, predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to development of nephropathy, neuropathy, retinopathy and angiopathy (Kristova *et al.,* 2008).

DM has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. Its pharmacotherapy however is over 80 years old. The word "Diabetes" was coined by the Greek physician Aeretaeus in the first century A.D. In the 17th century, Willis observed that the urine of diabetics as wonderfully sweet as if imbued with honey or sugar. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755 (Satoskar *et al.,* 1999). The affliction of DM is of two types: type 1 Diabetes mellitus (previously known as insulin-dependent Diabetes mellitus or IDDM) and type 2 Diabetes mellitus (previously known as non–insulin-dependent Diabetes mellitus or NIDDM). Type 2 diabetes mellitus (T2DM) is more common than type 1 diabetes mellitus (T1DM), with about 95% of diabetics being type 2 and 5% being type 1 (CDCP, 2011). Type 1 or IDDM or juvenile on-set diabetes involves autoimmune or idiopathic etiology. Type 2 or NIDDM or maturity on-set diabetes is basically due to predominant insulin resistant or predominant insulin secretary defects. Type 1 diabetes mellitus can only be controlled by insulin therapy. Type 2 diabetes is the most common one and usually starts at later life, generally over the age of 40 and mainly in obese individuals. In T2DM type, the insulin producing β-cells in the pancreas produce insulin, but the output is inadequate for the body's need or there is a defect in liberation and/or action of insulin. Type 2 is more common in elderly population and can be controlled with diet with herbs and spices, hypoglycemic drugs and insulin.

2.8.1. Pathology and etiology of Diabetes mellitus

Diabetes mellitus is a syndrome of disordered metabolic processes resulting from a variable interaction and environmental factors and is characterized by depleted insulin

secretion or action, hyperglycemia and altered metabolism of lipid, carbohydrates and proteins, in addition to damaged β-cells of pancreas and increased risk of complications of vascular diseases. In diabetes, products of lipid peroxidation, advanced glycation end products (AGEs), and damaged DNA accumulate and eventually result in pathological diabetic complications (Ugochukwu *et al.*, 2004). Furthermore, the development of diabetes is closely related to inflammatory processes. In the absence of an appropriate compensatory response from the endogenous antioxidant network against glucotoxicity and lipotoxicity caused by hyperglycemia and hyperlipidemia under diabetes, oxidative stress becomes marked, leading to activation of the stress-sensitive intracellular signaling pathway (Poitout *et al.*, 2002; Prentki *et al.*, 2002). Accordingly, the attenuation of oxidative stress and regulation of stress-sensitive signaling pathways have been considered as ways to alleviate diabetes and diabetic complications. Unfortunately, diabetes is often diagnosed relatively late in the course of the disease, at a point when many patients have already developed pathological complications. In addition, management efforts are labor intensive and challenging for both patients and physicians (Leena *et al.*, 2010).

Many predisposible factors are involved in the etiology of diabetes mellitus. Heredity, age, sex, obesity or over weight, and diet are the major contributors. Other factors which contribute in development of diabetic complications are sedentary life style, socio-economic status, hypertension and various stress related conditions. Different approaches have been used to reduce the incidence rate and to cure the disease. The most popular approaches are the drug therapy, dietary therapy and recently the natural herbal therapy. Drug therapy is the most common approach but is cost effective and has side effects too. The dietary therapy is the most natural, economical and more feasible. Proper dietary intake can stop the incidence of the disease and even can reduce the severity of existing conditions. The food quality and diabetes mellitus has a close association with each other. The broad aims of dietary prescription for people with diabetes remain, first, to abolish the primary symptoms, secondly to minimize the risks of hypoglycemia and thirdly to minimize the long-term vascular complications which altogether results in morbidity and shortened lifespan with all types of diabetes. Diet therapy in diabetic consists of basically of precaution concerning diet composition, the amount, distribution and timing of food intake. Precaution in

eating habits needs a very strong will power and many people may not restrict themselves to a particular way of eating.

Although several chemical and biochemical hypoglycemic agents, e.g., insulin, tolbutamide, phenformin, troglitazone, rosigitazone and repaglinide, are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they are often expensive and unaffordable and some have harmful side-effects and fail to significantly alter the course of diabetic complications (Li *et al.*, 2004). The inability of the modern therapy to control all the pathophysiological aspects of diabetes and its complications coupled with the enormous costs it poses on the economy of the developing nations of the World, underscore the alternative strategies urgently sought. Since time immemorial, medicinal and aromatic plants have been used in virtually all cultures and communities as a source of herbal medicines. It has been estimated that about 80-85% of population both in developed and developing countries rely on traditional medicine for their primarily health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles (Ignacimuthu *et al*., 2006). Due to lack of organized health care systems in developing countries like India, people with chronic diseases like diabetes are among the worst suffers in their communities today. Hence, majority of the populations still have limited access or no access, especially those in remote areas, to modern medicines. Instead they use traditional medicines for a range of diabetic complications (Kochhar and Nagi, 2005). Therefore traditional medicines have always been a new horizon for the development of novel antidiabetic drugs for improving the situations of diabetic pathological conditions.

2.8.2. Role of dietary plants in diabetic management

The use of dietary plants and plant foods to treat a specific disease and/or disease symptoms appears to have been part of medical care as observed for thousands of years in traditional system of medicine. Currently, choice of botanical remedies is highly practiced for the treatment of diabetes mellitus due to their less or no side effects over the use of oral antidiabetic drugs. The botanical medicines are mainly obtained from traditional ethnomedicinal plants which are used anciently in the management of diabetes mellitus. In recent years, exploration of dietary and medicinal plants as newer source of antidiabetic principles has gained greatest importance. It has been reported that more than 1200 plant species are being used as folk medicine to treat diabetes

(Jung *et al*., 2006). Botanical drugs or botanical extracts are rich in phenolic compounds, flavonoids, coumarins, terpenoids and bioactive compounds which help to reduce elevated levels of blood glucose. Several species of plants with potential antidiabetic therapeutics described by Hui *et al*. (2009), and Benzie and Wachtel-Galor (2011) are mentioned in Table 2.

2.8.3. Antidiabetic activity of herbs and spices

Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative medicine for the management of this disease. The active phytochemicals of many plant species are isolated and recognized for direct use as potent drugs, lead compounds or pharmacological agents. Different species of medicinal herbs and spices are used in the treatment of diabetes mellitus. Amongst such plants reported to have beneficial effects in the treatment of diabetes are cinnamon, cloves, bay leaves, ginger,turmeric, garlic amongst others (Broadhurst *et al*., 2000; Srinivasan, 2005). The hypoglycemic efficacy of sumac (*Rhus coriaria* L.) and black cumin (*Bunium persicum* Boiss) extracts were studied by Giancarlo *et al.* (2005) through inhibition of a glycoside hydrolase: alpha-amylase. On the basis of their result ethyl acetate extract of sumac suggested in the treatment and prevention of hyperglycaemia, diabetes and obesity, with an IC_{50} value of 28.7 mg mL^{-1}.

Table 2: Some antidiabetic plant species with their proposed mechanism of actions

Plant species	Scientific name	Proposed antidiabetic action
Aloe	*Aloe vera*	↑IS; ↓FPG
Bitter melon	*Momordica charantia*	↑IS; ↓FPG; ↓PPG; ↓LDL; ↓TG
Cinnamon	*Cinnamomum cassia*	↑IS; ↓FPG; ↓PPG; ↓BP; ↓LDL; ↓TG
Fenugreek	*Trigonella foenumgraecum*	↑IS; ↓FPG; ↓LDL; ↓TG
Garlic	*Allium sativum*	↓BP; ↓LDL
Ginseng	*Panax* spp.	↓BP
Ginkgo	*Ginkgo biloba*	↓BP
Gymnema	*Gymnema sylvestre*	↑IS; ↓FPG; ↓PPG ↓LDL; ↓TG; ↑Ins sec
Hoodia	*Hoodia gordonii*	WL, ↓AP
Ivy gourd	*Coccinia indica*	↑IS; ↓FPG
Indian kino	*Pterocarpus marsupium*	↓HK; ↓GK; ↓PFK

Little tree plant	*Biophytum sensitivum*	↑Ins sec
Pricklypear cactus	*Opuntia* spp.	↓LDL; ↓TG; ↓PPG; ↓IS
Russian tarragon	*Artemisia dracunculus* L.	↑IS; ↓PPG
Sweet potatoes	*Ipomoea batatas*	↓Ins insen; ↑Adipo; ↓Fibrg

Key: ↓AP = decreases appetite, ↓BP = lowers blood pressure; ↓LDL = lowers LDL cholesterol; ↓TG = lowers triglycerides; ↓FPG = lowers fasting blood glucose; ↓PPG = lowers postprandial blood glucose ↑IS = increases insulin sensitivity; WL = weight loss; ↑Ins sec = increases insulin secretion. ↓Ins insen = Decrease insulin insensitivity, ↑Adipo = increase adiponectin, ↓Fibrg = decrease fibrinogen levels, ↓HK = decrease hexokinase; ↓GK = decrease glucokinase; and ↓PFK = decrease phosphofructokinase (After Hui *et al.*, 2009; Benzie and Wachtel-Galor, 2011).

Chakraborty *et al.* (2010) investigated the anti-hyperglycemic activty of the aqueous extracts of *Cinnamomum tamala* (CTLEt) leaves on blood glucose of albino rats. CTLEt was administered at doses of 125 and 250 mg kg^{-1} body weight respectively on streptozotocin induced diabetic rats for 3 weeks. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 250 mg kg^{-1} body weight day^{-1} resulted in a marked decrease in the levels of fasting blood glucose and urine sugar, with a concomitant increase in body weight. The extract also produced a significant decrease in peroxidation products, *viz*., thiobarbituric acid reactive substances. Reduced glutathione and glycogen content, which had shown significant decrease following induction of diabetes, were found to be increased in the hepatic tissue of STZ-diabetic rats treated with CTLEt. STZ-diabetic rats treated with CTLEt (250 mg kg^{-1} body weight) significantly reversed all these changes to near normal. Quantification of antioxidants of the leaves-phenols, ascorbate and carotenoids revealed that *C. tamala* leaves had high antioxidants. These results suggested that CTLEt induce antihyperglycemic as well as antioxidant activities in STZ-diabetic rats.

Akah *et al.* (2011) had undertaken a work to provide the rationale for the use of the leaves of *Gogronema latifolium* as a traditional antidiabetic agent. Methanol extract (ME) of the leaves of *G. latifolium* was prepared by soxhlet extraction while the aqueous extract (AE) was prepared by cold maceration. The methanol extract was separated into fractions by column chromatography to yield methanol fraction (MF), n-hexane fraction (HF) and chloroform fraction (CF). The extract and the fractions were evaluated for antidiabetic effect in alloxan-induced diabetes in rats. The blood sugar

levels were assayed as indices of diabetes. The phytochemical analyses of the extracts and fractions as well as the LD_{50} of the ME were determined. The results indicated that intraperitoneal injection of AE, ME, CF, HF, and MF, (200-800 mg kg^{-1} body weight day^{-1}) exhibited a significant ($P<0.05$) anti-diabetic effect by ameliorating alloxan-induced increase in blood sugar. Antidiabetic potency of the extracts and fractions was in the order; MF> ME> AE>HF>CF. Phytochemical analysis of the extracts and fractions indicated high concentration of proteins, flavonoids, saponins, alkaloids, terpenoids, and steroids while tannins, reducing sugar and acidic compounds were absent. The LD_{50} of the methanol extract was calculated to be 900 mg kg^{-1} body weight. The results of this study lead credence to the use of *G. latifolium* in the management of diabetes mellitus.

Patil *et al.* (2012) reported that aqueous extracts of *Stevia rebaudiana, Momordicha charantia, Tamarindus indica, Gymnema sylvestre, Allium sativum* and *Murraya koenigii* were used for polyherbal combinations for their acute toxicity and 250 mg/kg dose was selected. OGTT, antidiabetic and anti-α amylase and α-glucosidase activity and liver function tests were performed for all the combinations. Reduction in blood glucose level was determined in antidiabetic activity for 0 to 20 days and histopathology of the pancreas was performed after 20^{th} day. IC_{50} value is determined in anti-α amylase activity. Results revealed that all combinations were safe and dose was selected at 250 mg kg^{-1} body weight. Polyherbal combinations II showed significant antidiabetic activity in OGTT and STZ-diabetic rats. Combination II showed sign-ificant anti-α amylase and α-glucosidase activity which is better than other combinations. Treatment with combination-II in diabetic animals produced beneficial improvement in lipid profile. Histopathological observations also showed improvement in the rat treated with combination-II. It may be concluded that combination-II was most effective and safe in comparison to other combinations. They reported flavonoids, tannins and sterols present in this combination might be responsible for this effect.

Ramkissoon *et al.* (2013) determined the contribution of total phenolic content (TPC) of some common tropical medicinal food and spices with potential antioxidative properties in glycation inhibition using *in vitro* glucose-bovine serum albumin (BSA) assay. Ethanolic extracts of ten common household condiments or herbs namely *Allium sativum, Zingiber officinale, Thymus vulgaris, Petroselinum crispum, Murraya koenigii, Mentha piperita, Curcuma longa, Allium cepa, Allium fistulosum* and

Coriandrum sativum were evaluated for antioxidant activity by 2,2-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). Total flavonoid and tannin contents were also estimated. Results showed good correlation between TPC and DPPH activity (r=0.8), TPC and FRAP (r=0.8), TPC and anti-glycation (r=0.9), DPPH and anti-glycation (r=0.6), FRAP and anti-glycation (r =0.9), flavonoid and anti-glycation (r=0.7) and tannins and antiglycation (r =0.8) and relatively fair correlation for TPC and flavonoids (r=0.5) and TPC and tannins (r=0.5). Results confirmed that these plant species are potential sources of natural antioxidants which have free radical scavenging activity and might be used for reducing oxidative stress.

The various mechanisms through which ginger (*Zingiber officinale*) and green tea (*Camellia sinensis*) exert their hypoglycemic effect were studied by Salim (2014). He mentioned that ginger and green tea share some mechanisms of action that reduce blood glucose level in diabetes mellitus. Pharmacokinetics studies revealed ample information about their absorption, distribution, and metabolism. Toxicological data exhibited their safe nature being used as complementary antidiabetic agents in the management of diabetes. In another study, Otunola and Afolayan (2015) studied *Allium sativum* (garlic), *Zingiber offcinale* (ginger) and *Capsicum frutescens* (cayenne pepper) for their antidiabetic activity combined form. This study evaluated the hypoglycaemic activity of aqueous extract of combined garlic, ginger and cayenne pepper (GGCP) at different doses in alloxan-induced diabetic rats. Diabetic rats were treated with GGCP at 200 and 500 mg kg^{-1} body weight day^{-1}, or glibenclamide at 5 mg kg^{-1} body weight day^{-1} for a week. GGCP extract significantly ($p < 0.05$) lowered the elevated fasting blood glucose level, lipid and haematological indices. The GGCP mixture markedly attenuated cellular toxicity, and reduced tubular degeneration and necrosis in the kidney, fatty degeneration and necrosis in the liver and pancreatic hyperplasia in alloxan-induced diabetic rats. Their results suggested that in addition to hypoglycaemic activity, GGCP protects the blood, kidney, liver and pancreas against diabetic injury.

Korou *et al.* (2016) studied plant species e.g. *Salvia officinalis, Rosmarinus officinalis, Lavandula stoechas, Melissa officinalis, Mentha piperita, Thymus vulgaris, Origanum majorana, Sideritis raeseri, Ocimum basilicum, Pistacia lentiscus, Crocus sativus,* and *Daucus carota* for their beneficial effects against lipid or carbohydrate metabolic disorders through their antioxidant, anti-inflammatory and antidiabetic activities, or due to the actions of their phytocompounds.

Thus, the review presented above provides an insight into the various properties of plants which have been and are being exploited by man in various ways- as food, medicines, spices etc. From traditional uses, focus has now shifted to various scientific validations of their medicinal properties to understand the basis underlying such activities.

CHAPTER 3

MATERIALS AND METHODS

3. Materials and methods

3.1. Plant material

Common dietary herbs and spices served as the plant materials which were surveyed, collected, identified, enlisted, selected and processed for biological evaluation in the following steps.

3.1.1. Survey and collection

Market survey of various herbs and spices was carried out in different districts/towns of West Bengal (mainly in the northern region) in between January 2011 and January 2012, and total 40 samples (15 herbs plus 25 spices) were purchased from different retail shops and market stalls (Table 3).

Table 3: GIS locations of different sampling sites for different herb and spice samples

Survey area	Sampling site	GIS location
Cooch Behar	Nutun Bazar	26° 18' 57.384" N and 89° 26' 38.167" E
Siliguri	City Centre	26° 43' 28.930" N and 88° 23' 42.342" E
	Shivmandir Bazar	26º42′29.63″ N and 88º21′40.52″ E
	Garden of Medicinal Plants, NBU	26° 42' 26.561" N and 88° 21' 14.363" E
Jalpaiguri	Din Bazar	26° 31' 44.592" N and 88° 43' 22.160" E
	Station Bazar	26° 31' 6.536" N and 88° 43' 29.518" E
Balurghat	Thana Bazar	25° 13' 21.662" N and 88° 46' 43.476" E
	Mini Market	25° 14' 58.359" N and 88° 51' 48.068" E
Malda	Chittaranjan Market	25° 0' 0.212" N and 88° 8' 41.444" E
	Bichitra Market	25° 0' 12.525" N and 88° 8' 12.351" E
Raiganj	Mohanbati Bazar	25° 37' 16.544" N and 88° 7' 41.133" E
	Debinagar Bazar	25° 35' 41.882" N and 88° 7' 32.208" E

Some of the dry spice samples were also purchased from Masala Centre, Broadway, Ernakulam, Cochin (9° 58' 39.986" N and 76° 16' 40.280" E). For authentication, samples were processed into botanical voucher collections. From retailers and

commoners, data on vernacular names, culinary and medicinal uses were collected to get an idea on the importance and the diversity of different dietary herbs and spices in human life.

3.1.2. Selection of plant material

Among the plants served so far, following were selected from the panel to study their phytochemical profiles and biological activities to establish or validate their traditional medicinal uses as these were commonly and extensively used herbs and spices (Table 4 and 5, Figure 3).

Table 4: Plant species selected for the present investigation

Herb samples selected for study	Plant parts tested	Spice samples selected for study	Plant parts tested
Mentha piperita	Whole leaf	*Illicium verum*	Whole fruit
Trigonella foenum-graecum	Whole leaf	*Myristica fragrans*	Aril
Coriandrum sativum	Whole leaf	*Ceiba pentandra*	Floral bud
Murraya koenigii	Whole leaf	*Capsicum annuum*	Whole fruit
Glinus oppositifolius	Aerial part	*Parmelia perlata*	Whole thallus
Foeniculum vulgare	Aerial part	*Dregea volubilis*	Stem bark

Table 5: Herb species along with the accession no. of the voucher specimens

Herb specimen	Accession number
Mentha piperita L.	09863
Trigonella foenum-graecum L.	09864
Coriandrum sativum L.	09865
Murraya koenigii (L.) Spreng.	09866
Glinus oppositifolius (L.) Aug. DC.	09867
Foeniculum vulgare Mill.	09868

3.2. Processing of plant material

A total of 12 plant samples of herbs and spices were selected. Immediately after collection, fresh samples were brought to the laboratory and washed thoroughly with tap water and then distilled water to remove the dirt and extraneous matter, blotted with filter paper, and then shade-dried on laboratory benches in plastic trays for two days and then air-dried in a ventilated oven for 24-48 h at 45 °C. The dry spices were washed only with distilled water, blotted with tissue paper and further dried in a hot air oven for 24 h at 45 °C, wherever to need (commercially available sealed and ready to use products were not required to wash) and used for the preparation of fine powder.

3.2.1. Preparation of fine powder

The dried plant samples were ground into fine powder using a household electric grinder (Bajaj GX8, India) and sieved through a fine mesh. The powdered samples were stored in air-tight screw-capped glass bottles in the dark at -20 °C until extraction.

3.2.2. Preparation of lyophilised extract

Each of the finely powdered samples of herbs and spices was processed for both aqueous and solvent extraction using standard procedures as described below:

3.2.2.1. Aqueous extraction process

Hot water extraction process as described by Aliakbarlu and Tajik (2012) was followed but with some modifications. Ten g of freshly washed and finely chopped leaf samples or powdered plant samples was extracted for 30 min under darkness (flask covered with aluminium foil) by refluxing with HPLC grade water (1:10, w/v) at 100 °C in a temperature controlled water bath shaker (REMI Equipment Pvt. Ltd., India) with gentle agitation. After cooling, sample was filtered through Whatman filter paper (Grade 1, Ø 90 mm) and the solid residues obtained were further treated with same procedure twice. The filtrate fractions from every extraction process were pooled and concentrated under reduced pressure at 40 ± 1°C in a rotary evaporator (RV 10 digital, IKA®, Germany) equipped with chiller (MC3i, IKA®, Germany), followed by lyophilisation in a vacuum freeze-dryer (EYELA FDU-506, Tokyo, Japan) to obtain the lyophilized hot water extracts (LHEs). The lyophilised extracts were weighed and re-dissolved in same fluid to prepare stock solutions of desired concentrations and subsequently stored in air tight vials at -20°C until use for analyses.

Figure 3. Herbs and spices selected for the bioassay. A: *Mentha piperita*, B: *Trigonella foenum-graecum*, C: *Coriandrum sativum*, D: *Murraya koenigii*, E: *Glinus oppositifolius*, F: *Foeniculum vulgare*, G: *Illicium verum*, H: *Myristica fragrans*, I: *Ceiba pentandra*, J: *Capsicum annuum*, K: *Parmelia perlata* and L: *Dregea volubilis*.

3.2.2.2. Solvent extraction process

The solvent extracts of plant materials were prepared by using methanol as solvent according to a method described previously by Cheesbrough (2000) with minor modifications. Under darkness and normal room temperature, a 10-15 g of fine powder was extracted thrice with HPLC grade methanol (1:10, w/v) by stirring at 150 r min^{-1} for 3 x 24 h intermittently with fresh solvent each time and filtered through Whatman filter paper (Grade 1, Ø 90 mm). The filtrate fractions from every single extraction process were pooled and concentrated*in vacuo*at 40 ± 1°C in a rotary evaporator (RV 10 digital, IKA®, Germany) equipped with chiller (MC3i, IKA®, Germany), followed by lyophilization in a vacuum freeze-dryer (EYELA FDU-506, Tokyo, Japan) to obtain the lyophilized methanolic extracts (LMEs). The lyophilized extracts were then weighed using a microbalance (Sartorius, Germany) and were dissolved in the same solvent (HPLC grade methanol) to make stock solutions of desired concentrations and preserved at -20°C until further use.

3.2.2.3. Determination of extractive value

The extractive value or yield is a measure of the solvent's efficiency to extract specific active compounds from the original plant material to be extracted and it is defined as the amount of extract recovered in mass compared with the initial amount of plant material used. It was presented in percentage (%) and was determined using the formula: Extractive value or % yield = (W_{CE} /W_{DP}) X 100; where W_{CE} = weight of lyophilised crude extract, W_{DP} = weight of dried powdered plant material used. The experiment was performed in triplicate (*n* = 3).

3.2.2.4. Determination of total moisture content

Moisture content of fresh and dried samples was determined using a laboratory oven kept at 105 ± 3°C for 24 h. The moisture content (%) was calculated on a weight basis from the difference between the wet and dry weight divided by the wet weight (AOAC, 1975), using the following formula: Total moisture content (%) = [(Initial weight of the sample before drying - Final weight of the sample after drying) / Initial weight of the sample before drying] x 100. One g of sample in pre-weighed Petri dish was placed in an oven for 24 h, cooled and reweighed. The dry weight of the sample was determined by repeated consistent weighing. The experiment was performed in triplicate (*n* = 3).

3.3. Analysis of nutritional components

3.3.1. Carbohydrate content

Total and reducing sugar was extracted by the method described by Harborne (1998) with minor changes. About 0.2 g of dried powdered or fesh sample was crushed properly in 10 mL of 95% ethanol. The alcoholic fraction was evaporated by keeping the beaker on a boiling water bath. The aqueous fraction was dissolved in 5 mL of distilled water and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and used for further estimation after making the final volume to 5 mL by addition of distilled water.

3.3.1.1. Total soluble sugar content

Total sugar estimation was done according to the method explained by Plummer (1978). In brief, 1 mL of test solution was added with 4 mL of Anthrone's reagent (0.2 % Anthrone in concentrated H_2SO_4). The reaction mixture was mixed thoroughly and allowed to incubate in boiling water for 10 min (precaution were taken to prevent the water loss). The reaction mixture was then cooled under running tap water and the absorbance was measured in a colorimeter at 620 nm against a suitable blank solution. Total soluble sugar content (TSC) was calculated from a D-glucose calibration curve and results were expressed as mg of glucose equivalents (GLE) g^{-1} of tissue weight. The experiment was performed in triplicate (n = 3).

3.3.1.2. Reducing sugar content

The estimation of reducing sugar was done following the Somogyi-Nelson's method as described by Plummer (1978). One mL of the test solution was mixed with 1 mL of alkaline copper tartarate solution (4 g of $CuSO_4.5H_2O$, 24 g of Na_2CO_3 anhydrous, 16 g of Na^+-K^+ tartarate, 180 g of Na_2SO_4 anhydrous in 1000 mL of distilled water) and heated over a boiling water bath for 20 min. After cooling the reaction mixture under running tap water, 1 mL of commercially available Nelson's arseno-molybdate reagent and 2 mL of distilled water was added sequentially and mixed thoroughly. The optical density values were recorded at the wavelength of 515 nm in a colorimeter against a suitable blank solution. The reducing sugar content (RSC) was calculated from a D-glucose calibration curve and results were expressed as mg of glucose equivalents (GLE) g^{-1} of tissue weight. The experiment was performed in triplicate (n = 3).

3.3.2. Total soluble protein content

Totalsoluble protein was extracted using the standard protocol given by Chakraborty *et al.* (1995) and quantification was done according to Lowry *et al.* (1951) using BSA as standard. One g of fresh or dried powdered sample was crushed in phosphate buffer (0.05M, pH 7.2) solution using mortar and pestle. The extracted material was then centrifuged in a cold centrifuge at 10,000 r min^{-1} for 15 min. The supernatant was collected and made up to 5 mL volume and stored at 4 ºC. For protein estimation, the sample was diluted 10 times or 100 times. To 1 mL of diluted solution 5 mL of alkaline reagent was added, mixed thoroughly and incubated at room temperature for 15 min. Then 0.5 mL of Folin-Ciocalteau solution was added, mixed thoroughly and incubated at room temperature for 20 min. Absorbance was measured in a colorimeter at 690 nm. Total soluble protein (TSP) content was calculated as BSA equivalents (BSAE) from a calibration curve of BSA and expressed as mg of BSAE g^{-1} of tissue weight. The experiment was performed in triplicates (n = 3).

3.3.3. Total lipid content

The total lipid extraction and estimation was performed following the method described by Bligh and Dyer (1959) with minor changes. Briefly, 1 g of dried powdered or fresh sample was macerated with 10 mL of distilled H_2O for 5min, followed by the addition of 30 mL of chloroform-methanol (2:1, v/v). The resultant mixture was mixed thoroughly in a beaker and kept overnight at room temperature. To this, 20 mL of chloroform and 20 mL of distilled H_2O was added and centrifuged at 1000 rpm for 10 min. Three distinct layers were formed after centrifugation, out of which the lowermost layer of chloroform containing lipid was collected in a beaker. The chloroform phase was then kept in an oven for one hour at 50 ºC for complete evaporation of chloroform. Weight of the beaker was taken and the lipid content was expressed as mg g^{-1}tissue weight.

3.3.4. Ascorbic acid content

Ascorbic acid (Vitamin C) was determined according to the method of Klein and Perry (1982). A fine powder of sample (200 mg) was extracted with *m*-phosphoric acid (1%, 10.0 mL) for 45 min at room temperature and filtered through Whatman filter paper (Grade 4, Ø 90 mm). The filtrate (1.0 mL) was mixed with 9.0 mL of50 mM 2, 6-dichloroindophenol (DCIP) and the absorbance was measured within 30 min at 515 nm

against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of standard L-ascorbic acid and the results were expressed as mg of ascorbic acid equivqlents g^{-1} of dry weight sample. The assay was performed in triplicate (n = 3).

3.3.5. α-Tocopherol content

α-Tocopherol (Vitamin E) in plant sample was extracted and estimated following the method of Jayaraman (1996) with minor modifications. The dried sample (0.5 g) was taken with 6 mL of hexane and shaken vigorously. The mixture was then filtered using whatman filter paper. The filtrate (2 mL) was taken in the test tube and 2 mL of absolute ethanol was added to it and mixed thoroughly. Further, 0.2 mL of 2, 2'-Bipyridyl solution (0.5 % in ethanol) and 0.2 mL of ferric chloride solution (0.2 % in ethanol) was added, shaken properly and incubated in dark for 15 min. After incubation, 4 mL of distilled water was added to the red colored solution and mixed well. Two distinct layers were formed which was then separated by the separating funnel. The red coloured aqueous layer was collected which was observed to be stable for 30 min. The absorbance was measured against the appropriate blank at wavelength 520 nm in a spectrophotometer. The α-tocopherol content was quantified using a standard curve of α-tocopherol and expressed as mg Tocopherol equivalent (TE) g^{-1}dry weight sample. The experiment was performed in triplicate (n = 3).

3.4. Analysis of major phytochemical constituents

3.4.1. Qualitative analysis

A number of chemical screening tests are in use to detect different biologically active chemical constituents present in different plant extracts and therefore help to characterize them phytochemically. Preliminary phytochemical analyses of the crude plant extracts were performed following standard test methods (Harborne, 1973; Trease & Evans, 1989; Sofowora, 1993; Kokate, 1994; Kokate *et al.*, 1995; Raman, 2006).

3.4.1.1. Test for reducing sugars

Fehling's test: To 1 mL of crude extract, 3 mL of distilled water was added. Then few drops of Fehling's A and Fehling's B reagents were mixed and gently boiled for few min. Formation of orange-red or brick-red precipitation at the bottom of the test tube confirmed the presence of reducing sugars.

Benedict's test: One mL of crude extract when mixed with few drops of Benedict's reagent and boiled for few min, a reddish-brown precipitation was formed indicating the positive result for reducing sugars.

3.4.1.2. Test for phenols

Ferric chloride test: One mL of crude extract was treated with 1 mL of neutral ferric chloride solution. Formation of a deep bluish-green solution gave an indication of the presence of phenols.

3.4.1.3. Test for flavonoids

Alkaline reagent test: To 1 mL of crude extract, 1 mL of dilute NaOH solution was added. Appearance of a yellow fluorescent color which turned colorless on addition of few drops of dilute HCl depicted the presence of flavonoids.

Shinoda test: To 1 mL of crude extract, 1-2 magnesium turnings were added followed by the addition of 1 or 2 drops of concentrated HCI and then boiled it for few min. Appearance of reddish pink or magenta-red or dirty brown or orange color indicated the positive result.

3.4.1.4. Test for resins

To 1 mL of crude extract, few drops of acetic anhydride solution and 1 mL of concentrated H_2SO_4 were added. Appearance of color ranging from orange to yellow suggested the presence of resins.

3.4.1.5. Test for free amino acids

Ninhydrin test: One mL of crude extract when boiled with few drops of 1% Ninhydrin (5.0 mg of ninhydrin in 100 mL of actone), would result in the formation of blue or violet colour indicating the presence of free amino acids.

3.4.1.6. Test for tannins

Lead acetate test: To 1 mL of crude extract, few drops of 10 % lead acetate solution was added to it. Formation of a bulky white precipitation confirmed the presence of tannins.

3.4.1.7. Test for alkaloids

Mayer's Test: To 1 mL of extract, a drop or two of Mayer's reagent were added along the side of the test tube. A white or creamy precipitation indicated the test as positive.

Wagner's test: To 1 mL of extracts, 2-3 drops of Wagner's reagent were added along the side of the test tube. A reddish-brown precipitation confirmed the presence of alkaloids.

3.4.1.8. Test for glycosides

NaOH test: To 1 mL of sample extract, 1 mL of distilled water and 1 mL of aqueous solution of NaOH were added. Formation of yellow color confirmed the presence of glycosides.

3.4.1.9. Test for steroids

One mL extract extract was taken in a test tube and 4 mL of acetic anhydride was added to it and shaken carefully. Then 2-3 drops of concentrated H_2SO_4 was added slowly. Brown ring at the junction of mixture suggested the presence of steroids. Test tube was kept in ice as it's an exothermic reaction.

3.4.1.10. Test for saponins

Frothing test: One mL of sample extract was added to 2 mL of distilled water. The mixture was shaken vigorously for few min. Formation of a stable persistent froth was indicated the positive result for saponins.

3.4.1.11. Test for cardiac glycosides

Keller-Kiliani test: To 1 mL of sample extract, 1 mL each of glacial acetic acid, ferric chloride ($FeCl_3$) solution and 1 mL of concentrated H_2SO_4 were added and mixed carefully. Formation of blue or green precipitation confirmed the presence of cardiac glycosides.

3.4.1.12. Test for phlobatannins

HCl test: To 1 mL of crude extract, few drops of 1 % HCl were added to it and heated for few min. Formation of red precipitation confirmed the presence of phlobatannins.

3.4.1.13. Test for cardenolides

Benzene test: One mL of crude extract was treated with 2 mL of benzene. Formation of a turbid brown color was indicated the presence of cardenolides.

3.4.2. Quantitativeanalysis

3.4.2.1. Total polyphenol content

The total polyphenol content was assayed with Folin-Ciocalteu reagent (FCR) using gallic acid as the standard (Taga *et al*., 1984). A 0.1 mL of extract was added to 2.0 mL of 2% aqueous solution of sodium carbonate. After 2 min, 0.1 mL of 50% FCR was added and the mixture was shaken thoroughly and left to stand for 30 min in the dark at RT. Absorbance was read spectrophotometrically at 750 nm. TPC was calculated as gallic acid equivalents (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of GAE g^{-1} of lyophilized extract. The experiment was performed in triplicate (n = 3).

3.4.2.2. Total flavonoid content

Total flavonoids were estimated using method described by Ordon-ez *et al*. (2006). To 0.5 mL of extract, 1.5 mL of methanol, 0.1 mL of 10 % aluminium trichloride hexahydrate, 0.1 mL of 1.0 M potassium acetate solution and 2.8 mL of ddH_2O water was added. After one and half hour of incubation at RT, the absorbance was measured spectrophotometrically at 420 nm against a blank. TFC was calculated as catechin equivalents (CAE) from a calibration curve of (+)-catechin standard solutions and expressed mg of CAE g^{-1} of lyophilized extract. The experiment was performed in triplicate (n = 3).

3.4.2.3. Total flavonol content

Total flavonols in the spice extracts were estimated using the method of Kumaran and Karunakaran (2007). To 2.0 mL of sample or standard, 2.0 mL of 2% aluminum trichloride (in methanol) solution and 3.0 mL of aqueous solution of sodium acetate (50 g L^{-1}) were added. The absorption at 440 nm was read spectrophotometrically after 2.5 h at 20 °C. Total flavonols (TFO) content was calculated as quercetin equivalents (QRE) and expressed as mg of QRE g^{-1} of lyophilized extract. The experiment was performed in triplicate (n = 3).

3.4.2.4. Total chlorophyll content

Chlorophylls were extracted from the samples following the method of Harborne (1998) with minor changes. About 1 g of the dried powder was crushed with 80% acetone in a mortar pestle and filtered through Whatman No.1 filter paper in a dark chamber. The residue was re-extracted with 80% acetone until it became completely

colorless attaining final volume up to 10 mL. Estimation for total chlorophyll, chlorophyll a and b was done using the method of Harborne (1998). The filtrate obtained (either crude or diluted) was taken directly in the test tube and the OD was measured at 645nm and 663 nm respectively in a UV-Vis spectrophotometer against a blank of 80% acetone. The content was calculated using the formula given by Arnon (1949): Total chlorophyll = (20.2 A_{645} + 8.02 A_{663}) mg g^{-1} fresh tissue weight. The experiment was performed in triplicate (n = 3).

3.4.2.5. Total carotenoid content

Carotenoids were extracted and estimated according to the method given by Lichtenthaler (1987). 1 g of the powdered material was soaked and crushed with 100% methanol in dark chamber and filtered using Whatman filter paper (Grade1). The total volume was made up to 10 mL with methanol and used for further analysis. Absorbances of the sample were observed spectrophotometrically at $\lambda_{663,}$ λ_{645}andλ_{480} against a blank in UV-Vis spectrophotometer 118 Systronics and the total carotenoid content (TCR) was calculated by using the formula: TCR = A_{480} – (0.114 A_{663} – 0.638 A_{645}) µg g^{-1}fresh tissue weight. The experiment was performed in triplicate (n = 3).

3.5. *In vitro* evaluation of antioxidant activity of herb and spice extracts

3.5.1. DPPH• free radical scavenging activity

The DPPH• free radical scavenging activity was monitored using the method of Yen & Duh (1994), with slight changes. Briefly, different aliquots (0.1 mL) of plant extract were added to 2.9 mL of freshly prepared solution of DPPH• (6 x 10^{-5} M in methanol). The mixtures were vortexed thoroughly for 15 s, and left in the dark at RT for 30 min. After incubation, the decrease in absorbance was recorded spectrophotometrically at 517 nm for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the radical without antioxidant (control) was measured daily. Ascorbic acid was used as reference. Free radical scavenging activity ($FRSA_{DPPH•}$)expressed as percentage inhibition (% I) of the DPPH• radical was calculated according to the formula given by Viuda-Martos *et al*. (2010): DPPH• radical scavenging activity (% I) = [(A_c – A_s)/ A_c] x 100, where A_c refers to the absorbance (λ_{517}) of control (t=0 min) and A_s is the absorbance (λ_{517})of sample plus DPPH• (t = 30 min). The experiment was performed in triplicate (n = 3). Further, IC_{50} values for each extract and ascorbic acid was also calculated.

3.5.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide (H_2O_2) scavenging assay was carried out following the procedure of Ruch *et al*. (1989). The principle of this method is that there is a decrease in absorbance upon oxidation of H_2O_2. A solution of 43.0 mM H_2O_2 was prepared in 0.1M phosphate buffer (*p*H 7.4). 1.0 mL extract in 3.4 mL phosphate buffer (0.1 M, *p*H 7.4) was added to 0.6 mL of 43.0 mM H_2O_2 solution and the absorbance of the reaction mixture was recorded at 230 nm after 10 min incubation at room temperature. A blank solution contained the sodium phosphate buffer (0.1 M, *p*H 7.4) without H_2O_2. The percentage of H_2O_2 scavenging by the extracts and standard were calculated using the following equation: H_2O_2 scavenging (HPS) activity (%) = $[(A_c - A_s) / A_c] \times 100$, where A_c was the absorbance (λ_{230}) of the control (without test sample) and A_s was the absorbance (λ_{230}) in presence of test sample. The experiment was performed in triplicate ($n = 3$).

3.5.3. Superoxide anion scavenging activity

The superoxide anion radicals ($O_2^{\bullet -}$) generated in a non-enzymatic PMS-NADH-O_2 system by oxidation of NADH and were detected by the reaction with NBT (Wang *et al*., 2002) based on the method described by Nishikimi *et al.* (1972). Reaction mixture contained 1.0 mL sample, 1.0 mL of NBT solution (312 µM prepared in phosphate buffer, *p*H 7.4) and 1.0 mL of NADH solution (936 µM prepared in phosphate buffer, *p*H 7.4). Finally, the reaction was accelerated by adding 0.1 mL PMS solution (120 µM prepared in phosphate buffer, *p*H 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was measured against a suitable blank. L-ascorbic acid was used as a positive control. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anions in the reaction mixture indicating the increased scavenging activity. Percentage of superoxide anion radicals scavenged was measured using the formula: Superoxide anion scavenging (SAS) activity (% I) = $[(A_c - A_s)/A_c] \times 100$, where A_c was the absorbance (λ_{560}) of the control, and A_s was the absorbance (λ_{560}) of the plant extract or L-ascorbic acid. The experiment was performed in triplicate ($n = 3$). Further, IC_{50} values for each extract and ascorbic acid was also calculated.

3.5.4. Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity of the extracts was determined using the protocol described by Packer and Ong (1998), and Jagetia *et al.* (2004). The reaction mixture containing 2 mL of sodium nitroprusside (10 mM), 0.5 mL of phosphate buffer saline (*p*H:7.4,0.1M) and 0.5 mL of the extract/standard (100-500 µg mL^{-1}) was incubated for 2.5 h at 25^{o}C. From the incubated solution, 0.5 mL solution was taken in the test tube and mixed with 1 mL of sulphanilic acid (0.33% in 20% glacial acetic acid). The mixture was allowed to stand for 5 min after which 1 mL of naphthylethylene diamine dihydrochloride (NED) (0.1% in distilled water) was added, mixed thoroughly and incubated for another 30 min at 25°C. The absorbance of the solution (pink chromophore) was read out at 540 nm against appropriate blank. Control was prepared taking all the reagents except the plant extract. L-ascorbic acid was taken as the reference standard. The nitric oxide scavenging percentage was calculated according to the formula: Nitric oxide (NO) scavenging activity (% I) = $[(A_c - A_s)/A_c] \times 100$, where A_c was the absorbance (λ_{540}) of the control, and A_s was the absorbance (λ_{540}) of the sample extract or L-ascorbic acid. The experiment was performed in triplicate ($n = 3$). Further, IC_{50} values for each extract and ascorbic acid was also calculated.

3.6. Evaluation of antibacterial activity of methanolic extracts of herbs and spices

The effect of methanolic extracts of herbs and spices on different bacterial strains was determined by standard Disc-agar diffusion (DAD) assay method. For quantitative determination of antibacterial activity, the minimum inhibitory dose (MID), *i.e.* minimum amount of extract per discrequired to inhibit the growth of the target bacterial strains was indicated by the presence of a zone of growth inhibition around the paper disc.

3.6.1. Preparation of media

Microbiological media, Nutrient agar, Mueller Hinton agar, Tryptone Soya broth used for the study were procured from Hi-Media, Mumbai, India. For antimicrobial assay, media were prepared according to the manufacturer's instructions. Media were suspended in distilled water and were dissolved completely by heating in microwave oven. The media were autoclaved for 15 min at 15 lbs pressure (121ºC) and then used in the experiments.

3.6.2. Preparation of filter-sterilized methanolic extract

The lyophilized crude extracts were reconstituted in the minimum volumes of the extracting solvent *i.e.*, methanol for desired concentrations. Prior to use, the reconstituted extracts were filter sterilized using disposable Millipore filter (pore size 0.22 µm) in the laminar air flowand further tested their sterility by streaking the extracts on sterile nutrient agar before being incubated for 24 h at 37 °C. A sterile extract was indicated by the absence of visible microbial growth in agar plate after incubation.

3.6.3. Target bacterial strains

Both Gram-positive and Gram-negative bacterial strains were used in the present study. The Gram-positive bacterial strains tested were *Bacillus cereus* MTCC 10655and *Baccilus pumilus* MTCC 1684 and Gram-negative bacterial strains tested were *Serratia marcescens* soilisolate (NCBI GenBank accession number JN 020963.1)and *Pseudomonas aeruginosa* MTCC 2453. The organisms were selected for antibacterial assay as these are the common pathogens either to animals or cause food spoilage. The pure culturess of these microbial strains were either obtained from the Microbial Culture Collection of Immuno-Phytopathology Laboratory, Department of Botany of University of North Bengal or from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

3.6.4. Growth conditions and maintenance

The stock cultures of bacterial strains were maintained on slants of Nutrient Agar (NA), stored at 4 °C, and routinely sub-cultured once in a month to prevent morphological and metabolic transformations. Working bacterial cultures were prepared on Mueller Hinton Agar (MHA) slants on the day before experiment and were incubated for 24 h at 37 °C.

3.6.5. Preparation of bacterial inoculum

Active cultures were generated by inoculating a loopful of cells from a 24 h-old pure culture of the respective bacterial strains into 15 mL of Tryptone Soya Broth (TSB). The broth suspension was then incubated for 6-8 h at 37 °C on an orbital shaker (150 r min^{-1}). Bacterial cell suspension in physiological saline (0.85 % NaCl)was prepared from the flask broth culture in such a way that yielded a cell concentration of about 10^8 cfu mL^{-1}. The bacterial suspension so obtained was used as the final inoculum for the

susceptibility assay. The suspension of the inoculum was tested within 10-15 min or stored temporarily at 4 ^{o}C until use. Besides, dilutions of the inoculum in saline solution were plated on solid medium (NA) to verify the absence of contamination and to check the validity of the inoculum.

3.6.6. Preparation of paper disc

Paper discs (Ø 5.5 mm) were prepared from Whatman filter paper (Grade 1) with the help of punching machine and were sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min. After sterilization, the moist discs were dried on hot air oven for 5-10 min at 50 ºC. The methanolic plant extracts of different concentrations (doses) ranging from 4-10 mg disc^{-1} were prepared by flooding the discs with adequate volumes of respective extracts from the stock solutions and evaporating the solvent from the discs completely in the laminar air flow. The commercially available discs of standard antibiotics were used as positive control and solvent disc so prepared as the negative control.

3.6.7. Disc-agar diffusion assay

The antibacterial activity of the methanolic extracts was carried out following standard Disc-agar diffusion method(Bauer *et al*., 1966). About 20 mL of MHA medium (45 ºC) was poured in the sterilized Petri dishes (Ø 70 mm) and allowed to stand about 15 min for solidify in the laminar air flow. A 0.1 mL of standardized inoculum suspension was pipetted out and swabbed to inoculate the entire surface of a solidified MHA plate using a sterile non-toxic cotton swab. About 5-10 min were allowed to dry and then previously prepared discs with specific concentration of plant extracts were aseptically placed onto the seeded medium with sterile forceps and gently pressed down to ensure complete contact of the discs with the agar surface. Each Petri plate contained four radially placed discs along with a disc of solvent control in the middle. All Petri plates were sealed with a strip of parafilm and incubated for 18-24 h at 37 ^{o}C. All the tests were performed in duplicate. Antibacterial activity was determined by measuring the diameter of the zones of inhibition, a clear area devoid of visible bacterial growth produced by the respective extracts. The diameter of the zone of inhibition around each disc was recorded in millimetre (mm).

3.6.8. Determination of minimum inhibitory dose

The minimum inhibitory dose (MID) is regarded as the lowest amount of an antimicrobial agent that produces a visible zone of growth inhibition. The MID of a

plant extract to a microbial strain gives a quantitative estimation in antimicrobial assay. In the present study, MID method was applied to the extracts that proved their high efficacy against the test bacterial strains. The MID of a bacterial strain was determined by impregnating paper discs with plant extracts at a concentration ranging from 10-0.5 mg $disc^{-1}$ (10, 9.5, 9.0, 8.5, 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.25, 1.0, 0.5 mg $disc^{-1}$).The discs loaded with plant extracts were then placed aseptically into MHA plates inoculated with the target bacterial strains for accessing growth inhibition (Kariba *et al*., 2001; Mwitari *et al*., 2013).

3.7. Evaluation of anti-quorum sensing activity of different herbs and spices

The anti-QS activity or QS-inhibitory activity of herb and spice extracts was evaluated using the *Chromobacterium violaceum* and *Pseudomonas aeruginosa* biosensor systems. *Chromobacterium violaceum* MTCC 2656, a typical pigmented indicator strain produces a short acyl-homoserine lactone (acyl-HSL) signaling molecule which regulates production of dark purple pigment, violacein under QS-control. Thus, inhibition of short acyl-HSL in *C. violaceum* MTCC 2656is indicated by inhibition of violacein production. Disc-agar diffusion (DAD) assay was performed to detect inhibition of violacein production by plant extracts, wherein a colorless, opaque, but viable halo due to loss of pigmentation around the discs against a purple lawn. The plant extracts which inhibited violacein production were further tested to explore their effects on QS-mediated virulence factors or QS-modulatory activities such as pigment production, motility pattern and biofilm-forming ability of *Ps. aeruginosa* MTCC 2453.

3.7.1. Preparation of media

Microbiological media such as Nutrient Agar, Nutrient Broth, Luria Bertani Broth and Luria Bertani Agar used for the anti-QS study were procured from Hi-Media, Mumbai, India. Media were prepared according to the manufacturer's instructions. Briefly, media were suspended in distilled water and were dissolved completely by heating in microwave oven. The media were autoclaved for 15 min at 15 lbs pressure (121ºC) and then used in anti-QS assay.

3.7.2. Preparation of filter-sterilized methanolic extract

The powdered samples were subjected to methanolic extraction following the modified method of Cheesbrough (2000) as described earlier. Extracts were weighted and reconstituted in HPLC grade methanol for making desired concentrations and stored at

-20°C until use. The reconstituted extracts were filter sterilized using disposable Millipore filter (Ø 0.22 µm) in the laminar air flowand further tested their sterility by streaking the extracts on sterilized agar medium before overnight incubation at 37 °C. A sterile extract was indicated by the absence of visible microbial growth on agar medium.

3.7.3. Growth conditions and maintenance

The pure cultures of *Chromobacterium violaceum* MTCC 2656 and *Pseudomonas aeruginosa* MTCC 2453 were maintained on slants of Nutrient Agar (NA) and stored at 4 °C, and routinely sub-cultured once in a month. Working bacterial cultures were prepared on Luria Bertani Agar (LB agar) slants from frozen stocks and incubated at 37 °C for 24 h before the day of experiment.

3.7.4. Preparation of standard inoculum

Active cultures were generated by inoculating a loopful of cells from a 24 h-old pure culture of *C. violaceum* 2656 and *Ps. aeruginosa*2453 into 15 mL of Luria Bertani broth (LB broth).The broth suspensions were then incubated for 6-8 h at 37 °C on an orbital shaker at 150 r min^{-1}. Bacterial cell suspensions in sterile physiological saline (0.85 % NaCl)were prepared from the flask broth cultures in such a way that yielded a cell concentration of aproximately 10^8 cfu mL^{-1}. The bacterial suspensions so obtained were used as the standard inoculum for the anti-QS activity test. The suspension of the inoculum was tested within 10-15 min or stored temporarily at 4 ^oC until use. The validity of inoculum was checked by pour plating.

3.7.5. Preparation of paper disc

Paper discs (Ø 5.5 mm) were prepared from Whatman filter paper (Grade 1) with the help of punching machine and were sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 min. After sterilization, the moistened discs were dried on hot air oven for 3-5 min at 50 ºC, whenever necessary. The methanolic plant extracts of different concentrations (doses) ranging from 3-10 mg $disc^{-1}$ were prepared by flooding the discs with adequate volumes of respective extracts from the stock, followed by the complete evaporation of the solvent part from the discs in the laminar air flow.

3.7.6. Preliminary screening test for anti-QS activity against *C. violaceum* 2656

Preliminary screening test for anti-QS potency was evaluated by placing the ethanol-washed whole or fragment of herb or spice sample directly onto a prepared *C.violaceum* plate. Briefly, LB agar plates (Ø 70 mm, 20 mL) were prepared and swabbed with 0.1 mL of standard suspension ofthe indicator strain *C. violaceum* 2656. Then surface sterilized plant sample (dipped in ethanol for 15 min and then dried in laminar air flow) was placed in agar plate and anti-QS activity was detected by the presence of an area of pigment inhibition. Control plate (without *C. violaceum* 2656) was also set for individual plant sample to check the surface sterilization. The test was performed in duplicate set.

3.7.7. Bioassay for anti-QS activity against *C.violaceum* 2656

3.7.7.1. Qualitative bioassay

3.7.7.1.1. Paper disc-agar diffusion assay

The integral paper disc-agar diffusion method proposed by Adonizio *et al.* (2006) was employed to detect anti-quorum sensing activity qualitatively. A 0.1 mL of standard inoculum suspension of *C. violaceum* 2656 was swabbed over the surface of LB agar using sterilized cotton swabs. After few minutes, previously prepared discs with specific concentration of plant extracts were aseptically placed onto the seeded medium with sterile forceps and gently pressed down to ensure complete contact of the discs with the agar surface. Each Petri plate contained a disc of solvent control (methanol) also. Petri plates were sealed with a strip of parafilm and placed in incubator for 18-24 h at 37 ^{o}C. The tests were performed in duplicate. After O/N incubation Growth and/or pigment inhibition around the disk was observed. A positive quorum-sensing inhibitory result was indicated by de-pigmentation of the indicator strain in the vicinity of the test extract. Digital photographs were taken of all the plates using a Nikon camera.

3.7.7.2. Quantitative bioassay

3.7.7.2.1. Violacein inhibition assay

Inhibition of violacein production in the presence of methanolic extracts was quantified using previously described protocol with some modifications (Choo *et al.*, 2006).Briefly, 15 mL LB broth containing different concentrations of plant extract (100-1000 μg mL^{-1}, 250 μL) was inoculated with 100 μL of *C. violaceum* 2656 and

incubated at 37 °C for 24 h under shaking at 150 r min^{-1}. After O/N incubation, bacterial cell culture were lysed by 10% SDS (1:1, v/v) and incubating them at room temperature for 5 min. Then 1 mL of cell lysed culture was centrifuged at 13,000 r min^{-1} for 10 min at 28 °C to precipitate the insoluble violacein. The culture supernatant was discarded and the cell pellet was re-suspended evenly in equal volume of water saturated n-butanol (n-butanol:distilled water, 5:1, v/v) and centrifuged again (13,000 r min^{-1}, 10 min, 28 °C) to remove the cell debris. The supernatant (upper n-butanol phase) containing violacein was quantified spectrophotometrically at 585 nm. The percentage of violacein inhibition was calculated by following the formula: % violacein inhibition = $(C_{OD585} - T_{OD585} / C_{OD585}) \times 100$, where C_{OD585} and T_{OD585}were optical density of control and test extract at 585 nm respectively (Packiavathy *et al*., 2012). The cell viability in the culture medium was tested by standard plate count method for validation. The experiment was performed in triplicate ($n = 3$).

3.7.8. Determination of effects on *PA* 2453 QS-mediated virulence factors

Effect of plant extracts on widely studied QS-mediated virulence factors such as pyocyanin synthesis, swarming motility and biofilm-forming ability was tested in *Ps. aeruginosa* MTCC 2453.

3.7.8.1. Pyocyanin inhibition assay

The quantitative estimation of pyocyanin is based fundamentally on the spectrophotometric absorbance of extracted pyocyanin at 520 nm in acidic medium. Pyocyanin was extracted and estimated from *Ps. aeruginosa* 2453culture supernatant as previously described method (Essar *et al*., 1990; Kong *et al*., 2005) with slight modifications. Briefly, 250 µL of extracts (100-1000 µg mL^{-1}) were mixed thoroughly with 15 mL of freshly prepared LB broth. Afterwards, the broth was inoculated with 100 µL of 24 h-old culture of *Ps. aeruginosa* 2453 (OD_{600}=0.1) and thus incubated for 48 h at 37 °C. Broth culture without plant extract was set as the control. After 48 h of cultivation, culture of *Ps. aeruginosa* 2453was centrifuged at 10,800 *g* for 5 min at 28 °C. The resulting supernatant was mixed vigorously with chloroform in a ratio of 5:3 (v/v) and centrifuged again at 4600 *g* for 10 min at 28 °C. The lower chloroform phase was transferred to another centrifuge tube, mixed with 1 mL of 0.2 M HCl by shaking and then centrifuged for another 10 min at 4600 *g*. The upper HCl phase (pink to deep red coloration indicating the presence of pyocyanin) was taken to read at OD_{520} in an UV-Vis spectrophotometer against a blank of 0.2 M HCl. The OD_{520} reading was

normalized by dividing the final OD_{600} value of the culture. The experiment was performed in triplicate set (n = 3).

3.7.8.2. Anti-swarming assay

A modified method after Rashid and Kornberg (2000) was adopted for anti-swarming assay. Briefly, 24 h-old single colony of *Ps. aeruginosa*2453 was point inoculated by using the sharp end of a sterilized toothpick at the center of the Petri plate containing LB agar (0.5% agar, supplemented with filter sterilized 1.0 % D-glucose) with or without varied concentrations of test extract (250 µL of extracts, 250-1000 µg mL^{-1}). After 24-48 h of incubation in upright position at 37 ℃, the extent of motility was determined by measuring the diameter of the bacterial colony and the mean value of the swarmer's diameter was assigned.Anti-swarming assay was performed in triplicate set (n = 3).

3.7.8.3. Protease inhibitory assay

To determine the efficacy of the plant extracts for inhibiting extracellular protease production, skim milk agar assay was followed. Briefly, 24 h-old single colony of *Ps. aeruginosa* 2453 was point inoculated by using the sharp end of a sterile toothpick at the center of the Petri plate containing 20 mL of skim milk agar with or without plant extract of different concentrations (250 µL, 200-500 µg mL^{-1}). After 24-48 h of incubation at 37 ℃ in upright position, the zone of proteolysis was determined by measuring the halo area around the colonies formed due to casein hydrolysis. Also diameter of the colony was measured and relative proteolytic activity was determined by the formula: diameter of the zone of proteolysis/diameter of the colony. The experiment was performed in triplicate set (n = 3).

3.7.8.4. Biofilm inhibition assay

Biofilm inihibition assay was performed following the method of Chong *et al.* (2011) with some modifications. An overnight broth culture of *Ps. aeruginosa* 2453 was adjusted to an OD_{600} of 0.4 and 0.1 mL of culture further grown in 15 mL of fresh LB medium supplemented with filter-sterilized 0.5% (w/v) D-glucose and 250 µL of test extract of different concentrations ranging from 100-1000 µg mL^{-1}. Cells grown in the absence of plant extract served as negative control. To facilitate biofilm formation, *Ps. aeruginosa* 2453 cells were incubated statically for 24 h at 37 ºC in sterile tissue culture tubes. The planktonic bacterial cells were discarded by washing with sterile distilled

water, and the tubes were air dried for 15 min and latter on stained with 1 mL of 0.1 % crystal violet (v/v) for 30 min. The stained biofilms were washed several times with sterile distilled water to rinse off the excess crystal violet, followed by the addition of 4 mL of ethanol (95%, v/v). The resulting solution (2 mL) was transferred to an ethanol-washed cuvette, and the absorbance was read spectrophotometrically at 590 nm against an ethanol blank. The experiment was performed in triplicate (n = 3).

3.8. Evaluation of *in vivo* antidiabetic activity of methanolic extracts of herb and spice

3.8.1. Selection of plant material

Two plant species selected for the screening of antidiabetic activities were *Glinus oppositifolius* (*Go*) and *Illicium verum* (*Iv*). Such plants were selected on the basis of the following reasons:

- traditionally used as antidiabetics in folk medicine;
- written records in Ayurvedic formulations;
- scientific validation of anecdotal evidence, and
- searching for new lead compounds for drug discovery, etc.

3.8.2. Reconstitution of lyophilized methanolic extract into aqueous extract

For evaluating the hypoglycaemic or antidiabetic activity powdered plant samples were subjected to solvent extraction process following the method of Cheesbrough (2000) as described earlier. Prior to experimentation, solvent free crude methanolic extracts were weighted and reconstituted in sterile distilled water to make stock solutions of desired concentrations ranging from 2000 mg mL^{-1} to 250 mg mL^{-1} and stored at 4 °C during the experiments.

3.8.3. *In vivo* test for antidiabetic activity

3.8.3.1. Animal and housing condition

Healthy Swiss albino adult male rats (*Rattus norvegicus*, 150-200 g) were procured from the Ghosh Enterprise, Kolkata-55, West Bengal. They were acclimatized for a week to the experimental room (Animal House, Department of Botany) at normal atmospheric temperature of 25±2 ^{0}C, humidity (40-60%) and normal 12 h light/12 h dark photoperiod (Niyonzima and Vlietinck, 1993). At the end of adaptation period, the animals were divided randomly into groups and housed in well aerated

polypropylene cages and were fed with standard food pellets (Hindustan Lever, Kolkata, and India.) alternating with some soaked cereals (*Cicer* seeds) and water *ad libitum*.

3.8.3.2. Animal ethical clearance

Institutional Animal Ethical Committee (NBU) obtained ethical clearance for conducting experiments on animals from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All procedures employed were reviewed and approved by the Animal's ethical Committee of University. The present work was carried out with a prior permission from the Animal's ethical Committee of University.

3.8.3.3. Acute TOX study

The extracts were studied for acute toxicity prior to the antidiabetic experimentation on animals according to OECD (Organization for Economic Cooperation and Development) guidelines (test 423: Acute oral toxicity- Acute toxic class method; 2002) (OECDiLibrary, 2002). Briefly, the rats were dosed once with 2000 mg kg^{-1} BW and monitored for 14 days for general clinical or toxicological signs and symptoms as well as mortality (Turner, 1965; Sundarranjan *et al.* 2011). Initially, the animals were observed continuously for 2 h under the following profiles a) Behavioural profile: alertness, restlessness, irritability, and fearfulness; b) Neurological profile: spontaneous activity-reactivity, touch response, pain response and gait; c) Autonomic profile: defecation and urination. After a period of 24 and 72 h they were observed for any lethality or death.

3.8.3.4. Induction of experimental diabetes

Experimental diabetes was induced by a single dose intraperitoneal (*i.p*) injection (1 mL kg^{-1} BW) of freshly prepared Streptozotocin (65 mg kg^{-1} BW) in citrate buffer (0.1M, pH 4.5) maintained at 37 ^{o}C, to rats fasted for 14 h (Siddique *et al.* 1987). Control rats received a similar volume of citrate buffer alone. STZ treated animals were allowed to drink 5% of D-Glucose for 2-4 h to prevent the potentially fatal hypoglycaemia shock as a result of partial destruction of pancreas. After 48 hours of streptozotocinization, fasting blood glucose levels of the animals were estimated using a glucometer (Accu-Chek, Active Blood Glucose meter, Roche Diagnostics India Pvt. Ltd.) through glucose oxidase-peroxidase method. Development of diabetes was

confirmed by elevated level of blood glucose, loss of body weight, polyuria, and glycosuria. Only rats that had fasting blood glucose levels above 250 mg dL^{-1} and glycosuria were considered as diabetic and were included in the present investigation.

3.8.3.5. Experimental design

In the present study, an experiment with 48 rats (6 normal plus 42 STZ-diabetic surviving rats) was conducted for three weeks (Table 6). The rats were distributed into eight groups each having six in number (n=6) as in the following manner:

Table 6: Experimental design followed in antidiabetic investigation

Group	Label	Treatment	Dose	Route	Schedule
I	NCR	CBS	1 mL kg^{-1} BW	*i.p*	Once in 3 Wk
II	DCR	STZ	65 mg kg^{-1} BW	*i.p*	Once in 3 Wk
III	DST	MET	10 mg kg^{-1} BW	*p.o*	OD for 3 Wk
IV	D*Iv*$_{250}$	*Iv*ME	250 mg kg^{-1} BW	*p.o*	OD for 3 Wk
V	D*Iv*$_{500}$	*Iv*ME	500 mg kg^{-1} BW	*p.o*	OD for 3 Wk
VI	D*Go*$_{250}$	*Go*ME	250 mg kg^{-1} BW	*p.o*	OD for 3 Wk
VII	D*Go*$_{500}$	*Go*ME	500 mg kg^{-1} BW	*p.o*	OD for 3 Wk

Note: NCR-Normal control rats, DCR-Diabetic control rats, DST-Diabetic rats with standard drug Metformin treatment, D*Iv*$_{250}$-Diabetic rats with *Iv*$_{250}$ treatment, D*Iv*$_{500}$-Diabetic rats with *Iv*$_{500}$ treatment, D*Go*$_{250}$-Diabetic rats with *Go*$_{250}$ treatment, D*Go*$_{500}$-Diabetic rats with *Go*$_{500}$ treatment, CBS-Citrate buffer solution, STZ-Streptozotocin, MET-Metformin, IvME-*Illicium verum*methanolic extract, GoME-*Glinus oppositifolius*methanolic extract, BW-Body weight, OD-Once a day, Wk-Week, *i.p*-intraperitoneal and *p.o*-per os/per orem.

The extracts were administered orally to the animals once daily for three weeks. For each group the dosage was adjusted every week according to the change in body weight to maintain similar dose per kg body weight of rat over the entire period of study. Blood glucose level (post-treatment) of rats was monitored on week 1, week 2, and week 3 as described earlier (pre-treatment).

3.8.3.6. Analytical procedure

3.9.3.6.1. Periodical measurement of body weight

Individual body weight (g) of all the experimental rats was recorded initially on week 0 and thereafter on week 1, week 2 and week 3 after the treatment.

3.8.3.6.2. Periodical estimation of blood glucose level

Fasting blood glucose level (mg dL^{-1}) was also recorded on week 0, week 1, week 2 and week 3 of the treatment. Blood was collected from the tail tip and the glucose levels were determined using glucometer-strip (Accu-Chek, Active Glucose meter) by glucose oxidase-peroxidase method.

3.8.3.6.3. Animal sacrifice

Three weeks after treatment, animals were fasted overnight and anaesthetized by dropping each in a transparent plastic jar saturated with chloroform vapour. Blood sample was collected through cardiac puncture and divided into plain and EDTA-containing centrifuge tubes. Humane procedure was adopted throughout the experiment.

3.8.3.6.4. Collection of blood and preparation of serum

Blood was collected in sterile Eppendorf tubes without anticoagulant and allowed to stand for 20-30 min at 4°C. Then, it was centrifuged at 1500 *g* for 10 min at 4°C. The serum got separated from the blood as an upper transparent liquid over the clotted blood. The serum was then collected in a separate sterile Eppendorf tubes without disturbing the residue and was store at –20 °C for studying different biochemical parameters.

3.8.3.6.5. Analysis of biological parameters

Sera were analyzed for the activities of alkaline phosphatise (ALP), alanine amino transferase (SGPT/ALT), aspartate amino transferase (SGOT/AST), total cholesterol, HDL-cholesterol, triglycerides, and for the bilirubin, creatinin and urea concentration.

3.8.3.6.5.1. Analysis of serum lipid profile

3.8.3.6.5.1.1. Estimation of total cholesterol

The total cholesterol level in the serum samples collected from all the groups of experimental rats were analysed using the manufacturer's instructions of

commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany). The estimation of cholesterol was performed by Dynamic extended stability with lipid clearing agent (CHOD-PAP method or modified Roeschlau's method) (Allian *et al.,* 1974; Roeschlau *et al.,* 1974).

Figure 4: Illustration of maintenance and handling of rats for antidiabetic test. A. Rats housed in the polypropylene cage, B: STZ injected intraperitonially (i.p.) C: Blood being withdrawn from the tail vein of rats with the help of sterile needle, D: Estimation of blood glucose with glucometer, E: Feeding the rats with standard drug (Metformin)/plant extracts, F: Measurement of body weight.

Prior to experimentation working reagent was prepared by mixing available Cholesterol reagent (*i.e.*, the mixture of Cholesterol esterase (pancreatic), cholesterol oxidase (microbial), peroxidase (horseradish), sodium phenolate, 4-aminoantipyrine, phosphate buffer (*p*H 6.5±0.1) and lipid clearing agent) with appropriate amount of Aqua 4 (double deionized, 0.2 micron, membrane filtered, particle free water). Prior to use, all available reagent were first allowed to attain the room temperature (15-30 ºC). Then, 2 mL of the working reagent was mixed well with 40 µL of test sample (unhaemolyzed serum of the experimental rats) and allowed to incubate for 37 ºC for 10 min. Blank and standard was prepared by taking distilled water and available cholesterol standard (200 mg dL^{-1}) instead of the serum. The reaction mixture was aspirated and the absorbance was recorded at 505 nm. The cholesterol content was calculated using the formula as provided in the kit:

$$\text{Cholesterol mg dL}^{-1} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Conc. of standard (mg dL}^{-1})$$

3.8.3.6.5.1.2. Estimation of triglycerides

Triglyceride content in the serum samples were analysed using the manufacturer's instructions of commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany). Triglycerides were estimated by Dynamic extended stability with lipid clearing agent (Glycerol Phosphate Oxidase-Trinder method) (Product Data sheet; McGowan *et al.*, 1983; Fossati and Prencipe, 1982; Trinder 1969). Briefly, supplied Triglycerides Des reagent (consisting of ATP, Mg^{2+}, 4-aminoantipyrine, 3-5 DHBS, Peroxidase, Glycerol Kinase, Glycerol Phosphate Oxidase (GPO), Lipoprotein Lipase, Buffer (*p*H 7.0±0.1 at 20 ºC) was mixed with Aqua 4 (double deionized, 0.2 micron, membrane filtered, particle free water) by gently swirling the mixture and was allowed to stand for 10min at room temperature. Following, reagent reconstitution 2 mL of the working reagent was then mixed with 20 µL of the serum/standard (200 mg dL^{-1}) and incubated for 10 min at 37 ºC). The absorbance was recorded at 505 nm. The triglycerides content was calculated using the following formula:

$$\text{Triglycerides (mg dL}^{-1}) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ x Conc. of standard (mg dL}^{-1})$$

3.8.3.6.5.1.3. Estimation of HDL-cholesterol

The estimation of HDL-cholesterol was done following the phosphotungstic acid method as described by Burstein *et al.* 1970 in manufacturer's instructions of commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany).The cholesterol working reagent (consisting the mixture of pancreatic Cholesterol esterase, microbial cholesterol oxidase, horseradish peroxidase, sodium phenolate, 4-aminoantipyrine, phosphate buffer (*p*H 6.5±0.1) and lipid clearing agent) was used to determine the concentration of HDL cholesterol in the sample. Prior, to the use of sample the HDL was precipitated from the sample using precipitating reagent (consisting of Phosphotungstic acid and magnesium chloride) was used to determine the concentration of HDL cholesterol in the sample. Briefly, 500 µL of sample was mixed thoroughly with 1000 µL of precipitating reagent and allowed to

stand for 10 min at room temperature (15-30 ºC). The reaction mixture was centrifuged at 4000 r min^{-1} (1800 *g*) for 10 min to obtain a clear supernatant. The clear supernatant (100 µL) was then mixed well with 2000 µL of cholesterol working reagent and incubated for 10 min at 37 ºC. The absorbance for each test samples and standard was read at 505 nm or 505/670 nm against appropriate reagent blank. The supplied HDL cholesterol standard was 25 mg dL^{-1}. The HDL cholesterol content in the serum sample was calculated using the following formula:

$$\text{HDL cholesterol (mg dL}^{-1}) = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \text{ x Conc. of standard (mg dL}^{-1}) \text{ x dilution factor}$$

3.8.3.6.5.2. Analysis of liver function test (LFT)

3.8.3.6.5.2.1. Estimation of SGPT/ALT (Alanine aminotransferase) level

The ALT level in the serum samples were analysed using IFCC method (International Federation of Clinical Chemistry method, 1980) given in the manufacturer's instructions of commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany). Briefly, 1000 µL of the working reagent was mixed thoroughly with 100 µL of the test sample and aspirated. Then, the absorbance was immediately recorded at 340nm at an interval of 1min. The working reagent was prepared by reconstituting the reagents supplied in the kit. The reagent was composed of L-Alanine, NADH (yeast), lactate dehydrogenase, 2-oxoglutarate and tris buffer. Prior, to the use all the reagent bottle and Aqua-4 (double deionized, 0.2 micron, membrane filtered, particle free water) was allowed to attain room temperature (15-30 ºC). About 20 mL of Aqua-4 was added to the reagent containing vial, swirled gently and was used for the estimation. The ALT activity was determined using the following formula as given with the kit. First, the mean absorbance change obtained was converted into International Units (IU) of activity using the general formula provided in the kit as follows:

$IU\ L^{-1} = (\Delta A\ min^{-1})$ x T.V. x 10^3/ S.V. x Absorptivity x P; Where:

T.V. = Total reaction volume in µL

S.V. = Sample volume in µL

Absorptivity = millimolar absorptivity of NADH at 340nm= 6.22

P = cuvette lightpath= 1cm

Activity of ALT at 37 ºC (IU L^{-1}) = ($\Delta A\ min^{-1}$) x Factor (1768)

3.8.3.6.5.2.2. Estimation of SGOT/AST (Aspartate aminotransferase) level

The IFCC method (International Federation of Clinical Chemistry method, 1980) provided in the manufacturer's instructions of commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany) was used to determine theAST level in the serum samples of all the experimental animals. The working reagent supplied had the composition of 2-oxoglutarate, L-aspartate, malate dehydrogenase, lactate dehydrogenase, NADH (yeast), tris buffer and EDTA. Prior to the use, the supplied reagent bottle and Aqua-4 (double deionized, 0.2 micron, membrane filtered, particle free water) was allowed to attain the room temperature (15-30 ºC). The working reagent was prepared mixing the content of one reagents bottle with 20mL of Aqua-4 (double deionized, 0.2 micron, membrane filtered, and particle free water). For estimation, 1000 µL of the working reagent was mixed thoroughly with 100 µL of the test sample (unhaemolysed serum) and aspirated. The absorbance was immediately recorded at 340 nm at an interval of 60 sec. The AST activity was determined using the following formula as given with the kit. First, the mean absorbance change obtained was converted into International Units (IU) of activity using the general formula provided in the kit as follows:

IU L^{-1} = ($\Delta A\ min^{-1}$) x T.V. x 10^3/ S.V. x Absorptivity x P; Where:

T.V. = Total reaction volume in µL;

S.V. = Sample volume in µL

Absorptivity = millimolar absorptivity of NADH at 340nm= 6.22

P = cuvette lightpath= 1cm

Activity of AST at 37 ºC (IU L^{-1}) = ($\Delta A\ min^{-1}$) x Factor (1768)

3.8.3.6.5.3. Analysis of kidney function test (KFT)

3.8.3.6.5.3.1. Estimation of urea level

The estimation of urea level in serum was done following the GLDH-Urease method, as described by Talke and Schubert (1965); Tiffany *et al.* (1972) and provided with the commercially available kit (Erba diagnostics Manheim GmbH, Mallaustr,

Manheim/Germany). The urea working reagent (consisting of a mixture of α-ketoglutarate, NADH, urease, glutamate dehydrogenase (GLDH), ADP, Tris buffer (*p*H 7.9±0.1) was used to determine the concentration of urea in the serum sample. Prior to the use, the supplied reagent bottle and Aqua-4 (double deionized, 0.2 micron, membrane filtered, particle free water) was allowed to attain the room temperature (15-30 ºC). The working reagent was then prepared by gently mixing the content of one reagent bottle with 20 mL of Aqua-4. For estimation, 2000 µL of the working reagent was mixed well with 20 µL of the standard/test serum and aspirated. Immediately, absorbance was recorded at 340nm at an interval of 60 sec against appropriate blank. The rate of decrease in absorbance is directly proportional to urea concentration in the sample.The concentration of urea in the serum sample was estimated using the following formula:

$$\text{Urea (mg dL}^{-1}) = \frac{\Delta\text{A of test}}{\Delta\text{A of standard}} \text{ x Conc. of standard (mg dL}^{-1})$$

Where, ΔA (absorbance change for the standard and test) = A_1-A_2.

3.8.3.6.5.3.2. Estimation of creatinine level

A modified method after Jaffe described by Slot (1965) and Bartel *et al.* (1972) that was provided with the commercial kit (Erba diagnostics Manheim GmbH, Mallaustr, Manheim/Germany) was employed for the estimation of creatinine level in the sera. The working reagent was prepared by mixing equal amount of picric acid and sodium hydroxide reagent. The mixture was then allowed to stand for 15 min. For estimation, 2000 µL of the working reagent was mixed with 200 µL of standard/serum sample and the initial absorbance (A_1) was read at 20 sec after mixing followed by final absorbance (A_2) at 80 sec after mixing at wavelength 505 nm. The absorbance of the orange-yellow colour (Jaffe's reaction) formed is directly proportional to the creatinine concentration.

The creatinine concentration was calculated by the formula:

$$\text{Creatinine (mg dL}^{-1}) = \frac{\Delta\text{A of test}}{\Delta\text{A of standard}} \text{ x Conc. of standard (mg dL}^{-1})$$

Where, ΔA (absorbance change) = A_2 (final) - A_1 (initial).

3.9. Isolation, partial purification and characterization of bioactive compounds

Since the methanolic extracts of *Illicium verum (Iv*ME*)* and *Glinus oppositifolius* (*Go*ME) were found to possess significant pharmacological activity compared to other extracts, an attempt has been made to partially purify and characterize the various chemical compounds present in these extracts through solvent partitioning followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

3.9.1. Preparation of methanolic extract by solid-liquid extraction

Twenty g of the fine powder of samples was extracted with 200 mL of HPLC grade methanol for 24 h under darkness and normal room temperature. The solution was filtered through a Whatman filter paper (Grade 1). This liquid phase of methanolic extract was used in solvent partitioning or liquid-liquid fractionation.

3.9.2. Solvent partitioning by liquid-liquid fractionation

The methanolic extract was transferred to a separating funnel and successively partitioned with n-hexane and ethyl acetate by liquid-liquid fractionation (LLF) based on polarity of the solvents. Briefly, the methanolic extract was poured in a separatingfunnel and n-hexane was added in equal quantity to the methanol phase and gently shaken. This process was repeated three times; hexane phase was separated from methanol phase and collected in a conical flask, and evaporated to dryness under reduced pressure at 40˚C. This fraction was further lyophilized to get solvent free mass fraction. Same procedure was also applied for ethyl acetate fraction. Thus three types of fractions i.e., methanolic fraction (ME), hexane fraction (HE) and ethyl acetate (EA) fraction were obtained.

3.9.3. Gas Chromatography-Mass Spectrometry analysis

In the present study, methanol, hexane and ethyl acetate fractions of *Illicium verum* and *Glinus oppositifolius* was used separately for carrying out the GC-MS analysis for various phytochemical compounds present in the plant samples. GC–MS analysis of the extract was carried out with GCMS-QP-2010 Ultra, Shimadzu, Japan with Thermal Desorption System TD 20. The instrument was equipped with programmable head space auto sampler and auto injector. The capillary column used was DB-1/RTXMS (30 m) with pure helium (99.999%) as a carrier gas, at a constant flow rate of 3 mL min^{-1} and 1 µL injection volume. Column oven temperature and injection temperature were set at 60 °C and 260 °C respectively. Samples were analyzed with the column

held at different temperatures. Pressure was established as 72.3 kPa and the sample was run for 60 min. Temperature and column flow for flame ionization detectors were set as 230 °C and 1.20 mL min^{-1}, correspondingly. MS parameters were as follows: scan range: 40 to 650 m/z with a scan speed of 3333. The relative percentage of the chemical constituents present in the samples was expressed as percentage by peak area normalization.

3.9.4. Identification of chemical compounds

The chemical compounds were identified on the basis of comparison of their retention time and mass spectra and computer matching with WILEY8.0 libraries and National Institute of Standards and Technology (NIST14.0) database provided with computer controlling the GC-MS system. The spectrum of the unknown component was compared with the spectrum of the known compounds stored in the library. The name, molecular weight and structure of the compounds of the test plants were ascertained.

3.10. Statistical analysis

All the data were subjected to various statistical analyses and expressed as mean ± standard deviation (SD). One way and two way analysis of variance (ANOVA) and LSD were employed for the statistical analysis of data using IBM SPSS statistic v. 21software. Level of significance *p*-value of less than 0.05 was considered to be statistically significant.

CHAPTER 4

RESULTS

4. Results

4.1. Enumeration of herbs and spices under survey

A total 40 plant samples comprising 15 herbs and 25 spices were collected during the survey and enumerated after proper taxonomic authentication. A panoramic view of different herbs and spices with their common English names, scientific names, family, status, usable plant parts, and medicinal properties are presented in Table 7.

4.2. Effect of solvent on soluble extractive of different samples

The powdered samples of selected herbs and spices were subjected to aqueous and solvent extraction using hot water and HPLC grade methanol. The extractive values of different lyophilized extracts are depicted in Figure 5. The data revealed that extractive value was more with methanol than hot water. The solvent soluble extractive value plays an important role in evaluation of crude plant extracts as different solvents has different level of polarity and thus capacity of phytochemical extraction. The extractive value of different plant extracts ranged from 2.05% to 34.61% depending on the initial amounts of plant materials and extracting solvents used. Generally methanolic extracts was found to have significantly higher percentage of extractive values in comparable to that of water extractive values in specific plant materials. Values of water soluble extractive of different herb and spice samples in the descending order are as follows: 27.06% in *P. perlata*> 25.98% in *M. fragrans*> 20.63% in *I. verum*> 18.76% in *C. pentandra*> 9.42% in *C. annuum*> 8.61% in *D. volubilis*> 7.92% in *G. oppositifolius*> 7.73% in *M. koenigii*> 5.75% in *M. piperita*> 5.03% in *F. vulgare*> 4.96% in *T. foenum-graecum*> 2.05% in *C. sativum*. The percentage of methanol soluble extractive of different herb and spice samples in the ascending order is as follows: 6.99% in *C. sativum*< 9.02% in *M. piperita*< 9.59% in *M. koenigii*< 12.44% in *T. foenum-graecum*< 12.93% in *D. volubilis*< 14.22% in *F. vulgare*< 14.60% in *C. annuum*< 22.77% in *G. oppositifolius*< 24.34% in *C. pentandra*< 29.88% in *I. verum*< 31.93% in *M. fragrans*< 34.61% in *P. perlata*.

4.3. Comparison of total moisture content of selected herbs and spices

Moisture is a quality factor in preservation and stability of herbs and spices. The dry matter that remains after removal of total moisture is generally referred to as total solids. This moisture or solids is often specified in packaging standards. The moisture content in herb and spice samples varies greatly as shown in Table 8.

Table 7: Enumeration of common dietary herbs and spices under survey and collection for the present study

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
SP01/2011	Pepper mint	*Mentha piperita* L.	Lamiaceae	Herb	Leaf	Fresh whole plant	Stimulant, stomachic, carminative, antiseptic, digestive, antispasmodic, contraceptive, used in vomiting, skin diseases, Amenorrhoea, dental caries.
SP02/2011	Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae	Herb/spice	Leaf, seed	Fresh whole plant and dried seeds	Leaves have carminative, tonic, antiulcer effects, hypoglycaemic activity, anti-inflammatory and antipyretic effects, Gastro protective efficiency; Seeds of fenugreek used to lower serum cholesterol, triglyceride, and low-density.
SP03/2011	Coriander	*Coriandrum sativum* L.	Apiaceae	Herb	Leaf	Fresh whole plant	Carminative, diuretic, tonic, stimulant, stomachic, refrigerant, aphrodisiac, analgesic, anti-inflammatory.
SP04/2011	Curry tree	*Murraya koenigii* (L.) Spreng.	Rutaceae	Herb	Leaf	Fresh leaves	Astringent, anthelmintic, febrifuge, stomachic, appetizing, carminative, constipating, anti inflammatory, antiseptic, used in skin diseases, in diarrhea, ulcers.
SP05/2011	Carpet weeds	*Glinus oppositifolius* (L.) Aug.DC.	Molluginaceae	Herb	Aerial part	Fresh whole plant	Useful as a bitter tonic for liver disorders, stomachic and antiseptic, anthelmintic, antioxidant, hepatoprotective, anti-

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
							inflammatory, antihyperglycemic activity
SP06/2011	Fennel	*Foeniculum vulgare* Mill.	Apiaceae	Herb/ spice	Aerial part, seeds	Fresh whole plant, seeds	Dried fruit have antioxidant, antimicrobial activity, seed oils are reported for hypoglycemic and hepatoprotective effects; antispasmodic and antifungal.
SP07/2011	Oregano	*Origanum vulgare* L.	Lamiaceae	Herb	Leaf	Dry leaves	Stimulant, carminative, stomachic, diuretic, diaphoretic and emmenagogue.
SP08/2011	Allspice	*Pimenta dioica* (L.) Merr.	Myrtaceae	Herb	Leaf	Dry leaves	Stimulant, digestive, carminative, anodyne against rheumatism & neuralgia.
SP09/2011	Basil	*Ocimum basilicum* L.	Lamiaceae	Herb	Leaf	Dry leaves	Stomachic, anthelmintic, diaphoretic, expectorant, antipyretic, carminative, stimulant, diuretic, demulcent, in skin diseases, asthma, opthalmia.
SP10/2011	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Herb	Leaf	Dry leaves	Astringent, nervine tonic, stomachic, antibacterial, protistocidal, rubefacient, used in headaches and hardy menstruation.
SP11/2011	Thyme	*Thymus vulgaris* L.	Lamiaceae	Herb	Leaf	Dry leaves	Antispasmodic, carminative, emmenagogue, anthelmintic, spasmodic, laxative, stomachic, tonic, vermifuge.

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
SP12/2011	Sage	*Salvia officinalis* L.	Lamiaceae	Herb	Leaf	Dry leaves	Mild tonic, astringent, carminative, deodorant, insecticidal,, antipyretic, used in gingivitis, dentifice, mouthwash, gargles.
SP13/2011	Bay leaf	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & Eberm.	Lauraceae	Herb	Leaf	Dry leaves	Carminative, used in colic, diarrhoea; have antioxidant and antidiabetic properties.
SP14/2011	Celery	*Apium graveolens* L.	Apiaceae	Herb	Seeds	Seeds	Stimulant, tonic, diuretic, carminative, emmenagogue, anti-inflammatory.
SP15/2011	Spring Onion	*Allium cepa* L.	Amaryllidaceae	Herb	Aerial part, Bulb	Fresh bulb, floral stick	Potent anti-oxidant, immuno-modulatory and anti-inflammatory, treating high cholesterol, diabetes, joint disorders, digestive ailments, loss of appetite, gallbladder diseases, angina pectoris, high blood pressure, atherosclerosis, sore throat, asthma, bronchitis, cough, intestinal gas and intestinal worms.
SP16/2011	Red chilli	*Capsicum annuum* L.	Solanaceae	Spice	Fruit	Fresh fruit	Digestive, thermogenic, carminative, stimulant, cardiotonic, antipyretic, serdorific, rubefacient & sialagogue.
SP17/2011	Mace/ nutmeg	*Myristica fragrans* Houtt.	Myristicaceae	Spice	Aril	Dry aril, seed	Fruit extract showed hypolipidemic activity and LDL-antioxidant activity, antibacterial activity.

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
SP18/2011	Cardamom (Small)	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Spice	Fruit, Seeds	Dry fruit	Effective against gastrointestinal disorders, enhance cytotoxic activity of natural killer cells indicating their potential anticancer effects; immune modulatory and antitumor activities; regulate inflammatory responses and prevent/attenuate carcinogenesis.
SP19/2011	Cardamom (Large)	*Amomum subulatum* Roxb.	Zingiberaceae	Spice	Fruit, Seeds	Fruit	Hypnotic, appetizer, astringent to bowels, tonic to heart and liver.
SP20/2011	Indian caper	*Ceiba pentandra* (L.) Gaertn.	Malvaceae	Spice	Floral bud	Dry floral bud	Astringent, diuretic herb that lowers fevers, relaxes spasms and controls bleeding, powdered fruit is taken with water as a remedy for intestinal parasites and stomach-ache.
SP21/2011	Stone flower	*Parmelia perlata* (Huds.) Ach.	Parmeliaceae	Spice	Thallus	Thallus	For dressing wounds, to treat ailments relating to either the lungs or the digestive system, treating sexually transmitted infections and ailments of the urinary system.
SP22/2011	Cumin	*Cuminum cyminum* L.	Apiaceae	Spice	Dried Fruit	Dried Fruit	In treatment of inflammation, pain, digestive disorders, blood purification, reducing inflammation of uterus and

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
							itching; seeds act as hypoglycaemic agent and reduce total serum cholesterol.
SP23/2011	Fennel	*Foeniculum vulgare* Mill.	Apiaceae	Spice	Seeds	Fresh aerial parts, seeds	Essential oils from dried fruits have antioxidant, antimicrobial activity.
SP24/2011	Cinnamon	*Cinnamomum verum* J.Presl	Lauraceae	Spice	Bark	Bark	Astringent, diuretic, carminative, aphrodisiac, deodorant, expectorant, febrifuge, stomachic.
SP25/2011	Turmeric	*Curcuma longa* L.	Zingiberaceae	Spice	Rhizome	Dried rhizome	In leprosy and diabetes, for stomach ache,carminative, tonic, antirheumatic, blood purifier, antiseptic and cure for liver ailments; raw juice of curcuma used to tear in gallstones, gall bladder complaints, and dental-troubles and for sore throat and common cold, parasitic skin diseases and to cure piles.
SP26/2011	Garlic	*Allium sativum* L.	Amaryllidaceae	Spice	Bulb	Bulb	Used for asthma, deafness leprosy, bronchial congestion, fevers, worms and liver gall bladder trouble; good for heart and hair; stimulant to appetite; in high blood pressure and skin disorder.
SP27/2011	Mustard	*Brassica nigra* (L.) K.Koch	Brassicaceae	Spice	Seeds	Seeds	Possess rubefacient properties, in disorders of the digestive organs, Irritant,

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
							stimulant, diuretic, emetic.
SP28/2011	Aniseed	*Pimpinella anisum* L.	Apiaceae	Spice	Seeds	Seeds	Mild expectorant, stimulating, carminative, diuretic, diaphoretic, in asthma powders, in veterinary medicine.
SP29/2011	Cotton milk plant	*Dregea volubilis* (Linn. f.) Benth ex. Hook.f.	Asclepidaceae	Spice	Stem bark	Stem bark	To cure various diseases such as diabetes mellitus, boils, abscesses, inflammations, eye ailments, tracheitis and stomach ache.
SP30/2011	Saffron	*Crocus sativus* L.	Iridaceae	Spice	Dried Stigmas and Styles	Dried Stigmas and Styles	Stimulant, tonic, stomachic, aphrodisiac, anodyne, anti spasmodic, emmenagogue, diuretic, laxative, used in bronchitis, fever, epilepsy, skin diseases.
SP31/2011	Clove	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Myrtaceae	Spice	Floral bud	Dry floral bud	Refrigerant, ophthalmic, digestive, carminative, stomachic, stimulant, antispasmodic, antibacterial, expectorant, rubefacient, Aphrodisiac, appetizer, emollient.
SP32/2011	Pepper	*Piper nigrum* L.	Piperaceae	Spice	Fruits	Dry fruit	To treat cough and cold, digestive problems and cholera; anti-influenza, anti-rheumatoid, antiarthritis, antispasmodic and antioxidant.
SP33/2011	Poppy seed	*Papaver somniferum*	Papaveraceae	Spice	Seeds	Seeds	Expectorant, sudorfic, sedative, nervine

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
		L.					tonic, constipating, aphrodisiac, used in internal haemorrhages, diarrhoea, dysentery.
SP34/2011	Sweet flag	*Acorus calamus* L.	Acoraceae	Spice	Root	Fresh root	Thermogenic, constipating, emmenagogue, intellect promoting, emetic, carminative, stomachic, expectorant, sudorfic, antipyretic, resusticative, tranquilising, sedative, nervine tonic.
SP35/2011	Caraway	*Carum carvi* L.	Apiaceae	Spice	Fruits	Dry fruit	Stomachic, carminative, anthemintic, lactagogue, adjuvant/ corrective for nauseating & griping effects of medicines.
SP36/2011	Asafoetida	*Ferula assa-foetida* L.	Apiaceae	Spice	Latex of rhizome and Root	Crystallized gum	As flatulence and constipation; asthma, excessive and painful menstruation, tooth ache, sexual impotency, fever, and whooping cough, anti-influenza A (H_1N_1).
SP37/2011	Black Cumin	*Nigella sativa* L.	Ranunculaceae	Spice	Seeds	Dry seed	Antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders, antidiabetic, anticancer, antimicrobial, antioxidant etc.
SP38/2011	Carom seeds	*Trachyspermum ammi* (L.) Sprague	Apiaceae	Spice	Fruit	Dry fruit	Gastro-protective, antifilarial, anti-inflammatory potential, detoxifier of

Sampling code	Common name	Scientific name	Taxonomic family	Plant type	Usable parts	Plant parts collected	Reported medicinal properties
							aflatoxins, antihypertensive, antispasmodic and broncho-dilating, hepatoprotective, antilithiasis and diuretic activity.
SP39/2011	Star anise	*Illicium verum* Hook. f.	Schisandraceae	Spice	Fruit	Dry fruit	Antimicrobial, antioxidant, insecticidal, analgesic, sedative and convulsive activities, antiflu drug (Tamiflu).
SP40/2011	Bird chilly	*Capsicum frutescens* L.	Solanaceae	Spice	Fruit	Fresh fruit	In upset stomach, intestinal gas, stomach pain, diarrhea, and cramps; used for conditions of the heart and blood vessels including poor circulation, excessive blood clotting, high cholesterol, and preventing heart disease.

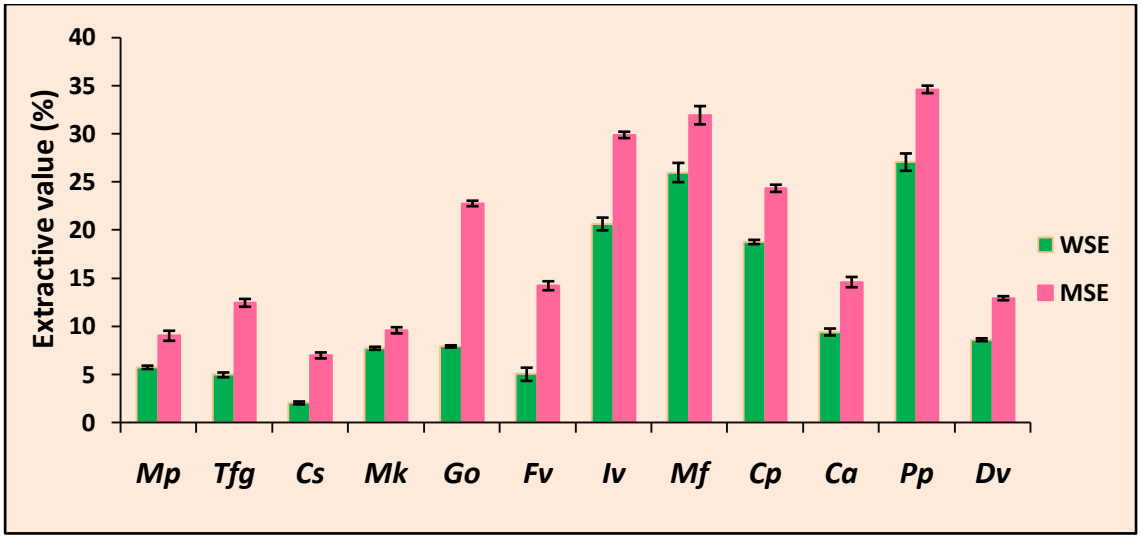

Figure 5.Water soluble extractive (WSE) and methanol soluble extractive (MSE) values of selected herbs and spices. Each value is expressed as mean ± standard deviation of triplicate analysis (n=3) and presented in percentage. Here, *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*.

Table 8: Moisture analysis of different herb and spice samples

Herb sample	% moisture content (wt/wt)	Spice sample	% moisture content (wt/wt)
Mentha piperita	89.56 ± 0.87	*Illicium verum*	7.44 ± 0.38
Trigonella foenum-graecum	70.41 ± 0.72	*Myristica fragrans*	10.12 ± 0.13
Coriandrum sativum	82.11 ± 0.67	*Ceiba pentandra*	8.54 ± 0.28
Murraya koenigii	78.83 ± 0.16	*Capsicum annuum*	80.33 ± 0.60
Glinus oppositifolius	80.20 ± 0.42	*Parmelia perlata*	11.20 ± 0.63
Foeniculum vulgare	87.97 ± 0.34	*Dregea volubilis*	5.710 ± 0.20
LSD value: 64.36			

Each value represents the mean ± standard deviation from triplicate analysis (n=3) and expressed in percentage on wet weight basis for fresh samples and on dry weight basis for commercially available dry samples. Values in the same column are significantly different ($p<0.05$), as determined using the Fisher's LSD test.

Table 8A: ANOVA of data presented in Table 8 (Total moisture content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	47076.56	11	4279.687	16773.4	8E-44	2.216309
R(A) (Within Groups)	6.123533	24	0.255147			
AR (Total)	47082.68	35				

Total moisture content of herbs and spices ranges from 5.71% to 89.56%. Fresh samples contained much higher moisture than the dried ones. The moisture contents of the samples in the ascending order are as follows: 5.71% in *D. volubilis*<7.44% in *I. verum*<8.54% in *C. pentandra*<10.12% in *M. fragrans* <11.20% in *P. perlata* <70.41% in *T. foenum-graecum* <78.83% in *M. koenigii* <80.33% in *C. annuum* <80.20% in *G. oppositifolius*<82.11% in *C. sativum*<87.97% in *F. vulgare*<89.56% in *M. piperita*.

4.4. Comparison of occurrences of phytochemicals in different herb and spice extracts

Both hot aqueous and methanolic extracts of were analysed for their major phytochemicals or secondary metabolites. Qualitative phytochemical analysis was carried out using at least two methods, in some cases with single standard method. This is required because of the varied sensitivities of the test methods employed in detecting specific chemical constituents that may occur with trace quantities in some cases. The results of this screening indicated that at least one phytochemical group was present in the plant extracts investigated so far. The data corresponding to Table 9 to Table 12 describes the comprehensive reports of qualitative phytochemical analysis of hot aqueous and methanolic extracts of different herbs and spices. The result reveals that methanolic extract was found to exhibit greater intensity of chemical compounds than hot aqueous extracts.

Table 9: Qualitative detection of major phytochemical groups present in lyophilized hot aqueous extracts of different herbs

Phytochemical Groups	*Mentha piperita*	*Trigonella foenum-graecum*	*Coriandrum sativum*	*Murraya koenigii*	*Glinus oppositifolius*	*Foeniculum vulgare*
Reducing sugars	+	+	+	++	++	+
Phenols	++	++	++	++	+++	+
Flavonoids	+	+	+	++	++	+
Resins	-	-	-	-	-	+
Free amino acids	+	++	+	++	++	+
Anthraquinones	-	+	-	-	-	-
Tannins	+	+	+	++	+++	-
Triterpenoids	-	-	-	+	+	-
Alkaloids	-	++	+	++	++	-
Glycosides	-	-	-	-	+	+
Steroids	-	+	+	++	++	+
Saponins	-	+	-	++	++	-
Cardiac glycosides	+	-	+	++	++	+
Phlobatannins	-	-	-	-	+	-
Cardenolides	-	-	-	-	-	-

Number of (+) sign denotes intensity of occurrence of phytochemicals present in lyophilized hot water extract (1 mg mL^{-1}), (-) sign denotes absence of a particular chemical group.

Table 10: Qualitative detection of major phytochemical groups present in lyophilized methanolic extracts of selected herbs

Phytochemical Groups	*Mentha piperita*	*Trigonella foenum-graecum*	*Coriandrum sativum*	*Murraya koenigii*	*Glinus oppositifolius*	*Foeniculum vulgare*
Reducing sugars	+	+	++	+	++	++
Phenols	++	++	++	++	+++	++
Flavonoids	+	++	+	+	++	+
Resins	-	+	-	-	-	++
Free amino acids	-	-	-	+	+	-
Anthraquinones	+	+	-	++	-	+
Tannins	++	+	-	+	++	+
Triterpenoids	+	-	-	+	+	+
Alkaloids	++	+	+	-	++	++
Glycosides	+	+	++	+	++	+
Steroids	-	+	+	+	-	+
Saponins	+	+	-	-	++	+
Cardiac glycosides	+	-	+	+	++	++
Phlobatannins	-	+	-	-	+	+
Cardenolides	-	-	-	-	+	+

Number of (+) sign denotes intensity of occurrence of phytochemicals present in lyophilized methanolic extract (1 mg mL^{-1}), (-) sign denotes absence of a particular chemical group.

Table 11: Qualitative detection of major phytochemical groups present in lyophilized hot aqueous extracts of different spices

Phytochemical Groups	*Illicium verum*	*Myristica fragrans*	*Ceiba pentandra*	*Capsicum annuum*	*Parmelia perlata*	*Dregea volubilis*
Reducing sugars	+++	++	+	++	++	+
Phenols	++	+	+	++	++	+
Flavonoids	+	+	+	+	+	+
Resins	-	++	+	-	-	+
Free amino acids	++	++	+	+	+	+
Anthraquinones	-	+	-	+	-	-
Tannins	+	+	+	+	++	-
Triterpenoids	+	-	-	-	+	-
Alkaloids	++	+	+	+	-	-
Glycosides	-	+	-	-	+	+
Steroids	++	++	+	-	-	+
Saponins	++	+	+	+	-	-
Cardiac glycosides	-	-	-	+	+	+
Phlobatannins	-	-	-	-	-	-
Cardenolides	-	-	-	-	-	-

Number of (+) sign denotes intensity of occurrence of phytochemicals present in lyophilized hot water extract (1 mg mL^{-1}), (-) sign denotes absence of a particular chemical group.

Table 12: Qualitative detection of major phytochemical groups present lyophilized in methanolic extracts from selected spices

Phytochemical Groups	*Illicium verum*	*Myristica fragrans*	*Ceiba pentandra*	*Capsicum annuum*	*Parmelia perlata*	*Dregea volubilis*
Reducing sugars	+++	++	++	+	++	+
Phenols	+++	++	++	++	+++	++
Flavonoids	+++	++	+	++	++	+
Resins	-	++	+	+	-	++
Free amino acids	++	+	-	-	+	-
Anthraquinones	-	++	-	+	-	+
Tannins	++	++	+	+	++	+
Triterpenoids	+	-	+	+	+	+
Alkaloids	+++	++	++	++	+	++
Glycosides	++	+	+	+	+++	+
Steroids	-	++	++	-	+	+
Saponins	++	+++	-	+	+	+
Cardiac glycosides	-	++	-	-	+	++
Phlobatannins	-	-	-	-	+	+
Cardenolides	-	-	-	-	-	+

Number of (+) sign denotes intensity of occurrence of phytochemicals present in lyophilized methanolic extract (1 mg mL^{-1}), (-) sign denotes absence of a particular chemical group.

4.5. Quantitative estimation of various biochemical components

4.5.1. Nutritional components

Three main nutrient components of food *i.e.* carbohydrates, proteins and lipids, along with vitamin C and E were quantified using standard methods and results are shown in Table 13 to Table 15.

Table 13: Quantitative estimation of soluble sugar content and reducing sugar content present in different herb and spice samples

Species of herbs	Soluble sugar content	Reducing sugar content
Mentha piperita	56.42 ± 0.07	29.68 ± 0.78
Trigonella foenum-graecum	54.17 ± 0.57	24.10 ± 0.89
Coriandrum sativum	62.55 ± 0.65	33.31 ± 0.58
Murraya koenigii	68.18 ± 0.87	41.03 ± 0.68
Glinus oppositifolius	70.70 ± 0.38	53.23 ± 0.59
Foeniculum vulgare	65.07 ± 0.41	48.88 ± 0.23
Illicium verum	51.64 ± 0.54	28.72 ± 0.44
Myristica fragrans	39.15 ± 0.71	21.09 ± 0.76
Ceiba pentandra	40.76 ± 0.62	12.18 ± 0.32
Capsicum annuum	29.57 ± 0.64	19.04 ± 0.80
Parmelia perlata	21.15 ± 0.68	10.36 ± 0.61
Dregea volubilis	25.09 ± 0.44	9.31 ± 0.43
LSD value:	27.958	24.775

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Values in the same column are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Total soluble sugar content and total reducing sugar content expressed in mg Glucose equivalent g^{-1} of tissue weight.

Table 13A: ANOVA of data presented in Table 13 (Soluble sugar content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	9773.921	11	888.5383	2618.954	3.77E-34	2.216309
R(A) Within Groups	8.142533	24	0.339272			
AR (Total)	9782.064	35				

Table 13B: ANOVA of data presented in Table 13 (Reducing sugar content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	6975.322	11	634.1202	1623.994	1.16E-31	2.216309
R(A) Within Groups	9.371267	24	0.390469			
AR (Total)	6984.694	35				

It was found that *G. oppositifolius* contained highest amount of total soluble sugars and reducing sugar content, while *P. perlata* and *D. volubilis* have the lowest amount of total soluble sugar and reducing sugar content respectively. Total soluble protein content and total lipid content were found to be maximum in *M. koenigii* while *D. volubilis* contained minimum protein content and *P. perlata* contained the the lowest lipid content among the plants studied. In case of vitamin C quantification *C. annuum* showed the highest value and *M. koenigii*with the lowest value. Vitamin E content was detected maximum in *I. verum* and lowest quantity was found in *M. piperita*. Among the dried spice samples *P. perlata* did not contain vitamin E at all.

Table 14: Quantitative estimation of soluble protein content and total lipid content present in different herb and spice samples

Species of herbs	Soluble protein content	Total lipid content
Mentha piperita	45.53 ± 0.61	10.27 ± 0.37
Trigonella foenum-graecum	35.03 ± 0.53	9.42 ± 0.13
Coriandrum sativum	35.00 ± 0.45	6.07 ± 0.16
Murraya koenigii	69.84 ± 0.39	11.35 ± 0.93
Glinus oppositifolius	31.19 ± 0.83	2.83 ± 0.75
Foeniculum vulgare	39.99 ± 0.84	3.33 ± 0.36
Illicium verum	4.66 ± 0.35	7.30 ± 0.38
Myristica fragrans	4.50 ± 0.46	8.48 ± 0.49
Ceiba pentandra	11.19 ± 0.55	2.84 ± 0.39
Capsicum annuum	19.01 ± 0.74	9.30 ± 0.33
Parmelia perlata	3.19 ± 0.20	0.47 ± 0.02
Dregea volubilis	2.47 ± 0.51	1.12 ± 0.15
LSD value:	36.110	5.197

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Values in the same column are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Total soluble protein content expressed in mg Bovine serum albumin equivalent g^{-1} of tissue weight and total lipid content in mg g^{-1} of dry tissue weight.

Table 14A: ANOVA of data presented in Table 14 (Soluble protein content)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Between Groups)	14817.41	11	1347.037	4178.876	1.39E-36	2.216309
R(A) Within Groups	7.736267	24	0.322344			
AR (Total)	14825.15	35				

Table 14B: ANOVA of data presented in Table 14 (Total lipid content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	306.925	11	27.90228	84.87277	1.26E-09	2.717331
R(A) Within Groups	3.94505	12	0.328754			
AR (Total)	310.8701	23				

Table 15: Quantitative estimation of vitamin C content and vitamin E content present in different herb and spice samples

Species of herbs	**Vitamin C content**	**Vitamin E content**
Mentha piperita	0.878 ± 0.032	0.028 ± 0.003
Trigonella foenum-graecum	0.597 ± 0.034	0.029 ± 0.002
Coriandrum sativum	0.864 ± 0.010	0.070 ± 0.003
Murraya koenigii	0.454 ± 0.028	0.057 ± 0.006
Glinus oppositifolius	1.167 ± 0.104	0.587 ± 0.045
Foeniculum vulgare	0.360 ± 0.016	0.013 ± 0.004
Illicium verum	16. 35 ± 0.48	1.83 ± 0.87
Myristica fragrans	6.92 ± 0.79	1.13 ± 0.21
Ceiba pentandra	3.68 ± 0.53	0.65 ± 0.16
Capsicum annuum	17. 72 ± 0.56	0.84 ± 0.04
Parmelia perlata	8.19 ± 0.32	Nd
Dregea volubilis	3.61 ± 0.13	0.51 ± 0.02
LSD value:	10.511	0.986

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Values in the same column are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Vitamin C content expressed in mg Ascorbic acid equivalent g^{-1} dry weight sample and Vitamin E content in mg α-Tocopherol equivalent g^{-1} dry weight sample.Nd: not detected.

Table 15A: ANOVA of data presented in Table 15 (Vitamin C content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	1255.569	11	114.1427	860.3712	2.32E-28	2.216309
R(A) Within Groups	3.184002	24	0.132667			
AR (Total)	1258.753	35				

Table 15B: ANOVA of data presented in Table 15 (Vitamin E content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	10.05979	10	1.005979	13.20872	3.92E-07	2.296696
R(A) Within Groups	1.675525	22	0.07616			
AR (Total)	11.73532	32				

4.5.2. Quantification of phytochemical components

Herbs and spices contain a variety of non-nutritive, biologically important, health promoting substances that enhance nutritional value of a diet, belong to a class of phytochemicals or plant secondary metabolites. The quantitative estimation of phenolic compounds such as total polyphenol content, total flavonoid content, total flavanol content, total flavonol content in different herb and spice materials is summarized in Table 16 to Table 18. Among the plant pigments which exert beneficial effects on human health are chlorophylls and carotenoids. Estimation of total chlorophyll content and total carotenoid content of green herbs is portrayed in Table 19. Total chlorophyll content was found to be highest in *G. oppositifolius* and lowest in *F. vulgare*. The descending order of total chlorophyll content present in the samples is as follows: *G. oppositifolius>M. koenigii>C. sativum>M. piperita>T. foenum-graecum >F. vulgare.*Total carotenoid content was found highest in *G. oppositifolius* among the samples. The total carotenoid content of the samples in the ascending order is as follows: *M. piperita<T. foenum-graecum<C. sativum<F. vulgare<M. koenigii <G. oppositifolius.*

Table 16: Quantitative determination of total polyphenol content present in lyophilized extracts of different herb and spice samples

Herb and spice samples	Total polyphenol content	
	LHE	LME
Mentha piperita	5.70 ± 0.20	8.46 ± 0.60
Trigonella foenum-graecum	3.45 ± 0.10	4.92 ± 0.26
Coriandrum sativum	2.55 ± 0.05	3.33 ± 0.36
Murraya koenigii	5.06 ± 0.02	7.43 ± 0.51
Glinus oppositifolius	12.08 ± 0.26	22.07 ± 0.27
Foeniculum vulgare	4.05 ± 0.82	6.16 ± 0.98
Illicium verum	13.72 ± 0.46	23.89 ± 0.34
Myristica fragrans	6.79 ± 0.81	15.78 ± 0.90
Ceiba pentandra	4.39 ± 0.15	10.68 ± 0.57
Capsicum annuum	10.27 ± 0.69	8.92 ± 0.13
Parmelia perlata	0.153 ± 0.034	0.215 ± 0.003
Dregea volubilis	0.942 ± 0.103	2.54 ± 0.03
LSD value (column): 39.044		
LSD value (row): 6.764		

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Total polyphenol content expressed in mg Gallic acid equivalents g^{-1} of lyophilized extract. Values in the same column and rows are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Lyophilized hot aqueous extract and lyophilized methanolic extract are abbreviated as LHE and LME respectively.

Table 16A: ANOVA of data presented in Table 16 (Total polyphenol content)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	85.1882	1	85.1882	10.53515	0.007793	4.844336
B (Columns)	727.7829	11	66.16208	8.182208	0.00079	2.81793
AxB (Error)	88.947	11	8.086091			
AB (Total)	901.9181	23				

Table 17: Quantitative determination of total flavonoid content present in lyophilized extracts of different herb and spice samples

Herb and spice samples	Total flavonoid content	
	LHE	LME
Mentha piperita	1.68 ± 0.26	3.19 ± 0.31
Trigonella foenum-graecum	1.33 ± 0.18	2.33 ± 0.16
Coriandrum sativum	0.66 ± 0.12	3.05 ± 0.11
Murraya koenigii	1.75 ± 0.18	2.76 ± 0.21
Glinus oppositifolius	4.94 ± 0.12	9.24 ± 0.47
Foeniculum vulgare	2.60 ± 0.19	3.74 ± 0.10
Illicium verum	9.82 ± 0.40	13.89 ± 0.34
Myristica fragrans	4.16 ± 0.85	7.83 ± 0.51
Ceiba pentandra	2.59 ± 0.26	5.17 ± 0.68
Capsicum annuum	5.52 ± 0.41	8.16 ± 0.84
Parmelia perlata	0.072 ± 0.003	0.098 ± 0.004
Dregea volubilis	0.635 ± 0.031	1.58 ± 0.13
LSD value (column): 24.770		
LSD value (row): 3.923		

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Total flavonoid content expressed in mg Catechin equivalents g^{-1} of lyophilized extract. Values in the same column and rows are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Lyophilized hot aqueous extract and lyophilized methanolic extract are abbreviated as LHE and LME respectively.

Table 17A: ANOVA of data presented in Table 17 (Total flavonoid content)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	26.62827	1	26.62827	27.72892	0.000266	4.844336
B (Columns)	244.8905	11	22.26278	23.18298	5.05E-06	2.81793
AxB (Error)	10.56338	11	0.960307			
AB (Total)	282.0822	23				

Table 18: Quantitative determination of total flavonol content present in lyophilized extracts of different herb and spice samples

Herb and spice samples	Total flavonol content	
	LHE	LME
Mentha piperita	0.85 ± 0.13	2.93 ± 0.14
Trigonella foenum-graecum	0.57 ± 0.06	1.12 ± 0.21
Coriandrum sativum	0.44 ± 0.07	2.68 ± 0.26
Murraya koenigii	0.98 ± 0.11	1.67 ± 0.24
Glinus oppositifolius	2.96 ± 0.11	5.68 ± 0.37
Foeniculum vulgare	1.65 ± 0.37	2.45 ± 0.46
Illicium verum	7.10 ± 0.28	9.73 ± 0.72
Myristica fragrans	3.08 ± 0.13	5.49 ± 0.51
Ceiba pentandra	1.68 ± 0.57	2.90 ± 0.21
Capsicum annuum	3.48 ± 0.46	6.50 ± 0.49
Parmelia perlata	0.05 ± 0.01	0.07 ± 0.01
Dregea volubilis	0.42 ± 0.03	0.74 ± 0.13
LSD value (column): 16.221		
LSD value (row): 2.808		

Content represented as mean ± standard deviation unit from triplicate analysis (n=3). Total flavonol content expressed in mg Quercetin equivalents g^{-1} of lyophilized extract. Values in the same column and rows are significantly different ($p<0.05$), as determined using the Fisher's LSD test. Lyophilized hot aqueous extract and lyophilized methanolic extract are abbreviated as LHE and LME respectively.

Table 18A: ANOVA of data presented in Table 18 (Total flavonol content)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	14.6797	1	14.6797	25.47787	0.000374	4.844336
B (Columns)	125.4596	11	11.40541	19.79506	1.13E-05	2.81793
AxB (Error)	6.337923	11	0.576175			
AB (Total)	146.4772	23				

Table19. Quantification of total chlorophyll and total carotenoid contents in different herbs

Herb samples	Total chlorophylls	Total carotenoids
Mentha piperita	2.040 ± 0.025	0.063 ± 0.002
Trigonella foenum-graecum	1.639 ± 0.058	0.066 ± 0.003
Coriandrum sativum	2.336 ± 0.096	0.116 ± 0.005
Murraya koenigii	2.557 ± 0.065	0.190 ± 0.006
Glinus oppositifolius	2.828 ± 0.042	0.199 ± 0.002
Foeniculum vulgare	1.276 ± 0.173	0.121 ± 0.004
LSD value:	0.923	2.2

Content expressed as mean ± standard deviation of triplicate determinations (n=3). Total chlorophyll content is presented as mg g^{-1} of fresh tissue weight and total carotenoid content is presented as µg g^{-1} of fresh tissue weight.

Table 19A: ANOVA of data presented in Table 19 (Total chlorophyll content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	4.397213	5	0.879443	106.8964	1.6E-09	3.105875
R(A) Within Groups	0.098725	12	0.008227			
AR (Total)	4.495938	17				

Table 19B: ANOVA of data presented in Table 19 (Total carotenoids content)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	0.051584	5	0.010317	719.02	1.98E-14	3.105875
R(A) Within Groups	0.000172	12	1.43E-05			
AR (Total)	0.051756	17				

4.6. *In vitro* antioxidant activities of hot aqueous and methanolic extracts of different herbs and spices

The extracts of all the plants were analysed for *in vitro* antioxidant activity. The antioxidant activity was determined by four different test models *i.e.*, DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, superoxide anion scavenging activity and nitric oxide radical scavenging activity. Among the test methods, Superoxide anion scavenging assay, hydrogen peroxide scavenging assay, and nitric oxide scavenging assay etc. involve hydrogen atom transfer (HAT) method, and the assays of total polyphenolic content (TPC), total flavonoid content (TFC) etc. are of electron transfer (ET) method, while DPPH• assay include both the method predominantly via single electron transfer (SET) method. Assays for total polyphenolic content (TPC) and total flavonoid content (TFC) were performed in quatification of phytochemicals section.

4.6.1. DPPH• free radical scavenging activity

The antioxidant activity of all the solvent extracts of herb and spice plant parts was measured through different concentrations. IC_{50} value which is widely used parameter to measure the free radical scavenging activity was also determined. A decrease by 50% of the initial radical concentration is defined as the IC_{50} value. Antioxidant activity is inversely proportional to the IC_{50} value and it is also noted that, lower the IC_{50} value higher is the antioxidant activity. DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH radical is determined by the decrease in the absorbance which is caused by antioxidants. The reaction between antioxidant molecules and free radicals results in the scavenging of the radicals by hydrogen donation. Free radical scavenging ability of the plant extracts was evaluated by change in the absorbance of reduced DPPH, visually noticed as a change in colour from purple to yellow. The ability of the extracts to neutralize hydroxyl radical was represented as 50% inhibitory concentration (IC_{50}) and expressed in μg mL^{-1}. All the selected plants were subjected for DPPH activity and the results revealed that among the different extracts tested, methanol extracts offered a maximum free radical scavenging activity. The control ascorbic acid showed 50% inhibition at 19.55 μg mL^{-1} concentration (Figure 6-9, Table 20-21).

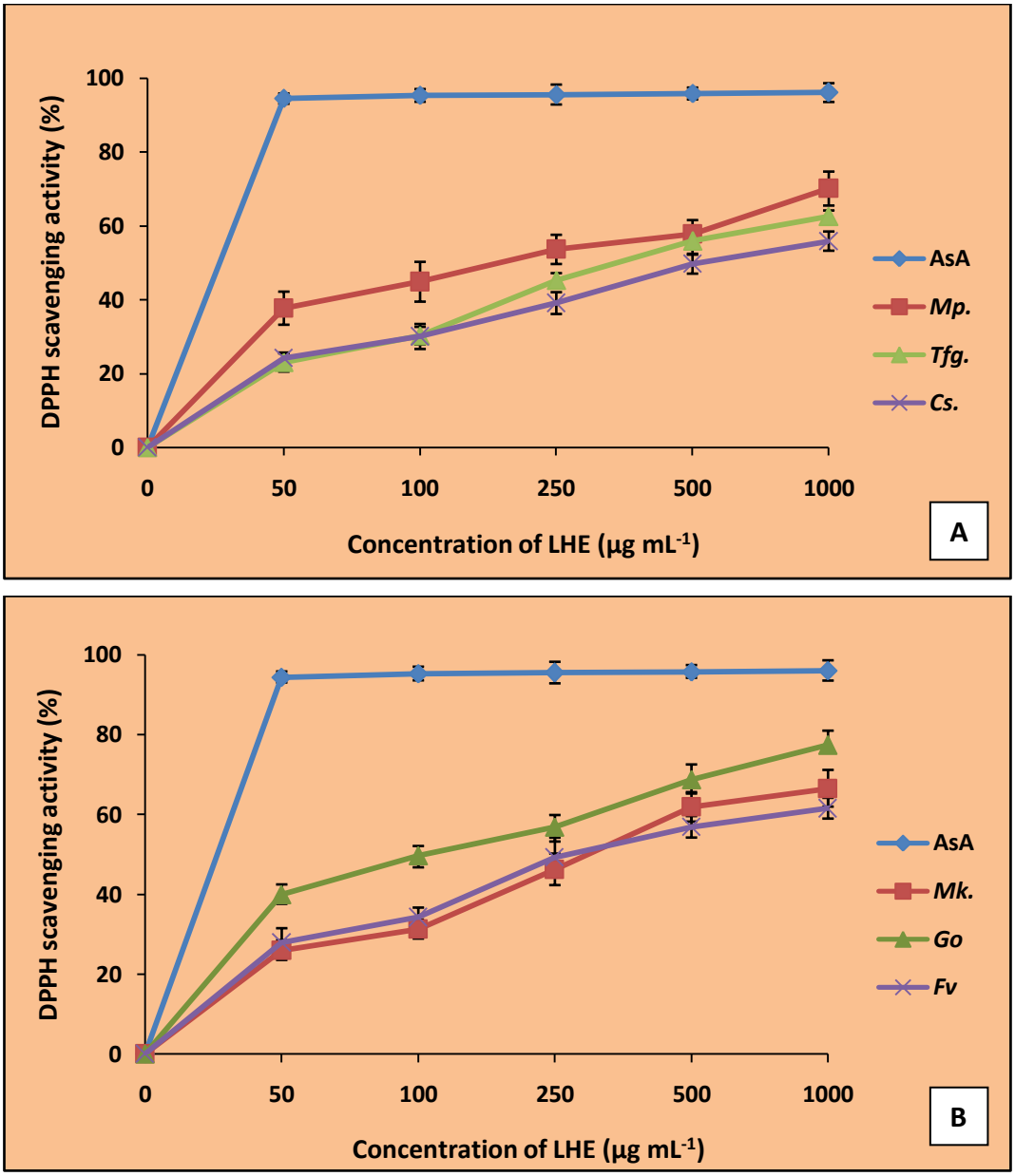

Figure 6. DPPH scavenging activity of lyophilized hot aqueous extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract.

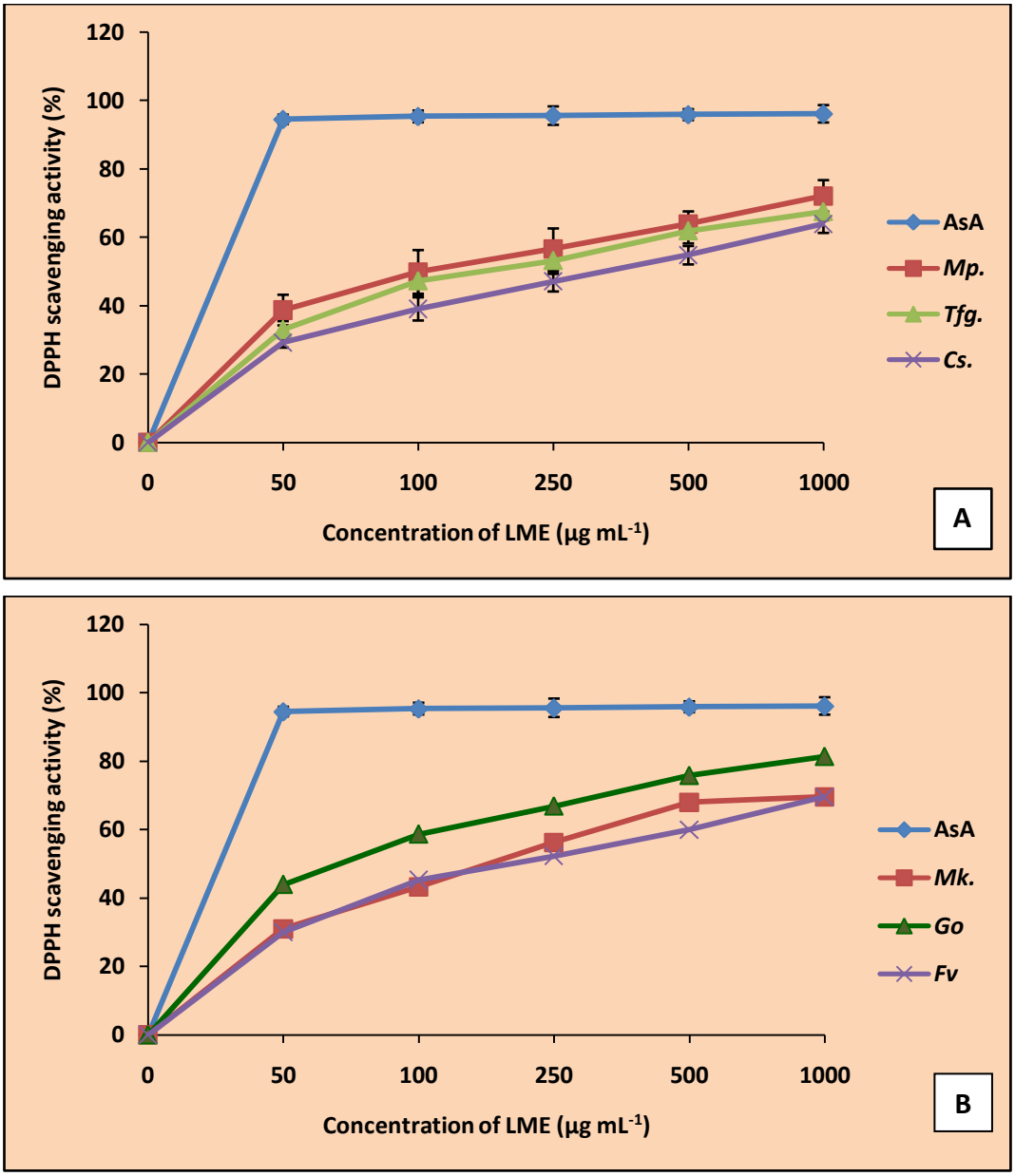

Figure 7. DPPH scavenging activity of lyophilized methanolic extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LME: Lyophilized methanolic extract.

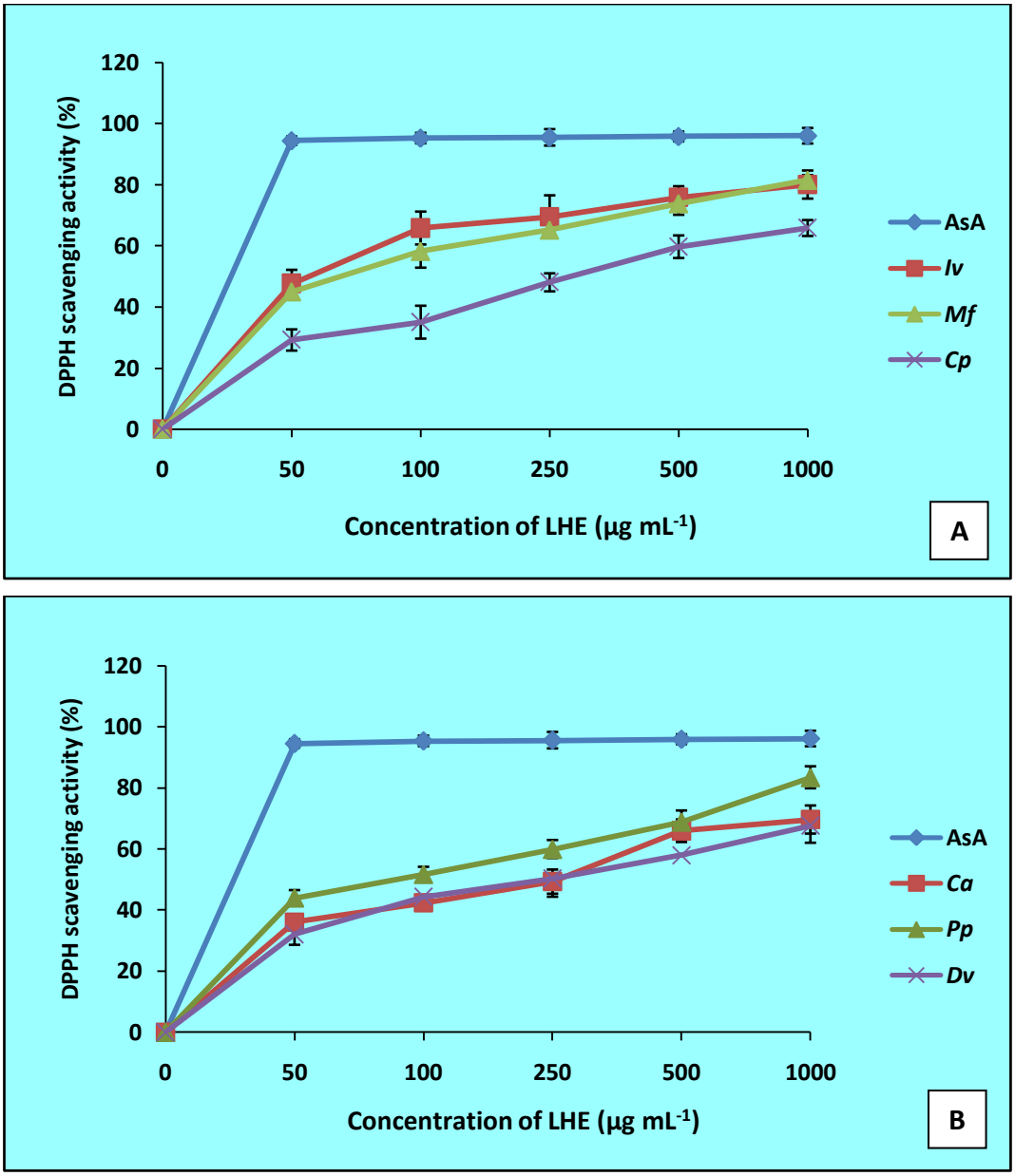

Figure 8. DPPH scavenging activity of lyophilized hot aqueous extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LHE: Lyophilized hot aqueous extract.

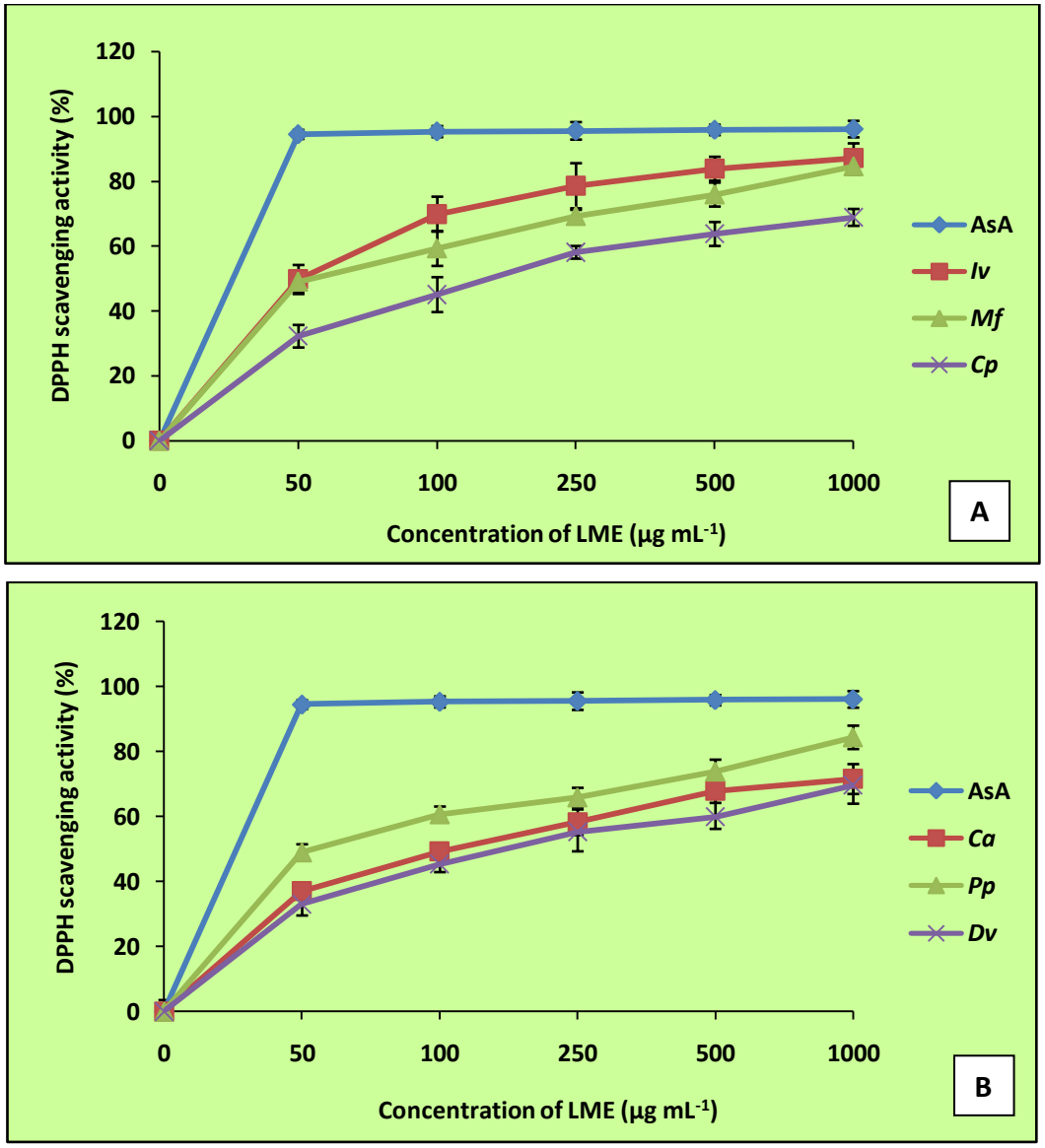

Figure 9. DPPH scavenging activity of lyophilized methanolic extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LME: Lyophilized methanolic extract.

Table 20: IC_{50} values (µg mL^{-1}) of DPPH scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different herbs

Standard/sample	LHE	LME
AsA	19.55 ± 0.46	19.55 ± 0.46
Mp	229.71 ± 2.90	129.24 ± 4.23
Tfg	384.87 ± 4.02	230.20 ± 5.01
Cs	515.16 ± 3.68	263.25 ± 4.34
Mk	330.12 ± 4.54	232.04 ± 2.26
Go	172.25 ± 4.36	79.28 ± 0.81
Fv	263.32 ± 3.74	217.39 ± 3.23
LSD value (column):	1190.496	
LSD value (row):	201.303	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3)and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*; LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 20A: ANOVA of data presented in Table 20 (IC_{50} values of DPPH scavenging activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	182277	6	30379.5	9.404407	0.007648	4.283866
B (Columns)	39542.18	1	39542.18	12.24085	0.012847	5.987378
AxB (Error)	19382.08	6	3230.347			
AB (Total)	241201.3	13				

Table 21: IC_{50} values (µg mL^{-1}) of DPPH scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different spices

Standard/sample	LHE	LME
AsA	19.55 ± 0.46	19.47 ± 0.46
Iv	70.23 ± 3.87	47.66 ± 2.88
Mf	76.20 ± 2.41	54.73 ± 3.14
Cp	307.18 ± 3.69	228.60 ± 4.20
Ca	262.19 ± 3.71	136.44 ± 3.35
Pp	96.99 ± 7.80	59.14 ± 1.33
Dv	253.87 ± 3.91	243.92 ± 5.77
LSD value (column):	474.009	
LSD value (row):	55.326	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3) and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*; LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 21A: ANOVA of data presented in Table 21 (IC_{50} values of DPPH scavenging activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	123917.3	6	20652.88	20.73674	0.000908	4.283866
B (Columns)	6268.721	1	6268.721	6.294174	0.045977	5.987378
AxB (Error)	5975.736	6	995.956			
AB (Total)	136161.8	13				

4.6.2. Superoxide anion scavenging activity

All the extracts were observed to be efficient superoxide anion radical scavengers which were evident by the ability of the extracts to scavenge the radical even at a very low concentration. Superoxide radical scavenging activity of all the plants tested exhibited increase in the activity in a dose dependent manner (Figure 10-13). However, the rise in the activity with the increase in concentration was not significantly different to each other. The IC_{50} values are presented in Table 22-23.

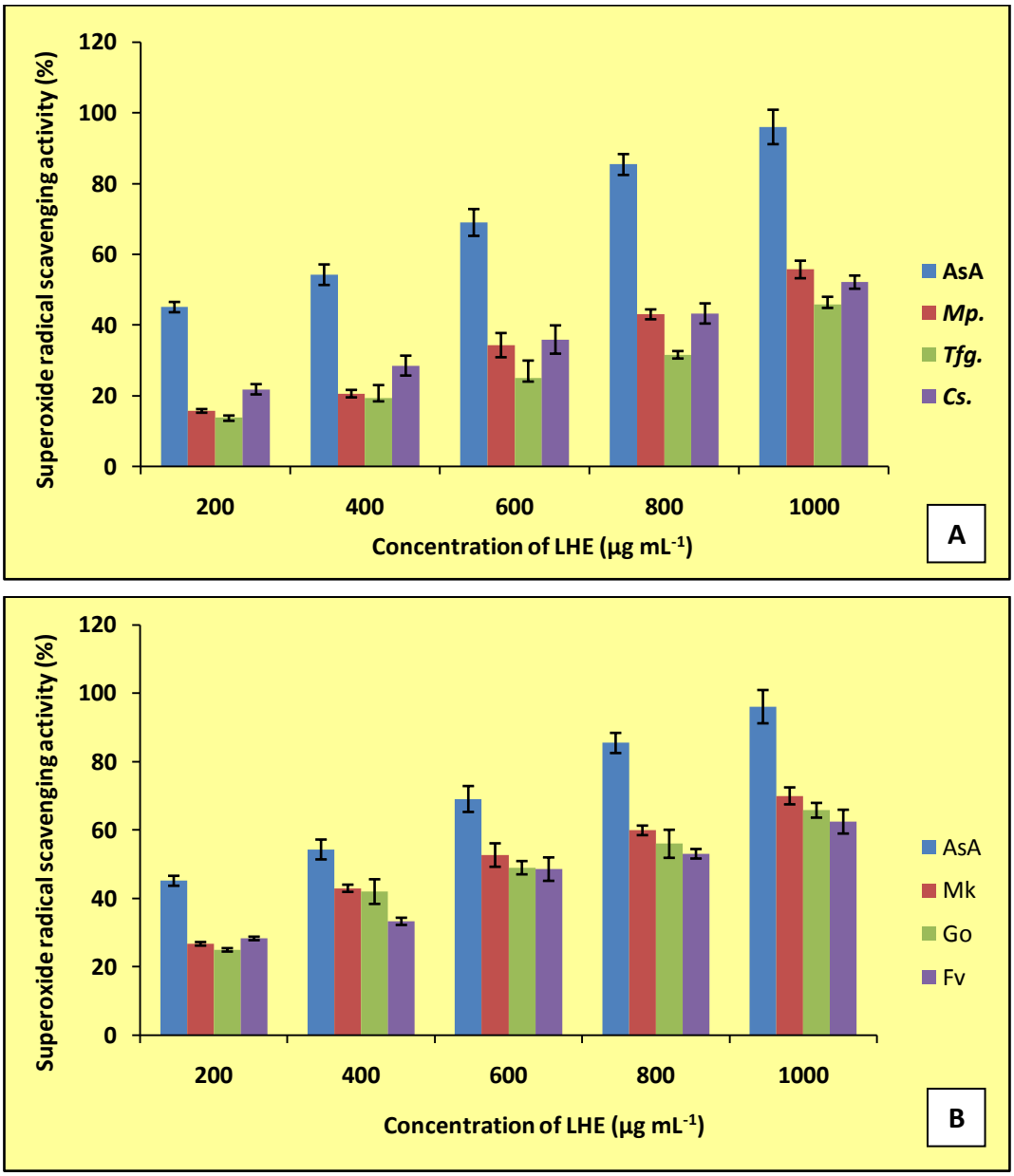

Figure 10. Superoxide radical scavenging activity of lyophilized hot aqueous extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract.

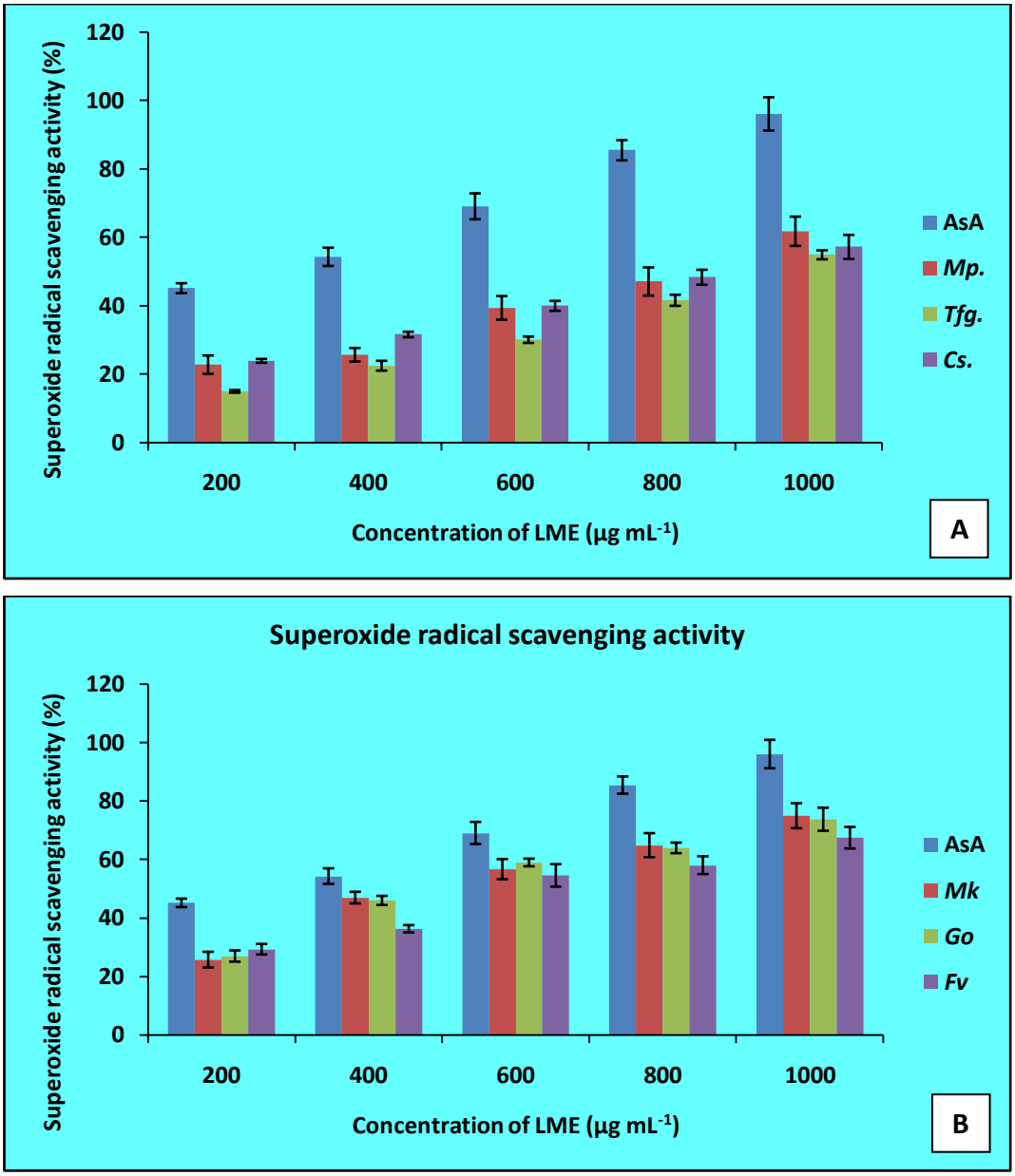

Figure 11. Superoxide radical scavenging activity of lyophilized methanolic extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LME: Lyophilized methanolic extract.

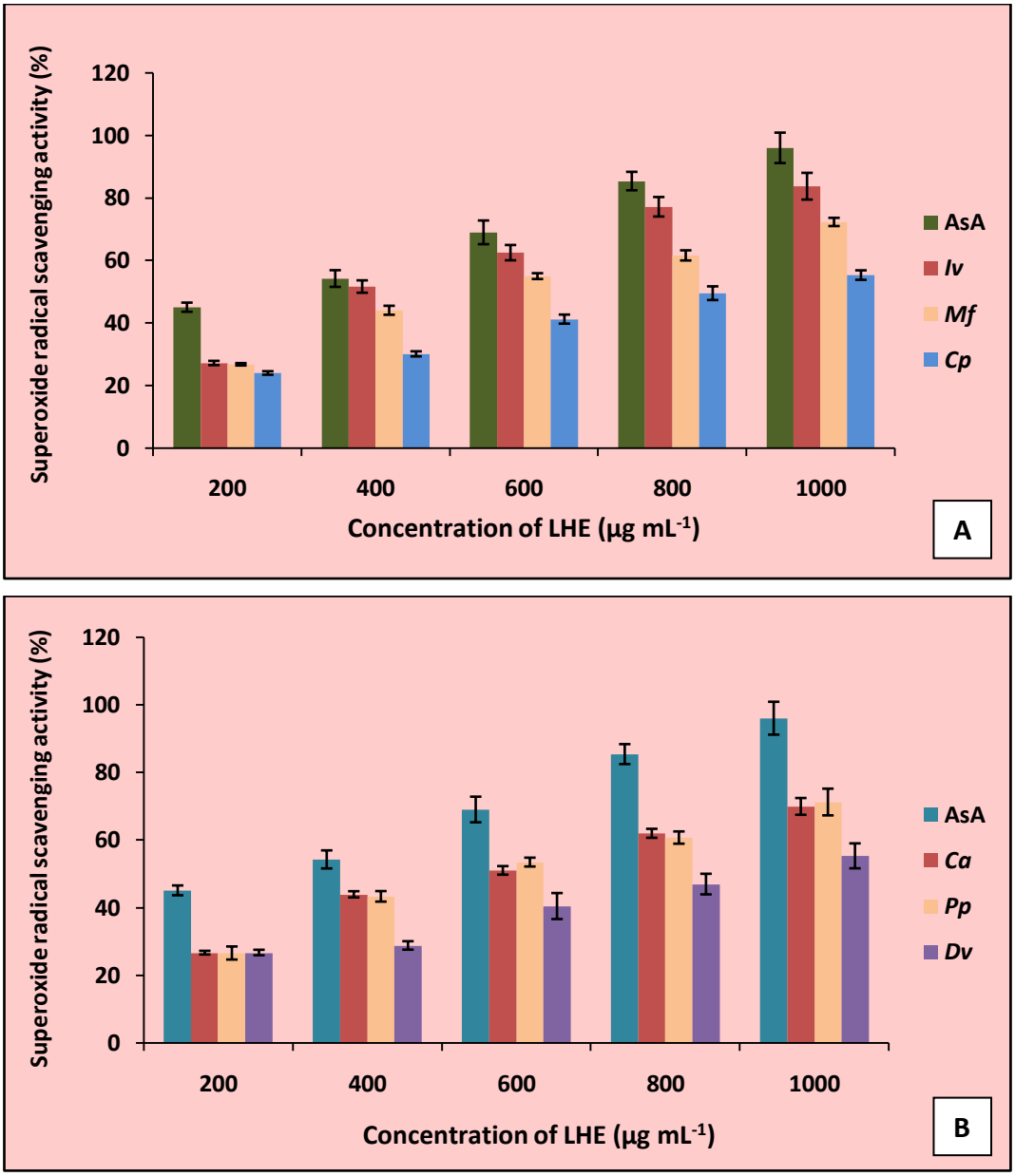

Figure 12. Superoxide radical scavenging activity of lyophilized hot aqueous extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LHE: Lyophilized hot aqueous extract.

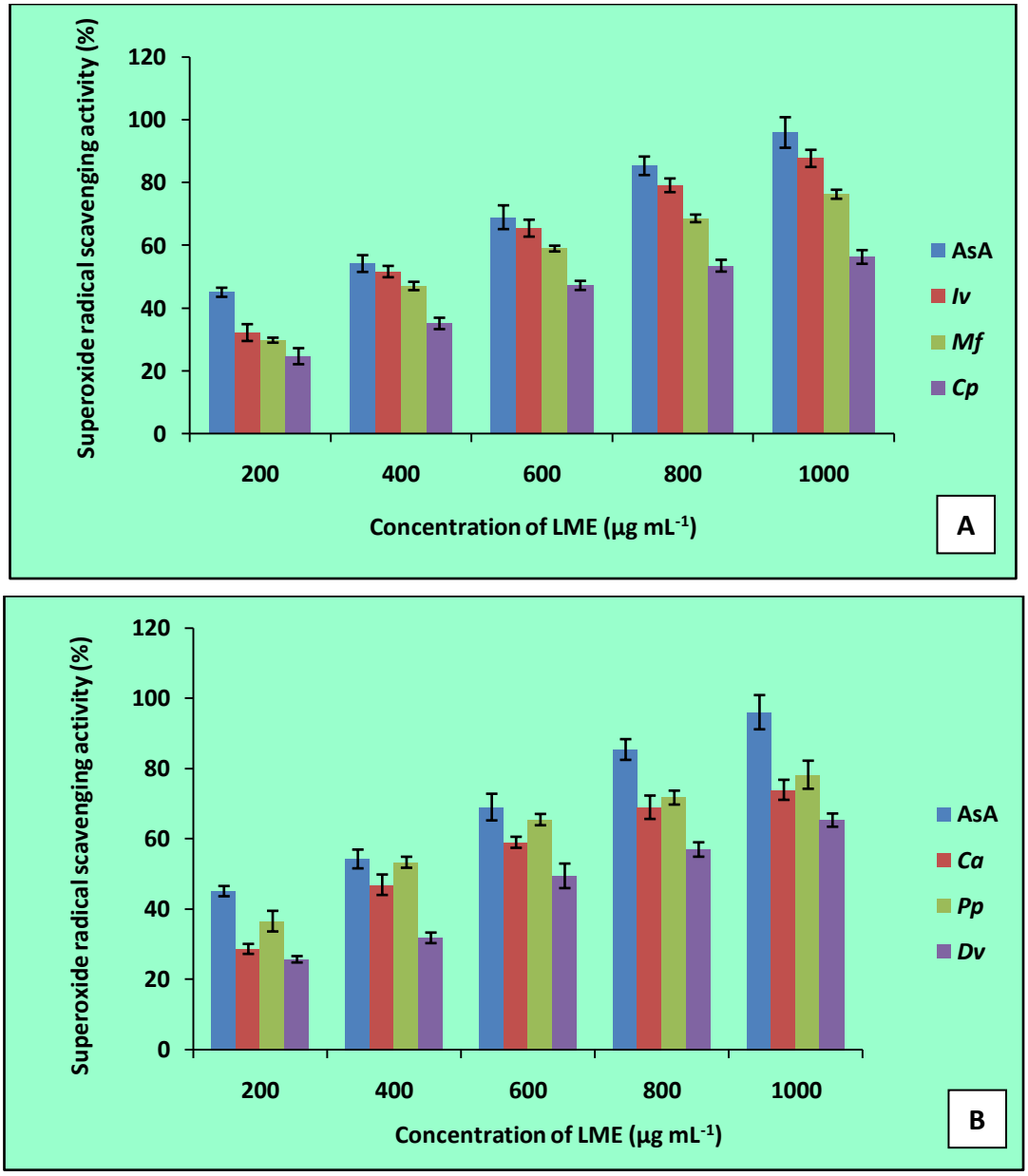

Figure 13. Superoxide radical scavenging activity of lyophilized methanolic extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LME: Lyophilized methanolic extract.

Table 22: IC_{50} values (µg mL^{-1}) of superoxide radical scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different herbs

Standard/sample	LHE	LME
AsA	253.51 ± 1.95	253.51 ± 1.95
Mp	920.11 ± 3.99	871.07 ± 2.20
Tfg	>1000	939.71 ± 1.20
Cs	928.90 ± 4.41	863.36 ± 3.29
Mk	531.59 ± 2.31	449.67 ± 2.60
Go	639.99 ± 4.07	481.43 ± 1.47
Fv	736.78 ± 3.39	562.91 ± 2.76
LSD value (column):	942.765	
LSD value (row):	426.871	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3)and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 22A: ANOVA of data presented in Table 22 (IC_{50} values of superoxide radical scavenging activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	819644.6	6	136607.4	71.83321	2.54E-05	4.283866
B (Columns)	24797.74	1	24797.74	13.03956	0.011217	5.987378
AxB (Error)	11410.39	6	1901.731			
AB (Total)	855852.7	13				

Table 23: IC_{50} values (µg mL^{-1}) of superoxide radical scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different spices

Standard/sample	LHE	LME
AsA	253.51 ± 1.95	253.51 ± 1.95
Iv	271.76 ± 2.90	263.63 ± 2.38
Mf	509.24 ± 2.36	447.43 ± 3.87
Cp	891.97 ± 3.34	729.52 ± 3.53
Ca	619.51 ± 1.76	473.64 ± 3.74
Pp	563.30 ± 3.19	337.35 ± 4.57
Dv	951.63 ± 2.93	664.87 ± 3.41
LSD value (column): 1425.618		
LSD value (row): 370.965		

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3) and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*; LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 23A: ANOVA of data presented in Table 23 (IC_{50} values of superoxide radical scavenging activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	619011.2	6	103168.5	17.33086	0.001494	4.283866
B (Columns)	56703.66	1	56703.66	9.525419	0.021488	5.987378
AxB (Error)	35717.27	6	5952.879			
AB (Total)	711432.1	13				

4.6.3. Hydrogen peroxide scavenging activity

Antioxidant activity by hydrogen peroxide method was carried out with all the plant extracts. Hydrogen peroxide is converted into free radical called hydroxyl radicals (-OH), which reacts with biomolecules leading to cell death and tissue damage. The results of the study revealed that all the plant extracts offered significant scavenging activity. Among the different plant extracts tested, maximum IC_{50} of 101.17 µg mL^{-1}

was obtained in hot aqueous extract of *I. verum* followed by *G. oppositifolius* (137.68 µg mL^{-1}) and methanolic extracts of *I. verum* and *G. oppositifolius* also have maximum IC_{50} values (85.61 µg mL^{-1} and 102.48 µg mL^{-1} respectively). The ascorbic acid control showed 50% inhibition at 25.45 µg mL^{-1} concentration (Figure 14-17, Table 24-25).

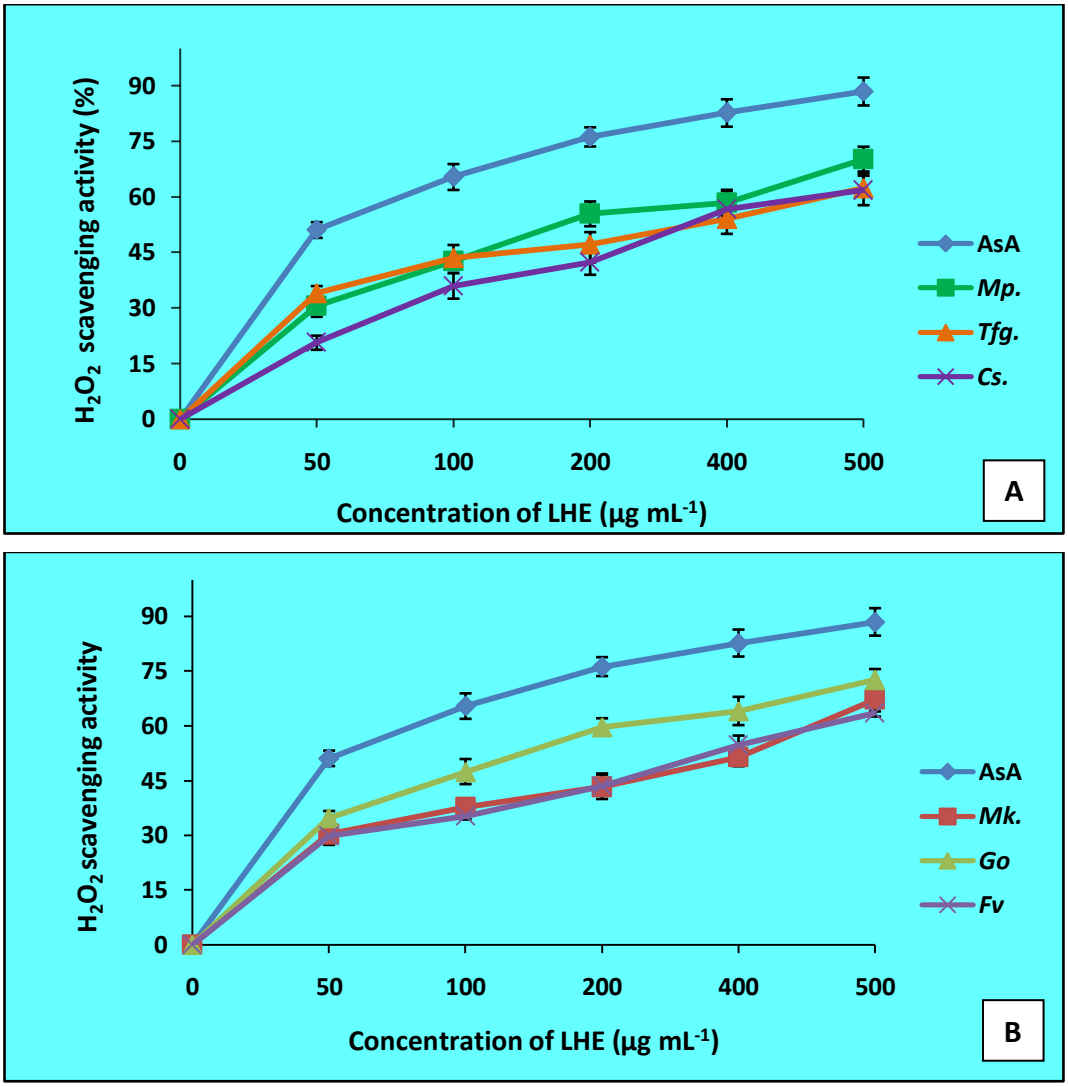

Figure 14. Hydrogen peroxide (H_2O_2) scavenging activity of lyophilized hot aqueous extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract.

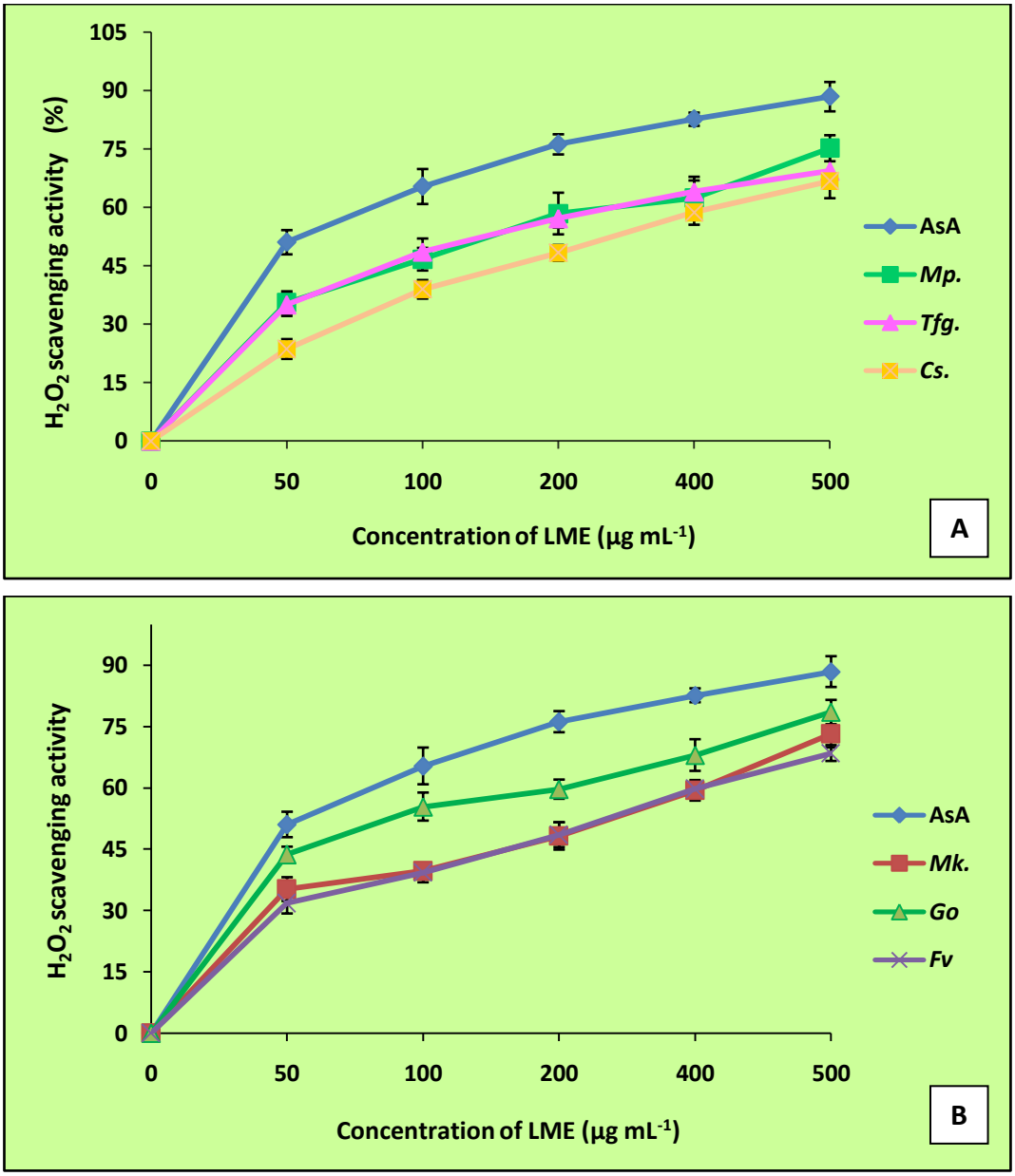

Figure 15. Hydrogen peroxide (H_2O_2) scavenging activity of lyophilized methanolic extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LME: Lyophilized methanolic extract.

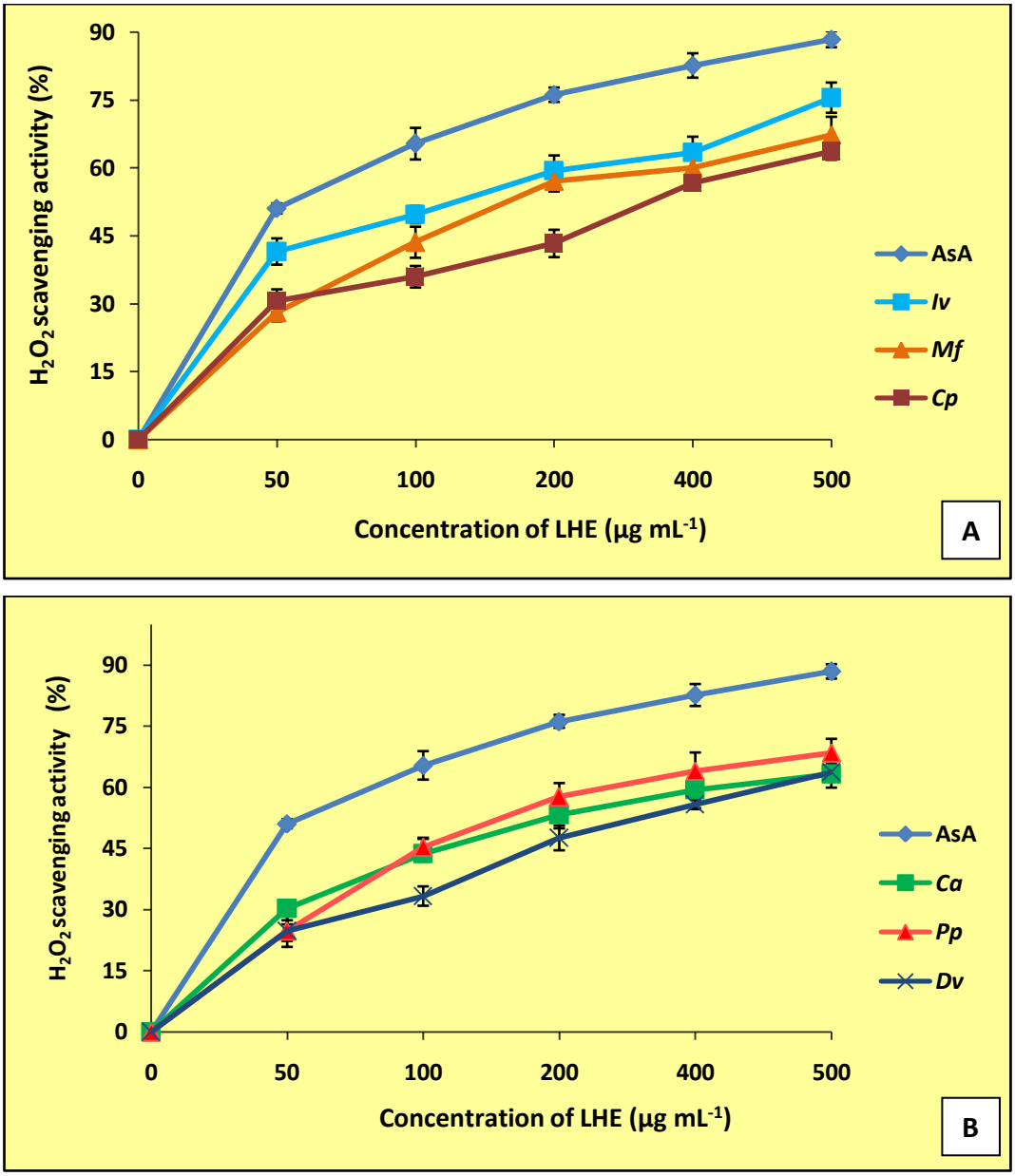

Figure 16. Hydrogen peroxide (H_2O_2) scavenging activity of lyophilized hot aqueous extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LHE: Lyophilized hot aqueous extract.

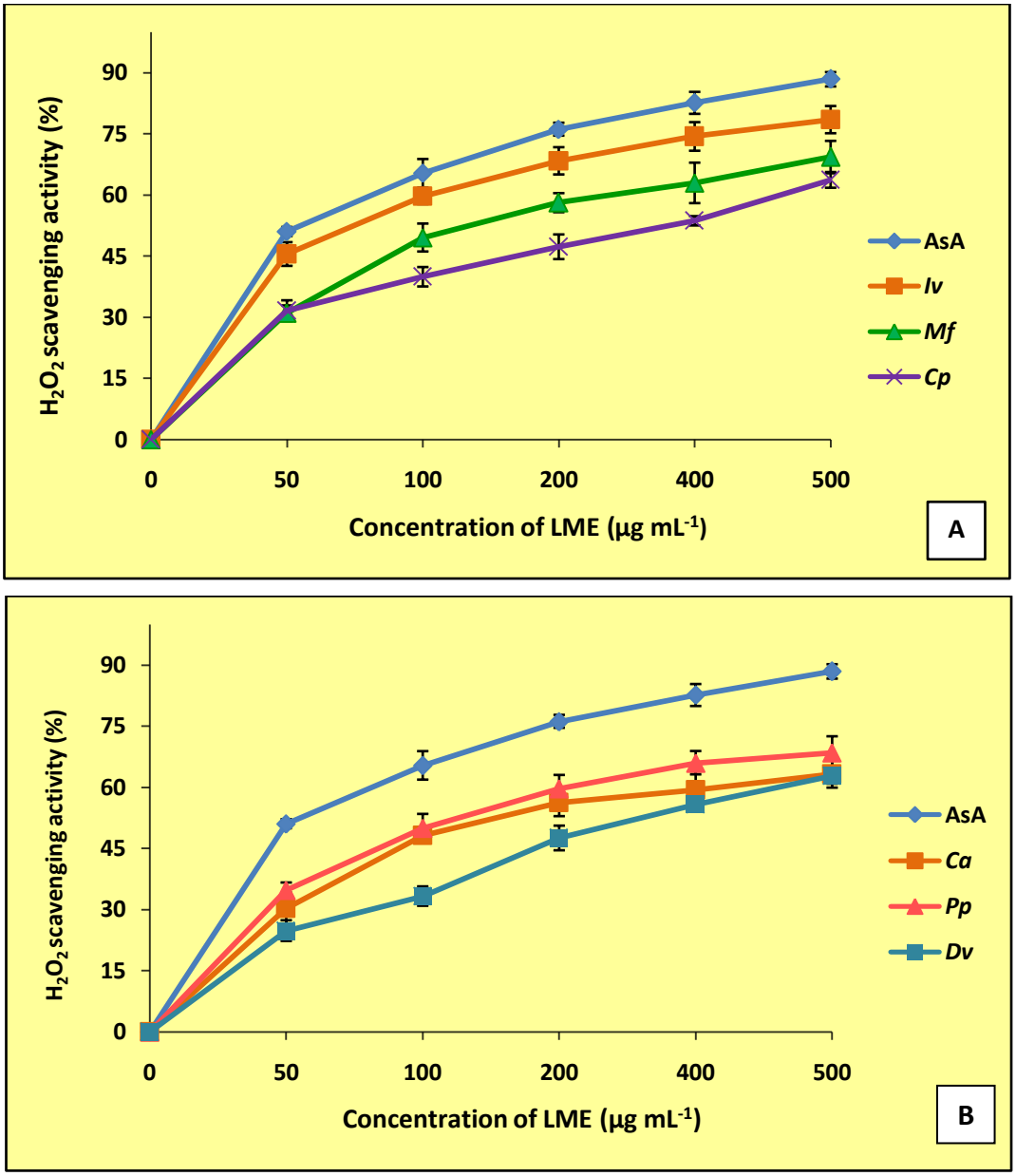

Figure 17. Hydrogen peroxide (H_2O_2) scavenging activity of lyophilized methanolic extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LME: Lyophilized methanolic extract.

Table 24: IC_{50} values (µg mL^{-1}) of hydrogen peroxide scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different herbs

Standard/sample	LHE	LME
AsA	47.92 ±1.74	47.92 ± 1.74
Mp	158.08 ± 1.08	128.42 ± 2.52
Tfg	261.14 ± 2.79	156.20 ± 2.34
Cs	408.66 ± 2.14	270.28 ± 1.39
Mk	378.45 ± 4.70	238.09 ± 3.04
Go	138.28 ± 3.51	110.50 ± 1.65
Fv	321.19 ± 2.10	225.61 ± 1.98
LSD value (column):	52.721	
LSD value (row):	427.253	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3)and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 24A: ANOVA of data presented in Table 24 (IC_{50} values of hydrogen peroxide scavenging activity)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	136818.2	6	22803.04	14.03352	0.002657	4.283866
B (Columns)	20574.75	1	20574.75	12.66218	0.01195	5.987378
AxB (Error)	9749.385	6	1624.898			
AB (Total)	167142.3	13				

Table 25: IC_{50} values (µg mL^{-1}) of hydrogen peroxide scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different spices

Standard/sample	LHE	LME
AsA	47.92 ± 1.74	47.92 ± 1.74
Iv	106.24 ± 2.34	88.44 ± 1.80
Mf	161.80 ± 5.52	112.92 ± 3.87
Cp	332.58 ± 2.97	228.43 ± 2.05
Ca	198.43 ± 3.31	152.12 ± 1.83
Pp	147.95 ± 3.57	109.40 ± 2.23
Dv	376.63 ± 2.87	252.72 ± 2.88
LSD value (column): 43.261		
LSD value (row): 427.253		

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3) and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*; LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 25A: ANOVA of data presented in Table 25 (IC_{50} values of hydrogen peroxide scavenging activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	112235.5	6	18705.91	18.77793	0.001197	4.283866
B (Columns)	10292.94	1	10292.94	10.33257	0.018265	5.987378
AxB (Error)	5976.988	6	996.1647			
AB (Total)	128505.4	13				

4.6.4. Nitric oxide scavenging activity

Nitric oxide scavenging activity was carried out for all the plant extracts in the present study. Nitric oxide is generated from sodium nitroprusside in solution which interacts with oxygen to produce stable products, leading to the production of nitrites. The extracts inhibited nitrite formation by directly competing with oxygen in reaction with nitric oxide and its efficacy of the extract in scavenging nitric oxide is expressed as IC_{50} values and compared with the control ascorbic acid. In the nitric oxide assay, IC_{50} values when compared among the extracts (LME and LHE) revealed LME to exhibit

higher nitric oxide scavenging activity than LHE over the range of concentrations tested (Figure 18-21, Table 26-27).

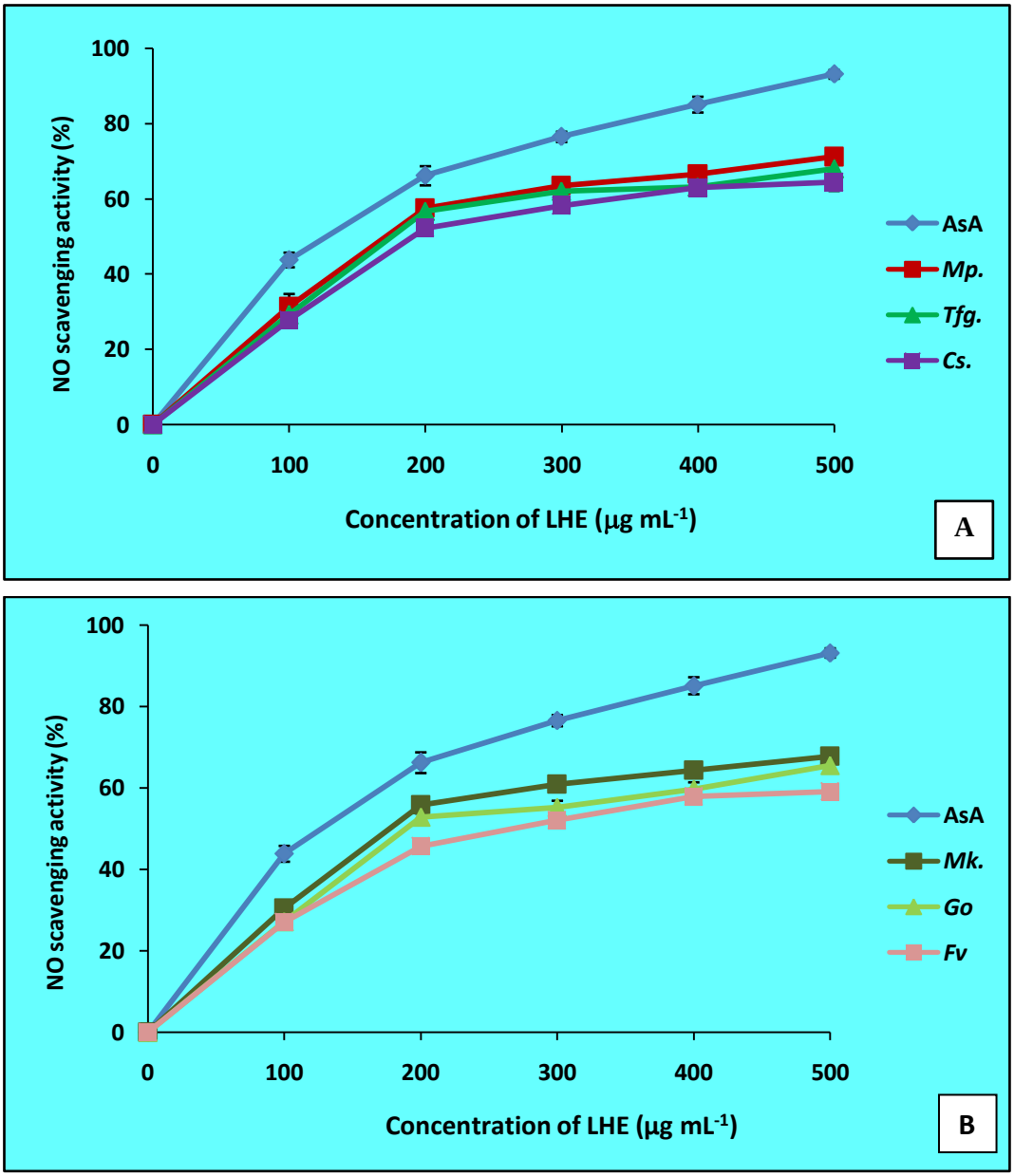

Figure 18. Nitric oxide (NO) scavenging activity of lyophilized hot aqueous extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract.

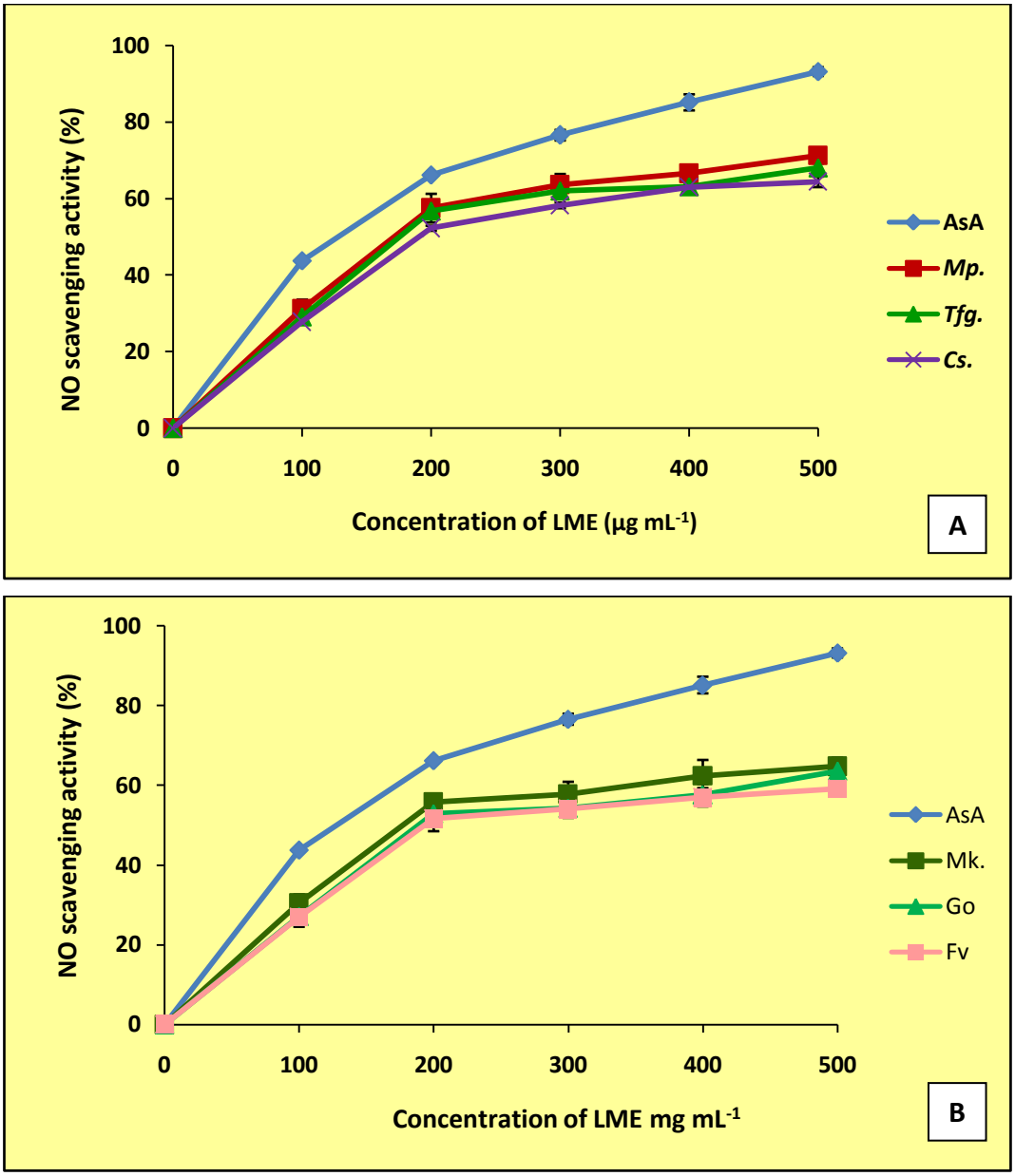

Figure 19. Nitric oxide (NO) scavenging activity of lyophilized methanolic extracts of different herbs. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LME: Lyophilized methanolic extract.

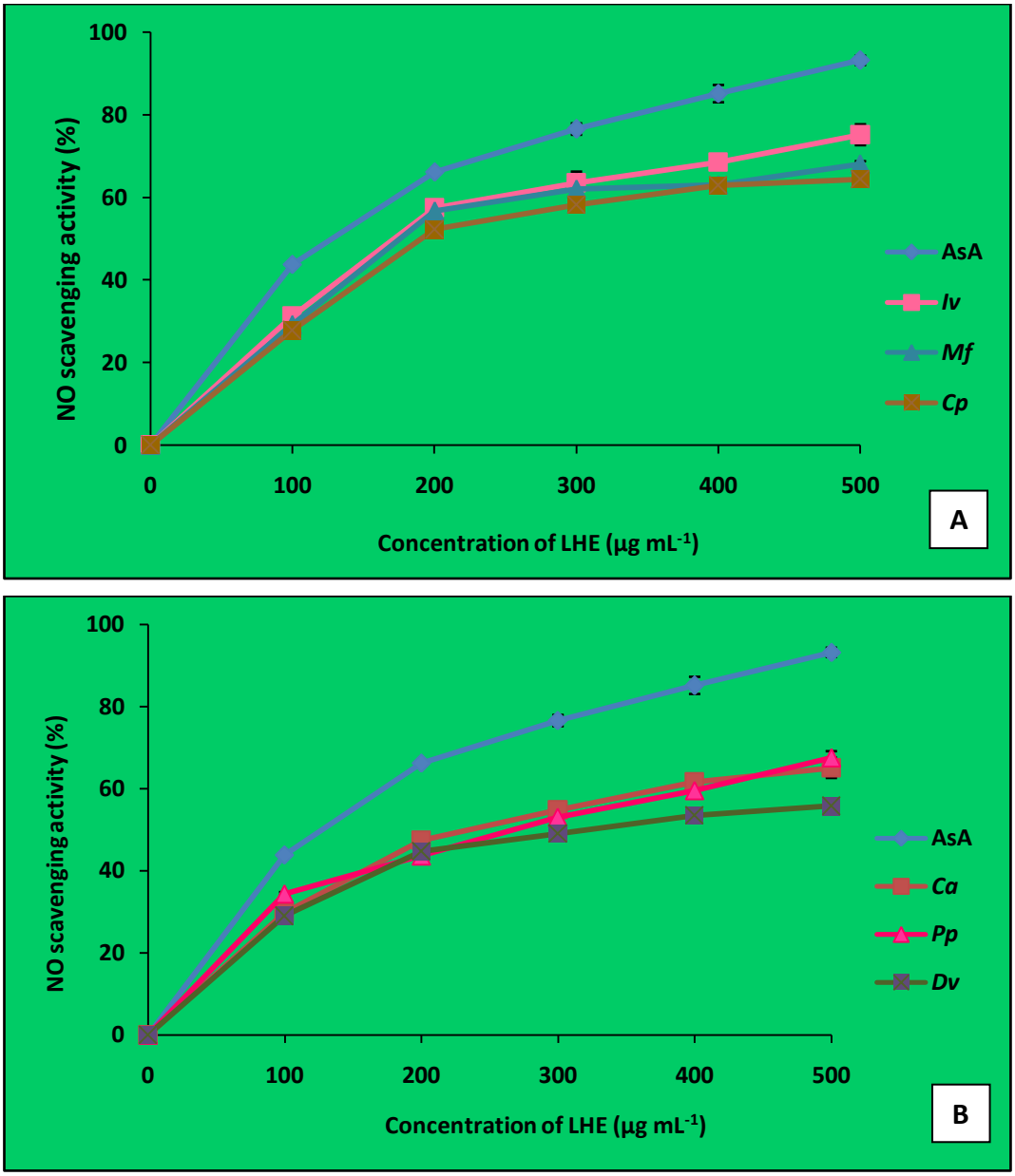

Figure 20. Nitric oxide (NO) scavenging activity of lyophilized hot aqueous extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LHE: Lyophilized hot aqueous extract.

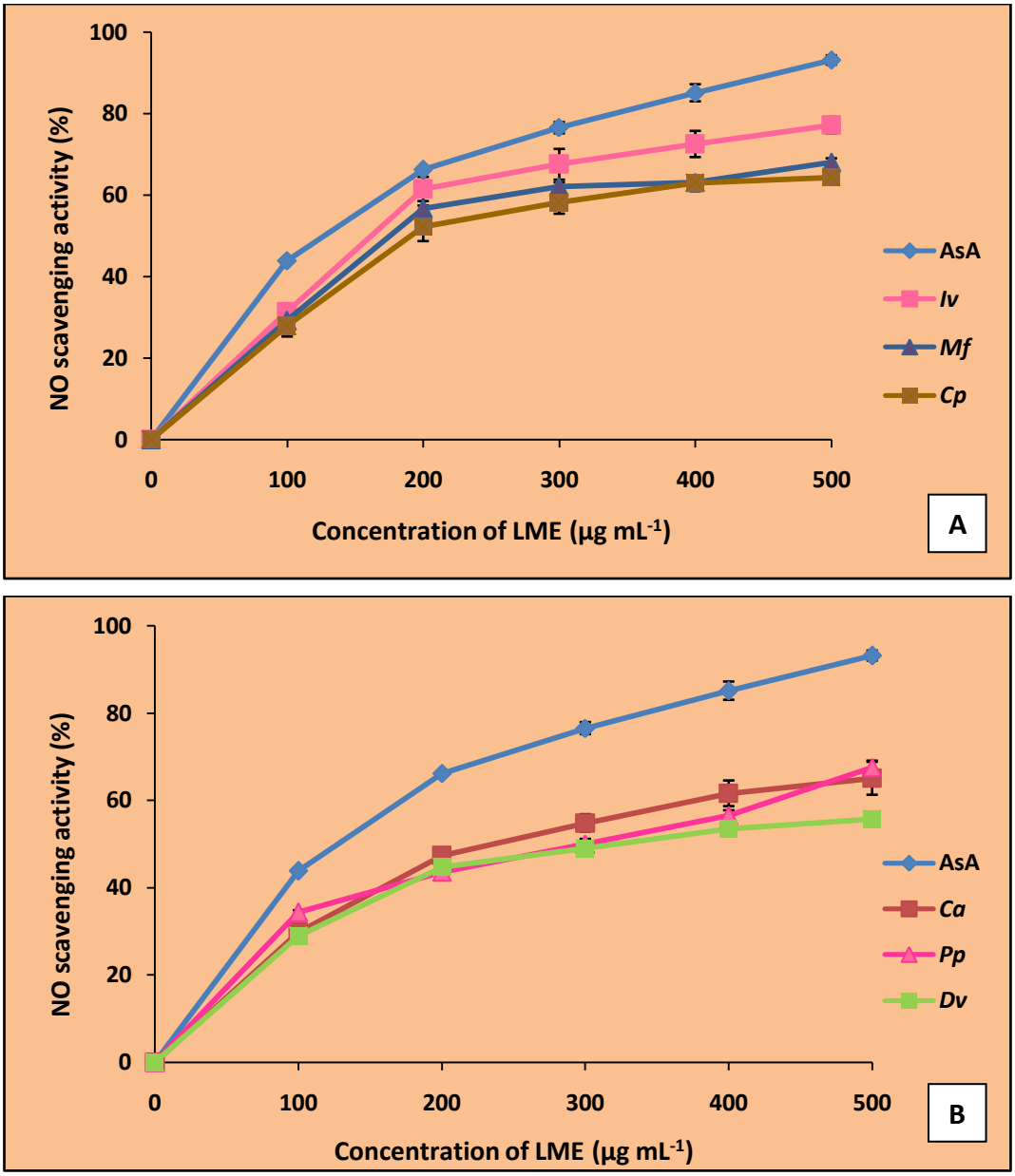

Figure 21. Nitric oxide (NO) scavenging activity of lyophilized methanolic extracts of different spices. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3) and expressed as percentage of inhibition. AsA: ascorbic acid (standard), *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*. LME: Lyophilized methanolic extract.

Table 26: IC_{50} values (µg mL^{-1}) of nitric oxide radical scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different herbs

Standard/sample	LHE	LME
AsA	127.08 ± 1.98	127.08 ± 1.98
Mp	182.84 ± 2.91	155.06 ± 2.64
Tfg	192.23 ± 2.12	163.41 ± 3.47
Cs	198.75 ± 3.20	179.73 ± 1.43
Mk	190.10 ± 2.41	166.92 ± 2.46
Go	196.92 ± 2.95	176.60 ± 2.56
Fv	283.55 ± 2.94	195.23 ± 2.25
LSD value (column):	331.914	
LSD value (row):	54.088	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3)and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Mp*: *Mentha piperita*, *Tfg*: *Trigonella foenum-graecum*, *Cs*: *Coriandrum sativum*, *Mk*: *Murraya koenigii*, *Go*: *Glinus oppositifolius*, *Fv*: *Foeniculum vulgare*, LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 26A: ANOVA of data presented in Table 26 (IC_{50} values of nitric oxide radical scavenging activity)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	13159.38	6	2193.23082	5.76242911	0.025588015	4.283866
B (Columns)	3073.668	1	3073.668114	8.075663744	0.029498303	5.987378
AxB (Error)	2283.652	6	380.6087291			
AB (Total)	18516.71	13				

Table 27: IC_{50} values (µg mL^{-1}) of nitric oxide radical scavenging activity exhibited by lyophilized hot aqueous and methanolic extracts of different spices

Standard/sample	LHE	LME
AsA	127.08 ± 1.98	127.08 ± 1.98
Iv	190.25 ± 2.35	132.3133 ± 2.26
Mf	174.76 ± 1.96	187.0733 ± 3.36
Cp	256.73 ± 2.78	199.61 ± 3.01
Ca	269.44 ± 1.03	248.7767 ± 2.16
Pp	283.24 ± 2.58	336.5167 ± 1.42
Dv	379.14 ± 1.21	342.0633 ± 2.74
LSD value (column):	171.536	
LSD value (row):	136.606	

Values are expressed as the mean ± standard deviation of triplicate analysis (n=3) and presented in µg mL^{-1}. Here, AsA: Ascorbic acid, *Iv*: *Illicium verum*, *Mf*: *Myristica fragrans*, *Cp*: *Ceiba pentandra*, *Ca*: *Capsicum annuum*, *Pp*: *Parmelia perlata* and *Dv*: *Dregea volubilis*; LHE: Lyophilized hot aqueous extract and LME: Lyophilized methanolic extract.

Table 27A: ANOVA of data presented in Table 27 (IC_{50} values of nitric oxide radical scavenging activity)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	83940.87	6	13990.14	17.18492	0.001529	4.283866
B (Columns)	820.9478	1	820.9478	1.008419	0.354047	5.987378
AxB (Error)	4884.565	6	814.0942			
AB (Total)	89646.38	13				

4.7. Antimicrobial activities of methanolic extracts of herbs and spices against different bacterial strains

The presence of antibacterial substances in higher plants is well established in recent years. Plants have provided sources for novel drug or principle compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of medicine, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural products as antimicrobial agents arise. Though soil microorganisms or fungi produce most of the clinically used antibiotics, higher plants can be very good source of antibiotics.

Among the herbs and spice samples, results revealed that methanolic extract of herbs are less effective in inhibiting the microbial growth, while antimicrobial action of methanolic spice extract against such microbial strains are promising. As because methanolic extracts of different herbs and spices showed comparatively better the antioxidant activities than hot aqueous extracts, thus chosen for antimicrobial studies. The antibacterial activity of different herb and spice extracts was studied against two Gram-positive and two Gram-negative bacterial strains. The Gram-positive organisms include *Bacillus cereus* (MTCC 10665) and *Bacillus pumilus* (MTCC 1684) whereas Gram-negative strains are *Serratia marcescens* field isolate (NCBI GENBANK acc no. JN020963) and *Pseudomonas aeruginosa* (MTCC 2453). Different concentrations or dose of lyophilized methanolic extracts *i.e*, 4-10 mg $disc^{-1}$were used to screen the antimicrobial activity. Extract of different plant samples investigated so far inhibited the growth of the microorganisms in a dose dependent manner for each extract and was evident with the formation varying diameter of inhibition zones (Table 28 and Table 29). Inhibition was not exhibited by solvent control (MeOH) in all the cases. In the same time, minimum inhibitory dose (MID) of different bacterial strains was also determined (Table 30 and Table 31). Among the herb extracts, *M. piperita* and *T. foenum-graecum*were found in inhibiting *B. cereus* and *B. pumilus.* Rest of the samples (*C. sativum, M. koenigii, G. oppositifolius* and *F. vulgare*) did not show any antimicrobial activity. Chloramphenicol (C_{25} µg), Kanamycin (K_{30} µg) and Ampicillin (A_{25} µg) exhibit inhibitory activity against specific organisms, not all the strains of microbial species (Figure 22-26).*B. cereus* and *B. pumilus* were inhibited only by the

M. piperita and *T. foenum-graecum* extracts at higher doses. No inhibition was observed by the other herbal extracts and against other test organisms. The MID value of *M. piperita* extract against *B. cereus* and *B. pumilus* was found to be 8.5 and 5.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID value of *T. foenum-graecum* extracts against *B. cereus* and *B. pumilus* was determined as 3.5 and 7.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID values of other herb extracts against respective organisms were found to be > 10 mg lyophilized methanolic extract $disc^{-1}$.

Table 28: Comparative antibacterial activities of different herbs against Gram positive and Gram negative bacterial strains at different doses of lyophilized extracts

Plant species/ Referent antibiotics*	**Dose (mg LME $disc^{-1}$)**	**Bacterial strains¥**	
		Diameter of zone of inhibition (mm)£	
		Bc	***Bp***
Mentha piperita	0[a]	Ni	Ni
	4	Ni	Ni
	6	Ni	Ni
	8	Ni	Ni
	10	9.75 ± 0.29	7.05 ± 0.10
Trigonella foenum-graecum	0[a]	Ni	Ni
	4	6.23 ± 0.26	Ni
	6	7.03 ± 0.37	5.50 ± 0.00
	8	10.13 ± 0.25	7.18 ± 0.24
	10	15.63 ± 0.48	9.08 ± 0.15
Ampicillin	A_{25}	Ni	Ni
Chloramphenicol	C_{25}	8.50 ± 0.58	12.02 ± 0.15
Kanamycin	K_{30}	19.63 ± 0.48	21.88 ± 0.25

£Each value represents the mean ± standard deviation from quadruplicate observations (n=4). Diameter of commercial antibiotic disc is 6 mm, and that of blank paper disc/solvent disc is 5.5 mm that correspond to 'not inhibited', Ni. 0[a] denoted as disc without extract *i.e.*, solvent control disc. ¥Bacterial strains, *Bc*: *Bacillus cereus* MTCC 10665, *Bp*: *Bacillus pumilus* MTCC 1684, *Sm*: *Serratia marcescens*, *Pa*: *Pseudomonas aeruginosa* MTCC 2453. *Antibiotics, A_{25}: Ampicillin at 25 µg $disc^{-1}$, C_{25}: Chloramphenicol at 25 µg $disc^{-1}$, K_{30}: Kanamycin at 30 µg $disc^{-1}$. Lyophilized methanolic extract is abbreviated as LME.

Table 29: Minimum inhibitory dose (MID) determination of herb extracts against target bacterial strainsusing DAD assay

Herb samples	Bacterial strains			
	MID value (mg LME disc^{-1})			
	Bc	*Bp*	*Sm*	*Pa*
Mentha piperita	8.5	5.5	>10.0	>10.0
Trigonella foenum-graecum	3.5	7.5	>10.0	>10.0
Coriandrum sativum	>10.0	>10.0	>10.0	>10.0
Murraya koenigii	>10.0	>10.0	>10.0	>10.0
Glinus oppositifolius	>10.0	>10.0	>10.0	>10.0

Minimum inhibitory dose (MID) expressed in mg Lyophilized methanolic extract disc^{-1}. Bacterial strains, *Bc*: *Bacillus cereus*, *Bp*: *Bacillus pumilus*, *Sm*: *Serratia marcescens*, *Pa*: *Pseudomonas aeruginosa*.

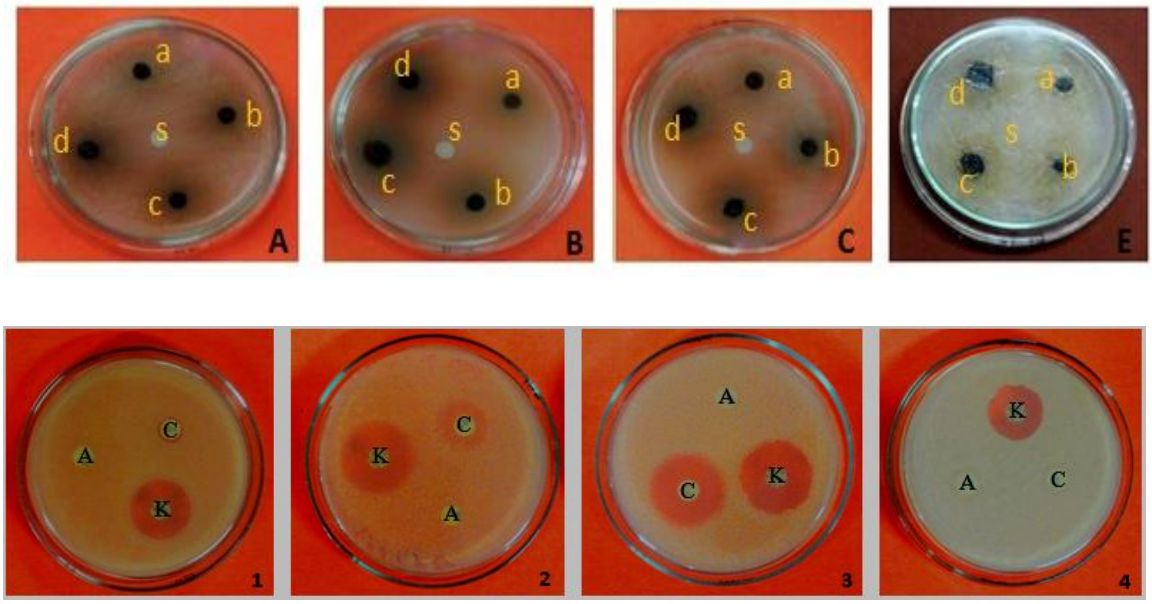

Figure 22. Antibacterial activity of *M. piperita* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

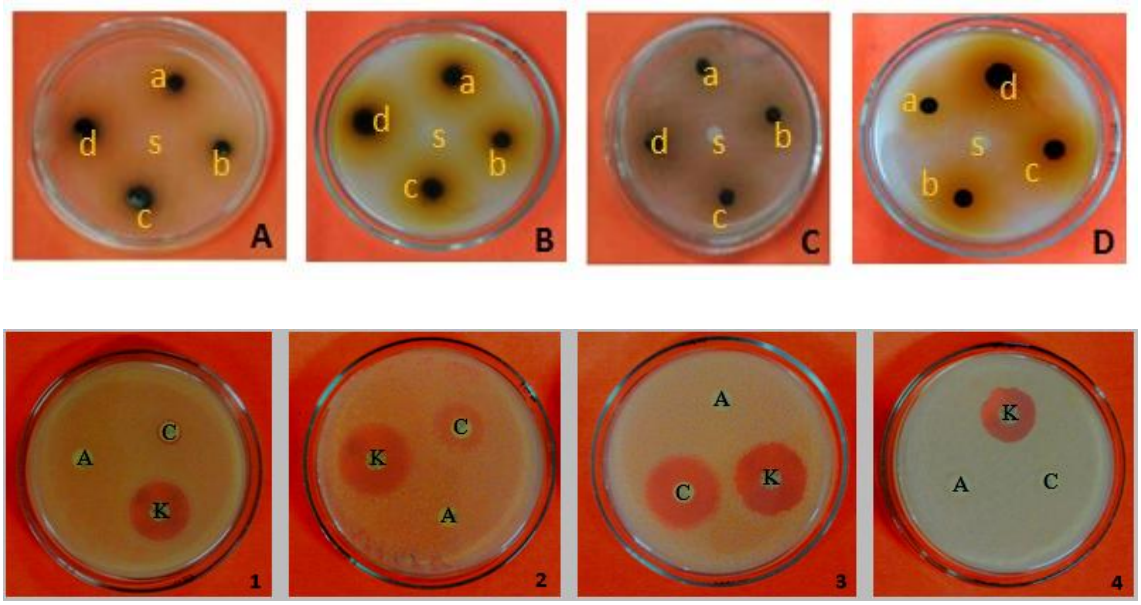

Figure 23. Antibacterial activity of *T. foenum-graecum* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa.*

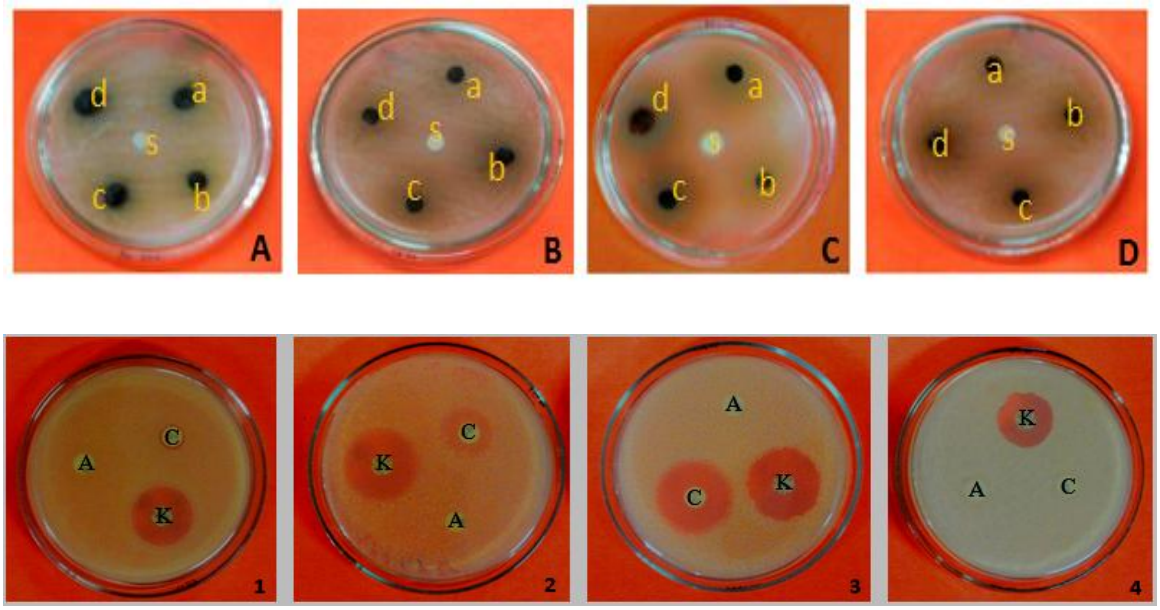

Figure 24. Antibacterial activity of *C. sativum* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

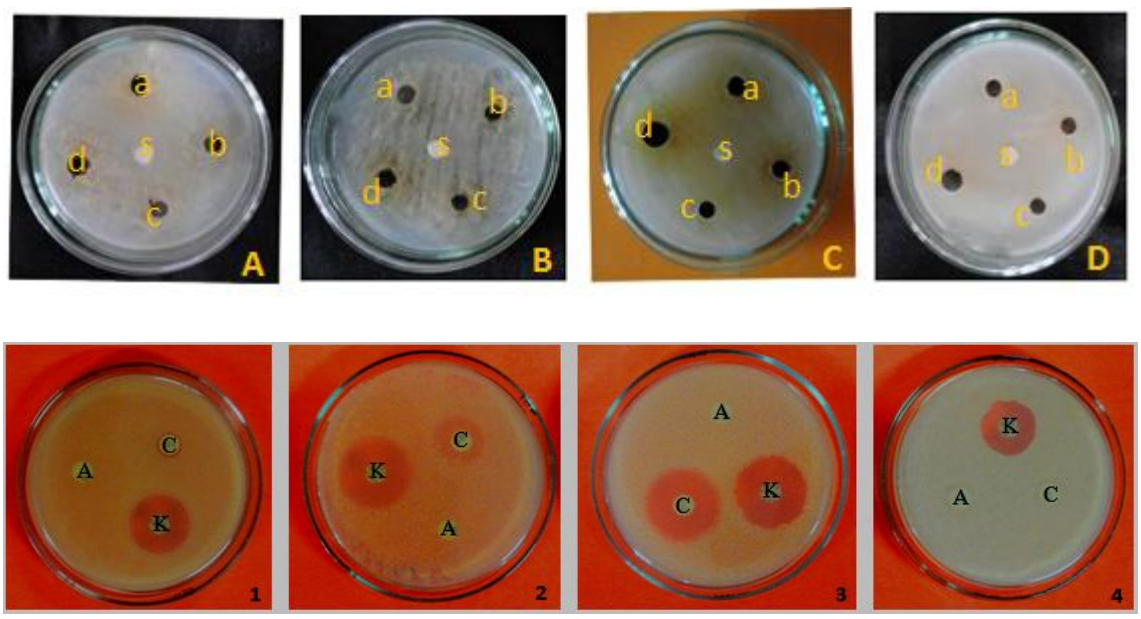

Figure 25. Antibacterial activity of *M. koenigii* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

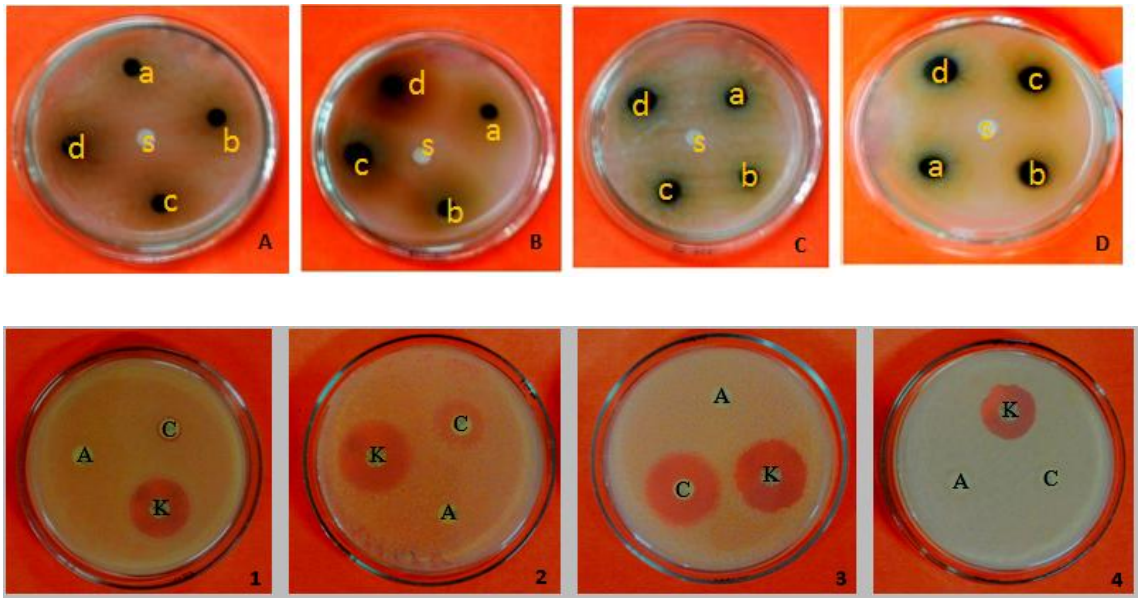

Figure 26. Antibacterial activity of *G. oppositifolius* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

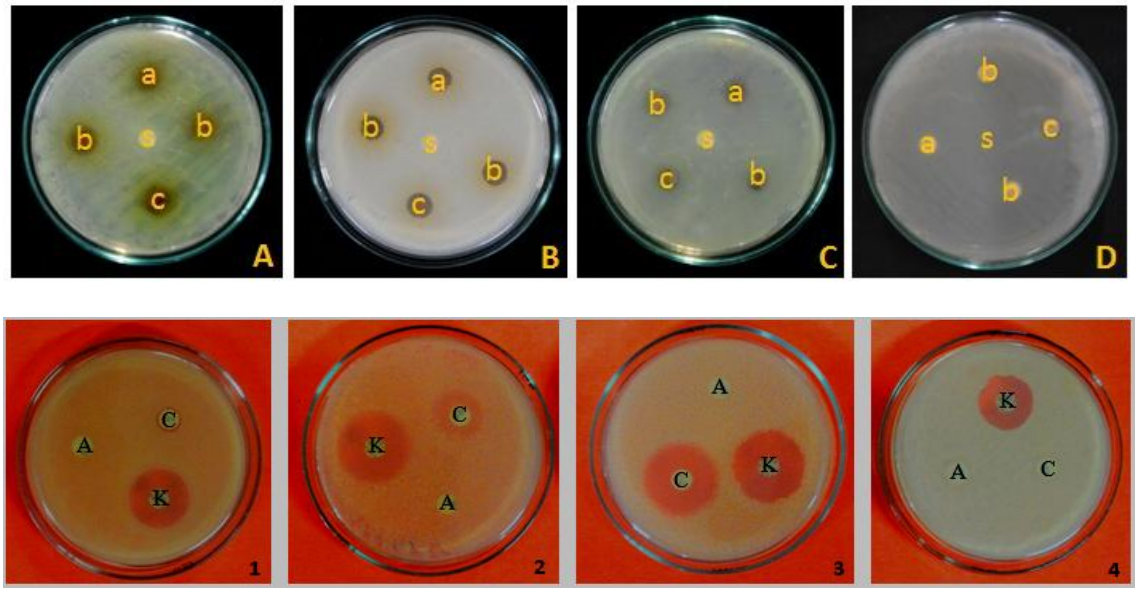

Figure 27. Antibacterial activity of *I. verum* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

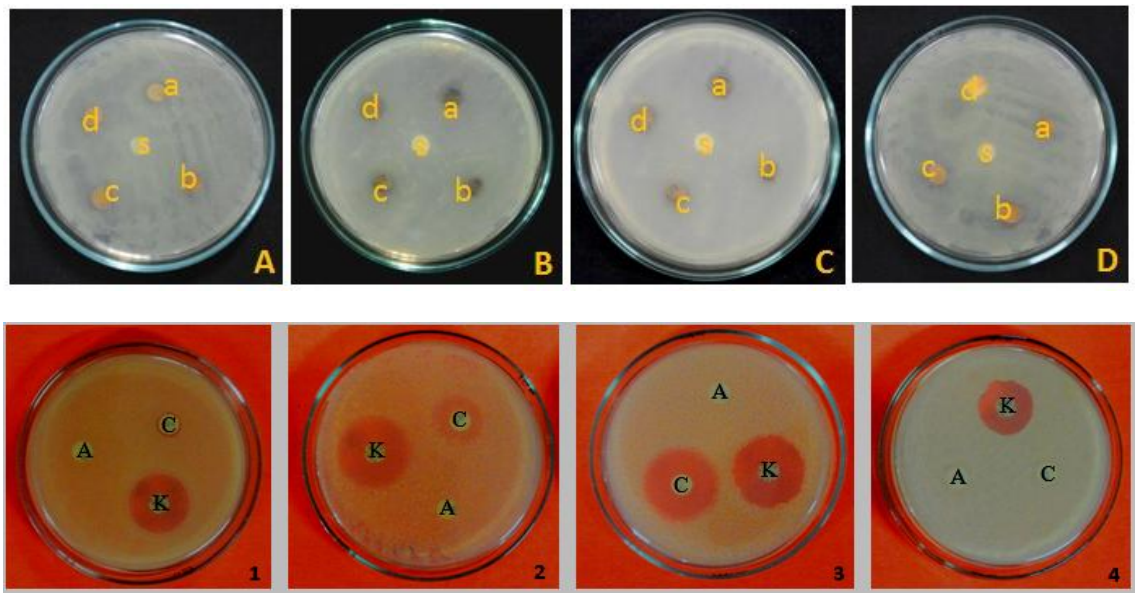

Figure 28. Antibacterial activity of *M. fragrans* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

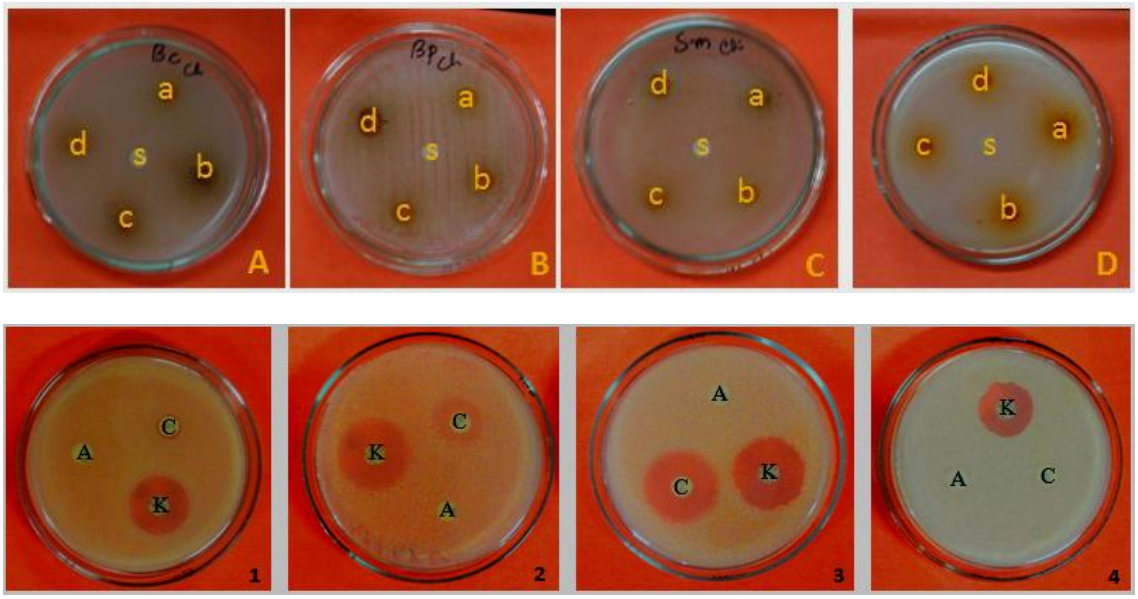

Figure 29. Antibacterial activity of *C. annuum* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

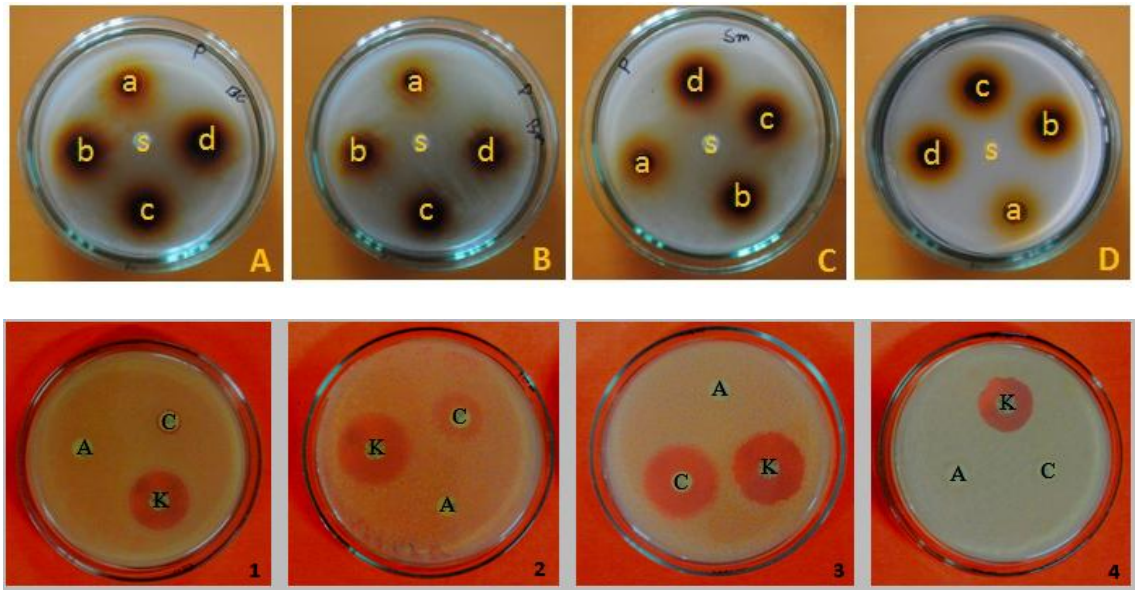

Figure 30. Antibacterial activity of *P. perlata* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa*.

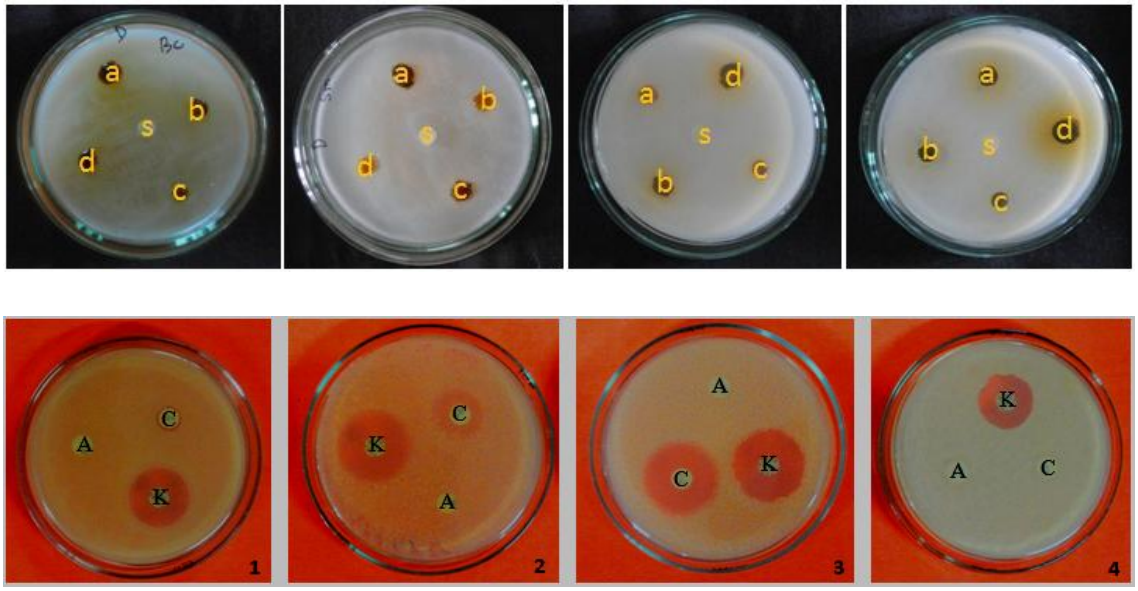

Figure 31. Antibacterial activity of *P. perlata* methanolic extract. (a: 4 mg disc^{-1}; b: 6 mg disc^{-1}; c: 8 mg disc^{-1}; d: 10 mg disc^{-1}, s: solvent control) against A. *B. cereus*, B. *B. pumilus*, C. *S. mercescens* and D. *Ps. aeruginosa*. Standard antibiotics (C= Chloramphenicol; K=Kanamycin; A=Ampicillin) against 1. *B. cereus*, 2.*B. pumilus*, 3.*S. mercescens* and 4. *Ps. aeruginosa.*

Among the spice extracts, extract of *I. verum* was found to be most potent showing highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg lyophilized methanolic extract disc^{-1} respectively

Table 30: Comparative antibacterial activities of different spices against Gram positive and Gram negative bacterial strains at different doses of extracts

Plant species/ Referent antibiotics*	Dose (mg LME disc^{-1})	Bacterial strains			
		Diameter of zone of inhibition (mm)			
		Bc	***Bp***	***Sm***	***Pa***
Illicium verum	0[a]	Ni	Ni	Ni	Ni
	4	11.25 ± 0.29	5.87 ± 0.01	6.00 ± 0.02	6.58 ± 0.42
	6	12.85 ± 0.30	6.08 ± 0.15	6.18 ± 0.13	7.65 ± 0.24
	8	15.13 ± 0.25	7.10 ± 0.20	8.93 ± 0.15	8.38 ± 0.25
	10	18.35 ± 0.24	14.78 ± 0.26	10.23 ± 0.26	11.13 ± 0.75

Plant species/ Referent antibiotics*	Dose (mg LME disc^{-1})	Bacterial strains			
		Diameter of zone of inhibition (mm)			
		Bc	***Bp***	***Sm***	***Pa***
Myristica fragrans	0[a]	Ni	Ni	Ni	Ni
	4	Ni	7.03 ± 0.05	Ni	Ni
	6	6.85 ± 0.30	8.05 ± 0.10	Ni	Ni
	8	8.38 ± 0.48	10.00 ± 0.20	9.10 ± 0.12	Ni
	10	12.25± 0.29	12.05 ± 0.17	10.30 ± 0.36	7.28 ± 0.32
Ceiba pentandra	0[a]	Ni	Ni	Ni	Ni
	4	Ni	Ni	Ni	Ni
	6	Ni	Ni	Ni	Ni
	8	Ni	Ni	Ni	Ni
	10	8.73 ± 0.32	6.15 ± 0.24	Ni	Ni
Capsicum annuum	0[a]	Ni	Ni	Ni	Ni
	4	Ni	6.05 ± 0.06	6.03 ± 0.05	Ni
	6	Ni	7.08 ± 0.09	7.05 ± 0.10	Ni
	8	Ni	8.10 ± 0.12	8.15 ± 0.12	6.20 ± 0.23
	10	9.80 ± 0.36	10.05 ± 0.42	9.00 ± 0.34	7.78 ± 0.26
Parmelia perlata	0[a]	Ni	Ni	Ni	Ni
	4	7.85 ± 0.30	7.15 ± 0.19	6.73 ± 0.32	6.30 ± 0.36
	6	8.88 ± 0.25	8.35 ± 0.24	7.75 ± 0.29	7.35 ± 0.24
	8	10.23 ± 0.26	14.00 ± 0.16	12.75 ± 0.29	10.25 ± 0.29
	10	11.25 ± 0.29	15.60 ± 0.27	14.50 ± 0.41	12.23 ± 0.26
Dregea volubilis	0[a]	Ni	Ni	Ni	Ni
	4	Ni	Ni	Ni	Ni
	6	Ni	Ni	Ni	Ni
	8	Ni	Ni	Ni	Ni
	10	8.10 ± 0.20	Ni	Ni	Ni
Ampicillin	A_{25}	Ni	Ni	Ni	Ni
Chloramphenicol	C_{25}	8.50 ± 0.58	12.02 ± 0.15	24.13 ± 0.25	Ni
Kanamycin	K_{30}	19.63 ± 0.48	21.88 ± 0.25	22.00 ± 0.41	17.88 ± 0.25

£Each value represents the mean ± standard deviation from quadruplicate observations (n=4). Diameter of commercial antibiotic disc is 6 mm, and that of blank paper disc or solvent disc is 5.5 mm that correspond to 'no activity'. 0[a] denoted as disc without extract. ¥Bacterial strains, *Bc*: *Bacillus cereus*, *Bp*: *Bacillus pumilus*, *Sm*: *Serratia marcescens*, *Pa*: *Pseudomonas aeruginosa*. *Antibiotics, A_{25}: Ampicillin at 25 µg disc^{-1}, C_{25}: Chloramphenicol at 25 µg disc^{-1}, K_{30}: Kanamycin at 30 µg disc^{-1}. Lyophilized methanolic extract is abbreviated as LME.

Table 31: Minimum inhibitory dose (MID) determination of spice extracts against target bacterial strainsusing DAD assay.

Spice samples	Bacterial strains			
	MID value (mg LME disc^{-1})			
	Bc	*Bp*	*Sm*	*Pa*
Illicium verum	1.25	2.5	3.5	1.5
Myristica fragrans	5.5	3.0	6.5	8.5
Ceiba pentandra	9.0	9.0	>10.0	>10.0
Capsicum annuum	8.5	3.5	3.5	7.5
Parmelia perlata	2.0	2.0	3.0	2.0
Dregea volubilis	9.0	>10.0	>10.0	>10.0

Minimum inhibitory dose (MID) expressed in mg Lyophilized methanolic extract disc^{-1}. Bacterial strains, *Bc*: *Bacillus cereus*, *Bp*: *Bacillus pumilus*, *Sm*: *Serratia marcescens*, *Pa*: *Pseudomonas aeruginosa*.

4.8. Anti-quorum sensing activities of different herbs and spices

Quorum sensing is a system of stimulus and response correlated to population density. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population. In the present study, anti-quorum sensing activities of *C. annuum, C. pentandra, C. sativum, G. oppositifolius, I. verum, M. piperita, M. koenigii, M. fragrans, P. perlata, T. foenum-graecum, F. vulgare* and *D. volubilis* hadbeen carried out using *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. Preliminary screening for anti-QS activity was done through inhibition of violacein synthesis by ethanol sterilized whole dried plant plant parts and results were revealed in Table 32. Among the plant samples *I. verum* showed significant inhibition of violacein synthesis, followed by *P. perlata, C. annuum* and *M. fragrans.* This result was further justified by disc-agar diffusion assay for pigment inhibition.

4.8.1. Comparison of anti-QS activity among different spice extracts

In DAD assay, among the plant extracts tested, the methanolic extract of *I. verum* showed consistent as well as maximum inhibition of violacein production in *C. violaceum* MTCC 2656 (Figure 32) followed by second maximum pigment inhibitor

was *P. perlata*, whereas, *C. annuum* and *M. fragrans* showed very minimal and inconsistent reductions (Table 33).

4.8.2. Inhibitory effect of *Iv*ME on violacein production in CV2656

The methanolic extract of *I. verum* which showed effective inhibitory activity on violacein production in *C. violaceum* MTCC 2656 without any impact on its growth were selected for the quantitative assessment of violacein inhibition at a concentration of 100 -1000 µg mL^{-1}. The results obtained revealed that test extract exerted a dose dependent inhibitory activity on violacein pigment production. Compared to the control, methanolic extract of *I. verum* showed a significant drop in violacein production.

Table 32: Preliminary screening for anti-QS activity by intact herb and spice sample against *C. violaceum* 2656

Herb and spice samples	Plant parts tested	Violacein inhibition
Mentha piperita	Whole leaf	Nd
Trigonella foenum-graecum	Whole leaf	Nd
Coriandrum sativum	Whole leaf	Nd
Murraya koenigii	Whole leaf	Nd
Glinus oppositifolius	Aerial part	Nd
Foeniculum vulgare	Aerial part	Nd
Illicium verum	Whole fruit	+++
Myristica fragrans	Aril	+
Ceiba pentandra	Floral bud	Nd
Capsicum annuum	Whole fruit	+
Parmelia perlata	Whole thallus	++
Dregea volubilis	Stem bark	Nd

(+) sign showed varying level of violacein pigment inhibition, Nd: Not detected.

The methanolic extract of *I. verum*at the concentration of 1 mg mL^{-1} inhibited the violacein production by 78.57% as depicted in Figure 33 and 34**.** Hence, the extract of *I. verum* alone was used in quantitative assessment of violacein inhibition with CV2656 and subsequent assays in the present study.

Table 33: Comparative anti-QS activities of selected herb and spice extracts on *C. violaceum* 2656 by DAD assay

Plant species	Effective dose (mg LME $disc^{-1}$)	CV2656	
		AM activity	anti-QS activity
Illicium verum	0	Ni	Nil
	3	7.30 ± 0.24	4.31 ± 0.37
	5	9.70 ± 0.36	3.90 ± 0.43
	7	11.13 ± 0.48	3.87 ± 0.66
	10	13.60 ± 0.27	2.90 ± 0.39
Myristica fragrans	0	Ni	Nil
	3	Ni	0.38 ± 0.05
	5	6.10 ± 0.22	0.70 ± 0.36
	7	6.50 ± 0.35	1.60 ± 0.18
	10	7.40 ± 0.12	1.30 ± 0.27
Capsicum annuum	0	Ni	Nil
	3	Ni	1.08 ± 0.13
	5	Ni	1.90 ± 0.36
	7	6.80 ± 0.56	2.80 ± 0.48
	10	8.60 ± 0.30	0.78 ± 0.23
Parmelia perlata	0	Ni	Nil
	3	6.90 ± 0.30	3.90 ± 0.41
	5	9.60 ± 0.10	3.78 ± 0.36
	7	10.10 ± 0.25	3.65 ± 0.50
	10	12.6 ± 0.48	2.45 ± 0.52
LSD value (column):		11.978	4.845
LSD value (row):		14.667	4.0541

Values represent the mean ± standard deviation from quadruplicate observations (n=4). Antimicrobial (AM) activityis expressed as zone of growth inhibition in mm and anti-quorum sensing activity is measured as zone of pigment inhibition in mm. Anti-QS activity is determined by subtracting the antimicrobial zone from de-pigmented opaque zone around the disc. Diameter of blank paper disc or solvent disc is 5.5 mm. 0 denoted as disc without extract. Ni: no growth inhibition, Nil: no pigment inhibition activity.

Table 33A: ANOVA of data presented in Table 33 (Antimicrobial activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	278.35678	4	69.5892	16.7747	7.42E-05	3.259167
B (Columns)	106.405135	3	35.46838	8.549766	0.00262	3.490295
AxB (Error)	49.78154	12	4.148462			
AB (Total)	434.543455	19				

Table 33B: ANOVA of data presented in Table 33 (Anti-QS activity)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Rows)	21.26665	4	5.316663	7.774423	0.002483	3.259167
B (Columns)	17.40806	3	5.802687	8.485124	0.0027	3.490295
AxB (Error)	8.20639	12	0.683866			
AB (Total)	46.8811	19				

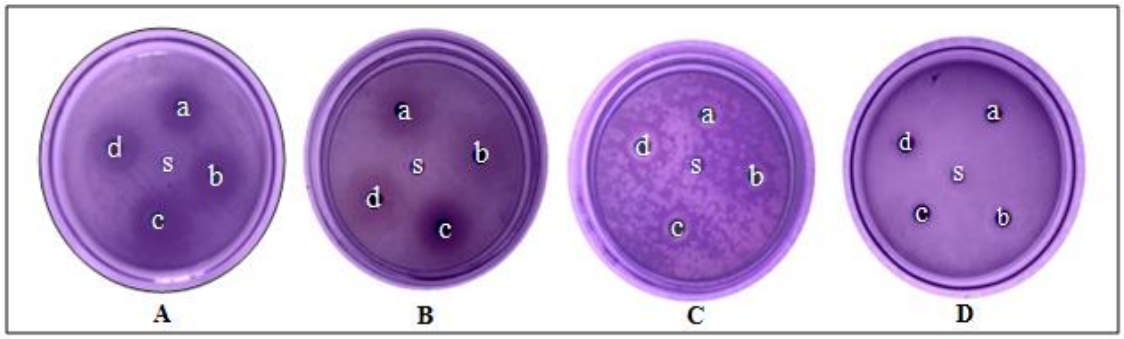

Figure 32. Effect of different spice extracts on violacein synthesis in C. violaceum at different doses (3-10 mg disc^{-1}) evaluated by disc-agar diffusion assay. CV treated with A. *I. verum*, B. *M. fragrans*, C. *C. annuum* and D. *P. perlata*. a. 3 mg disc^{-1}, b. 5 mg disc^{-1}, c. 7 mg disc^{-1}, d. 10 mg disc^{-1}, and s. solvent control (methanol).

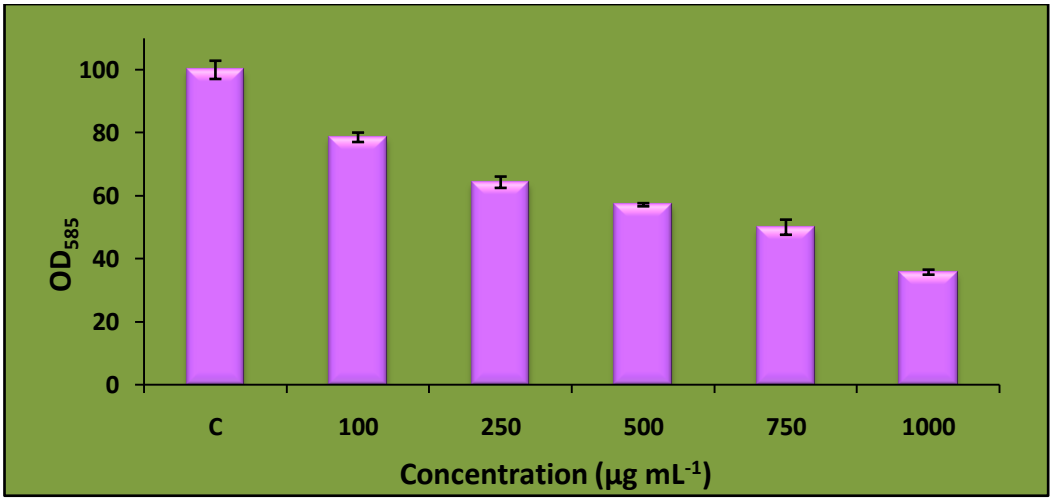

Figure 33. Graphical representation showing the effect of *I. verum* on violacein production by wild-type *C. violaceum*. Violacein production in presence of different concentrations (100-1000 µg mL^{-1}) of lyophilized methanolic extract of *I. verum* fruit. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3).

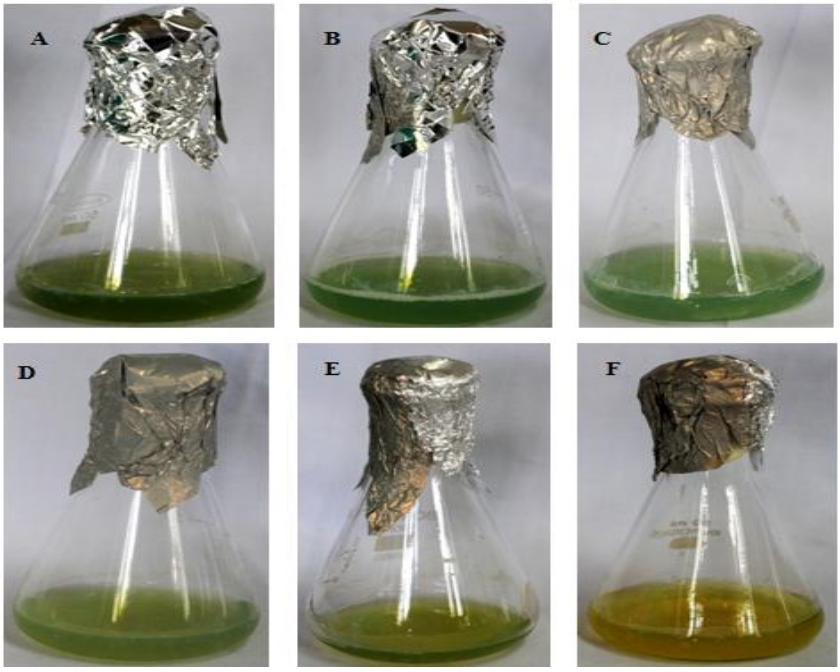

Figure 34. Effect of *I. verum* on violacein production by wild-type *C. violaceum*. A is Control (untreated); B, C, D, E, and F are treated with extract at 100, 250, 500, 750 and 1000 µg mL^{-1} respectively.

4.8.3. Reduced secretion of *Ps. aeruginosa* virulence factors upon *Iv*ME treatment

Secretion of bacterial virulence factors like pyocyanin and proteases upon treatment with *Iv*ME were measured. Inhibition of the quorum sensing activity in the form of altered pyocyanin synthesis by *I. verum* extract is presented in Figure 37 and 38.

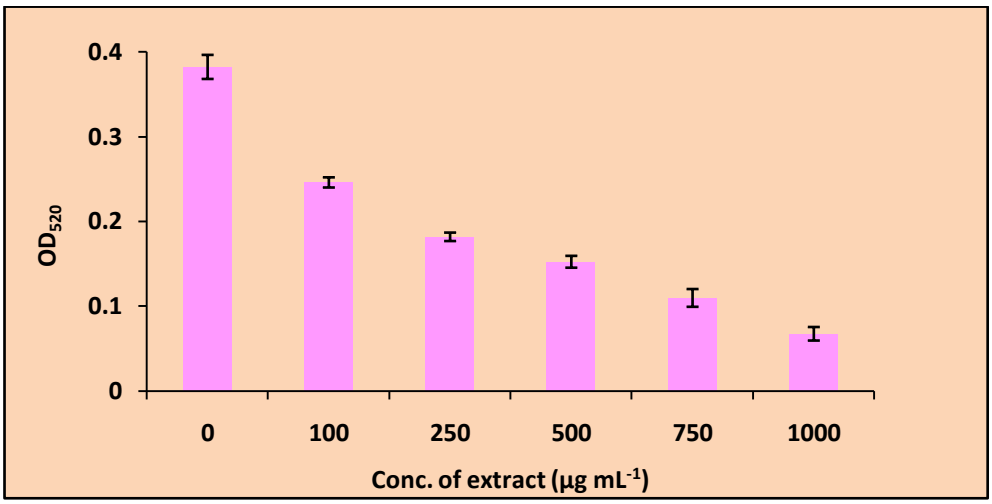

Figure 35. Graphical representation of the effect of *I. verum* on pyocyanin production by *P. aeruginosa* at different concentrations (100-1000 µg mL^{-1}). Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3).

Figure 36. Effect of *I. verum* methanolic extract on synthesis of pyocyanin by *Ps. aeruginosa*. C (control): 0; 1: 100; 2: 250; 3: 500; 4: 750 and 5: 1000 µg mL-1 concentrations.

Reduction in the production of pyocyanin pigment in *P. aeruginosa* is an indicator of anti-QS activity by plant methanolic extract. In comparison to the *Iv*ME-untreated microbial cells, *Iv*ME-treated cells produce lesser amounts of pyocyanin. Pyocyanin production is decreased with the increasing concentration of *Iv*ME concentration.

On the other hand, the result of azocaseinolytic protease assay showed that in comparison to the *Iv*ME-untreated cells, *Iv*ME-treated microbial cells exhibited reduced level of protease activity wherein the proteolytic activity was maximally and minimally inhibited by 200 µg mL^{-1} and 500 µg mL^{-1} concentrations respectively. Upon calculation of relative proteolytic activity it was found that 500 µg mL^{-1} of *Iv*ME gave a value of 0.067 in release of proteases by *Ps. aeruginosa* (Table 34 and Figure 39).

Table 34: Inhibitory effect of *Illicium verum* methanolic extract on protease production by *Ps. aeruginosa*

Plant species	Effective conc. (mg LME mL^{-1})	Relative proteolytic activity
Illicium verum	Control	0.193 ± 0.023
	200	0.088 ± 0.005
	250	0.072 ± 0.001
	500	0.067 ± 0.003

Values represent the mean ± standard deviation from quadruplicate observations (n=4).
Relative proteolytic activity was determined by ratio of diameter of zone of proteolysis (mm) to diameter of bacterial colony (mm).

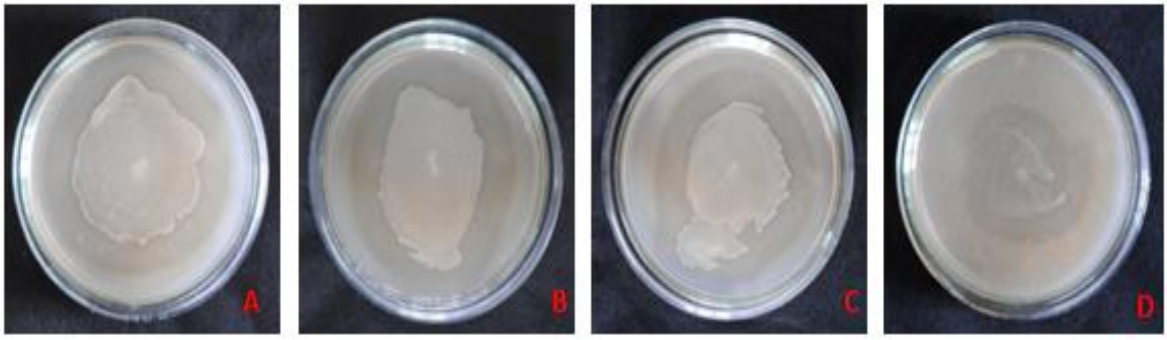

Figure 37. Effect of *I. verum* methanolic extract on protease production by *Ps. aeruginosa* at different concentrations. A is Control (untreated); B, C, and D are treated with extract at 200, 250, and 500 µg mL^{-1} respectively.

4.8.4. Reduction in swarming motility after *Iv*ME treatment

Methanolic extract of *I. verum* was further explored for its effect on another bacterial virulence property *i.e.*, swarming motility. The efficacy of *Iv*ME over swarming motility of *Ps. aeruginosa* MTCC 2453 was further examined. The attained results lucidly revealed that the extracts of *I. verum*effectively inhibited QS-dependent swarming migration in *Ps. aeruginosa* MTCC 2453at different concentrations. Result revealed that the swarming motility was minimally and maximally inhibited when the bacterial cells were treated with 200 µg mL^{-1} and 800 µg mL^{-1}concentrations of *Iv*ME respectively in comparison to untreated control. The reduction in swarming motility was dose dependent and extract of *Iv*MEat 200 µg mL^{-1} and 800 µg mL^{-1} concentrations had the efficiency to reduce 74.14% and 90.10% swarming motility respectively (Table35, Figure 40)

Table 35: Effect of *I. verum* methanolic extract on swarming motility of *Ps. aeruginosa*

Plant species	Effective conc. (µg LME mL^{-1})	Diameter of swarm (mm)	% reduction in swarming motility
Illicium verum	Control	38.67 ± 0.29	
	200	10.25 ± 0.66	74.14 ± 1.29
	400	7.13 ± 0.85	81.90 ± 2.59
	600	6.13 ± 0.63	84.91 ± 0.75
	800	3.88 ± 0.63	90.10 ± 1.97

Values represent the mean ± standard deviation from quadruplicate observations (n=4).

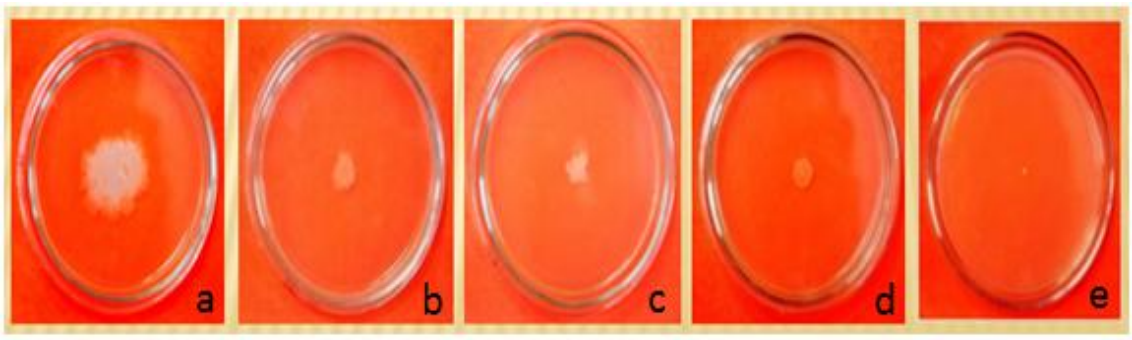

Figure 38. Effect of *I. verum* methanolic extract on swarming behaviour of *Ps. aeruginosa*. (a) Untreated control, (b-e) treated with *I. verum* extract (150, 200, 250 and 500 µg mL^{-1}) respectively.

All results of virulence factors showed that all the tests at different doses of *Iv*ME exhibited significant reduction in virulence property compared to the extent of virulence factors produced by the *Iv*ME-untreated bacterial cells.

4.8.5. Inhibitory effect of *Iv*ME on biofilm forming ability

The methanolic extract of *I. verum* showed **s**trong anti-biofilm avtivity while compared with control in crystal violate assay. The test tube with less violet pigmention indicated the higher antibiofilm activity. The control tube appeared as dark violet coloration**.** Figure 35-36 is showing the quantitative measurements of adherent biofilm stained by crystal violate dye.

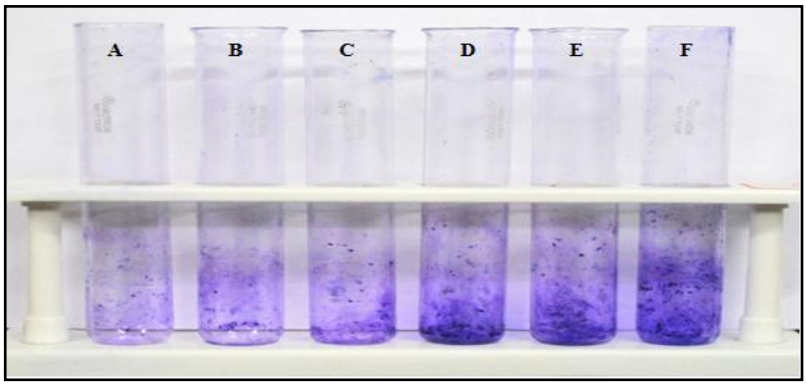

Figure 39. Effect of *I. verum* methanolic extract on *in vitro* biofilm formation by *Ps. aeruginosa*. A: 1000; B: 750; C: 500; D: 250 and E: 100 µg mL^{-1}. F: Control. Test tubes were stained with crystal violet to visualize the biofilm.

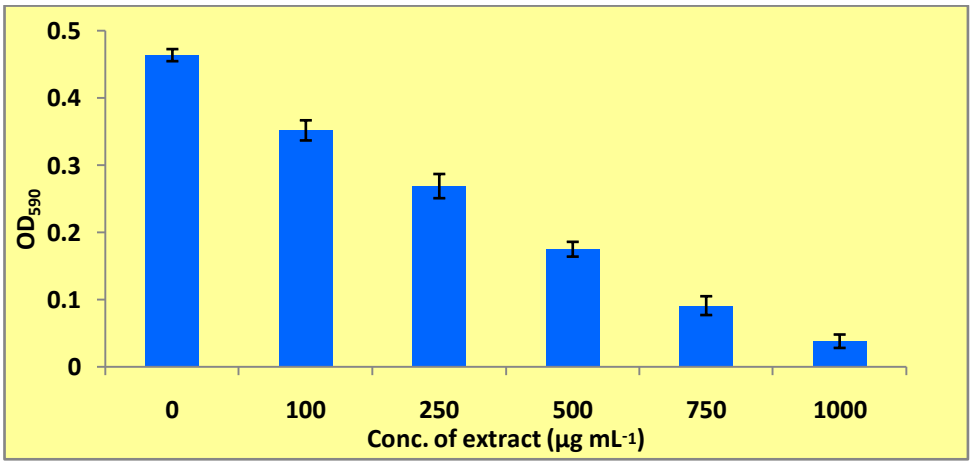

Figure 40. Graphical representation of biofilm inhibition by methanolic extract of *I. verum* at different concentrations. Values are presented as mean ± standard deviation from analysis of three independent replicates (n=3).

4.9. Antidiabetic activities of methanolic extracts of selected herb and spice

4.9.1. Acute TOX study of*Illicium verum*and *Glinus oppositifolius* extracts

In acute toxicity study, oral administration of *I. verum* extract (*Iv*ME) and *G. oppositifolius* extract (*Go*ME) with a single dose of 2000 mg kg^{-1} BW prior to the use of these extracts for *in vivo* antidiabetic studies did not produce any toxicological signs. Mortality was not observed till the end of 21 days of experiment. Thus, $1/4^{th}$ and $1/8^{th}$ doses of 2000 mg kg^{-1} BW *i.e.* 500 mg kg^{-1} BW and 250 mg kg^{-1} BW were selected for subsequent studies.

4.9.2. Effect of different extracts on diabetic induced rats

4.9.2.1. Changes in body weight

At the end of 21^{st} day *i.e.*, on last day of 3^{rd} week of experimentation, the body weight of the diabetic control was found to be decreased significantly in diabetic control (Group II) when compared to the normal rats (Group I). However, an oral administration of *Iv*ME and *Go*ME at 500 mg kg^{-1} BW and Metformin to diabetic rats reversed the body weight changes to near normal range (Table 36).

4.9.2.2. Changes in blood sugar level

The antidiabetic effect of *I. verum* and *G. oppositifolius* extracts was assessed by measuring the fasting blood glucose levels on 0 day, 7^{th} day, 14^{th} day and 21st day of the treatment. As shown in Table 37, the fasting blood glucose levels of diabetic control rats were increased significantly after the injection of Streptozotocin, in comparison to normal control rats (NCR). Before administration of the respective drug and plant extracts, the fasting blood glucose levels of different diabetic groups (DR, DR+Met, DR+ *Iv*ME 250 mg kg^{-1} BW, *Iv*ME 500 mg kg^{-1} BW, *Go*ME 250 mg kg^{-1} BW and *Go*ME 500 mg kg^{-1} BW) were almost similar except for DR+Met groups which had significantly higher blood glucose levels than the DCR groups.It was observed that treatment with respective extracts for 21 days reduced the fasting blood glucose level to near normal. Compared with the diabetic control group (DCR), administration of both plant extracts to STZ-induced diabetic rats showed anti-hyperglycemic effect. However, *Iv*ME showed significant reduction in blood glucose levels than that of *Go*ME at the end of the experimental period when compared to diabetic control. Decrease in the blood glucose level was related to dose of the extracts and duration of the treatment.

Table 36: Effect of *I. verum* and *G. oppositifolius* methanolic extracts on body weight in diabetic rats

Gr	Treatment	Body weight (g)		
		Initial (0 day)	**Final (21^{th} day)**	**% difference**
I	Normal control rats (NCR)	182.33 ± 10.79	199.87 ± 3.67	+ 9.62
II	Diabetic control rats (DCR)	190.67 ± 5.03	153.53 ± 8.30	- 13.23
III	DR + Met (10 mg kg^{-1} BW)	186.00 ± 8.19	203.30 ± 8.35	+ 9.3
IV	DR + *Iv*ME (250 mg kg^{-1} BW)	181.33 ± 12.58	191.67 ± 14.29	+ 7.27
V	DR + *Iv*ME (500 mg kg^{-1} BW)	184.66 ± 13.32	200.23 ± 8.65	+ 8.43
VI	DR + *Go*ME (250 mg kg^{-1} BW)	176.67 ± 6.03	187.50 ± 5.68	+ 6.13
VII	DR + *Go*ME (500 mg kg^{-1} BW)	178.00 ± 10.58	192.50 ± 11.95	+ 8.14
LSD value (column): 85.763 LSD value (row): 14.350				

Valuesare presented as mean ± standard deviation (n=6) and expressed in gram (g). Values in the same column and rows are significantly different ($p<0.05$), as determined using the Fisher"s LSD test.DR + Met: Diabetic rats treated with antidiabetic standard drug Metformin; *Iv*ME: *Illicium verum* methanolic extract; *Go*ME: *Glinus oppositifolius* methanolic extract.

Table 36A: ANOVA of data presented in Table 36 (body weight change in experimental rats)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	926.2730159	6	154.378836	1.502915	0.316635	4.283866
B (Columns)	205.2114286	1	205.2114286	1.997783	0.207251	5.987378
AxB (Error)	616.3174603	6	102.7195767			
AB (Total)	1747.801905	13				

4.9.2.3. Changes in lipid profiles

Lipid profiles of both normal and STZ-induced diabetic rats were assessed by monitoring the parameters like cholesterol, triglycerides and HDL-cholesterol. It was observed that in diabetic rats the levels of cholesterol and triglycerides were increased while HDL-cholesterol level was decreased compared to normal control rats (Table 38). However, administration of extracts (*Iv*ME at 250 and 500 mg kg^{-1} BW; *Go*ME at 250 and 500 mg kg^{-1} BW) and standard drug Metformin (10 mg kg^{-1} BW) showed significant reduction in elevated cholesterol and triglyceride levels compared to diabetic control groups. On the contrary, significant increase in the level of HDL-cholesterol was observed in diabetic rats treated with the extracts and standard drug compared to diabetic control rats.

Table 37: Effect of *I. verum*and *G. oppositifolius* methanolic extracts on blood glucose level in diabetic rats

Gr	Treatment	Blood glucose (mg dL^{-1})			
		0 day	**7th day**	**14th day**	**21st day**
I	Normal control rats (NCR)	108.75 ± 4.11	110.25 ± 3.86	108.50 ± 4.65	108.25 ± 4.27
II	Diabetic control rats (DCR)	286.50 ± 7.54	297.75 ± 8.06	318.75 ± 22.62	343.50 ± 12.26
III	DR + Met (10 mg kg^{-1} BW)	280.50 ± 4.36	177.00 ± 17.26	146.50 ± 16.66	132.50 ± 7.72
IV	DR + *Iv*ME (250 mg kg^{-1} BW)	291.75 ± 7.82	274.75 ± 12.04	263.25 ± 11.33	173.25 ± 18.00
V	DR + *Iv*ME (500 mg kg^{-1} BW)	286.25 ± 11.30	271.50 ± 10.97	258.75 ± 11.67	167.50 ± 10.15
VI	DR + *Go*ME (250 mg kg^{-1} BW)	293.50 ± 14.46	28.75 ± 16.15	260.50 ± 13.68	183.50 ± 11.24
VII	DR + *Go*ME (500 mg kg^{-1} BW)	296.25 ± 11.96	284.25 ± 11.18	270.75 ± 12.23	180.75 ± 18.01
LSD value (column):		220.016			
LSD value (row):		178.732			

Values are presented as mean ± standard deviation (n=6) and expressed in mg dL^{-1}. Values in the same column and rows are significantly different ($p<0.05$), as determined using the Fisher‟s LSD test.DR + Met: Diabetic rats treated with antidiabetic standard drug Metformin; *Iv*ME: *Illicium verum* methanolic extract; *Go*ME: *Glinus oppositifolius* methanolic extract.

Table 37A: ANOVA of data presented in Table 37 (blood glucose level change in experimental rats)

Source of Variation	***SS***	***Df***	***MS***	***F(cal)***	***P-value***	***F crit (0.05)***
A (Rows)	175625.0278	6	29270.83797	7.592113	0.000359	2.661305
B (Columns)	78984.06564	3	26328.02188	6.828821	0.002869	3.159908
AxB (Error)	69397.69174	18	3855.427319			
AB (Total)	324006.7852	27				

Table 38: Effect of *I. verum* and *G. oppositifolius* methanolic extracts on serum lipid profile in normal and Streptozotocin-induced diabetic rats

Gr	**Treatment**	**Total cholesterol (mg/dL)**	**Total triglycerides (mg/dL)**	**HDL cholesterol (mg/dL)**
I	Normal control rats (NCR)	84.43 ± 7.72	81.39 ± 8.42	40.15 ± 3.43
II	Diabetic control rats (DCR)	164.77 ± 7.56	182.12 ± 7.33	25.11 ± 5.33
III	DR+Met (mg kg^{-1} BW)	86.21 ± 5.30	76.57 ± 5.13	38.15 ± 2.69
IV	DR+*Iv*ME (250 mg kg^{-1} BW)	114.47 ± 7.85	90.36 ± 7.87	32.04 ± 3.19
V	DR+*Iv*ME (500 mg kg^{-1} BW)	98.70 ± 2.51	85.99 ± 11.11	34.64 ± 4.97
VI	DR+*Go*ME (250 mg kg^{-1} BW)	117.35 ± 5.28	95.74 ± 10.88	29.37 ± 3.25
VII	DR+*Go*ME (500 mg kg^{-1} BW)	109.42 ± 9.50	87.97 ± 9.50	31.03 ± 3.21
LSD value:		51.491	69.689	9.815

Values are presented as mean ± standard deviation (n=6) and expressed in mg dL^{-1}. Values in the same column are significantly different ($p<0.05$), as determined using the Fisher‟s LSD test.DR + Met: Diabetic rats treated with antidiabetic standard drug Metformin; *Iv*ME: *Illicium verum* methanolic extract; *Go*ME: *Glinus oppositifolius* methanolic extract.

Table 38A: ANOVA of data presented in Table 38 (Total cholesterol)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	13250.92	6	2208.487	46.74483	1.82E-08	2.847726
R(A) (Within Groups)	661.4383	14	47.24559			
AR (Total)	13912.36	20				

Table 38B: ANOVA of data presented in Table 38 (Total triglycerides)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	24272.49871	6	4045.416452	51.97946	9.01E-09	2.847726
R(A) (Within Groups)	1089.580867	14	77.82720476			
AR (Total)	25362.07958	20				

Table 38C: ANOVA of data presented in Table 38 (HDL cholesterol)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	481.4222	6	80.23703	5.444161	0.004292	2.847726
R(A) (Within Groups)	206.3345	14	14.73818			
AR (Total)	687.7567	20				

4.9.2.4. Changes in liver enzyme (SGPT and SGOT) levels

In the present study, liver enzymes like SGPT and SGOT level was found to be significantly elevated in diabetic control group compared to the normal control group. However, increased level of SGPT and SGOT in the diabetic induced rats was significantly decreased with subsequent administration of the standard drug and the plant extracts (*Iv*ME at 250 and 500 mg kg^{-1} BW; *Go*ME at 250 and 500 mg kg^{-1} BW), results are shown in Table 39.

4.9.3.5. Kidney function test (urea and creatinine level)

The elevated levels of serum urea and creatinine of the diabetic rats was observed to be significantly reduced to almost normal range on treating the animals with both standard drug (Metformin) and the extracts (*Iv*ME at 250 and 500 mg kg^{-1} BW; *Go*ME at 250

and 500 mg kg^{-1} BW). On the other hand, the untreated diabetic control group showed significantly high level of serum urea and creatinine with respect to normal and treated rats (Table 40).

Table 39: Effect of *I. verum* and *G. oppositifolius* extracts on liver enzymes (SGPT and SGOT level) in normal and streptozotocin induced diabetic rats

Gr	Treatment	SGPT ($U\ dL^{-1}$)	SGOT ($U\ dL^{-1}$)
I	Normal control rats (NCR)	42.74 ± 3.67	76.62 ± 3.88
II	Diabetic control rats (DCR)	75.53 ± 5.47	146.81 ± 3.38
III	DR + Met (mg kg^{-1} BW)	46.45 ± 4.08	79.65 ± 2.15
IV	DR + *Iv*ME (250 mg kg^{-1} BW)	60.86 ± 6.24	91.51 ± 6.37
V	DR + *Iv*ME (500 mg kg^{-1} BW)	51.15 ± 2.60	84.33 ± 4.30
VI	DR + *Go*ME (250 mg kg^{-1} BW)	68.69 ± 2.08	94.12 ± 5.30
VII	DR + *Go*ME (500 mg kg^{-1} BW)	59.21 ± 4.68	89.63 ± 2.12
LSD value:		22.509	45.260

Values are presented as mean ± standard deviation (n=6) and expressed in U dL^{-1}. Values in the same column are significantly different ($p<0.05$), as determined using the Fisher‟s LSD test. DR+Met: Diabetic rats treated with antidiabetic standard drug Metformin; *Iv*ME: *Illicium verum* methanolic extract; *Go*ME: *Glinus oppositifolius* methanolic extract.

Table 39A: ANOVA of data presented in Table 39 (SGPT level)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	2532.34	6	422.0567	22.39007	2.02E-06	2.847726
R(A) (Within Groups)	263.9024	14	18.85017			
AR (Total)	2796.243	20				

Table 39B: ANOVA of data presented in Table 39 (SGOT level)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	10238.21936	6	1706.37	97.25777	1.34E-10	2.847726
R(A) (Within Groups)	245.6274667	14	17.54482			
AR (Total)	10483.84683	20				

Table 40: Effect of *I. verum* and *G. oppositifolius* extracts on serum urea and creatinine level of normal and Streptozotocin-induced diabetic rats

Group	Treatment	Serum urea (mg/dL)	Serum creatinine (mg/dL)
I	Normal control rats (NCR)	21.65 ± 3.34	0.36 ± 0.04
II	Diabetic control rats (DCR)	65.48 ± 2.70	1.14 ± 0.16
III	DR + Met (mg kg-1 BW)	26.25 ± 5.16	0.52 ± 0.06
IV	DR + *Iv*ME (250 mg kg-1 BW)	38.46 ± 2.43	0.66 ± 0.02
V	DR + *Iv*ME (500 mg kg-1 BW)	31.13 ± 2.74	0.59 ± 0.03
VI	DR + *Go*ME (250 mg kg-1 BW)	43.86 ± 5.11	0.69 ± 0.05
VII	DR + *Go*ME (500 mg kg-1 BW)	37.47 ± 3.87	0.64 ± 0.02
LSD value:		27.305	0.458

Values are presented as mean ± standard deviation (n=6) and expressed in U dL^{-1}.Values in the same column are significantly different ($p<0.05$), as determined using the Fisher‟s LSD test. DR+Met: Diabetic rats treated with antidiabetic standard drug Metformin; *Iv*ME: *Illicium verum* methanolic extract; *Go*ME: *Glinus oppositifolius* methanolic extract.

Table 40A: ANOVA of data presented in Table 40 (Serum urea)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	3726.279181	6	621.0465302	43.63199029	2.85548E-08	2.847726
R(A) (Within Groups)	199.2724	14	14.23374286			
AR (Total)	3925.551581	20				

Table 40B: ANOVA of data presented in Table 40 (Serum creatinine)

Source of Variation	*SS*	*Df*	*MS*	*F(cal)*	*P-value*	*F crit (0.05)*
A (Between Groups)	1.046925238	6	0.17448754	20.96749982	3.02659E-06	2.847726
R(A) (Within Groups)	0.116505333	14	0.00832181			
AR (Total)	1.163430571	20				

4.10. Partial characterization of active principles isolated from *Iv*ME and *Go*MEby GC-MS analysis

Different fractions of *I. verum (Iv*ME*)* and *G. oppositifolius* (*Go*ME) were subjected to GC-MS analysis for partial characterization of the chemical compounds present in them (Figure 41). Numerous compounds were identified and the variation of these compounds for respective plants has been presented in Table 41-46, Figure 42-64. Further, GC-MS total ion chromatogram and mass spectra for all the identified compounds with its core structures. The spectra of unknown compounds were compared with spectra of known compounds stored. The identification of compounds was confirmed based on the active principle, Molecular Weight (MW), Concentration (%), Retention Time (RT), Molecular Formula (MF) and Peak Area (PA). GC-MS profiling revealed the presence of multitude chemical compounds containing volatile compounds, phenolics, terpenoids, fatty acids, phytosterol etc.

Some of the compounds identified through GC-MS analysis of methanolic fraction of *I. verum* wereLinalool, Estragole, Benzaldehyde, 4-methoxy-; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; Benzhydrazide, 4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-; cis-Vaccenic acid; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; Octadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-. The chemical compounds identified through GC-MS analysis of hexane fraction of *I. verum* were Benzaldehyde, 4-methoxy-; Anethole; Anisaldehyde dimethyl acetal; 2-Propanone, 1-(4-methoxyphenyl)-; 4-(p-Methoxyphenyl)-1-butanol; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; cis-Vaccenic acid; (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-; 4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and Ethanone, 2-

hydroxy-1,2-bis(4-methoxyphenyl)-. Some of the compounds identified through GC-MS analysis of ethyl acetate fraction of *I. verum* were Linalool; Estragole; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; n-Hexadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and cis-Vaccenic acid.

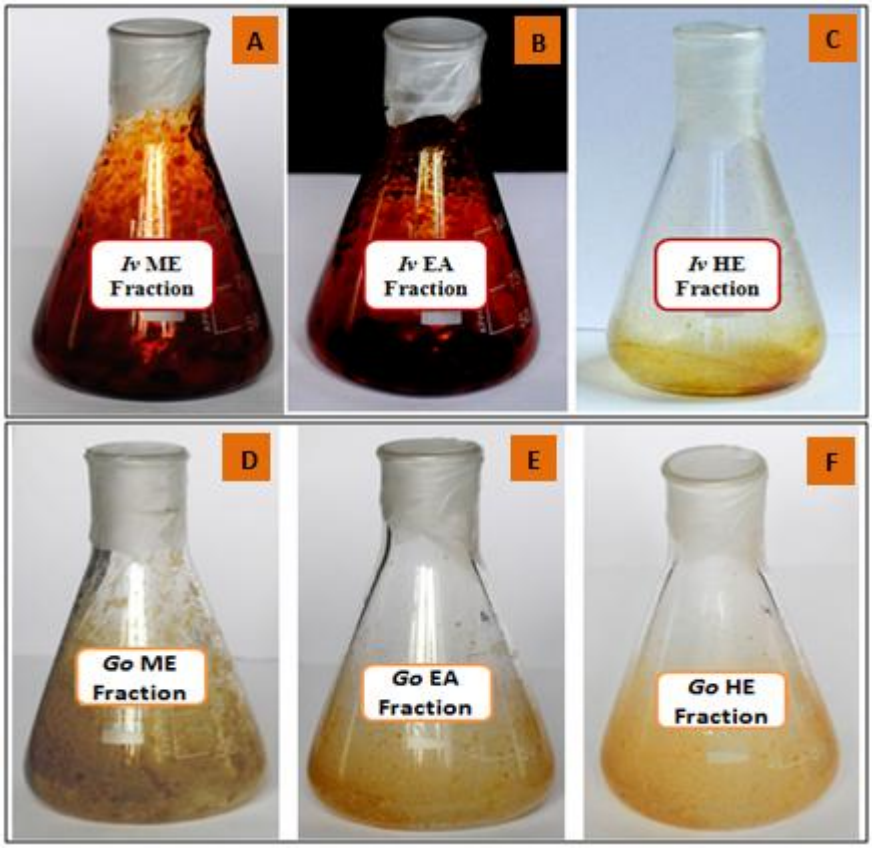

Figure 41. Partially purified different fractions of *I. verum* and *G. oppositifolius*. A. Methanolic fraction, B. Ethyl acetate fraction, C. Hexane fraction of *I. verum*; D. Methanolic fraction, E. Ethyl acetate fraction and E. Hexane fraction of *G. oppositifolius*.

GC-MS analysis of different fractions of *G. oppositifolius* also revealed different types of chemical compounds. Compounds identified through GC-MS analysis of methanolic fraction of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; 1-Deutero-2,2,5,5-tetramethylcyclopentanol; n-Hexadecanoic acid; Phytol; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d]-naphthalene;(4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-exahydronaphthalen-2-yl and Retinol, acetate. Compounds identified through GC-MS analysis of hexane

fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; Mome inositol; Hexadecanoic acid, methyl ester, n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; Linoelaidic acid; Octadecanoic acid; Squalene and Chondrillasterol. Chemical compounds identified through GC-MS analysis oethyl acetate fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; DL-Proline, 5-oxo-, methyl ester; Mome inositol; n-Hexadecanoic acid; Phytol; Lanosterol; Lup-20(29)-en-28-ol; Beta.-copaen-4 .alpha.-ol and Retinol, acetate.

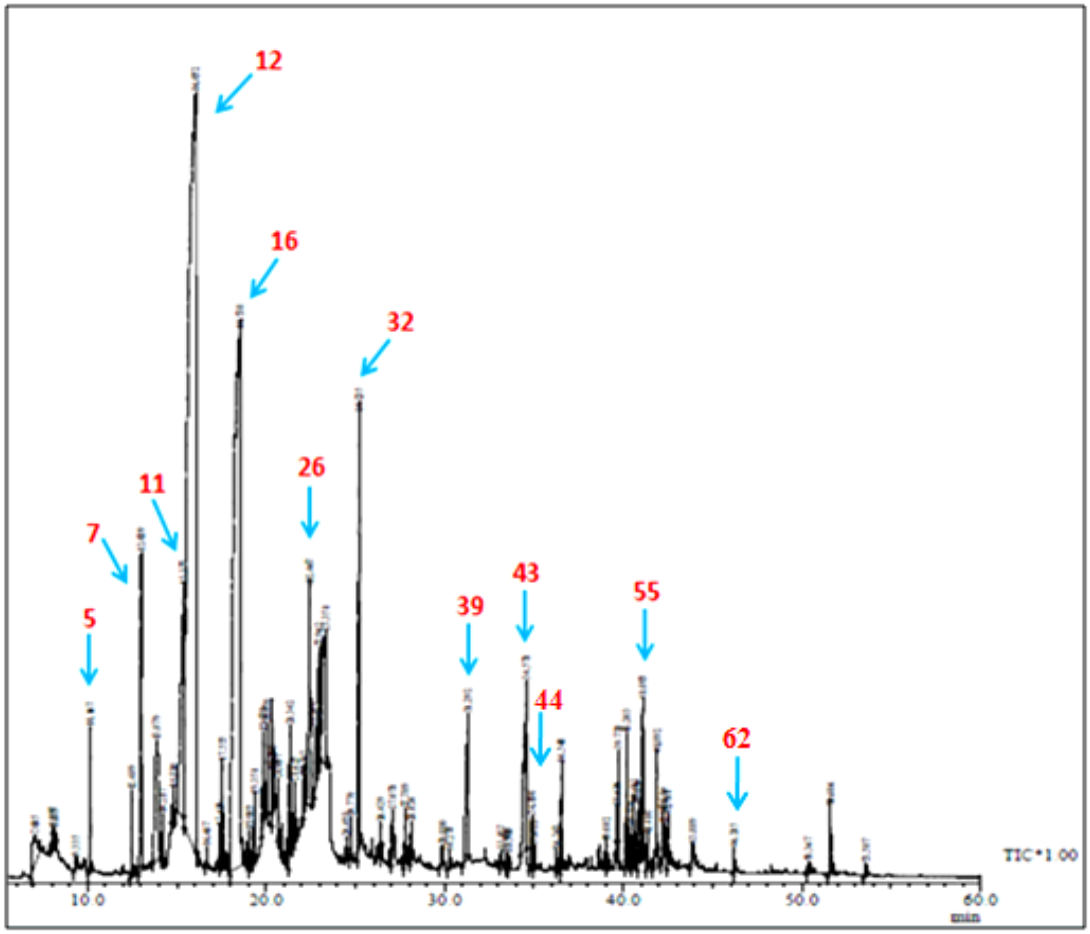

Figure 42. GC-MS Total Ion Chromatogram (TIC) of methanolic fraction of *I. verum* (*Iv*ME).

Table 41: Compounds identified in the methanolic fraction of *I. verum (Iv*ME*)*

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
1	7.085	cis-1,2-Dihydrocatechol	$C_6H_8O_2$	112	0.94
2	8.037	o-Cymene	$C_{10}H_{14}$	134	0.07
3	8.175	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-	$C_{10}H_{16}$	136	0.06
4	9.335	trans-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$	170	0.06
5	10.167	Linalool	$C_{10}H_{18}O$	154	0.65
6	12.489	1-Isopropyl-4-methyl-3-Cyclohexen-1-ol	$C_{10}H_{18}O$	154	0.40
7	13.009	Estragole	$C_{10}H_{12}O$	148	2.47
8	13.879	Catechol	$C_6H_6O_2$	110	2.75
9	14.217	Phenol, 4-methoxy-	$C_7H_8O_2$	114	0.40
10	14.814	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	0.24
11	15.377	Benzaldehyde, 4-methoxy-	$C_8H_8O_2$	136	4.05
12	16.052	Benzene, 1-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O$	148	36.13
13	16.667	3-Hydroxymandelic acid, dimethyl ether, methyl ester	$C_{11}H_{14}O_4$	210	0.08
14	17.430	Benzene, 1-methoxy-4-(1-methylpropyl)-	$C_{11}H_{16}O$	164	0.15
15	17.555	Anisole, o-(1-ethylvinyl)-	$C_{11}H_{14}O$	162	0.69
16	18.538	2-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	22.99
17	19.069	Caryophyllene	$C_{15}H_{24}$	204	0.13

18	19.351	trans-alpha-Bergamotene	$C_{15}H_{24}$	204	0.26
19	19.839	1-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	0.49
20	20.070	3,3'-Dimethoxybenzil	$C_{16}H_{14}O_4$	270	0.46
21	20.390	3-Hydroxymandelic acid, dimethyl ether, methyl ester	$C_{11}H_{14}O_4$	210	0.67
22	20.697	3-Hydroxymandelic acid, dimethyl ether, methyl ester	$C_{11}H_{14}O_4$	210	0.22
23	21.342	trans-Isoeugenol	$C_{10}H_{12}O_2$	164	1.00
24	21.625	1-Benzoxepin-3-ol, 2,3,4,5-tetrahydro-	$C_{10}H_{12}O_2$	164	0.33
25	22.142	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	208	0.24
26	22.467	Benzhydrazide, 4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-	$C_{17}H_{19}F_3N_2O_3$	356	2.35
27	22.823	Benzeneacetic acid, 4-methoxy-, methyl ester	$C_{10}H_{12}O_3$	180	0.59
28	22.962	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	0.53
29	23.351	Benzenemethanol, 4-methoxy-	$C_8H_{10}O_2$	138	3.16
30	24.493	Geranyl formate	$C_{11}H_{18}O_2$	182	0.06
31	24.770	alpha-Cadinol	$C_{15}H_{26}O$	222	0.21
32	25.235	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	4.19
33	26.429	2aS,3aR,5aS,9bR)-2a,5a,9-Trimethyl-2a,4,5,5a,6,7,8,9b-octahydro-2H-naphtho	$C_{15}H_{22}O_2$	234	0.11
34	27.071	4,8-Dimethyl-1,3,7-Nonatriene	$C_{11}H_{18}$	150	0.19
35	27.789	2-Phenyl-2-butanol	$C_{10}H_{14}O$	150	0.36

36	28.126	2-Phenyl-2-butanol	$C_{10}H_{14}O$	150	0.25
37	29.880	5-(3-Hydroxypropyl)-2,3-Dimethoxyphenol	$C_{11}H_{16}O_4$	212	0.17
38	30.278	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.07
39	31.292	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.77
40	33.157	1H-2-Indenol, 2,3,4,5,6,7-hexahydro-1-(2-hydroxy-2-methylpropyl)	$C_{13}H_{22}O_2$	214	0.09
41	33.458	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.05
42	33.590	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.06
43	34.571	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	2.58
44	34.890	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.24
45	35.022	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	0.12
46	36.263	(E)-1-(4-Methoxyphenyl)propene	$C_{10}H_{12}O$	148	0.09
47	36.548	(S)-1,1-bis(4-Methoxyphenyl)propan-2-ol	$C_{17}H_{20}O_3$	272	0.69
48	39.082	Carbamic acid, n-(2,5-Dimethylphenyl)-, propyl ester	$C_{12}H_{17}NO_2$	207	0.24
49	39.636	Benzoic acid, 4-methoxy-, 4-Ethylphenyl ester	$C_{16}H_{16}O_3$	247	0.14
50	39.728	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.44
51	40.203	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.74
52	40.354	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.26
53	40.612	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.37

54	40.878	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.29
55	41.093	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.87
56	41.410	4-Methoxybenzoic acid, 2,6-dimethyl-4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethylethyl) 57	$C_{19}H_{16}F_6O_4$	422	0.20
57	41.892	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.93
58	42.216	4,4'-Dimethoxybenzil	$C_{16}H_{14}O_4$	270	0.23
59	42.301	1,4-Dimethyl 2-[(4-methoxyphenyl)methyl]butanedioate	$C_{14}H_{18}O_5$	266	0.05
60	42.511	(Trans)-2-(2-(3-ethoxy-phenyl) ethylidene)-6-methyl-7-methyl-	$C_{20}H_{22}O_2$	294	0.22
61	43.889	1,3-Heptadiin-6-ol, 1-trimethyl silyl-	$C_{10}H_{16}OSi$	180	0.37
62	46.215	Carinol	$C_{20}H_{26}O_7$	358	0.12
63	50.367	Ergost-5-en-3-ol, (3.beta.)-	$C_{28}H_{48}O$	400	0.06
64	51.606	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414	0.53
65	53.587	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	0.08

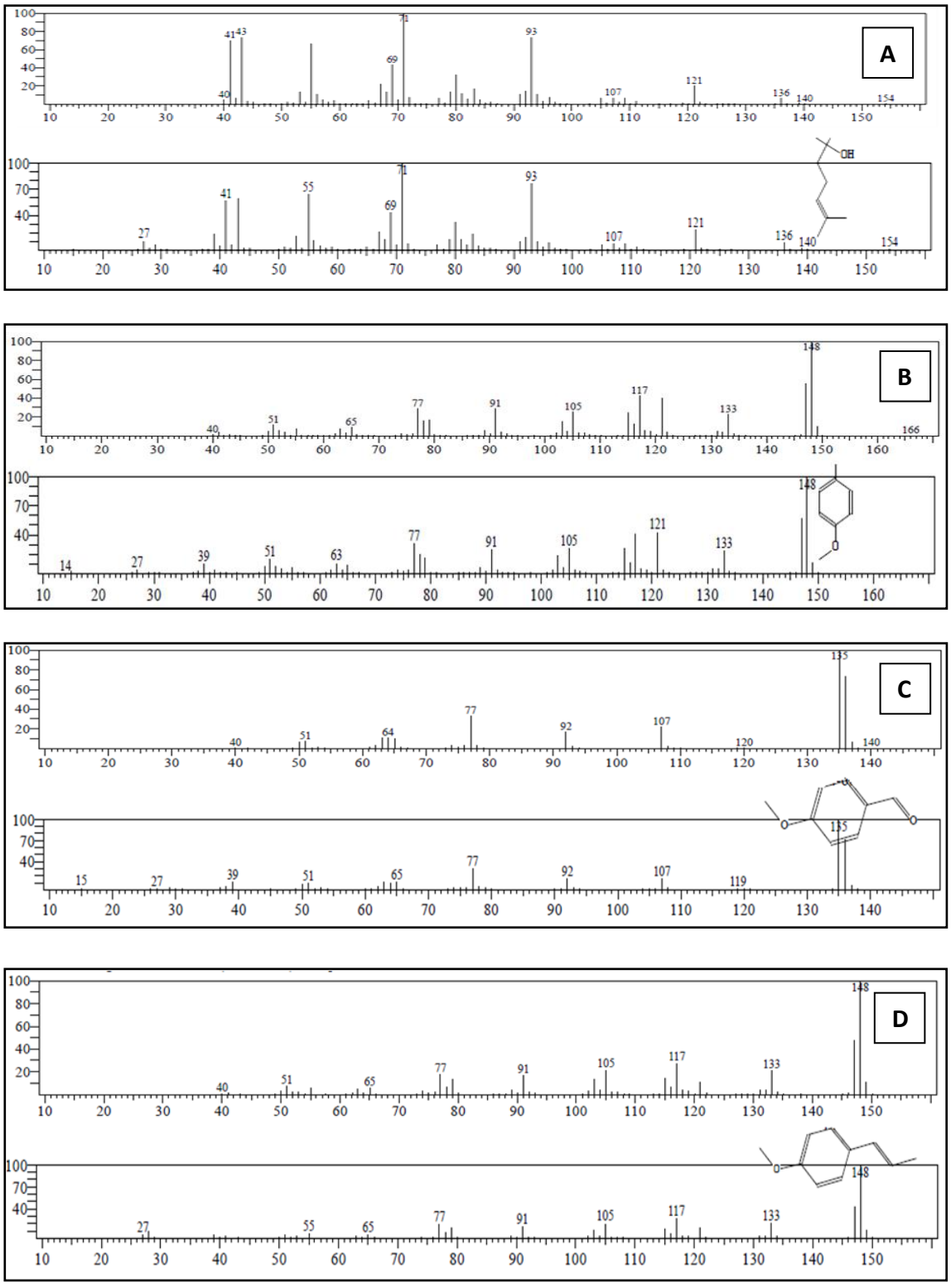

Figure 43. Mass spectra of the compounds in the GC peaks of retention time of 10.167, 13.009, 15.377 and 16.052 min, which were identified as A. Linalool; B. Estragole, C. Benzaldehyde, 4-methoxy- and D. Benzene, 1-methoxy-4-(1-propenyl)- or cis-Anothole respectively by the MS library.

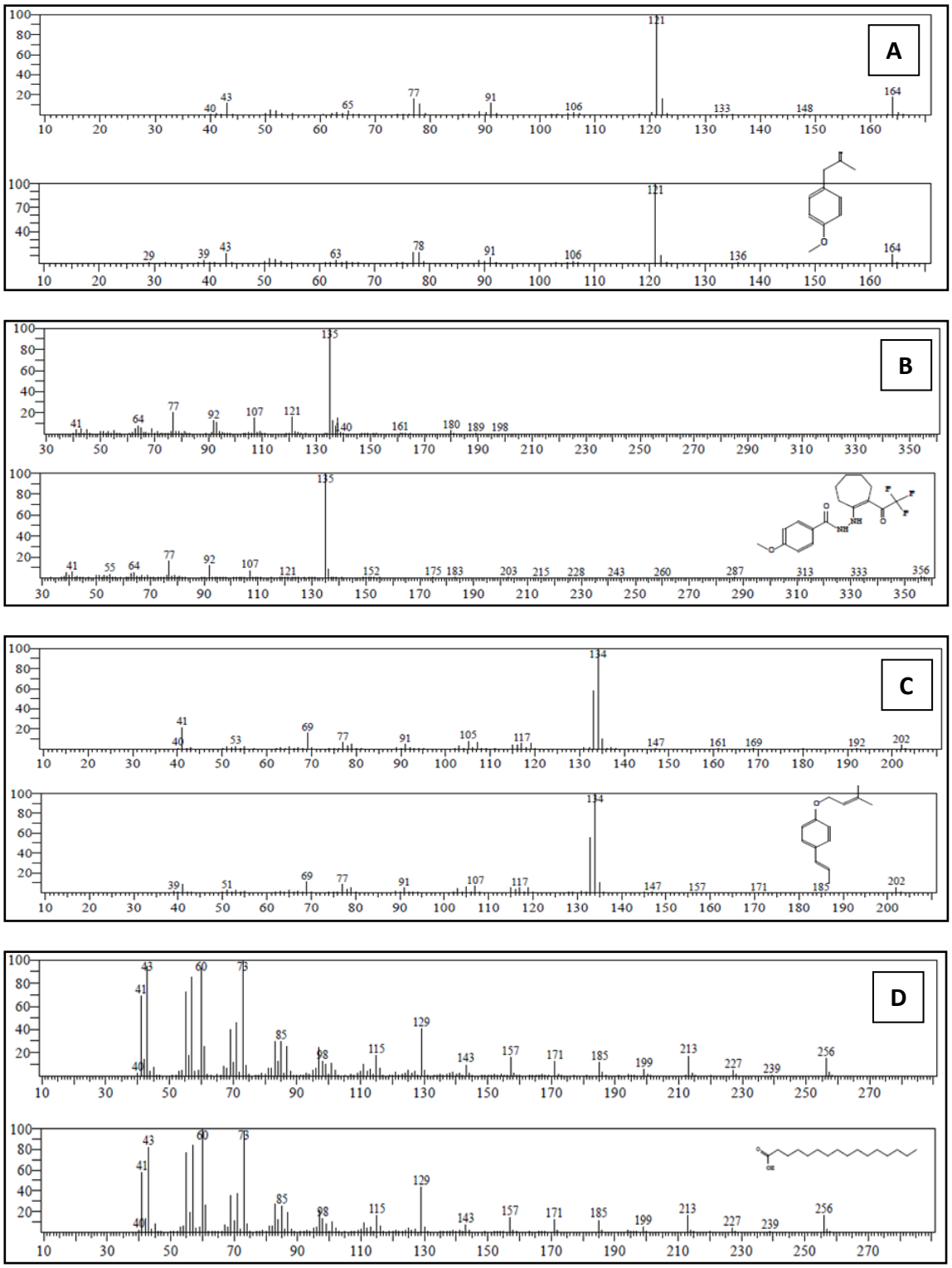

Figure 44. Mass spectra of the compounds in the GC peaks of retention time of 18.538, 22.467, 25.235 and 31.292 min, which were identified as A. 2-Propanone, 1-(4-methoxyphenyl)-, B. Benzhydrazide, 4-methoxy-N2-(2-trifluoroacetyl cyclohepten-1-yl)-, C. 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene and D. n-Hexadecanoic acid respectively by the MS library.

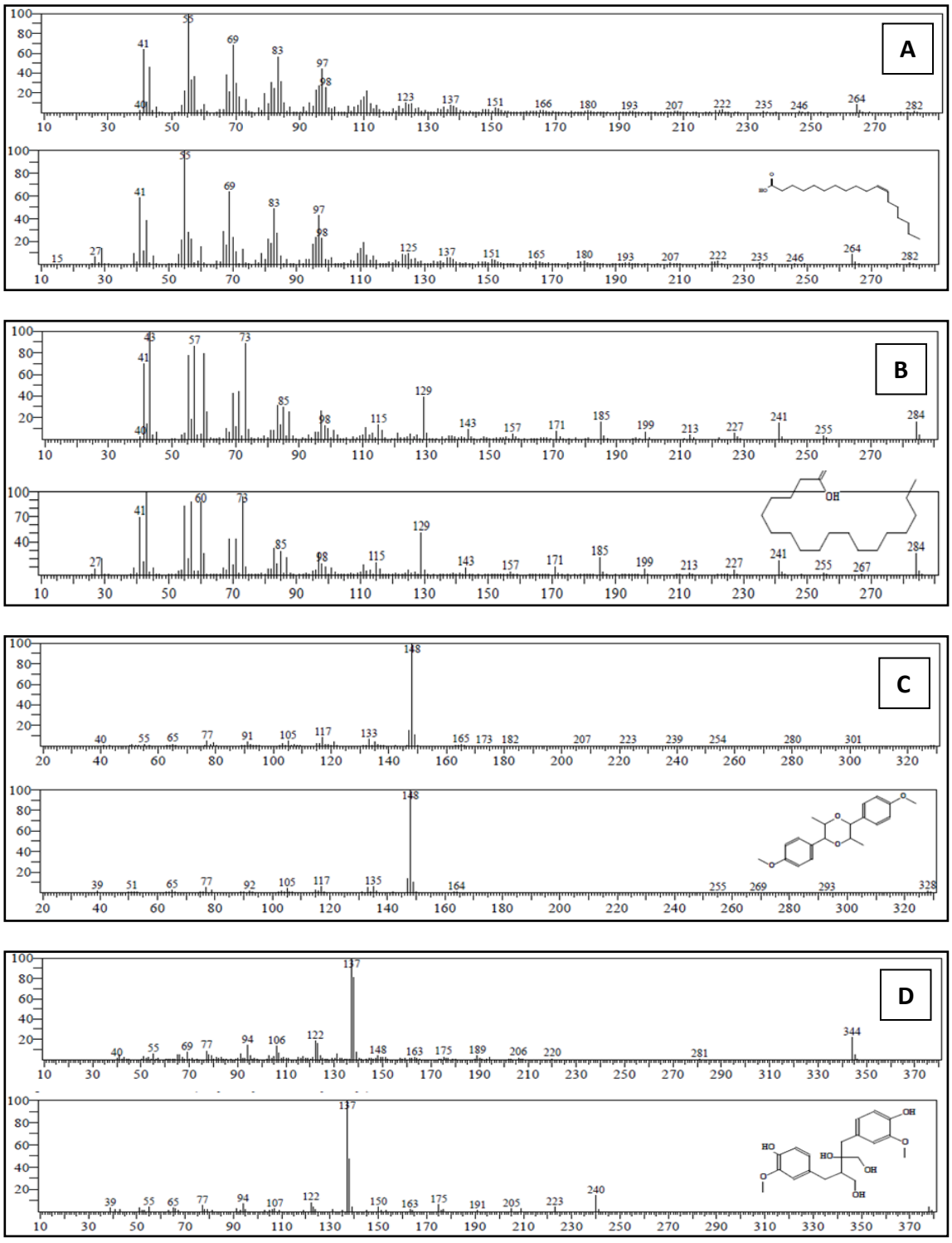

Figure 45. Mass spectra of the compounds in the GC peaks of retention time of 34.571, 34.890, 41.093 and 46.215 min, which were identified as A. cis-Vaccenic acid, B. Octadecanoic acid, C. (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-, and D. Carinol respectively by the MS library.

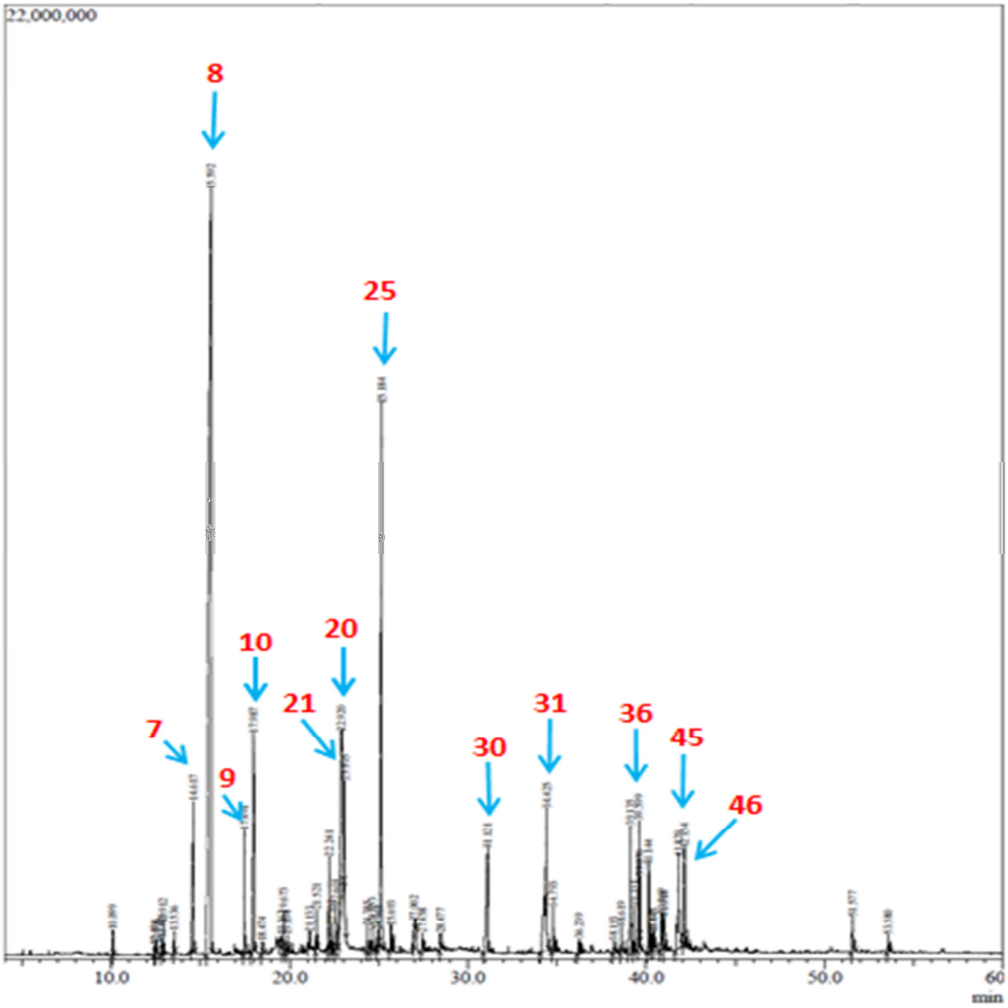

Figure 46. GC-MS Total Ion Chromatogram (TIC) of hexane fraction of *I. verum* (*Iv*HE).

Table 42: Compounds identified in the hexane fraction of *I. verum* (*Iv*HE)

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
1	10.099	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154	0.24
2	12.434	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{18}O$	154	0.08
3	12.536	Ethanol, 2-(2-butoxyethoxy)-	$C_8H_{18}O_3$	162	0.10
4	12.846	(+)-Alpha-terpineol (p-menth-1-en-8-ol)	$C_{10}H_{18}O$	154	0.13

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
5	12.912	Benzene, 1-methoxy-4-(2-propenyl)-	$C_{10}H_{12}O$	148	0.28
6	13.536	Phenol, 4-methoxy-	$C_7H_8O_2$	124	0.27
7	14.617	Benzaldehyde, 4-methoxy-	$C_8H_8O_2$	136	3.56
8	15.592	Anethole	$C_{10}H_{12}O$	148	34.22
9	17.491	Anisaldehyde dimethyl acetal	$C_{10}H_{14}O_3$	182	1.58
10	17.987	2-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	4.09
11	18.474	4-(p-Methoxyphenyl)-1-butanol	$C_{11}H_{16}O_2$	180	0.17
12	19.562	Benzoic acid, 4-methoxy-	$C_8H_8O_3$	152	0.47
13	19.673	1-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	0.55
14	19.894	3-Methoxy-n-(4h-1,2,4-triazol-4-yl)benzamide	$C_{10}H_{10}N_4O_2$	218	0.30
15	21.133	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene	$C_{10}H_{16}$	136	0.26
16	21.521	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	0.59
17	22.241	2-Hydroxy-1-(4-methoxyphenyl)propan-1-one	$C_{10}H_{12}O_3$	180	1.53
18	22.392	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	222	0.34
19	22.601	trans-4-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	162	0.98
20	22.920	4-(p-Methoxyphenyl)-1-butanol	$C_{11}H_{16}O_2$	180	7.19
21	23.105	1-(4-Methoxyphenyl)propane-1,2-diol	$C_{10}H_{14}O_3$	182	2.20
22	24.385	Geranyl formate	$C_{11}H_{18}O_2$	182	0.47
23	24.653	alpha.-Cadinol	$C_{15}H_{26}O$	222	0.47

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
24	24.961	1-Hydroxy-1-(4-methoxyphenyl)propan-2-one	$C_{10}H_{12}O_3$	180	0.25
25	25.184	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	12.90
26	25.693	2-Hydroxy-2-(4-methoxy-phenyl)-N-methyl-acetamide	$C_{10}H_{13}NO_3$	195	0.50
27	27.002	2H-1-Benzopyran, 3,4-dihydro-2,2-dimethyl-	$C_{11}H_{14}O$	162	0.31
28	27.458	1,3-Dioxolane, 2-(4-methoxyphenyl)-	$C_{10}H_{12}O_3$	180	0.40
29	28.477	(4-Methoxyphenyl)(2-methylenecyclohexyl)methanol	$C_{15}H_{20}O_2$	232	0.20
30	31.121	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.34
31	34.425	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	5.30
32	34.793	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.70
33	36.239	(E)-1-(4-Methoxyphenyl) propene	$C_{10}H_{12}O$	184	0.20
34	38.193	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.26
35	38.619	Acetic acid, (5-formyl-2-methoxybenzyl) ester	$C_{11}H_{12}O_4$	208	0.38
36	39.135	(2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-	$C_{18}H_{20}O_4$	300	2.21
37	39.331	(2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-	$C_{18}H_{20}O_4$	300	0.65
38	39.599	(2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-	$C_{18}H_{20}O_4$	300	1.84
39	39.670	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.68
40	40.144	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	1.28

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
41	40.303	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.16
42	40.443	alpha.,.alpha.-Diethyl-o-methoxybenzyl alcohol	$C_{12}H_{18}O_2$	194	0.26
43	40.869	alpha.,.alpha.-Diethyl-o-methoxybenzyl alcohol	$C_{12}H_{18}O_2$	194	0.95
44	41.018	(E)-1-(4- Methoxyphenyl) propene	$C_{18}H_{36}O_2$		0.93
45	41.829	4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide	$C_{14}H_{18}O$		2.90
46	42.154	Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-	$C_{10}H_{12}O$		2.09
47	51.577	gamma.-Sitosterol	$C_{17}H_{20}O_3$		0.88
48	53.580	Stigmast-4-en-3-one	$C_{12}H_{17}NO_2$		0.35

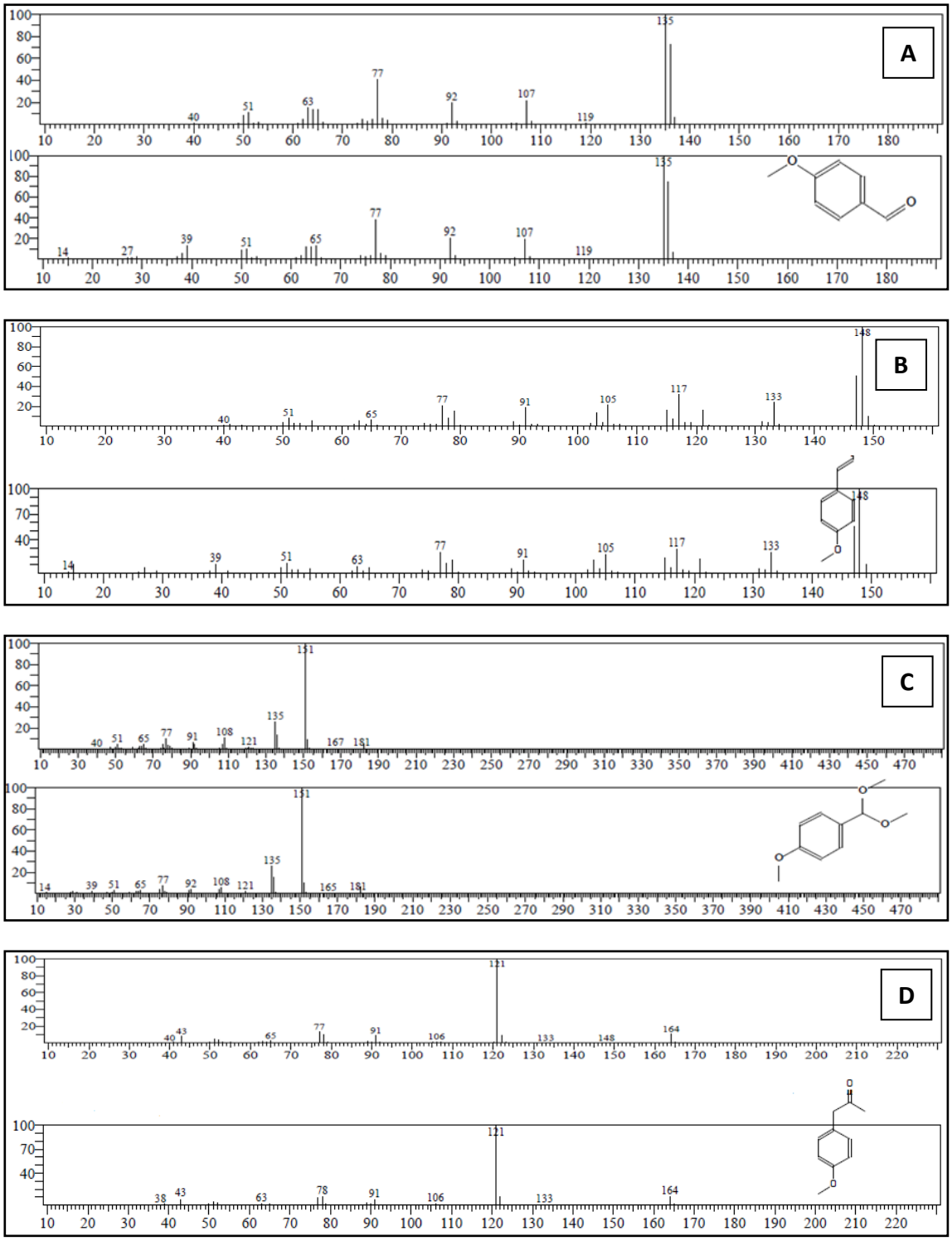

Figure 47. Mass spectra of the compounds in the GC peaks of retention time of 14.617, 15.592, 17.491 and 17.987 min, which were identified as A. Benzaldehyde, 4-methoxy-; B. Anethole, C. Anisaldehyde dimethyl acetal, and D. 2-Propanone, 1-(4-methoxyphenyl)- respectively by the MS library.

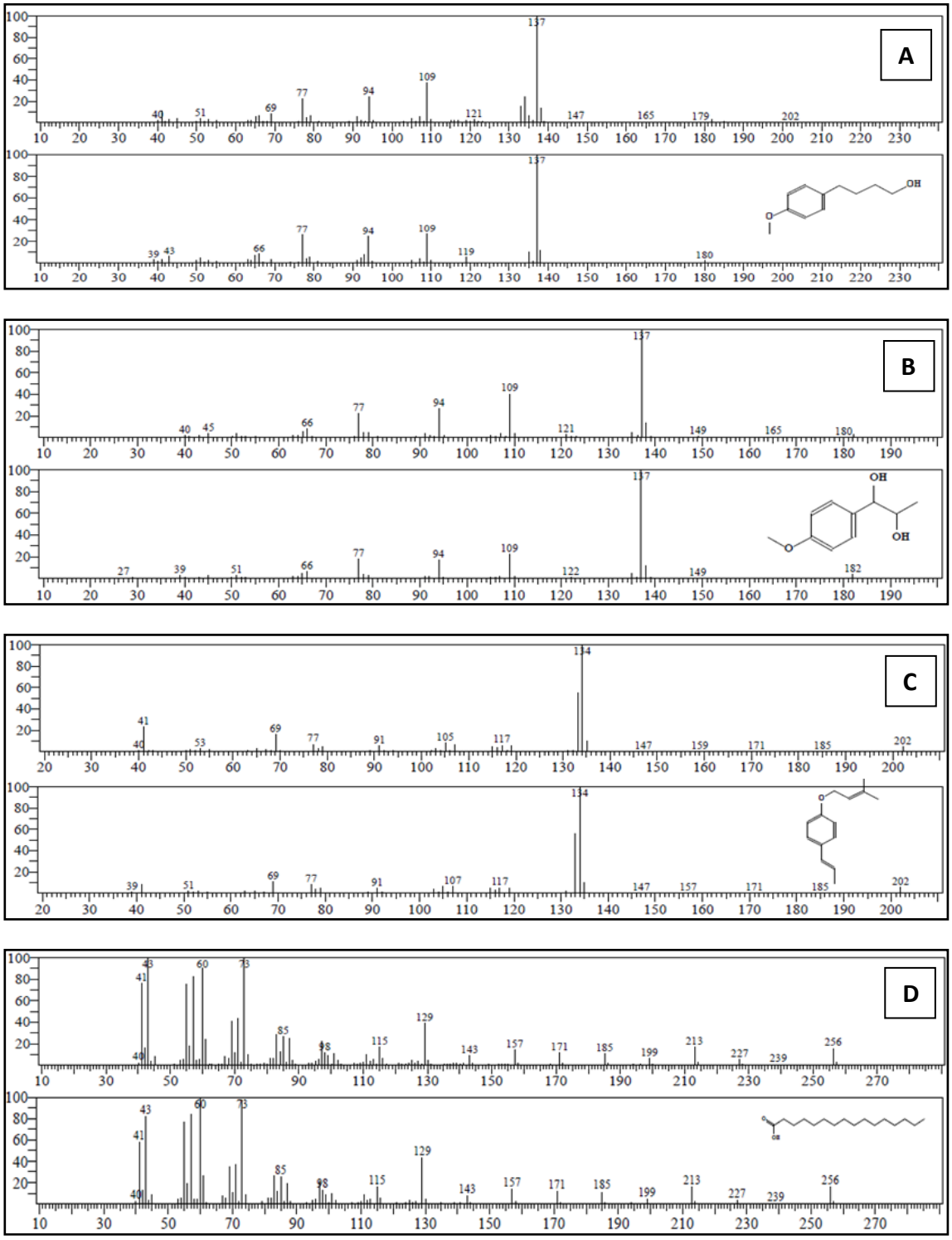

Figure 48. Mass spectra of the compounds in the GC peaks of retention time of 22.920, 23.105, 25.184 and 31.121 min, which were identified as A. 4-(p-Methoxyphenyl)-1-butanol, B. 1-(4-Methoxyphenyl) propane-1,2-diol; C. 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene and D. n-Hexadecanoic acid respectively by the MS library.

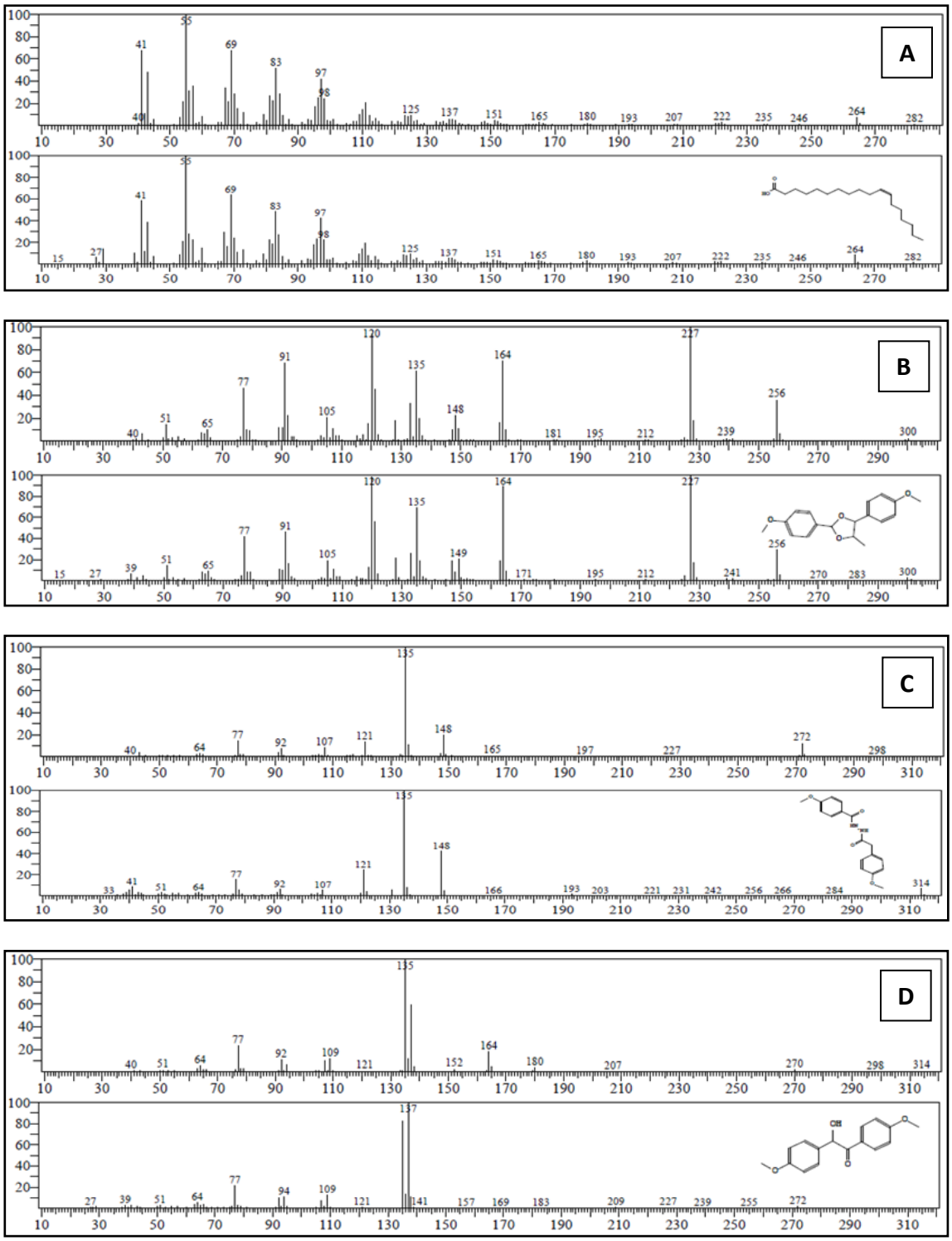

Figure 49. Mass spectra of the compounds in the GC peaks of retention time of 34.425, 39.135, 41.829 and 42.154 min, which were identified as A. cis-Vaccenic acid, B. (2R,4R,5S)-2,4-bis (4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-; C. 4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and D. Ethanone, 2-hydroxy-1,2-bis (4-methoxyphenyl)- respectively by the MS library.

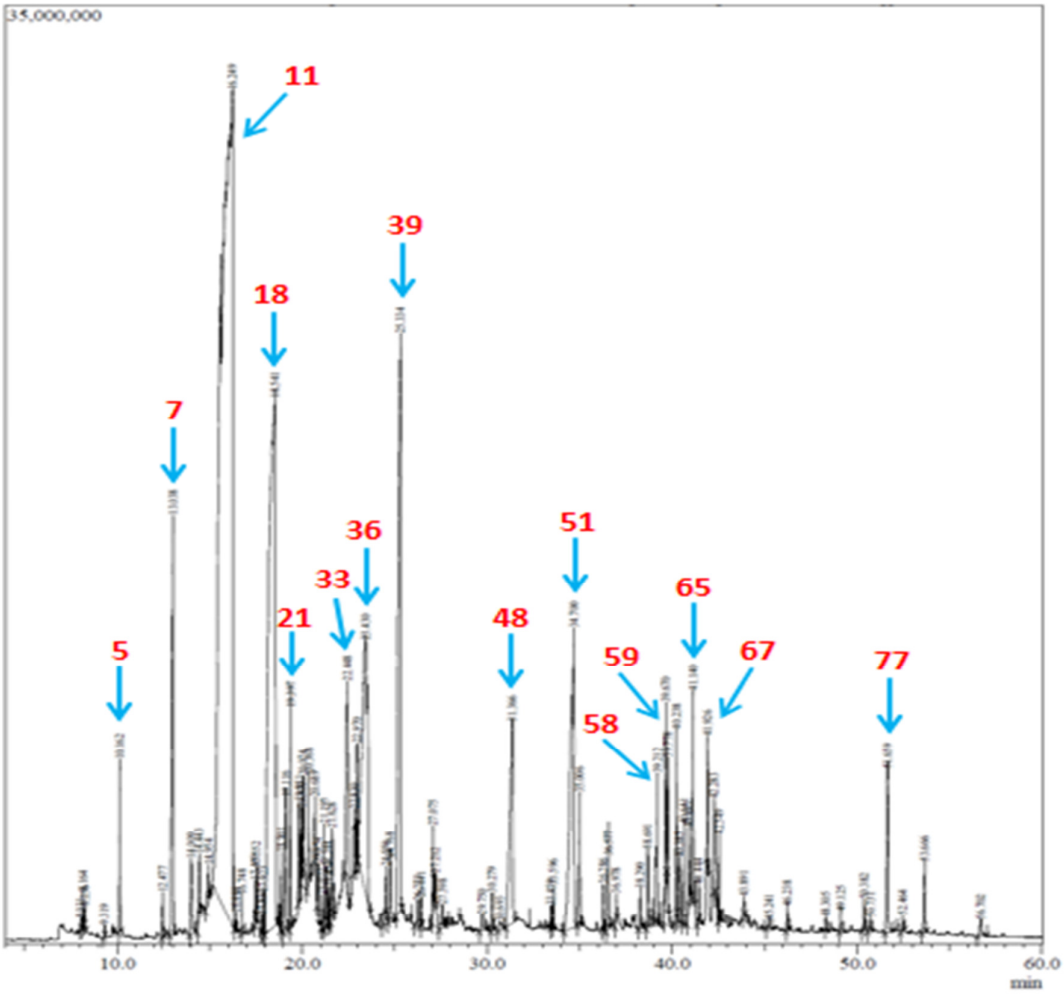

Figure 50. GC-MS Total Ion Chromatogram (TIC) of ethyl acetatefraction of *I. verum* (*Iv*EA).

Table 43: Compounds identified in the ethyl acetate fraction of *I. verum (Iv*EA)

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
1	8.031	o-Cymene	$C_{10}H_{14}$	134	0.03
2	8.164	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (s)-	$C_{10}H_{16}$	136	0.10
3	8.256	2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-	$C_{10}H_{18}O$	154	0.04
4	9.319	trans-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$	170	0.03

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
5	10.162	Linalool	$C_{10}H_{18}O$	154	0.54
6	12.477	1-Isopropyl-4-methyl-3-cyclohexen-1-ol	$C_{10}H_{18}O$	154	0.17
7	13.038	Estragole	$C_{10}H_{12}O$	148	2.72
8	14.009	Catechol	$C_6H_6O_2$	110	0.51
9	14.443	Mequinol	$C_7H_8O_2$	124	0.30
10	14.954	5-hydroxymethylfurfural	$C_6H_6O_3$	126	0.23
11	16.249	Benzene, 1-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O$	148	41.05
12	16.444	Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl-	$C_{10}H_{20}O_2$	172	0.07
13	16.748	3-Hydroxymandelic acid, dimethyl ether, methyl ester	$C_{11}H_{14}O_4$	210	0.12
14	17.469	Benzene, 1-(1,3-dimethyl-3-butenyl)-3-methoxy-	$C_{13}H_{18}O$	190	0.10
15	17.552	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	134	0.27
16	17.830	Benzoic acid, 4-methoxy-, methyl ester	$C_9H_{10}O_3$	166	0.04
17	17.923	Tricyclo[4.4.0.0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl	$C_{15}H_{24}$	204	0.12
18	18.541	2-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	14.88
19	18.901	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	$C_{15}H_{24}$	204	0.25
20	19.116	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,	$C_{15}H_{24}$	204	0.57
21	19.397	Trans-.alpha.-bergamotene	$C_{15}H_{24}$	204	0.79
22	19.851	1-Propanone, 1-(4-methoxyphenyl)-	$C_{10}H_{12}O_2$	164	0.41

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
23	19.970	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,	$C_{15}H_{24}$	204	0.18
24	20.074	3,3'-Dimethoxybenzil	$C_{16}H_{14}O_4$	270	0.29
25	20.368	3-Hydroxymandelic acid, dimethyl ether, methyl ester	$C_{11}H_{14}O_4$	210	0.39
26	20.689	Anisaldehyde dimethyl acetal	$C_{10}H_{14}O_3$	182	0.23
27	20.854	4-Methoxyphenyl methyl carbinol	$C_9H_{12}O_2$	152	0.22
28	21.077	.alpha.-Farnesene	$C_{15}H_{24}$	204	0.09
29	21.195	.beta.-Bisabolene	$C_{15}H_{24}$	204	0.29
30	21.344	3-Octen-5-yne, 2,2,7,7-tetramethyl-, (E)-	$C_{12}H_{20}$	164	0.21
31	21.459	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	204	0.08
32	21.628	Acetamide, 2-(hydroxyimino)-n-phenyl-	$C_8H_8N_2O_2$	164	0.33
33	22.448	S-(p-Methoxybenzoyl) thiohydroxylamine	$C_8H_9NO_2$	183	1.84
34	22.830	4-(4-Methoxyphenyl)-1-butanol	$C_{11}H_{16}O_2$	180	0.40
35	22.970	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	0.37
36	23.430	1-(4-Methoxyphenyl)propane-1,2-diol	$C_{10}H_{14}O_3$	182	5.92
37	24.550	Geranyl formate	$C_{11}H_{18}O_2$	182	0.32
38	24.768	.alpha.-Cadinol	$C_{15}H_{26}O$	222	0.39
39	25.334	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	$C_{14}H_{18}O$	202	6.32
40	26.273	2-Propenoic acid, 3-(3-methoxyphenyl)-	$C_{10}H_{10}O_3$	178	0.17

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
41	26.441	2aS,3aR,5aS,9bR)-2a,5a,9-Trimethyl-2a,4,5,5a,6,7,8,9b-octahydro-2H-naphtho[1,2-b] oxireno [2,3-c] furan	$C_{15}H_{22}O_2$	234	0.07
42	27.075	Benzene, 1-ethoxy-4-ethyl-	$C_{10}H_{14}O$	150	0.22
43	27.212	Desoxyanisoin	$C_{16}H_{16}O_3$	256	0.10
44	27.598	Bicyclo[2.2.1]heptane, 2-ethylidene-1,7,7-trimethyl-, (Z)-	$C_{12}H_{20}$	164	0.20
45	29.750	4,4,8-Trimethyl-tricyclo[6.3.1.0 1,5]dodecane-2,9-diol	$C_{15}H_{26}O_2$	238	0.12
46	30.279	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.10
47	30.693	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	0.09
48	31.366	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.46
49	33.459	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.06
50	33.596	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.11
51	34.700	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	4.92
52	35.006	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.68
53	36.286	(E)-1-(4-Methoxyphenyl) propene	$C_{10}H_{12}O$	148	0.15
54	36.577	(S)-1,1-bis(4-Methoxyphenyl)propan-2-ol	$C_{17}H_{20}O_3$	272	0.48
55	36.978	Trihexadecyl borate	$C_{48}H_{99}BO_3$	734	0.10
56	38.290	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.18
57	38.691	Acetic acid, (5-formyl-2-methoxybenzyl) ester	$C_{11}H_{12}O_4$	208	0.28
58	39.212	(2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-	$C_{18}H_{20}O_4$	300	0.90

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
59	39.670	Benzene, 1-methoxy-4-(1-methylpropyl)-	$C_{11}H_{16}O$	164	0.79
60	39.778	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.41
61	40.238	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.74
62	40.385	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	$C_{20}H_{24}O_3$	312	0.34
63	40.641	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.45
64	40.897	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.32
65	41.140	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.88
66	41.444	4-Methoxybenzoic acid, 2,6-dimethyl-4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethylethyl	$C_{19}H_{16}F_6O_4$	422	0.22
67	41.926	(2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-	$C_{20}H_{24}O_4$	328	0.43
68	42.283	1,2-bis(4-methoxyphenyl)-1,2-Ethanedione	$C_{16}H_{14}O_4$	270	0.31
69	42.549	(Trans)-2-(2-(3-ethoxy-phenyl)ethylidene)-6-methyl-7-methyl-70	$C_{20}H_{22}O_2$	294	0.27
70	43.891	Benzenemethanol, .alpha.-ethyl-4-hydroxy-	$C_9H_{12}O_2$	152	0.09
71	45.241	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	$C_{15}H_{22}O$	218	0.06
72	46.238	Benzenepropanol, 4-hydroxy-3-methoxy-	$C_{10}H_{14}O_3$	182	0.11
73	48.305	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-trans)-	$C_{20}H_{18}O_6$	354	0.06
74	49.125	Vitamin E	$C_{29}H_{50}O_2$	430	0.10

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
75	50.382	Ergost-5-en-3-ol, (3.beta.)-	$C_{28}H_{48}O$	400	0.13
76	50.731	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412	0.08
77	51.659	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414	1.01
78	52.464	Cyclopropa [5,6] stigmast-22-en-3-one, 3',6-dihydro-, (5.beta.,6.alpha.,22E)-	$C_{30}H_{48}O$	424	0.07
79	53.666	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	0.37
80	56.702	Stigmastane-3,6-dione, (5.alpha.)-	$C_{29}H_{48}O_2$	428	0.15

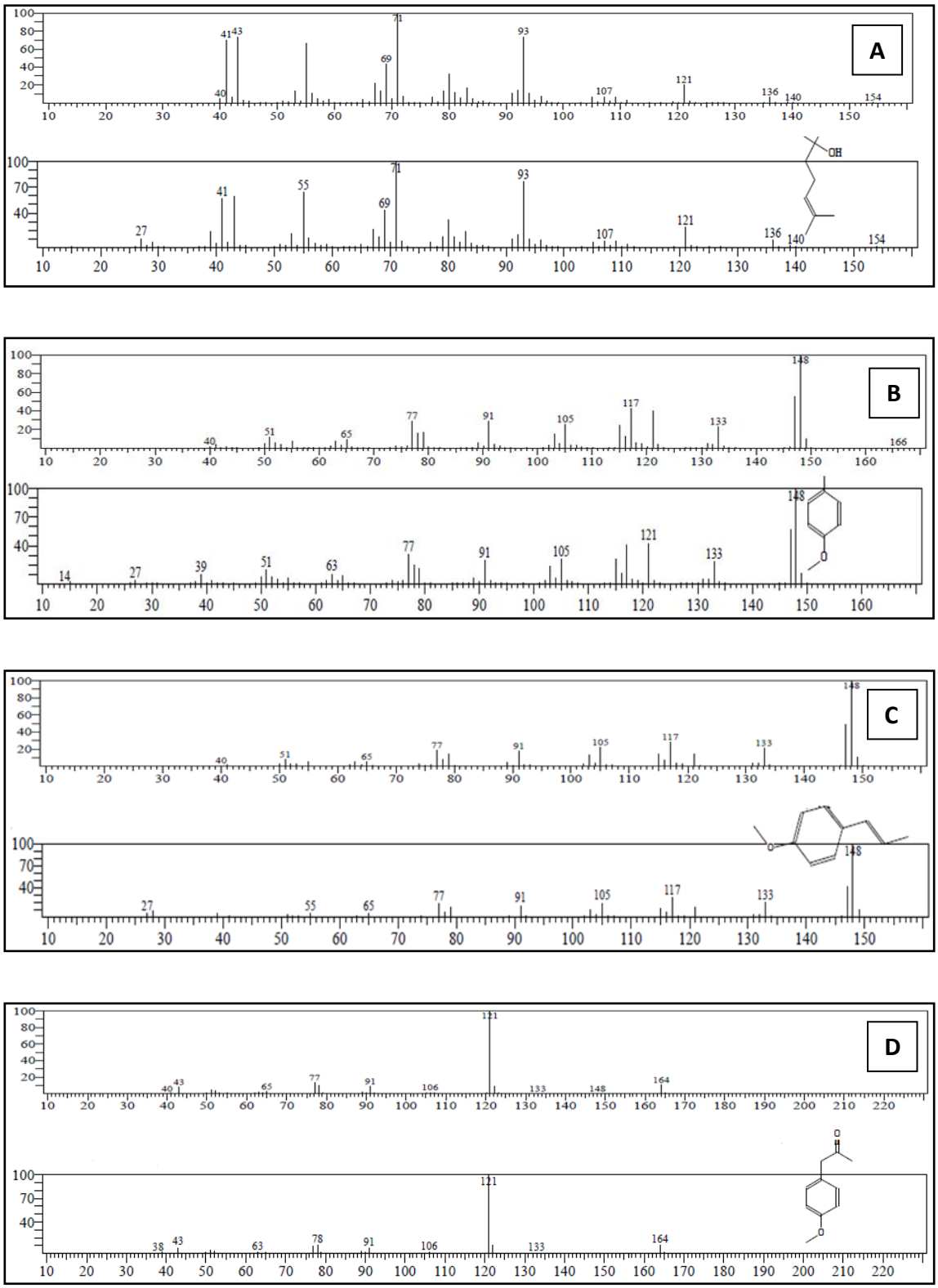

Figure 51. Mass spectra of the compounds in the GC peaks of retention time of 10.162, 13.038, 16.249 and 18.541 min, which were identified as A. Linalool, B. Estragole, C. Benzene, 1-methoxy-4-(1-propenyl)- and D. 2-Propanone, 1-(4-methoxyphenyl)- respectively by the MS library.

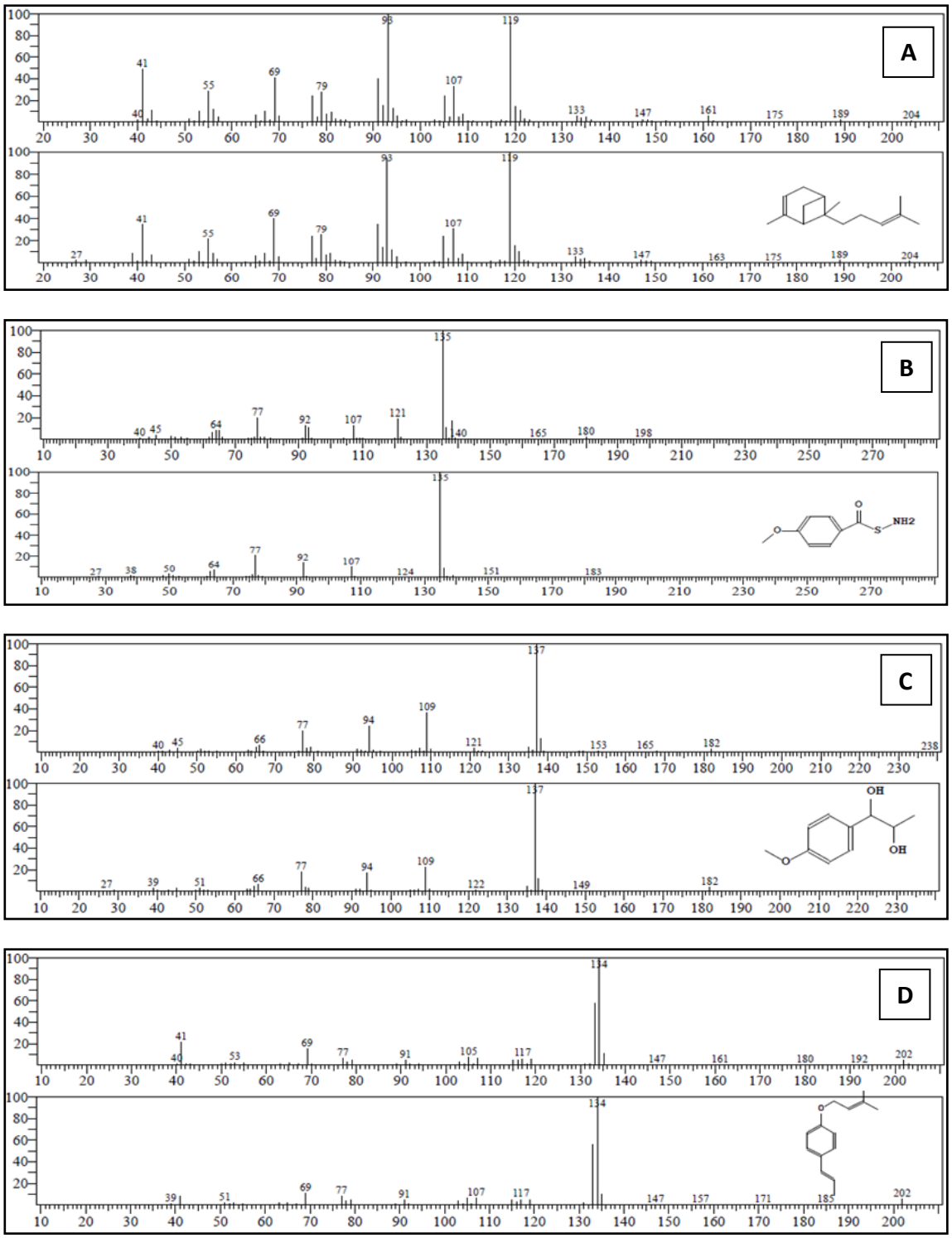

Figure 52. Mass spectra of the compounds in the GC peaks of retention time of 19.397, 22.448, 23.430 and 25.334 min, which were identified as A. Trans-.alpha.-bergamotene, B. S-(p-Methoxybenzoyl) thiohydroxylamine, C. 1-(4-Methoxyphenyl)propane-1,2-diol and D. 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene respectively by the MS library.

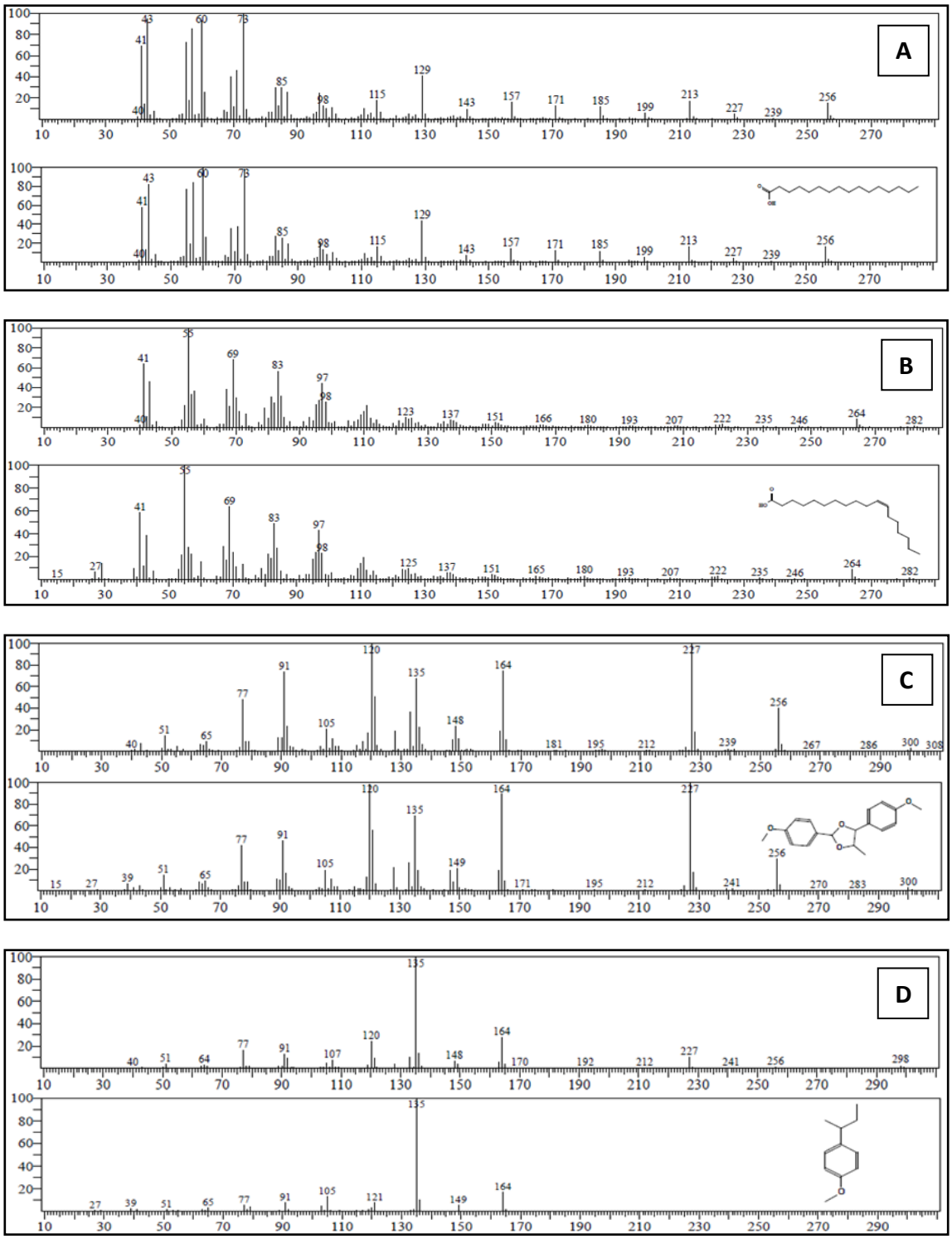

Figure 53. Mass spectra of the compounds in the GC peaks of retention time of 31.366, 34.700, 39.212 and 39.670 min, which were identified as A. n-Hexadecanoic acid, B. cis-Vaccenic acid, C. (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel- and D. Benzene, 1-methoxy-4-(1-methylpropyl)- respectively by the MS library.

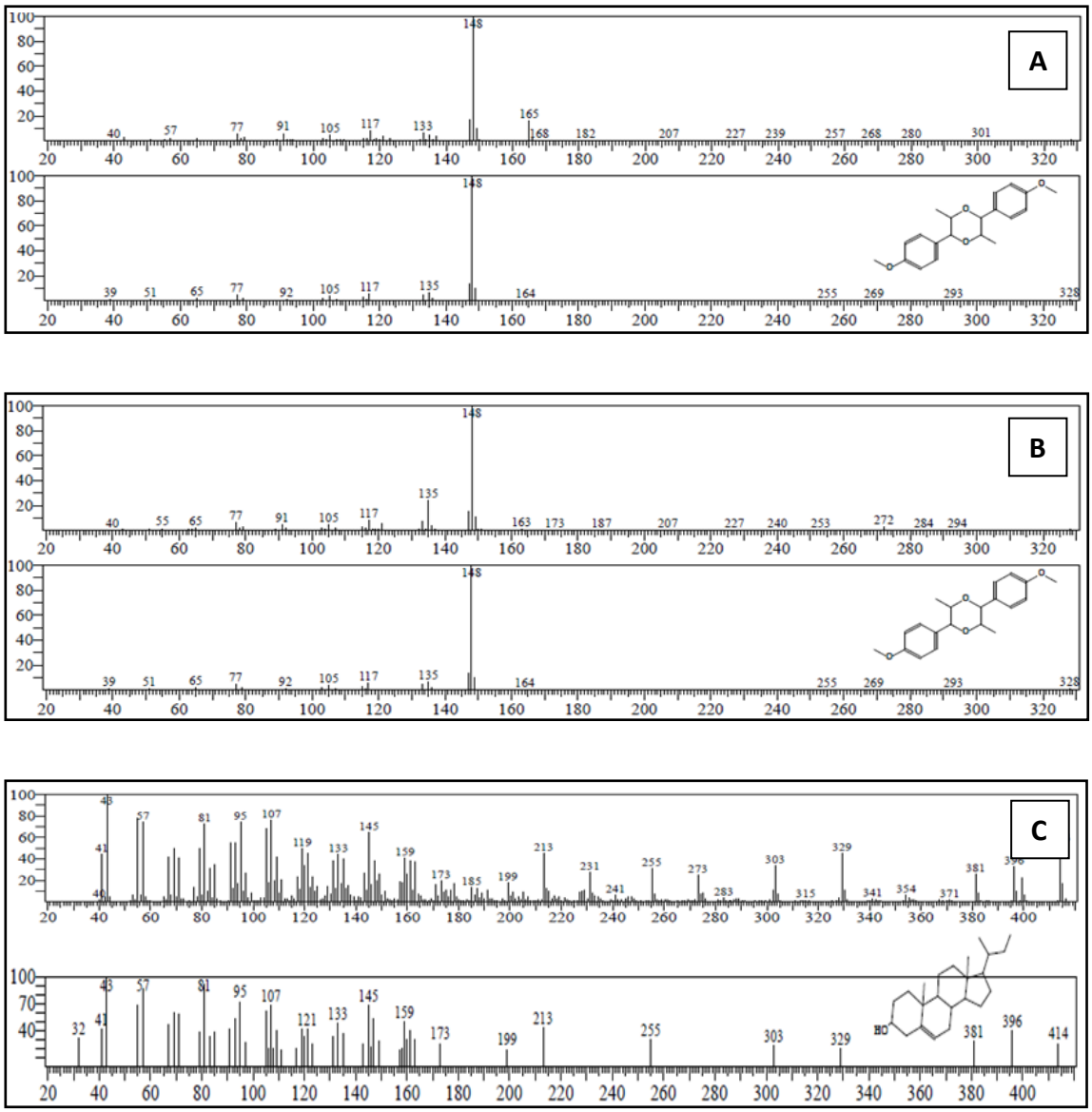

Figure 54. Mass spectra of the compounds in the GC peaks of retention time of 41.140, 41.926 and 51.659 min, which were identified as A. (2R,3S,5S,6R)-2,5-bis (4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-, B. (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and C. Stigmast-5-en-3-ol, (3.beta.)- respectively by the MS library.

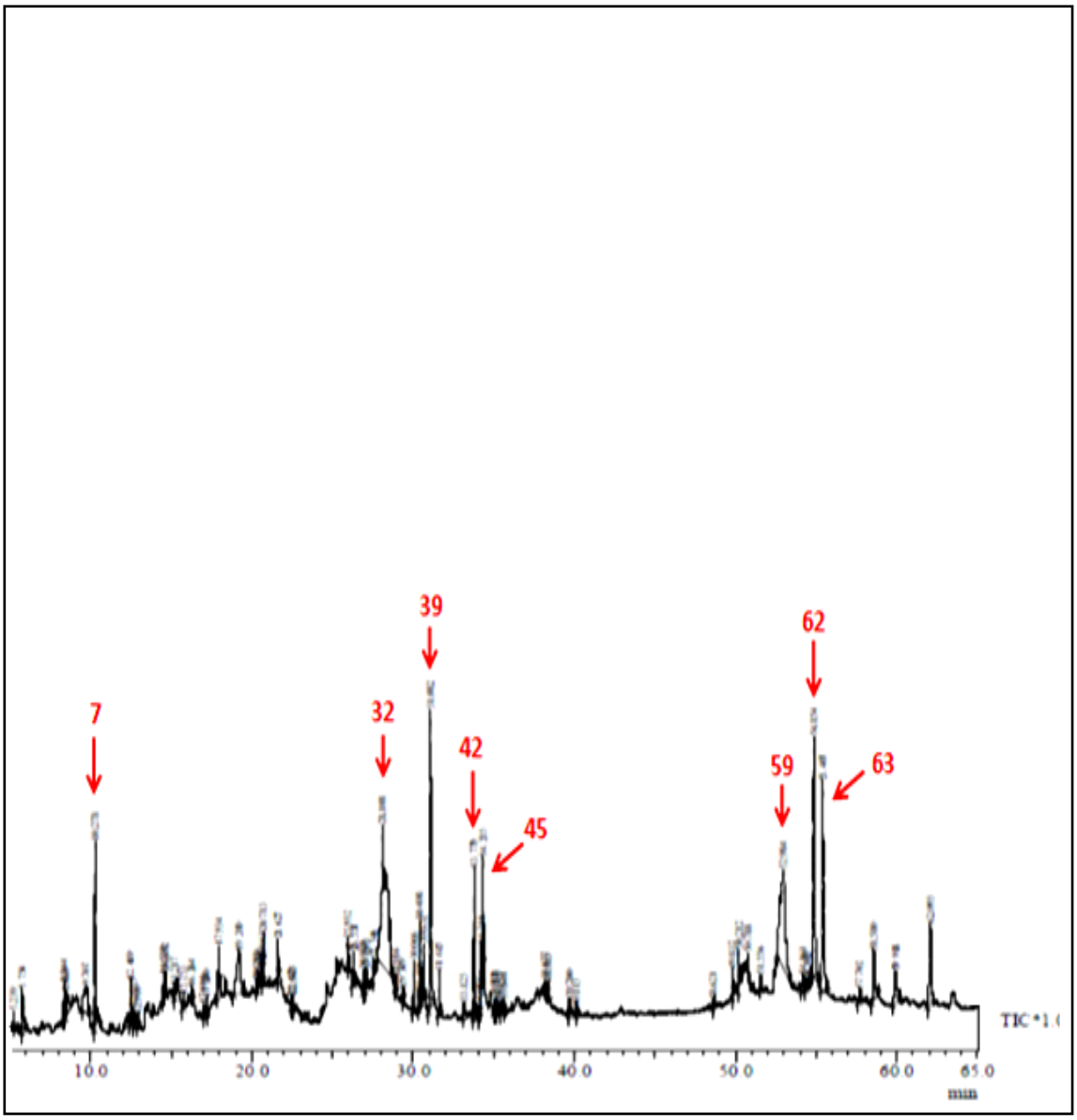

Figure 55. GC-MS Total Ion Chromatogram (TIC) of methanolic fraction of *G. oppositifolius* (*Go*ME).

Table 44: Compounds identified in the methanolic fraction of *G. oppositifolius(Go*ME*)*

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
1	4.083	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102	0.31
2	5.239	Methane, sulfonylbis-	$C_2H_6O_2S$	94	0.17

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
3	5.754	N-(2-Methylbutylidene) isobutylami	$C_9H_{19}N$	141	1.46
4	8.344	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	$C_{10}H_{21}N$	155	0.77
5	8.469	2,4-dihydroxy-3,3-dimethyl butanoic acid gamma.-lactone	$C_6H_{10}O_3$	130	0.34
6	9.707	2-pyrrolidinone	C_4H_7NO	85	2.71
7	10.278	1H-Pyrrole, 2,5-dihydro-	C_4H_7N	69	3.16
8	12.469	2-n-Butylacrolein	$C_7H_{12}O$	112	0.63
9	12.655	Pyridine, 1-acetyl-1,2,3,4-tetrahydro-	$C_7H_{11}NO$	125	0.25
10	12.910	Benzene, 1-methoxy-4-(2-propenyl)-	$C_{10}H_{12}O$	148	0.11
11	14.538	Propanedioic acid, phenyl-	$C_9H_8O_4$	180	0.46
12	14.652	Ethosuximide	$C_7H_{11}NO_2$	141	0.35
13	15.217	Vitispirane	$C_{13}H_{20}O$	192	0.11
14	15.753	Phenol, 5-methyl-2-(1-methylethyl)-	C10H14O	150	0.14
15	16.264	4-Heptanol, 2,4-dimethyl-	$C_9H_{20}O$	144	0.14
16	17.010	1,2-Dihydrosiline	C_5H_8Si	96	0.32
17	17.200	(E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB, 1)	$C_{13}H_{16}$	172	0.20
18	17.934	5-Oxo-pyrrolidine-2-carboxylic acid methyl ester	$C_6H_9NO_3$	143	1.03
19	19.200	1-Deoxy-d-arabitol	$C_5H_{12}O_4$	136	4.16
20	20.293	1-Dodecanol	$C_{12}H_{26}O$	186	0.13
21	20.403	2,5,5,8A-Tetramethyl-3,5,8,8A-Tetrahydro-2H-Chromene	$C_{13}H_{20}O$	192	0.08

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
22	20.663	Bicyclo[4.4.0]dec-5-en-4-one-1-carboxylic acid	$C_{11}H_{14}O_3$	194	0.08
23	20.713	2,5-Difluorobenzoic acid, 5-pentadecyl ester	$C_{22}H_{34}F_2O_2$	368	0.39
24	21.627	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180	0.52
25	22.473	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.14
26	22.565	4-Methylcyclohexyl propylphosphonofluoridoate	$C_{10}H_{20}FO_2P$	222	0.20
27	25.932	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{16}O$	152	0.34
28	26.331	N-[2-[Cyclooctylamino] ethyl] aziridine	$C_{12}H_{24}N_2$	196	0.64
29	26.928	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.27
30	27.093	2(4H)-benzofuranone, 5,6,7,7A-tetrahydro-6-hydroxy-4,4,7A	$C_{11}H_{16}O_3$	196	0.39
31	27.546	2,6,8-Trimethyl bicycle [4.2.0]oct-2-ene-1,8-diol	$C_{11}H_{18}O_2$	182	0.48
32	28.108	1-Deutero-2,2,5,5-tetramethylcyclopentanol	$C_9H_{17}DO$	143	17.59
33	28.916	1,2-Benzene dicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	0.21
34	29.349	2-(4-Hydroxy-butyl)-Cyclohexanol	$C_{10}H_{20}O_2$	172	0.17
35	30.046	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210	0.99
36	30.408	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210	1.46
37	30.490	5H,10H-Dipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione, octahydro	$C_{10}H_{14}N_2O_2$	194	0.12
38	30.632	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	1.18

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
39	31.082	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.60
40	31.615	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240	0.78
41	33.125	Cyclopropanecarboxylic acid,4-methylpentyl ester	$C_{10}H_{18}O_2$	170	0.40
42	33.779	Phytol	$C_{20}H_{40}O$	296	2.54
43	34.043	(3S,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1-ene	$C_{10}H_{16}O_2$	168	0.16
44	34.211	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252	0.90
45	34.335	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.14
46	35.018	Dodecanamide	$C_{12}H_{25}NO$	199	0.15
47	35.173	Bicyclo[3.3.1]non-2-en-9-ol, syn-	$C_9H_{14}O$	138	0.33
48	35.457	2-(Methyl-D3)-Cycloheptanone	$C_8H_{11}D_3O$	129	0.35
49	35.644	(1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d] naphthalene	$C_{15}H_{22}O$	218	0.18
50	38.177	1-beta.-d-Ribofuranosyl-4-imidazolin-2(3H)-one	$C_8H_{12}N_2O_5$	216	0.16
51	38.383	Cycloheptanol, 1-allyl-2-methylene-	$C_{11}H_{18}O$	166	0.42
52	39.700	N1-Isopropyl-2-methyl-1,2-propanediamine	$C_7H_{18}N_2$	130	0.25
53	40.113	2,6,6-Trimethylbicyclo[3.1.1] Heptan-2-ol	$C_{10}H_{18}O$	154	0.10
54	48.621	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	206	0.14
55	49.837	1,2-Dihydro-1,1,2,6-Tetramethyl naphthalene	$C_{14}H_{18}$	186	0.48
56	50.212	Unidendified	$C_{31}H_{52}O_3$	472	0.75

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
57	50.788	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	$C_{31}H_{52}O_3$	472	0.43
58	51.556	Retinal	$C_{20}H_{28}O$	284	0.30
59	52.984	Lanost-8-en-3-ol, (3.beta.)-	$C_{30}H_{52}O$	428	12.55
60	54.161	Beta.-copaen-4 alpha.-ol	$C_{15}H_{24}O$	220	0.43
61	54.465	(3aR,6R,8aR)-7,7-Dimethyl-8-methylenehexahydro-1H-3a,6-methanoazulen	$C_{14}H_{20}O$	204	0.31
62	54.854	(4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl	$C_{15}H_{24}O$	220	8.61
63	55.407	Retinol, acetate	$C_{22}H_{32}O_2$	328	8.49
64	57.702	Spirosolan-3-ol, 28-acetyl-, acetate (ester), (3.beta.5.alpha.)-	$C_{31}H_{49}NO_4$	499	0.46
65	58.580	(7AR,8R,13AS,15S)-11-Ethoxy-1,1,7A,8,13A-Pentamethyl octadeca hydro-	$C_{29}H_{48}O_4$	460	2.40
66	59.918	D:A-Friedo-2,3-secooleanane-2,3-dioic acid, dimethyl ester, (4R)-	$C_{32}H_{54}O_4$	502	1.99
67	62.093	(1R,4aR,4bS,7S,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-	$C_{20}H_{30}O$	286	3.99

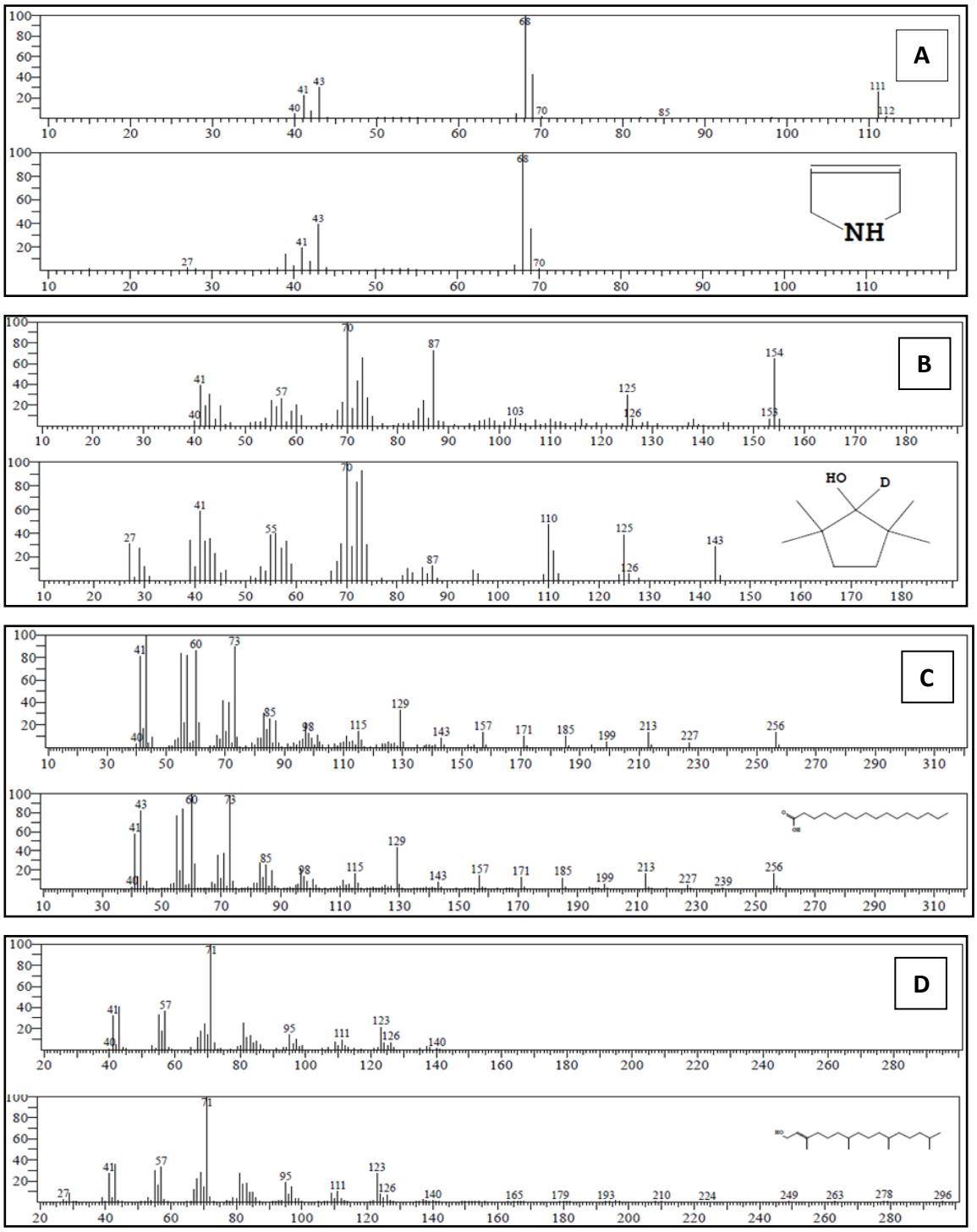

Figure 56. Mass spectra of the compounds in the GC peaks of retention time of 10.278, 28.108 31.082 and 33.779 min, which were identified as A. 1H-Pyrrole, 2,5-dihydro-; B. 1-Deutero-2,2,5,5-tetramethylcyclopentanol, C. n-Hexadecanoic acid and D. Phytol respectively by the MS library.

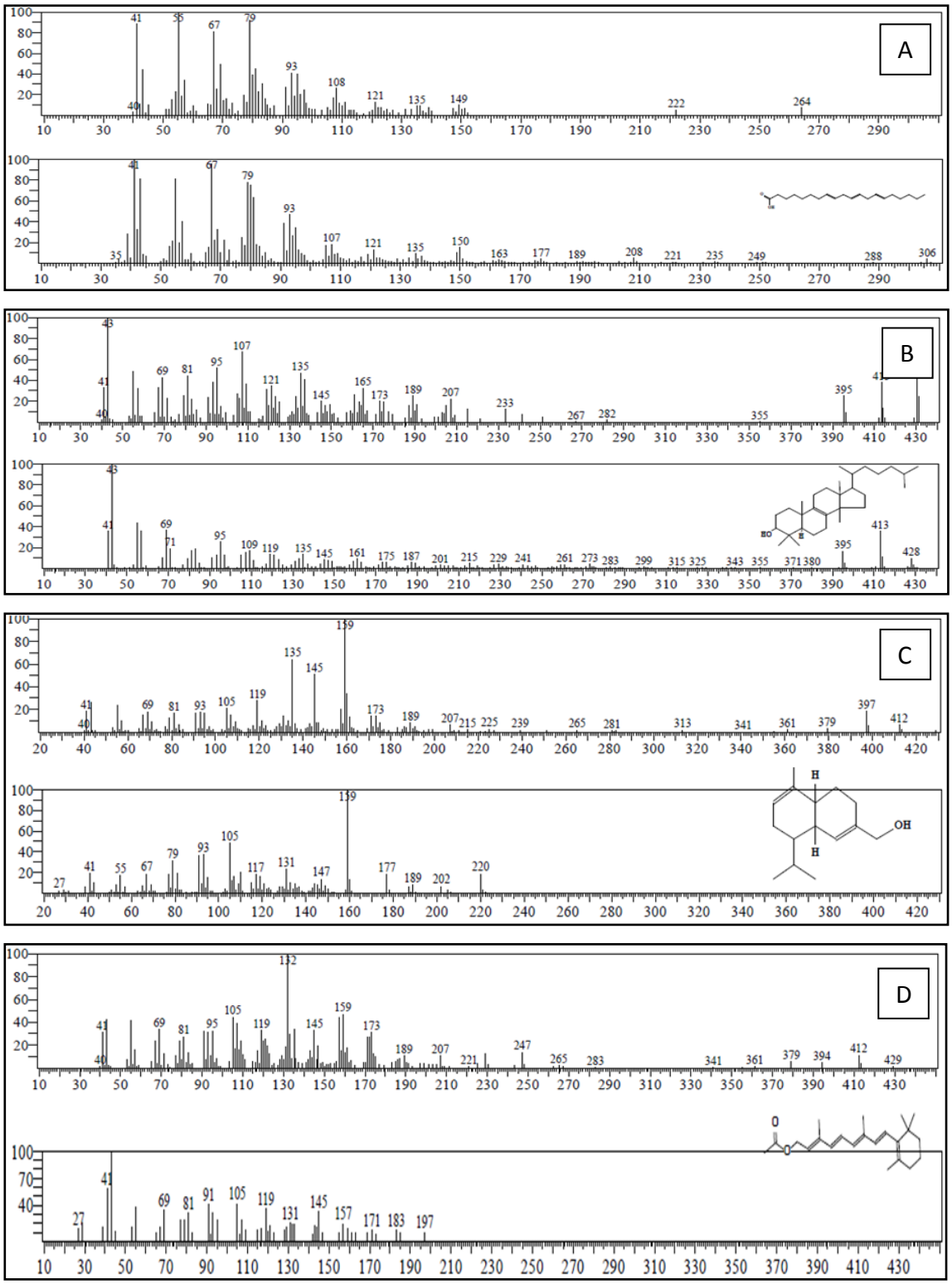

Figure 57. Mass spectra of the compounds in the GC peaks of retention time of 34.335, 52.984, 54.854 and 55.407 min, which were identified as A. 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; B. Lanost-8-en-3-ol, (3.beta.)-; C. (4aS, 8S, 8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl; and D. Retinol, acetate respectively by the MS library.

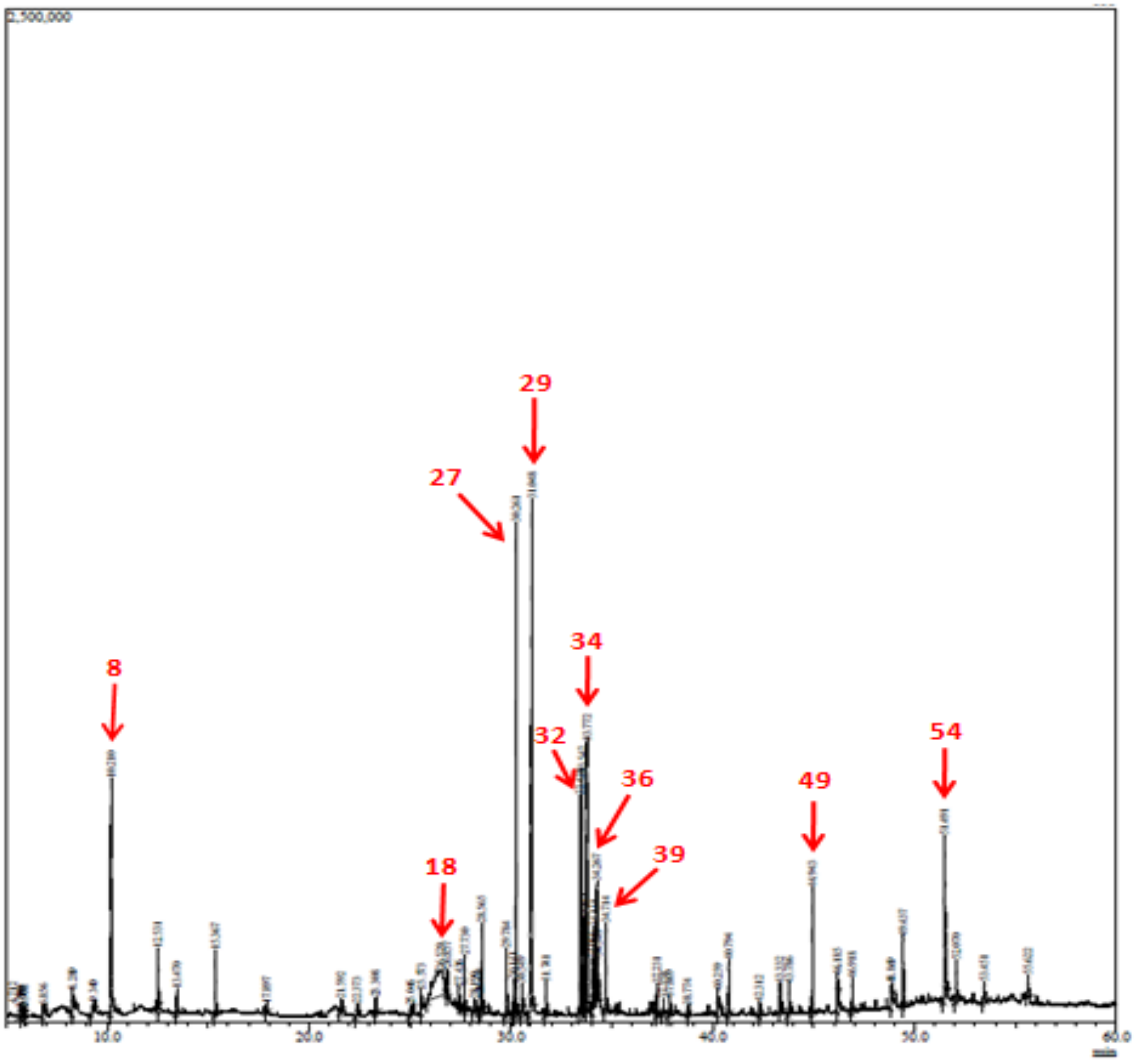

Figure 58. GC-MS Total Ion Chromatogram (TIC) of hexane fraction of *G. oppositifolius* (*Go*HE).

Table 45: Compounds identified in the hexane fraction of *G. oppositifolius* (*Go*HE)

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight ($g\ mol^{-1}$)	Relative abundance (%)
1	4.712	Ethanone, 1-(3,3-dimethyloxiranyl)-	$C_6H_{10}O_2$	114	0.17
2	5.700	N-(2-Methylbutylidene)isobutylami	$C_9H_{19}N$	141	0.08
3	5.789	1-octene, 3-ethyl-	$C_{10}H_{20}$	140	0.20
4	5.908	4-Piperidone	C_5H_9NO	99	0.14

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
5	6.856	2-Dimethylsilyloxytetradecane	$C_{16}H_{36}OSi$	272	0.52
6	8.289	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99	0.42
7	9.340	2-pyrrolidinone	C_4H_7NO	85	0.39
8	10.210	1H-Pyrrole, 2,5-dihydro-	C_4H_7N	69	5.36
9	12.531	Ethanol, 2-(2-butoxyethoxy)-	$C_8H_{18}O_3$	162	1.23
10	13.470	L-Proline, 1-acetyl-	$C_7H_{11}NO_3$	157	0.50
11	15.367	(E)-1-(4-methoxyphenyl)propene	$C_{10}H_{12}O$	148	1.18
12	17.897	Ethyl 3-oxo-4-(4-methoxyphenyl)butanoate	$C_{13}H_{16}O_4$	236	0.15
13	21.592	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.21
14	22.373	n-Decanoic acid	$C_{10}H_{20}O_2$	172	0.22
15	23.308	Pentadecane	$C_{15}H_{32}$	212	0.28
16	25.046	1-((2,2-dimethyl-5-hexenyl)oxy)-1-phenyl-1-propene	$C_{17}H_{24}O$	244	0.17
17	25.573	Tridecane	$C_{13}H_{28}$	184	0.38
18	26.529	Mome inositol	$C_7H_{14}O_6$	194	7.02
19	26.857	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.53
20	27.436	N-maleic acid 2,2,6,6-Tetramethyl,-4-piperidylmonoamide	$C_{13}H_{22}N_2O_3$	254	0.33
21	27.730	Pentadecane	$C_{15}H_{32}$	212	1.06
22	28.190	.Alpha.-d-galactopyranoside, methyl 3,6-anhydro-	$C_7H_{12}O_5$	176	0.36
23	28.474	Neophytadiene	$C_{20}H_{38}$	278	0.31

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
24	28.565	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	1.70
25	29.784	Nonadecane	$C_{19}H_{40}$	268	1.54
26	30.141	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	0.68
27	30.261	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.35
28	30.549	Palmitoleic acid	$C_{16}H_{30}O_2$	254	0.69
29	31.048	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.46
30	31.741	2-Bromo dodecane	$C_{12}H_{25}Br$	248	0.66
31	33.438	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	4.10
32	33.542	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	5.09
33	33.671	2-Nonadecanone	$C_{19}H_{38}O$	282	1.65
34	33.772	Phytol	$C_{20}H_{40}O$	296	7.27
35	34.066	Methyl stearate	$C_{19}H_{38}O_2$	298	0.78
36	34.151	Linoelaidic acid	$C_{18}H_{32}O_2$	280	1.56
37	34.267	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	2.59
38	34.349	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	0.61
39	34.714	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.88
40	37.231	2-Nonadecanone	$C_{19}H_{38}O$	282	0.64
41	37.558	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	0.28
42	37.869	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.38

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
43	38.774	Tetracosane	$C_{24}H_{50}$	338	0.16
44	40.259	n-Heptadecanol-1	$C_{17}H_{36}O$	256	0.63
45	40.794	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	1.03
46	42.312	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466	0.24
47	43.332	1-Octadecanol	$C_{18}H_{38}O$	270	1.03
48	43.786	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	0.63
49	44.943	Squalene	$C_{30}H_{50}$	410	2.53
50	46.185	Octadecyl trifluoroacetate	$C_{20}H_{37}F_3O_2$	366	0.67
51	46.918	Phytyl decanoate	$C_{30}H_{58}O_2$	450	0.66
52	48.869	Phosphonic acid, dioctadecyl ester	$C_{36}H_{75}O_3$	586	0.47
53	49.437	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.53
54	51.491	Chondrillasterol	$C_{29}H_{48}O$	412	6.58
55	52.070	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278	1.25
56	53.451	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	0.76
57	55.622	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278	0.74

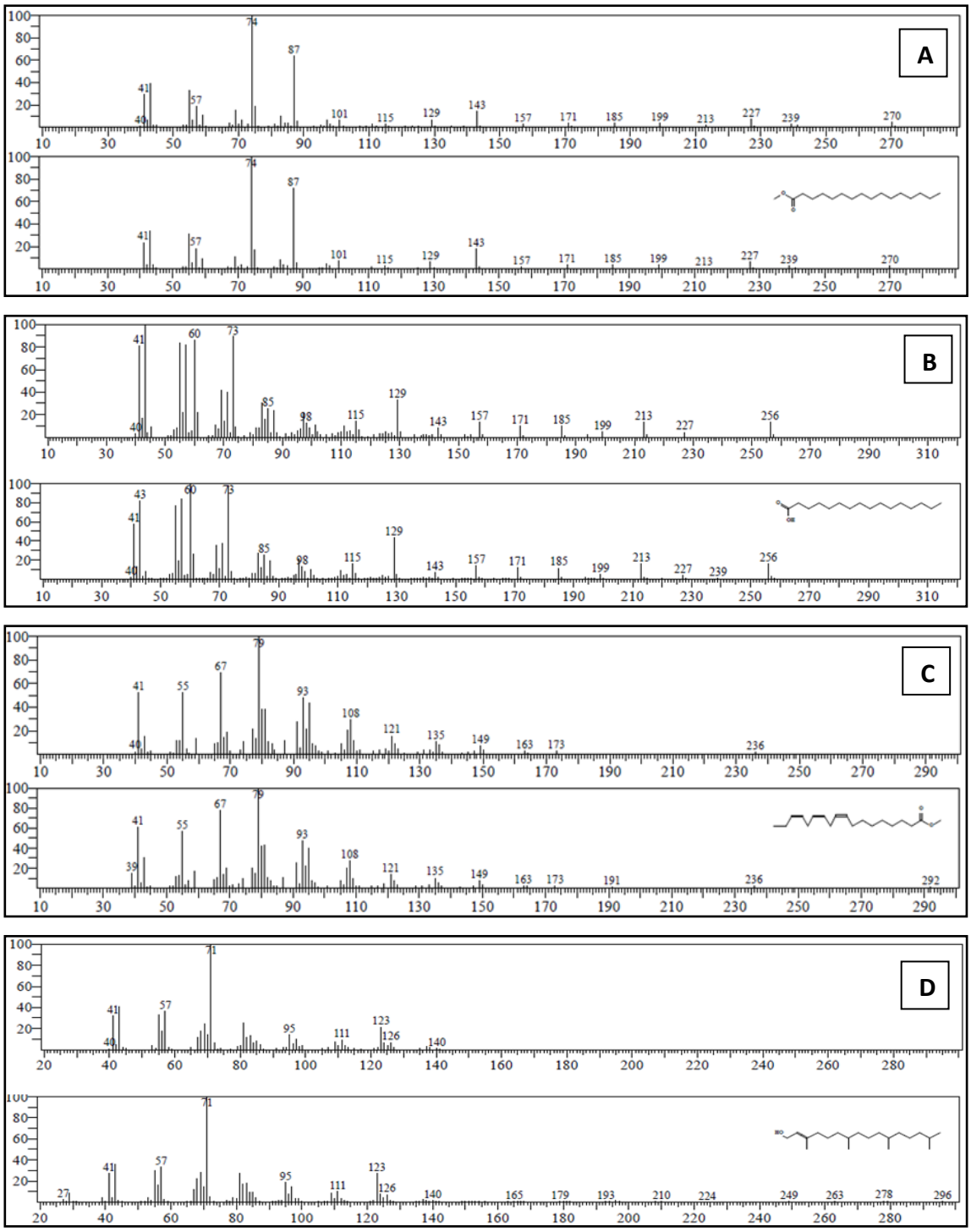

Figure 59. Mass spectra of the compounds in the GC peaks of retention time of 30.261, 31.048, 33.542 and 33.772 min, which were identified as A. Hexadecanoic acid, methyl ester; B. n-Hexadecanoic acid; C. 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; and D. Phytol 34 respectively by the MS library.

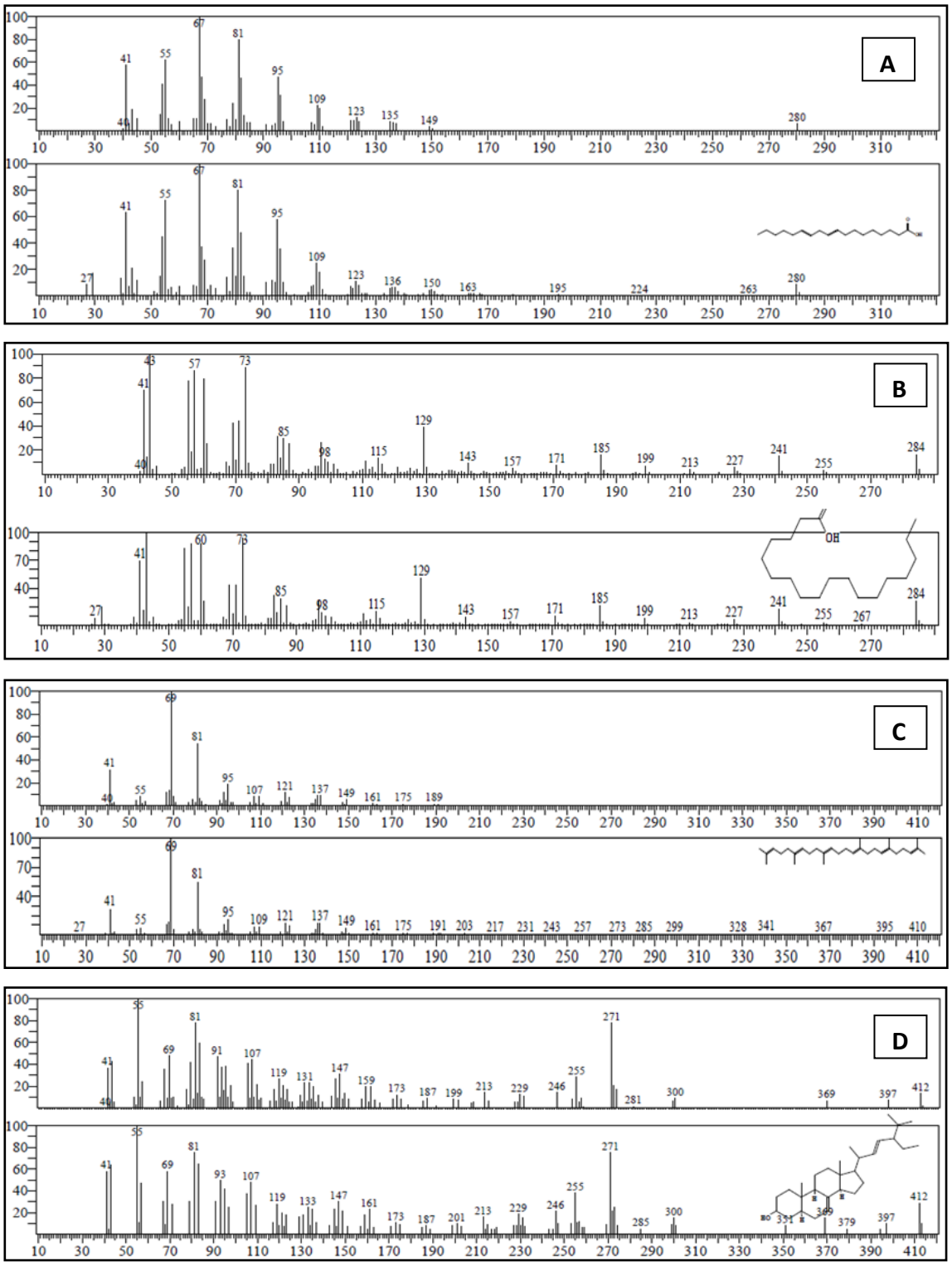

Figure 60. Mass spectra of the compounds in the GC peaks of retention time of 34.151, 34.714, 44.943 and 51.491 min, which were identified as A. Linoelaidic acid, B. Octadecanoic acid, C. Squalene and D. Chondrillasterol respectively by the MS library.

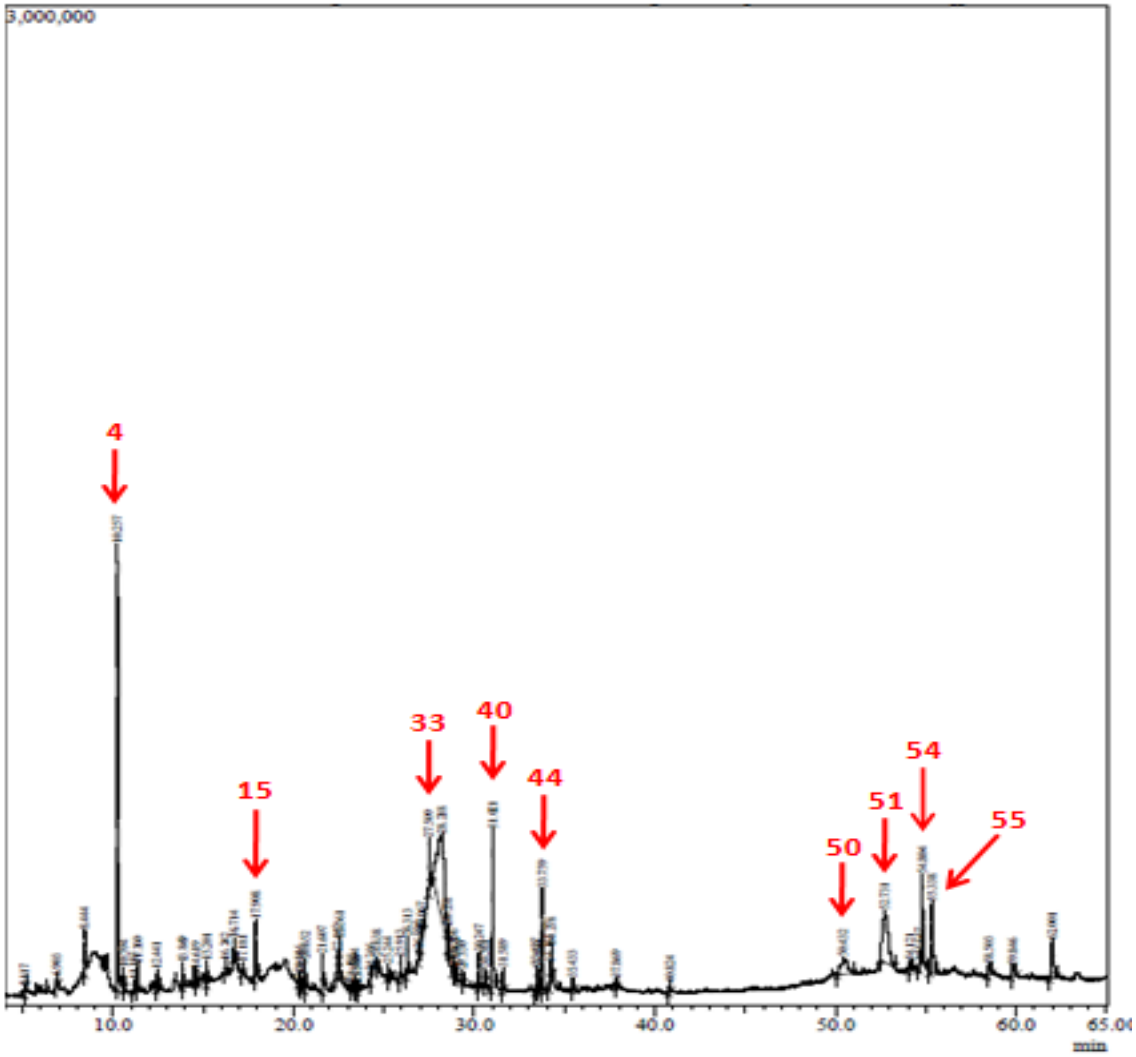

Figure 61. GC-MS Total Ion Chromatogram (TIC) of ethyl acetate fraction of *G. oppositifolius* (*Go*EA).

Table 46: Compounds identified in the ethyl acetate fraction of *G. oppositifolius* (*Go*EA)

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
1	5.117	Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$	110	0.09
2	6.903	1-Butanol, 2-amino-3-methyl-, (+/-)-	$C_5H_{13}NO$	103	0.37
3	8.444	2,4-Dihydroxy-3,3-dimethylbutanoic acid .gamma.-lactone	$C_6H_{10}O_3$	130	0.94
4	10.257	1H-Pyrrole, 2,5-dihydro-	C_4H_7N	69	13.70

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
5	10.594	Methyl ester of 5-hydroxy-hexanoic acid	$C_7H_{14}O_3$	146	0.49
6	11.183	Succinimide	$C_4H_5NO_2$	99	0.34
7	11.360	1,5-Anhydro-6-deoxyhexo-2,3-diulose	$C_6H_8O_4$	144	0.82
8	12.441	7-oxabicyclo[4.1.0]heptane, 3-methyl-	$C_7H_{12}O$	112	0.53
9	13.869	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_9NO_2$	139	0.52
10	14.619	Ethosuximide	$C_7H_{11}NO_2$	141	0.35
11	15.201	Vitispirane	$C_{13}H_{20}O$	192	0.48
12	16.242	Ethanedioic acid, bis(3-methylbutyl) ester	$C_{12}H_{22}O_4$	230	0.35
13	16.714	Ethanol, 2-(dimethylamino)-	$C_4H_{11}NO$	89	1.00
14	17.181	Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	$C_{13}H_{16}$	172	0.22
15	17.908	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	3.11
16	20.316	Piperidine, 4-(4-methylphenyl)-	$C_{12}H_{17}N$	175	0.36
17	20.455	3-Buten-2-ol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{22}O$	194	0.18
18	20.652	3-Buten-2-one, 4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-, 19	$C_{14}H_{22}O$	206	1.03
19	21.607	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180	0.72
20	22.442	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.45
21	22.561	Propylphosphonic acid, fluoroanhydride, decyl ester	$C_{13}H_{28}FO_2P$	266	0.75
22	23.193	2-Norpinene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-, trans-(-)-	$C_{15}H_{24}$	204	0.24

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
23	23.404	Cyclohexene, 3-(3-methyl-1-butenyl)-, (E)-	$C_{11}H_{18}$	150	0.31
24	23.540	2-Octensaeure, 4-isopropyliden-7-methyl-6-methylen-, methylester	$C_{14}H_{22}O_2$	222	0.21
25	24.246	Campholaldehyde	$C_{10}H_{16}O$	152	0.24
26	24.638	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{13}H_{22}O_3$	226	0.33
27	25.244	Limonene dioxide 1	$C_{10}H_{16}O_2$	168	0.51
28	25.913	Nona-2,3-dienoic acid ethyl ester	$C_{11}H_{18}O_2$	182	0.70
29	26.313	N-[2-[Cyclooctylamino] ethyl]aziridine	$C_{12}H_{24}N_2$	196	1.17
30	26.896	Docosanoic acid	$C_{22}H_{44}O_2$	340	0.27
31	27.067	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O$	196	0.57
32	27.509	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	$C_{11}H_{18}O_2$	182	1.64
33	28.218	Mome inositol	$C_7H_{14}O_6$	194	21.36
34	28.558	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.53
35	28.856	3,5-Octadienoic acid, 7-hydroxy-2-methyl-, [R*,R*-(E,E)]-	$C_9H_{14}O_3$	170	0.56
36	28.962	2-Tridecyne	$C_{13}H_{24}$	180	0.21
37	29.330	1-Octadecyne	$C_{18}H_{34}$	250	0.41
38	30.247	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.83
39	30.581	9-Octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282	0.44
40	31.021	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.93

Peak no.	Retention time (min)	Name of the chemical compound	Molecular formula	Molecular weight (g mol^{-1})	Relative abundance (%)
41	31.589	Cyclohexanecarboxylic acid, octyl ester	$C_{15}H_{28}O_2$	240	0.69
42	33.427	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.58
43	33.530	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.59
44	33.759	Phytol	$C_{20}H_{40}O$	296	2.84
45	34.165	Linoelaidic acid	$C_{18}H_{32}O_2$	280	0.68
46	34.278	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl	$C_{22}H_{38}O_2$	334	0.96
47	35.433	Cyclopropanemethanol, 2-isopropylidene-.alpha.-methyl-	$C_8H_{14}O$	126	0.44
48	37.869	3,7-Dimethyl-1-octyl methylphosphonofluoridate	$C_{11}H_{24}FO_2P$	238	0.22
49	40.824	4-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde	$C_{10}H_{16}O_2$	168	0.28
50	50.432	Lanosterol	$C_{30}H_{50}O$	426	3.74
51	52.731	Lup-20(29)-en-28-ol	$C_{30}H_{50}O$	426	9.96
52	54.121	((4as,8S,8ar)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)	$C_{15}H_{24}O$	220	0.52
53	54.647	Contrunculin-b	$C_{16}H_{24}O_2$	248	0.38
54	54.804	.Beta.-copaen-4 .alpha.-ol	$C_{15}H_{24}O$	220	5.04
55	55.338	Retinol, acetate	$C_{22}H_{32}O_2$	328	4.76
56	58.503	1-Coprosten-3-one semicarbazone	$C_{28}H_{47}N_3O$	441	0.79
57	59.846	(-)-Isolongifolol, methyl ether	$C_{16}H_{28}O$	236	1.08
58	62.001	(1R,4ar,4bs,7S,10ar)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-	$C_{20}H_{30}O$	286	3.20

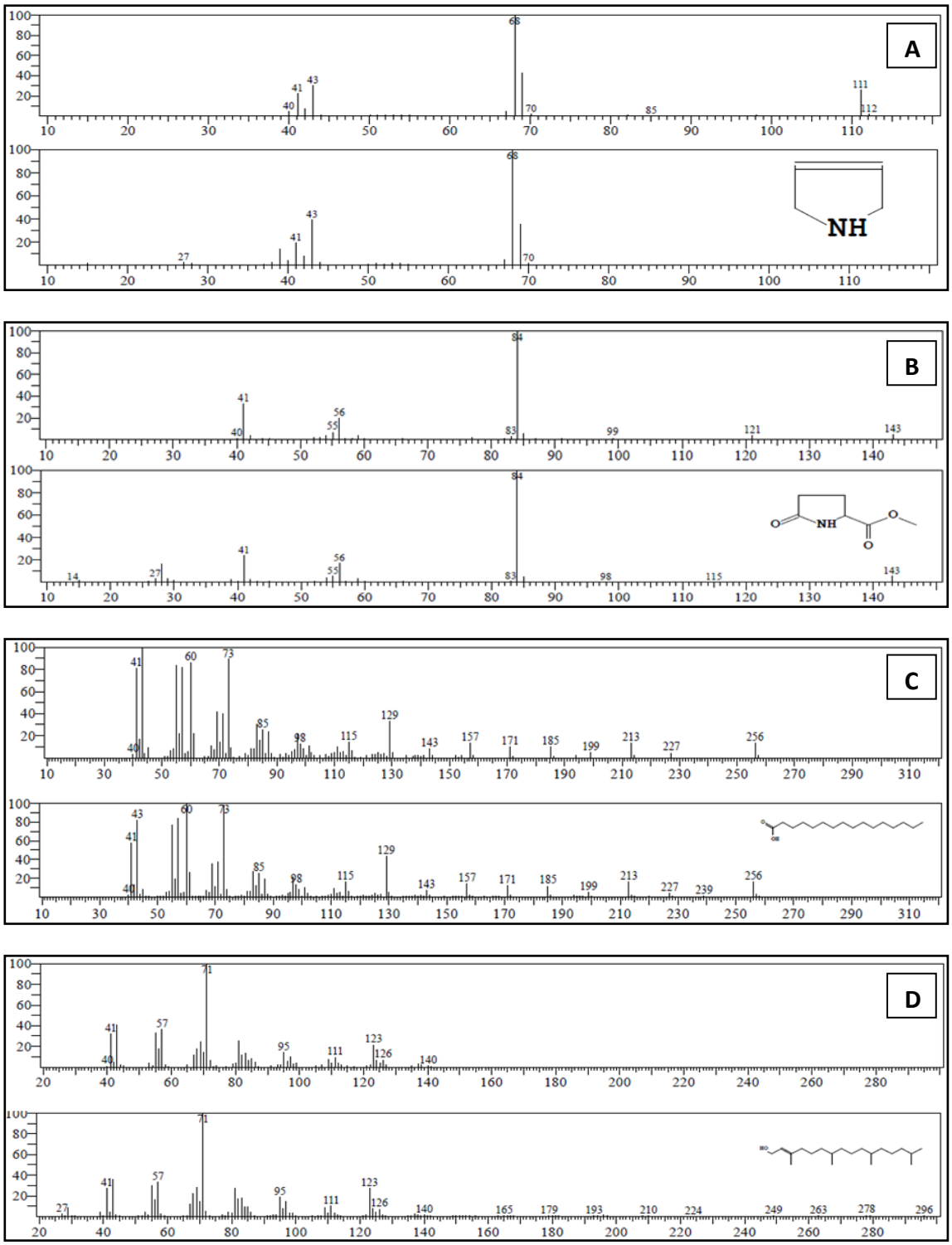

Figure 62. Mass spectra of the compounds in the GC peaks of retention time of 10.257, 17.908, 31.021 and 33.759 min, which were identified as A. 1H-Pyrrole, 2,5-dihydro-, B. DL-Proline, 5-oxo-, methyl ester, C. DL-Proline, 5-oxo-, methyl ester and D. Phytol respectively by the MS library.

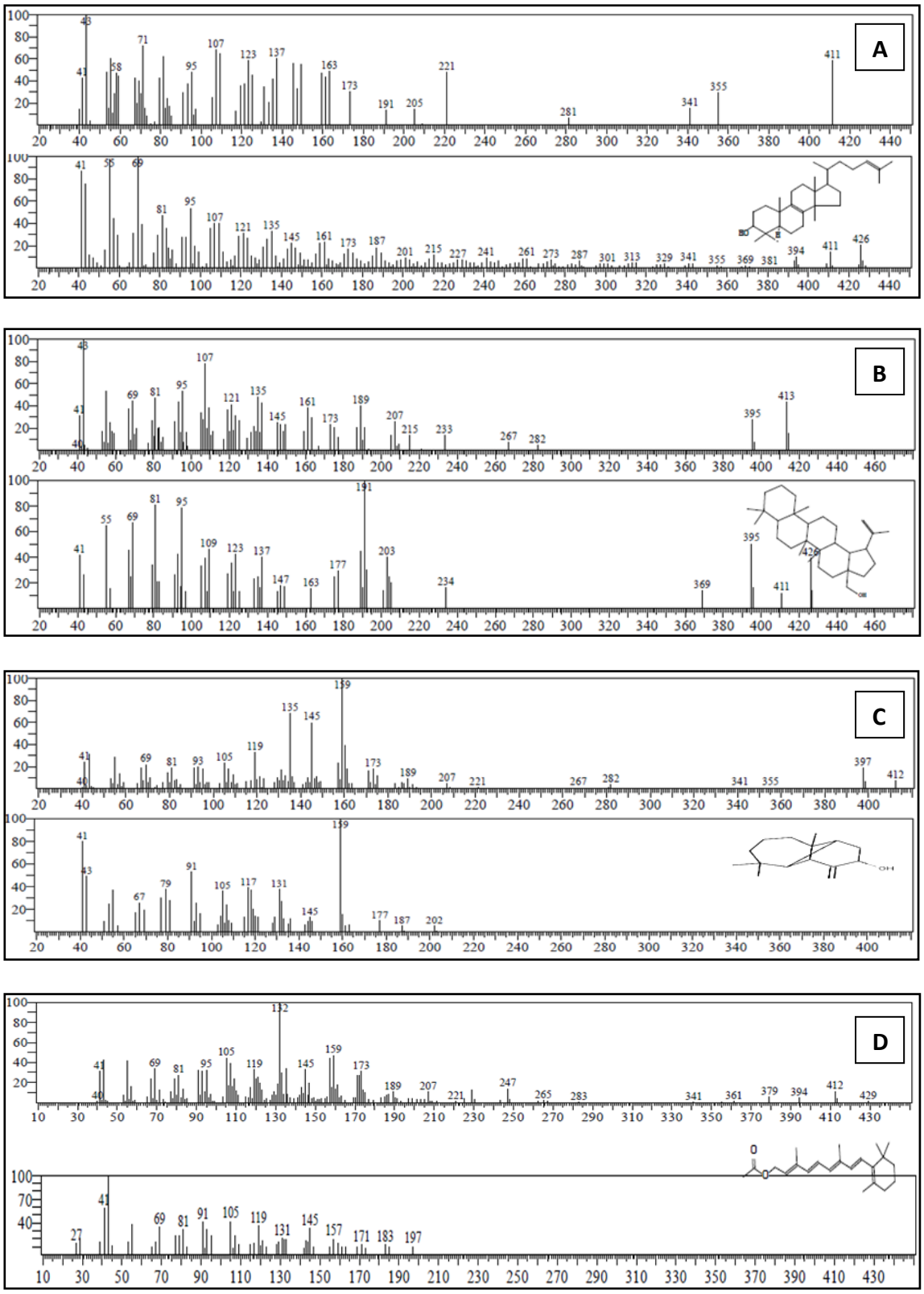

Figure 63. Mass spectra of the compounds in the GC peaks of retention time of 50.432, 52.731, 54.804 and 55.338 min, which were identified as A. Lanosterol, B. Lup-20(29)-en-28-ol, C. Beta.-copaen-4 .alpha.-ol and D. Retinol, acetate respectively by the MS library.

CHAPTER 5

DISCUSSION

5. Discussion

Currently, one of the most numerously pronounced keyword related to food-health-disease concept is ROS i.e., Reactive Oxygen Species. ROS, surprisingly besides playing super roles in energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds, hammer the living systems when produced in excess, resulting in oxidative stress and causing oxidation of cellular biomolecules *viz*. carbohydrates, proteins, lipids and nucleic acids; membrane damage, decreasing membrane fluidity. Such damage in turn leads to several ROS-mediated diseases or disorders such as cancer, acquired immunodeficiency syndrome, infectious diseases, cardiovascular disease, stroke, gastric ulcer, diabetes, malignant tumours, rheumatic joint inflammation, arthritis, cataracts, Parkinson's and Alzheimer's disease, old-age symptoms and aging etc. (Ivanov *et al*., 2017). Scientific reports advocate that these diseases or disorders can be controlled or cured by regular intake of dietary antioxidants as antioxidant molecules scavenge free radicals or suppressing the formation of free radicals, quenching hydrogen peroxide and superoxide and singlet oxygen (Hajhashemi *et al*., 2010). Natural antioxidants present in dietary food-adjuncts or foodstuffs have only recently attracted attention because of their presumed safety concern as well as high therapeutic attributes. Different parts and products of plantsuch as fruits, vegetables, culinary herbs and spice etc. contain a wide variety of phytochemicals and provide a storehouse of natural antioxidants. Phytochemicals such as carotenoids, phenolic compounds, flavonoids, terpenoids, alkaloids, nitrogen compounds, vitamins, and some enzymes show remarkable antioxidant potential.

Of late, much attention has been paid to explore the role of natural antioxidants mainly phenolic compounds for their health benefits and therapeutic attributes. Medicinal plants are a rich source of different phytochemicals, including polyphenols and flavonoids which have been reported as scavenger of free radicals due to their redox properties and chemical structures. Flavonoids, including flavonols and proanthocyanidins, on the other hand, a diverse group of plant secondary metabolites, exhibit high antioxidant activity owing to the presence of free hydroxyl (OH) groups, especially 3-OH (Geetha *et al*., 2003).

In most of the contemporary cuisine culinary herbs and leafy spices have been employed to impart diverse flavor, aroma, color and taste to various foods and drinks around the world. Foods and drinks in combination with culinary herbs are full of

pharmacological agents; they act as drugs in the body and strengthen body's defense system. Ineffective defense system may lead to a high-risk of various disease developments. Majority of the present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic conditions in the body.Herbs and spices are known for their characteristic pungent aroma and delightful flavor and are used indispensably in various culinary preparations. They are reputed to possess several medicinal and pharmacological properties and used in the preparation of a number of Ayurveda medicines. The medicinal and beneficial properties of such plants are due to the presence of various chemical substances that include phenols, flavonoids, terpenoids, tannins, carotenoids, antioxidant vitamins and some minerals. Most recently, herbs and spices have been reported to possess avaried group of phytochemicals from which valuable drugs are developed. Consequently, across the world research on herbs and spices has been initiated to verify their biological functions.

Natural antioxidants present in culinary herbs and leafy spices are responsible for inhibiting or preventing the deadly effects of oxidative stress. Herbs and spices contain free radical scavengers like polyphenols, phenolic acids and flavonoids. Polyphenols form a complex group of molecules associated with the cell walls of most plant species. This group of compounds ranges from simple phenolic acids (e.g., caffeic acid) to high-molecular-weight tannins. Polyphenols have various applications, such as in the production of paints, paper, cosmetics, as tanning agents, and as natural colorants and preservatives in the food industry. In addition, some phenolic compounds act as antibiotics and anti-diarrheal, antiulcer, and anti-inflammatory agents and can be used in the treatment of diseases such as hypertension, vascular fragility, allergies, and hypercholesterolemia (Bravo, 1998; Higdon and Frei, 2003). In addition, herbs are widely used in traditional medicine particularly widespread for example in China. The determination of the total phenolic content and antioxidant-related activity of such medicinal herbs revealed that most of them are much stronger potential antioxidants than dietary fruits and vegetables (Cai *et al*., 2004). Dietary herbs seem to be remarkable sources of antioxidants as well (Dragland *et al*., 2003). However, there can be significant differences among the antioxidant concentrations in various species.

As an important source of antioxidants, herbs and spices are considered to have a great potential as food preservatives (Hinneburg *et al*., 2006; Szabo *et al*., 2010). For

example, the extract obtained from a mixture of culinary herbs namely basil, lovage, milfoil, oregano, rosemary, marjoram and thyme has proven to be potential food preservative which may replace synthetic antioxidants in some food products (Szabo *et al*., 2010). Unfortunately, strong aroma of natural herb-derived antioxidants may restrict their use to some extent and duration ofantioxidant activity may be limited to less than two years (Szabo *et al*., 2010).

In the present investigation effort has been made to evaluate twelve commonly used herb and spice samples collected from various areas of North Bengal region, for the presence of diversified phytochemicals along with their biological activities like antioxidant, antimicrobial and antidiabetic through *in vitro* and *in vivo* test models. In the present study, plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively. The phytochemical screening of the sample extracts of *M. piperita*, *T. foenum-graecum*, *C. sativum*, *M. koenigii*, *G. oppositifolius, F. vulgare, I. verum*, *M. fragrans, C. pentandra*, *C. annuum*, *P. perlata* and *D. volubilis* revealed the presence of different bioactive phytochemicals. In most of the cases, the presence of different phytochemicals was more prominent in methanolic extract than in the hot aqueous extract. The crude plant extracts contain a mixture of various active phytocompounds which may act synergistically and their overall biological activity is supposed to be usually superiortothe individual compounds.Preliminary phytochemical study of the herb and spiceextracts from *M. koenigii*, *C. sativum*, *T. foenum-graecum*, *M. piperita, G. oppositifolius*,*F. vulgare*,*I. verum*, *M. fragrans, C. pentandra*, *C. annuum*, *P. perlata* and *D. volubilis*showed the presence of major plant secondary metabolites *viz*. reducing sugars, phenolic compounds, flavonoids and cardiac glycosides, etc. but tannins, alkaloids, saponins, steroids and anthraquinones occurred in some of them. Some of the phytochemicals were present in both hot aquous and methanolic extracts. The present study revealed the presence of secondary metabolites considered as medicinally active phytochemical constituents. Most important phytochemicals such as reducing sugars, phenols, flavonoids and free amino acids were present in all the plant samples. The result of the phytochemical analysis showed that *T. foenum-graecum*, *C. sativum*, *M. koenigii*, *G. oppositifolius*, *F. vulgare* are rich in at least one of alkaloids, flavonoids, terpenoids, reducing sugars and phlobatannins. Saponins and cardiac glycosides were absent in the extracts of *C. sativum* and *T. foenum-graecum* respectively. Phlobatannins were absent in all the hot aqueous

extracts of herb samples, except *G. oppositifolius*, but phlobatannins were present in methanolic extracts of *T. foenum-graecum*, *G. oppositifolius* and *F. vulgare*. In case of cardenolides, hot aqueous extracts of all the herb species did not show the positive result, except methanolic extracts of *G. oppositifolius* and *F. vulgare*. On the other hand, Resins was absent in the extracts of *I. verum* and *P. perlata*. In *I. verum*, *C. pentandra* and *P. perlata* anthraquinone was not ditected. Triterpenoid was absent in *M. fragrans* among the spice samples. Cardiac glycoside was also in some spice extracts such as *I. verum* and *C. pentandra*. Further in case of spice extracts, cardenolides and phlobatannins were not detected in all the samples, except *D. volubilis*. Methanolic extracts of *P. perlata* showed positive result of phlobatannin test.Such secondary metabolites contribute significantly towards the biological activities of plant extracts such as antioxidant, antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory activities etc. (Negi *et al*., 2011). Studies by Sohail *et al*., 2016 revealed the presence of reducing sugars in *Syzygium cumini, Mentha arvensis, Trigonella foecum-graecum* and *Ocimum tenuiflorum*.In addition, Singh *et al*., 2015alsoreported that leaf extracts of *Mentha piperita* which contained phenols, flavonoids, terpenoids, steroids and tannins showed remarkable antioxidant potential.

The quantitative estimation of total moisture content, total soluble sugar, total soluble protein contents, total chlorophyll content, total carotenoid content present in the herb samples had been done. Total moisture content among the herb samples was found in a range of 89% to 70%.The total soluble sugar content of was found highest in *M. koenigii* (68.18 mg glucose equivalents g^{-1} of fresh tissue weight) and lowest in *T. foenum-graecum* (53.17 mg glucose equivalents g^{-1} of fresh tissue weight), while content of soluble protein was highest in *M. koenigii* (69.507 mg BSAE g^{-1} of fresh tissue weight) and lowest in *C. sativum*(35.027 mg BSAE g^{-1} of fresh tissue weight). Pigment analysis revealed that *M. koenigii* contained highest amount of total chlorophyll (6.223 mg g^{-1} of fresh tissue weight) and carotenoid (0.190 µg g^{-1} of fresh tissue weight) content. *T. foenum-graecum* and *M. piperita* showed the lowest chlorophyll content (1.639 mg g^{-1} of fresh tissue weight) and lowest carotenoid content (0.063 µg g^{-1} of fresh tissue weight) respectively. Garg *et al*., 2012 also reported that chlorophyll content was higher in curry leaves due to the darker shade of green, then the coriander. Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl (Khalaf *et al*., 2008). Carotenoids that include xanthophylls and carotenes have the ability to detoxify

various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. Dietary carotenoids are thought to provide health benefits due to their role as antioxidant molecules.

The study also revealed that among the herbs *M. koenigii* ranked highest and *C. sativum* was lowest in total phenolic content and total flavonoid content.Total phenolic content and total flavonoid content in extracts of *M. koenigii* was foundas 5.70 mg GAE g^{-1} of lyophilized extract and 1.68 mg CAE g^{-1} of lyophilized extract. Leaf extract from *C. sativum* contained 2.55 mg GAE g^{-1} of lyophilized extract as total phenolic content and 0.66 mg CAE g^{-1} of lyophilized extract as total flavonoid content. The health benefit of phenolics is directly linked to their antioxidant potentiality. Phenolic compounds act as effective antioxidant molecules is mainly due to their redox properties, which allow them to behave as hydrogen donors, singlet oxygen quenchers as well as reducing agents. The potential hazard from oxidative stress in the body may be compensated through the consumption of a diet exclusively rich in antioxidant phenolics including polyphenols, phenolic acids and flavonoids. According to Scalbert and Williamson (2000) the amount of total human intake of phenolic compounds is about 1 g day^{-1} consisting two-thirds of flavonoids and one-thirds of phenolic acids.

Study of stem bark extractsof *Dregea volubilis* revealed that the methanol soluble extractive (MSE) value (12.93%) was quite higher than that of water soluble extractive (WSE) value (8.61%). The total moisture content of *Dv* stem bark was analysed and it was observed the total moisture content (TMC) was 5.71%. Screening of phytochemical constituents showed the presence of major plant secondary metabolites viz. reducing sugars, phenolic compounds, flavonoids, resins, glycosides, cardiac glycosides and steroids in both methanolic and hot water extracts. The contents of total polyphenols, total flavonoids and total flavonols were more or less within the range of values reported earlier. It was found that one milligram of methanolic fruit extracts of *D. volubilis* contained 95.03 μg of pyrocatechol equivalents of phenols(Biswas *et al.*, 2010). Generally, same species of medicinal plants but with different habitat and growth conditions shows difference in their composition of phytocomponents and contents. Furthermore, the extraction process is another critical factor to determine the various phytochemical contents in the resulting crude extract.

Extraction of different phytochemicals from plant species and biological activities of the extracts aresignificantly influenced by various factors including solvent types, solvent concentration, extraction methods used, extracting time, geographical

origin of plant materials, harvest time, drying methods, storage conditions and rout extract (Cervenka *et al*., 2006, Bernard *et al*., 2014).

In this study, methanol was selected as the extraction solvent, because methanol iswidely used to extract greater amount of different phytochemicals and thus showing significant bioactivities. Water, on the other hand, is a universal solvent generally used to obtain crude extracts of plant constituents from medicinal and aromatic plant species in the herbal medicine industry for therapeutic applications.Further, antioxidant and antipathogenic activities, and other bioactivity solely depend on the concentration of the solvent. So, it is necessary to choice the appropriate solvent type and its concentration for better results.

The methods of evaluation for various activities shown by the plant extracts were selected based on the availability of the laboratory standardised protocols. For anti-oxidant activity test, DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, superoxide anion scavenging activity and nitric oxide radical scavenging activity;for anti-microbial activity standard disc-agar diffusion method was employed along with MIC determination; for anti-quorum sensing activity disc-agar diffusion method, pigment inhibition assay, protease inhibition assay, anti-swarming assay and anti-biofilm activity were tested; and anti-diabetic activity through the analysis of biological parameters including periodical measurement of body weight, estimation of blood glucose levels, andanalysis of alkaline phosphatise (ALP), alanine amino transferase (SGPT/ALT), aspartate amino transferase (SGOT/AST), total cholesterol, HDL-cholesterol, triglycerides, and for the bilirubin, creatinin and urea concentration.

In vitro antioxidant activity, principally,can be determined by hydrogen atom transfer (HAT) method and single electron transfer (SET) or electron transfer (ET) method (Joon and Shibamoto, 2009).HAT based methods measure the ability of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET based methods detect the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals (Prior *et al*., 2005, Huang *et al*., 2005). Superoxide anion scavenging (SAS) assay, hydrogen peroxide scavenging (HPS) assay, etc. involve HAT method, and the assays of total polyphenolic content (TPC), total flavonoid content (TFC) etc. are of ET method, while DPPH• assay include both the method predominantly via SET method (Karadag *et al*., 2009; *Badarinath et al*., 2010). The relatively stable radical DPPH has been used widely for

the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, medicinal herb and fruit extracts and food materials (Purohit *et al*., 2005). Superoxide anion appears as a weak oxidant that generates powerful and harmful hydroxyl radicals as well as dangerous singlet oxygen, both of which contribute to the oxidative stress.Superoxide anion scavenging activity is correlated to the total flavonoids (Thaipong *et al*., 2006). Hydrogen Peroxide radical scavenging activity is correlated to the presence of phenolic compounds. Generally, extracts that contain a high amount of phenolic compounds exhibit high antioxidant activity.

Antioxidant activity in terms of scavenging potentiality of hot water extracts of *M. koenigii*, *C. sativum*, *T. foenum-graecum* and *M. piperita* have been evaluated. All of them could act as potential radical scavengers in a concentration oriented fashion. Interestingly *M. koenigii* showed the highest antioxidant activities scoring 77.354 % mg^{-1} of lyophilized extract in DPPH scavenging, 60.205 % mg^{-1} of lyophilized extract in superoxide anion radical scavenging and 57.209 % mg^{-1} of lyophilized extract in hydrogen peroxide scavenging assay, followed by *M. piperita*. Extract from *T. foenum-graecum*showed least scavenging activity in DPPH scavenging (33.145 % mg^{-1} of lyophilized extract) and superoxide anion radical scavenging (25.364 % mg^{-1} of lyophilized extract) system while *C. sativum*had the least activity in hydrogen peroxide scavenging assay (43.695 % mg^{-1} of lyophilized extract).The DPPH· activity of *D. volubilis* stem bark extracts was estimated as 70.70% and 61.35% for methanolic extract and hot water extract respectively. Superoxide anion radical scavenging activity of methanolic extract of *D. volubilis* stem bark was observed 59.63% and that of hot water extract was 56.62%. Hydroxyl radical scavenging potentiality was measured as 63.41% for methanolic extract and 53.50% for hot water extract, whereas hydrogen peroxide scavenging efficiency was found to 61.48% for methanolic extract and 56.84% for hot water extract. The results showed that the scavenging activity of methanolic extract showed significantly higher than the hot water extract. It may be of the reason that methanol have higher polarity than water that enable it to extract greater amount of phytochemicals. However, the radical scavenging potentiality for both the extracts at different concentrations was quite low as compared to standard reference, L-ascorbic acid (AsA). A study on methanolic fruit extract of *D. volubilis* showed scavenging efficiency of 84.81% and 89.92% in DPPH· and superoxide anion scavenging assays (Biswas *et al*., 2010). Results revealed that

stem showed comparatively less efficiency than fruits of the plant due to accumulation of lesser amount of secondary metabolites in that part of plants.In a research by Dragland *et al*. (2003) dried greenhouse herbs containing the highest levels of antioxidants were arranged in higher to lower concentrations as oregano > sage > peppermint > thyme > lemon balm. But commercially dried spices showed a different order of sequence: rosemary and thyme having higher antioxidant content than oregano, and total antioxidant concentrations were mostly lower than those of dried culinary herbs due to different varieties used as well as different drying methods and storage conditions.

The antioxidant activity may vary not only in different plant species, but also in different varieties of the same species (Dragland *et al*., 2003). Further, there can be some seasonal variations as well, thus it is difficult to compare and contrust the antioxidant activities of the same herbs harvested at different times of the year or even in different years (Dragland *et al*., 2003). Plant habitat may also influence the concentrations of active compounds and activities. Studies by Hossain *et al*. (2010) showed that the antioxidant capacity of herbs do not decrease with air-drying methods. On the contrary, fresh herbsamples seem to lose phenolic compounds and flavonoids and their antioxidant activities due to enzymatic degradation and atmospheric O_2-promoted oxidation. That's why herbal materials dried with standard techniques are suitable both for providing the organism with greater antioxidants and for the preservation of food products. In case of paprika (a spice), the situation is different. During drying process, paprika loses primarily vitamins C and E, consequently exhibiting insignificant antioxidant activity (Daood *et al*., 1996). In addition, during storage for few months, antioxidant properties of ground paprika also deteriorate. Thus bioavailability of antioxidant molecules from some herbs and spices may sometimes be quite uncertain.

Phenolic compounds are ubiquitously present in all the plants and plant parts at varying concentrations and contribute immensely towards the medicinal properties of the plants. Plant phenolics are effective vasodilators, help in reducing inflammation and serve as anticancerous, antioxidant, antidiabetic and antimutagenic agents. Further, its preventive role in various neurodegenerative diseases are encouraging and exploring (Kusirisin *et al*., 2009; Zhang *et al*., 2011; Mohanlal *et al*., 2013). Despite the beneficial health effects of phenolics, it is only in the recent years that enormous

attention has been paid towards the role of phenolics (Manach *et al.*, 2004). Currently, structures of more than 8000 plant phenolics have been elucidated.

Plant phenolics are the most abundantly distributed secondary metabolites bearing a common aromatic ring with one or more hydroxyl groups (Chirinos *et al.*, 2009). Naturally occurring phenols are soluble in water and may occur in combination with a sugar molecule, as glycoside (Harbone, 1998). In plants, it is synthesized mainly during physiological or environmental stresses such UV radiation, injuries or pathogen attack. Few years back, Quideau *et al.* (2011) proposed that only those secondary metabolites that are produced through shikimate/phenylpropanoid pathway or 'polyketide' acetate/malonate pathway should be termed as "Plant phenolics". Depending on the number of phenol units in the molecule polyphenols are classified as simple phenols, phenolic acids and flavonoids, lignins, lignans, coumarins, condensed and hydrolysable tannins (Soto-Vaca *et al.*, 2012).

Flavonoids, the chief compounds among the polyphenols are known to possess multiple biological activities such as antibacterial, antifungal, antiviral, anticancer, anti-allergic and anti-inflammatory activities (Montro *et al.*, 2005). Structurally, it has a flavan nucleus with 15 carbon atom. The carbon atoms are arranged in a ring of three as C6-C3-C6 which are labeled as A, B and C. They are known to possess an inherent effective ability to scavenge most of the harmful oxidizing molecules or reactive oxygen/nitrogen molecules involved in various life threatening diseases (Bravo, 1998). Flavonoids have been also reported to exhibit protection against various cardiovascular diseases and cancers. It represses the development of cancer by inhibiting the enzymes involved in estrogen production. For example, flavonoids inhibit estrogen synthetase involved in coupling estrogen to its receptor (Okwu and Omadamiro, 2005). They have also been designated as "biological response modifiers" (Cushnie and Lamb, 2005).

Tannins have been shown to possess antioxidant, antibacterial, antiviral, antiparasitic, antiinflammatory and antiulcer activity. Since, tannin can precipitate the proteins from the exposed tissues forming a protective layer; it is widely used in the treamtment of burns. Other medicinal applications are in treating gonorrhoea, leucorrhoea, piles, inflammation and even used as an antidote. Studies also revealed the HIV replication inhibitory activity of tannin. Most of the drugs containing tannin are used as an astringent and diuretic in medicine (Kolodziej *et al.*, 2005).

Terpenoids have been used in pharmaceutical industries since long years as antibiotics, antiseptic, insecticidal and anthelmintic (Parveen *et al*., 2010). Terpenoidsare natural compounds with muti-cyclic in structural configuration and are derived from 5-hydrocarbon isoprene units (CH_2=C(CH_3)-CH=CH_2) (Elbein *et al.*, 1999). These natural lipids are ubiquitously present in all forms of living things (Elbein *et al.*, 1999). Commercially, terpenoids are used to add flavours and fragrances in cosmetics, foods and agricultural products (Harborne *et al.*, 1991).

Anthraquinones are the naturally occurring aromatic compounds and are known to possess antioxidant, antimutagenicity and antitumor activities (Lee *et al.*, 2005; Kimura *et al.*, 2008).

Alkaloids are the natural compounds bearing mostly basic nitrogen atoms and are known to have anticonvulsant, hypotensive, antiprotozoal, antimalarial and antimicrobial activities (Frederich *et al.*, 2002).

Saponins belong to the group of glycosides and possess a property of foaming like soap in an aqueous solution, thereby making them an efficient foaming and surface active agent. Besides industrial applications, saponins have been known to precipitate and coagulate RBCs (Okwu, 2004) and exhibit antihyperglycemic (Vats *et al.*, 2003), antioxidant (Gulcin *et al.*, 2004) and antimicrobial (Mandal *et al.*, 2005). Plant extracts rich in saponins have been reported by Sani *et al.* (2007) and Govindappa *et al.* (2011).

Various environmental stresses have been known to be responsible for the production of phenolic compounds in plants; for example light has been shown to greatly influence the synthesis of flavonoids. Increased levels of flavonoids (antioxidants) have been reported in the plants available in higher altitudes as many prevailing stresses such as decreased pressure, low atmospheric temperature, exposure to higher UV rays etc augments its synthesis (Chanishvili *et al.*, 2007). This possibly could be one of the reasons behind higher phenolic content in some plant samples.Further, there are reports suggesting the protective role of phenolics against UV-B damage and successive cell death. Phenolics are known to protect DNA against dimerization and breakage which in turns protects the cell death that may arise through UV damages (Strack, 1997).

Differences in the phenolic content between the intraspecific and/or interspecific samples studied by different workers may be attributed to the method used for determination. Presence of diverse natural phenolics in the plant material and other oxidized substrates prevalent in the extract may interfere in the measurement of total

phenols either by inhibiting, enhancing or adding the content (Singleton and Rossi, 1965; Singleton *et al.,* 1999).

Protein content was found to be highest in *M. koenigi* and *I. verum* and lowest in *G. oppositifolius* and *D. volubilis.* Almost all the herbs and some spices were observed to have considerableamount ofprotein in them. In addition to antioxidant property, use of proteins or its hydrolysates in cosmetics and food may add on to its functional and nutritional ability (Moure *et al.,* 2006). Thus, presence of appreciable amount of protein in these edible plants or plant parts may possibly promote health benefits.

In the present study, total sugar content reducing sugar content was found to be highest in *G. oppositifolius.* Carbohydrates are abundantly available biomolecules and find its application in various natural products that needs to be glycosylated, many of which have been used as anticancerous and antimicrobial drugs. For example, iminosugars like nojirimycin, aminoglycosides like streptomycin etc are some commonly used glycosylated natural products (Asano, 2003). Further, carbohydrates have been reported to exhibit antioxidative activity. Basu *et al.* (2012) reported Pusa Basmati polished seeds containing higher sucrose and starch content revealed better superoxide and hydroxyl scavenging capacity. Many of the polysaccharides isolated and purified from medicinal herbs of China have been reported to act as an effective immunomodulatory (Jayabalan *et al.*, 1994). In addition, proteins have been identified as a potent antioxidant.

Photosynthetic pigments like chlorophylls have been known to possess several beneficial properties for example chlorophyllin (water soluble analogue of chlorophyll) was proven to be more efficient than the parent compound (Dashwood *et al*, 1998). Numerous reports have suggested strong antioxidant activity of chlorophyllin and its usage to treat number of human diseases without evident harmful effects (Kumar *et al.*, 2001). In the present study highest amount of total chlorophyll and chlorophyll a was found in *G. oppositifolius* followed by *M. koenigii* while chlorophyll b in *F. vulgare* was found to be lowest. On the other hand, lowest level of carotenoids was found in *M. piperita* and highest in *G. oppositifolius.* Considerable amount of chlorophyll was found in almost all the herbs studied. Carotenoids are also known to exhibit anti-carcinogenic, anti-oxidative, antihypertensive, antimicrobial and anti-mutagenic activities (Yen *et al.,* 2002)

Lipid content was found to be highest in *M. koenigii* and *C. annuum* and lowestin *G. oppositifolius* and *P. perlata.* Biologically lipids are important for energy storage, signaling and cell membrane development.

In the present study vitamins such as vitamin C and E was quantified spectophotometrically. Among the herbs, *M. koenigii* contained lowest amount of vitamin C and *M. piperita* contained lowest amount of vitamin E, whereas both vitamin C and E found to be highest in *G. oppositifolius*.Among the spices, *C. annuum* and *I. verum* contained highest amount of vitamin C and E respectively. *D. volubilis* contained both the vitamins in lowest amount. Vitamins are organic compounds which cannot be produced *in vivo* and are required in a very small quantity for performing several biochemical functions. Thus, it is essential to obtain from the diet or consume as the supplement (Peter, 1990). Vitamin C is known for its antioxidative activity and for its potential to prevent arteriosclerosis (Addo, 2004). Similarly, vitamin E or tocopherols (α, β, γ, and δ) are the potent free radical, lipid peroxy and superoxides scavengersand an anti-hyperglycemic agent (Celik *et al.*, 2002).

The antibacterial activity of different herb and spice extracts was studied against two Gram-positive and two Gram-negative bacterial strains. The Gram-positive organisms include *B. cereu* and *B. pumilus* whereas Gram-negative strains are *S. marcescens* field isolate and *Ps. aeruginosa*. Different concentrations or dose of lyophilized methanolic extracts *i.e*, 4-10 mg $disc^{-1}$were used to screen the antimicrobial activity. Extract of different plant samples investigated so far inhibited the growth of the microorganisms in a dose dependent manner for each extract and was evident with the formation varying diameter of inhibition zones. Parekh and Chanda (2007) reported that methanol was a better solvent for the extraction of antimicrobial compounds from medicinal and aromatic plants compared to other solvents such as water, ethanol and hexane. Our investigation also supported this conclusion. Therefore methanol was used for phytochemical extraction in our study as well as antimicrobial and anti-quorum sensing activities were quantitatively assessed by the presence or absence of zone of inhibition. Among the herb extracts, *M. piperita* and *T. foenum-graecum* was found in inhibiting *B. cereus* and *B. pumilus.* Rest of the samples (*C. sativum, M. koenigii, G. oppositifolius* and *F. vulgare*) did not show any antimicrobial activity. Chloramphenicol (C_{25} µg), Kanamycin (K_{30} µg) and Ampicillin (A_{25} µg) exhibit inhibitory activity against specific organisms, not all the strains of microbial species. *B. cereus* and *B. pumilus* were inhibited only by the *M. piperita* and *T. foenum-graecum*

extracts at higher doses. No inhibition was observed by the other herbal extracts and against other test organisms. The MID value of *M. piperita* extract against *B. cereus* and *B. pumilus* was found to be 8.5 and 5.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID value of *T. foenum-graecum* extracts against *B. cereus* and *B. pumilus* was determined as 3.5 and 7.5 mg lyophilized methanolic extract $disc^{-1}$ respectively. The MID values of other herb extracts against respective organisms were found to be >10 mg lyophilized methanolic extract $disc^{-1}$. Among the spice extracts, extract of *I. verum* was found to be most potent showing highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. Several studies have been executed to confirm the antimicrobial activity of the major groups of chemical compounds identified from different medicinal plants (Jamuna Bai *et al*., 2011; Bibi *et al*., 2011). Chouksey *et al*. (2010) and Huang *et al*. (2010) also reported the antimicrobial properties of *I. verum*. Methanolic extract of dry fruit of *I. verum* had antibacterial activity against *E. coli, B. cereus* and *S. aureus* (Shan *et al*., 2007). The present study showed that methanolic extract of fruits of *I. verum* inhibited the growth of *B. cereu,B. pumilus, S. marcescens* and *Ps. aeruginosa*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *Ps. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg lyophilized methanolic extract $disc^{-1}$ respectively.

Our findings suggest that Gram positive bacterial strains are susceptible to the plant extracts than Gram negative bacterial strains. These results were consistent with previous reports shown by other herbs and spices (Ceylan *et al*., 2004; Lopez *et al*., 2005). A possible explanation of the fact lies in the structural differences in Gram positive and Gram negative bacteria. Generally Gram negative bacteria have an outer membrane (LPS) and a periplasmic space not common in Gram positive bacteria (Duffy and Power, 2001). The drug resistance of the Gram negative bacteria towards commercial antimicrobials is related to the lipopolysaccharide outer membrane which is hydrophilic in nature and serving as a barrier to the cellular permeability of antibiotic molecules and is also associated with the enzymes present in the periplasmic space, which are capable of degrading antibacterial drugs directly introduced from outside (Gao *et al*., 1999). On the other hand, Gram positive bacteria do not possess such a lipopolysaccharide outer membrane and cell wall structure. Antimicrobial substances can easily disrupt the bacterial cell wall composition through enzymatic degradation

and leakage of the cytoplasmic components and its coagulation (Kalemba and Kunicka, 2003).

Antimicrobial activity of plant extracts thought to be related to the polyphenolic compounds present in them. Thousands of phenolic compounds are synthesized by plants in response to biotic and abiotic factors of environment including microbial infections. It is therefore possible that phenolics can act as effective antimicrobial agents against a variety of microbial species. However, antimicrobial activity showed by plant extracts depends not only on phenolic compounds but also on other secondary metabolites (Gordana *et al*., 2007).

The present study provide with an additional and plant-based sources of compounds, in the form of plant extracts, to inhibit quorum sensing in *C. violaceum* MTCC 2656 and *Ps. aeruginosa* MTCC 2453system. Disruption of QS can occur by various means, inactivating transcriptional activators by competitive binding, or directly affecting expression of QS genes or decrease in AHL production by inactivation of AHL synthases. Most of the known QS inhibitors (QSIs) have been found to act by competitive inhibition of the LuxR homologous proteins. Halogenated furanones, the first isolated natural QSIs identified in 1996 (Givskov *et al*. 1996), have been shown to displace the native AHLs binding to the LuxR protein in*Vibrio fischeri* (Manefield *et al*. 1999). Analogues of AHLs, namely N-acyl cyclopentylamides were found to inhibit QS and its regulated phenotypes in *P. aeruginosa*, probably by interfering with the interactions of LasR and RhlR with their native AHLs (Ishida *et al*. 2007). Natural compounds such as salicylic acid, nifuroxazide and chlorzoxazone that exhibit configurational dissimilarity to AHLs, showed attenuation of QS in *Ps. aeruginosa*, and was thought to function by binding to the LasR protein, as evident by the molecular docking studies (Yang *et al*. 2009). Tateda *et al*. (2001) reported that very few QSI molecules such as azithromycin, has been explored to act by inactivation of LasI enzyme.

In our study, preliminary screening of twelveplant species of herb and spice was performed to investigate their ability to inhibit synthesis of violacein production in *C. violaceum*. Studies based on the anti-QS activity of plants by inhibition of violacein production highlightedonly the ability to affect short acyl-HSLs in *C. violaceum* (Zahin *et al*. 2010). In this study, the focus was to screen the anti-QS activity and anti-biofilm

ability of plant extracts to explore the possibility of finding new scope for preventing or treating biofilm-associated infectious diseases.

The effect of methanolic extract of *I. verum* on the biofilm formation was tested using the crystal violet method. It is inexpensive and easily adopted, and can be repeated for several times to get accurate results. Sauer (2003) reported that adhesion of bacterial cells to a surface is an essential step to form a biofilm matrix. The basic disadvantage is that crystal violet stains both the living and dead cells, because it binds to the polysaccharides and negatively charged surface molecules in theextracellular matrix. Thus it is difficult to differentiate the cells types in a biofilm (Burton *et al*., 2007).

Extract of *I. verum* showed strong anti-QS activity against *P. aeruginosa* compared to the untreated control. The spice could be used to manage *Pseudomonas* pathogenesis and prevent its dissemination. Vasavi *et al*., 2014 suggested that plant extractsthat inhibited motility of *Ps. aeruginosa explored* as an alternate strategy for controllingbacterial virulence. The swarming motilityis considered to be an important factorinvolved at early stages of biofilm formation for development of cystic fibrosis and nosocomial infections. A close relation between swarming motility and biofilm formation in *Ps aeruginosa* is also reported by Jimenez *et al.* (2012). *I. verum* is rich in linalool which is chiefly responsible for its antioxidant, antibacterial, anti-QS, and analgesic properties.

The increasing evidence from experimental as well as clinical studies suggested that diabetes is associated with oxidative stress that leads to an increased production of ROS, including superoxide radical, hydrogen peroxide and hydroxyl radical (Gokce and Haznedaroglu, 2008; Bagri *et al*., 2009). It has been suggested that ROS/oxidative stress constitutes the common events of different diabetic complications (Sepici-Dincel *et al*., 2007). Several studies and evidences indicate that free radicals may play an essential role in the mechanism of β-cell damage and diabetogenic effect of Streptozotocin (STZ) (Ohkuwa *et al*., 1995). Streptozotocin induced diabetic condition induces generation of free radicals which further leads to the damage or degradation of cellular macromolecules like DNA damage, protein degradation, lipid peroxidation and finally culminating into damage of the heart, kidney, liver, brain and eyes (Yazdanparast *et al*., 2007).

The present study also shows that methanolic extracts of *I. verum* and *G. oppositifolius* at the doses of 250 and 500 mg kg^{-1} BW are able to produce a consistent reduction in blood glucose, serum cholesterol and serum triglyceride. The extracts have also shown presence of active constituents responsible for various biological activities.From our findings, it is suggested that the methanolic extract of *I. verum* and *G. oppositifolius* can be chosen as primary antihyperglycemic, antihyperlipidemic and antioxidant supplement. Further, clinical evaluation will throw more light on clinical usefulness, safety, and efficacy of these plant extracts. Methanolic extract of *G. oppositifolius* whole plant at single oral doses 200 and 400 mg kg^{-1} have shown significant antidiabetic activity in glucose-overloaded hyperglycemic mice (Hoque *et al*., 2011) and rats (Behera *et al*., 2010; Panigrahi *et al*., 2012) compared to the standard antidiabetic drug metformin and glibenclamide treatment, respectively. Studies by Sahu *et al*. (2012) have exhibited that ethanolic extract at 200 and 400 mg kg^{-1}, *p.o*. of the aerial parts of *G. oppositifolius* produced significant reduction in the blood glucose level compared with the controls and glibenclamide treatment in alloxan-induced Wistar Albino diabetic rats. Aqueous and methanolic extracts of *G. oppositifolius* were found to have significant anti-hyperlipidemic activity in Streptozotocin-induced diabetic rats when treated with 200 and 400 mg kg^{-1} for 14 days and 28 days (Behera *et al*., 2010). The methanolic extract of *G. oppositifolius* was tested for anti-hyperlipidemic activity in Triton-induced hyperlipidemic rats at 200 and 400 mg kg^{-1} dose. Such a treatment exhibited a significant reduction in serum lipid profile like triglycerides, total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and increase in high-density lipoprotein (HDL) in hyperlipidemic rats compared to the controls (Panigrahi *et al*., 2012). Our results supported this observation. *G. oppositifolius* is reported to be a good source of carbohydrates, polysaccharides, phenols, flavonoids, alkaloids, saponins, steroids and several other aromatic compounds. In previous studies, triterpenoidal saponins were reported to act as hypoglycemic and hypolipidemic agents (Behera *et al*., 2010; Hoque *et al*., 2011; Panigrahi *et al*., 2012; Sahu *et al*., 2012). It is established that inhibition of α-glucosidase enzyme plays a vital role in the management of hyperglycaemic conditions, as it delays the digestion process of carbohydrates. Kumar *et al*., 2013 reported that chemicals like hopanoid triterpene saponins from *G. oppositifolius* were observed to exert α-glucosidase inhibitiory activity. The plausible mechanism, by which the plant

extracts and/or phytochemicals lowered the blood glucose levels in experimentally induced diabetic rats, may be by inhibiting the absorption of glucose from the intestinal cells, inhibiting gluconeogenesis in the liver cells, increasing the glycogenesis process or stimulation of the insulin secretion (Mowla *et al*., 2009).

The results of antidiabetic study validate the use of fruit of *I. verum* and the aerial part of *G. oppositifolius* for treating diabetes as suggested in the folklore remedies. The comparable effect of the methanolic extract with streptozotocin may suggest similar mode of antidiabetic action, since streptozotocin permanently damages the pancreatic ß-cells and the extract have the capacity to lowerthe blood sugar level in STZ-rats, indicating that the extract might be contain phytochemicals that have extra pancreatic effects.

Partial characterization of the volatile compounds of the two plant extracts were carried out by GC-MS analysis and it was revealed that among the two, star anise – *I verum* contained much more volatiles. Comparison of their biological activities with similar compounds indicated that the compounds such as cis-1,2-Dihydrocatechol, trans linalool oxide, estragole, benzaldeyde, 3-methoxy and several others have antimicrobial, anti-quorum sensing, antioxidant and antidiabetic activities. Hence the presence of such compounds is important for the biological activities of the spices and herbs.

CHAPTER 6

CONCLUSION

6. Conclusion

- During survey 40 different herbs and spices were collected and authenticated.
- Among them six popular herbs (*Mentha piperita, Trigonella foenum-graecum, Coriandrum sativum, Murraya koenigii, Glinus oppositifolius* and *Foeniculum vulgare*) and six aromatic spices (*Illicium verum, Myristica fragrans, Ceiba pentandra, Capsicum annuum, Parmelia perlata* and *Dregea volubilis*) hadbeen selected for the evaluation of biological activities.
- Plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively.
- Preliminary phytochemical screening to detect the presence or absence of some significant phytochemicals viz. phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard protocols. Phytochemical analysis revealed that phenol, flavonoid, reducing sugar, free amino acids, tannins were detected in all plants tested; anthraquinone was absent in *I. verum, C. pentandra* and *P. perlata*; triterpenoid was not detected in *C. sativum, T. foenum-graecum* and *M. fragrans*;cardiac glycosides were detected in all samples except *T. foenum-graecum, I. verum* and *C. pentandra*;alkaloid was detected in all the test plants; saponin was absent in *C. sativum*; steriod was absent in *C. annuum*; phlobatannin was present only in *P. perlata* and *D. volubilis* and cardenolide was presen only in *D. volubilis.*
- Samples were evaluated for the total soluble sugar content, reducing sugar content, soluble protein content and total lipid content, total phenol, flavonoid and flavonol content along withvitamin C and E.*G. oppositifolius and I. verum had h*ighest amount of total phenol, total flavonoid, total flavonol, total sugar and vitamin C.Highest amount of reducing sugar and total and vitamin E was present in *G. oppositifolius and I. verum.* Protein content was found to be highest in *M. koenigii,* lowest in *G. oppositifolius.*

- Quantity of different plant pigments like total chlorophyll content and total carotenoid content was estimated and found highest in *G.oppositifolius* for both the pigments. Carotenoid content was lowest in *M. piperita*.

- Extraction of the samples with methanol revealed higher yield than hot aqueous which may be due to the higher solubility potential of phytochemicals in methanol. Amongst the samples, *P. perlata* showed the highest yield.

- In vitro antioxidant activity in terms of free radical scavenging activity revealed that both the sample extracts of *I. verum* and *O. oppositifolius* had highest scavenging activity. In all the cases, gradual rise in the activity with the increase in the concentration was observed but it was insignifcantly different to each other in case of majority of herb extracts.

- Different biological activities reveals that methanolic extract was most active in compared to aqueous extract. Hence, methanol extract was thus selected for further studies on antimicrobial activity, anti-quorum sensing activity and antidiabetic activity.

- Antimicrobial activity of methanolic extracts of different herbs and spices against both Gram positive bacteria and Gram negative bacteria by disc-agar diffusion method. Revealed that among the herb extracts, *M. piperita* and *T. foenum-graecum* were found inhibiting *B. cereus* and *B. pumilus.* The MID values of *M. piperita* and *T. foenum-graecum* against *B. cereus* were 8.5 and 3.5 mg extract $disc^{-1}$ respectively, whereas MID values of the same plant extracts against *B. pumilus* was found to be 5.5 and 7.5 mg extract $disc^{-1}$ respectively. Rest of the herb samples (*C. sativum*, *M. koenigii*, *G. oppositifolius* and *F. vulgare*) did not show antimicrobial activity. Among the spice extracts, extract of *I. verum* was found to be most potent showing highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *P. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg extract $disc^{-1}$ respectively.

- Anti-quorum sensing activities of different herbs and spices evaluatedthrough preliminary screening for inhibition of violacein synthesis by whole plant parts.Among the plant tested *I. verum* was found to be most potent in inhibiting the violacein production in *C. violaceum,* followed by *P. perlata*. Methanolic extract of *I. verum* was also able in reducing the virulance phenotypes such as pyocyanin synthesis, ptotease production, swarming motility and biofilm formation in *Ps. aeruginosa*.

- Evaluation of *in vivo* anti-diabetic activity was performed in Streptozotocin-induced rats using *I. verum* and *G. oppositifolius* methanolic extracts as they were traditionally reported as antidiabetics.
  - The methanolic extracts were reconstituted in sterile distilled water and used to determine the toxicity and pharmacological effects on rats.
  - Before performing *in vivo* assay, the crude extracts were tested fot their acute toxicity at a concentration of 2000 mg kg^{-1} BW and analyzed the lethal and safer doses of extracts.
  - For anti-diabetic assay 500 mg kg^{-1} BW and 250 mg kg^{-1} BW doses were selected as safer and non-toxic.
  - Streptozotocin-induced diabetic rats treated orally with both the sample extracts and Metformin were able to reverse the diabetic conditions to near normal.
  - Various biological markers such as fasting blood sugar level, cholesterol, triglycerides liver enzymes (SGPT and SGOT), serum urea and creatinine were reduced to nearly normal level while significant increase in body weight and HDL-cholesterol level was observed in compared to the diabetic controls.
  - Among the plant extracts, *I. verum* extract (*Iv*ME) showed comparatively better *in vivo* antidiabetic activity than *G. oppositifolius* extract (*Go*ME). This may due to various antidiabetic compounds presents in the extract.

- Further, characterization of bioactive compounds present in the different fractions of *I. verum* (*Iv*ME) and *G. oppositifolius* (*Go*ME) were performed by GC-MS analysis. GC-MS profiling revealed the presence of multitude chemical

compounds containing volatile compounds, phenolics, terpenoids, fatty acids, phytosterol etc.

- Compounds identified through GC-MS analysis of methanolic fraction of *I. verum* wereLinalool, Estragole, Benzaldehyde, 4-methoxy-; Benzene, 1-methoxy-4-(1-propenyl)- or cis-Anothole; 2-Propanone,1-(4-methoxyphenyl)-; n-Hexadecanoic acid; Benzhydrazide,4-methoxy-N2-(2-trifluoroacetylcyclohepten-1-yl)-; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel-, cis-Vaccenic acid; Octadecanoic acid.
- Some of the compounds identified through GC-MS analysis of hexane fraction of *I. verum* were Benzaldehyde, 4-methoxy-; Anethole; Anisaldehyde dimethyl acetal; 2-Propanone, 1-(4-methoxyphenyl)-; 4-(p-Methoxyphenyl)-1-butanol; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene; n-Hexadecanoic acid; cis-Vaccenic acid; (2R,4R,5S)-2,4-bis(4-Methoxyphenyl)-5-methyl-1,3-dioxolane-rel-;4-Methoxy-benzoic acid N'-[2-(4-methoxy-phenyl)-acetyl]-hydrazide and Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-.
- Some of the compounds identified through GC-MS analysis of ethyl acetate fraction of *I. verum* were Linalool; Estragole; Benzene, 1-methoxy-4-(1-propenyl)-; 2-Propanone, 1-(4-methoxyphenyl)-; 1-(4-Methoxyphenyl) propane-1,2-diol; 1-(3-Methyl-2-butenoxy)-4-(1-propenyl) benzene; n-Hexadecanoic acid; (2R,3S,5S,6R)-2,5-bis(4-Methoxyphenyl)-3,6-dimethyl-1,4-dioxane-rel- and cis-Vaccenic acid.
- GC-MS analysis of different fractions of *G. oppositifolius* also revealed different types of chemical compounds. Compounds identified through GC-MS analysis of methanolic fraction of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; 1-Deutero-2,2,5,5-tetramethylcyclopentanol; n-Hexadecanoic acid; Phytol; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydro cyclopropa [d]-naphthalene and Retinol, acetate.

- Compounds identified through GC-MS analysis of hexane fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; Mome inositol; Hexadecanoic acid, methyl ester, n-Hexadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; Linoelaidic acid; Octadecanoic acid; Squalene and Chondrillasterol.
- Compounds identified through GC-MS analysis oethyl acetate fraction extract of *G. oppositifolius* were 1H-Pyrrole, 2,5-dihydro-; DL-Proline, 5-oxo-, methyl ester; Mome inositol; n-Hexadecanoic acid; Phytol; Lanosterol; Lup-20(29)-en-28-ol; Beta.-copaen-4 .alpha.-ol and Retinol, acetate.

❖ Finally, it can be concluded that the two selected spice and herb showed high potential in the different activities tested. *I. verum*, being used as a spice has a large number of volatiles which would be responsible for the flavour and *G. oppositifolius*- a commonly consumed leafy vegetable with so many different phytochemical constituents contributed to beneficial wholesomeness. The health benefits and therapeutic potentials of the investigated herbs and spices might be attributed to the presence of various phytochemical compounds in plant extracts.

CHAPTER 7

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CHAPTER 8

APPENDICES

APPENDIX A

List of Thesis related Publications

I. **Dev Chaudhuri, S**, Shah, A and Chakraborty, U. Comparative analysis of antioxidant activities and phytochemical properties of some culinary herbs.***NBU Journal of Plant Sciences*** Vol. 10, Issue 1, March 2016 p. 55-65. (ISSN No. 0974-6927)

II. **Dev Chaudhuri, S** and Chakraborty, U. Phytochemical constituents and radical scavenging **a**ctivities of stem bark extractsof *Dregea volubilis* (Linn. f.) Benth ex. Hook.f. ***International Journal of Pharmaceutical sciences and research***. Vol. 8, Issue 11, November, 2017. Accepted for publication. (ISSN No. 0975-8232/2320-5148)

APPENDIX B

List of Abbreviations

µg- Micro gram

µg mL- Micro gram per mililiter

µL- Micro liter

A_{25}- Ampicillin

$AlCl_3.6H_2O$- Aluminium chloride hexa hydrate

AsAE- Ascorbic acid equivalents

BSA- Bovine serum albumin

BSAE- Bovine serum albumin equivqlents

BW- Body weight

C- Control

C_{25}- Chloramphenicol

Ca- Capsicum annuum

Cp- Ceiba pentandra

Cs- Coriandrum sativum

$CuSO_4.5H_2O$- Cupper sulphate penta hydrate

dH_2O- Distilled water

Dv- Dregea volubilis

$FeCl_3$- Feric chloride

Fv- Foeniculum vulgare

g- Gram

Go- Glinus oppositifolius

Gr- Group

h- Hour

H_2O- Water

H_2O_2- Hydrogen peroxide

H_2SO_4- Sulphuric acid

HCL- Hydrochloric acid

IC_{50}- Inhibitory concentration at which 50% inhibition occurs

Iv- Illicium verum

K- Kanamycin

K^+- Potassium ions

KOH- Potassium hydroxide

LB agar- Luria Bertani Agar

LB broth- Luria Bertani Broth

LHE- Lyophilized hot aqueous extract

LME- Lyophilized methanolic extract

M- Molar

Mf-Myristica fragrans

mg g^{-1}- Milligram per gram

MHA- Mueller Hinton Agar

min- Minute

Mk- Murraya koenigii

mM- Millimolar

Mp- Mentha piperita

NA- Nutrient Agar

Na^+- Sodium ions

Na_2CO_3- Sodium carbonate

Pp- Parmelia perlata

SDA- Sabouraud dextrose agar

TCA- Trichloro acetic acid

TFC- Total flavonoid content

Tfg- Trigonella foenum-graecum

TPC- Total polyphenol content

TSB- Tryptone Soya Broth

UV-Vis- Ultra violet-Visible wave length

Vit C- Vitamin C

Vit E- Vitamin E

APPENDIX C

List of Chemicals and Reagents

0.1 M sodium acetate buffer (*p*H 4.7)

0.1 M sodium phosphate buffer (*p*H 7.4)

0.2 M sodium acetate buffer (*p*H 4.7)

0.2 M sodium acetate buffer (*p*H 6.6)

1% Ferric chloride

1% Hydrochloric acid

10% Ammonium solution

2, 2'-Bipyridyl

2, 6-Dichloroindophenol (DCIP)

2,2-Diphenyl-1-picrylhydrazyl

20% Sodium hydroxide

Acetone

Agar

Alkaline copper tartarate reagent

Aluminium chloride

Aluminium trichloride hexahydrate

Aluminum trichloride

Anthrone's reagent

α-tocopherol

BSA

Catechin

Catechin

Chloroform

Cholesterol reagent

Crystal violet

D-Glucose

Dinitrophynyl hydrazine

Dinitrosalicylic acid

DNSA reagent

DPPH•

Ethanol

Ferric chloride

Ferrous chloride ($FeCl_2$)

Ferrozine

$FeSO_4$

Folin Ciocalteu's Phenol reagent

Folin-Ciocalteau reagent

Gallic acid

Gallic acid monohydrate

Glacial acetic acid

Glucose

H_2O_2

H_2SO_4

HCl

Hydrogen peroxide

L-Ascorbic acid

Mayer's Reagent:

Metformin

Methanol, HPLC grade

m-Phosphoric acid

Na_2EDTA

NaCl

NADH

Napthylethylene diamin dihydrochloride

NBT

n-Butanol

Nelson's arseno molybdate reagent

Nelson's Arseno molybdate reagent

n-Hexane

Phloroglucinol

PMS

Potassium acetate

Potassium ferricyanide

Quercetin

SDS- Sodium dodecyl sulphate

Sodium carbonate

Sodium salicylate

Streptozotocin

Trichloroacetic acid

Vanillin

Wagner’s reagent:

APPENDIX D

List of Microbiological Media

Luria Bertani Agar:

Composition	g L^{-1}
Casein enzymic hydrolysate	10.00
Yeast extract	5.00
Sodium chloride	10.00
Agar	15.00
Final *p*H (at 25°C)	7.5±0.2

Luria Bertani Broth:

Composition	g L^{-1}
Casein enzymic hydrolysate	10.00
Yeast extract	5.00
Sodium chloride	10.00
Final *p*H (at 25°C)	7.5±0.2

Mueller Hinton Agar:

Composition	g L^{-1}
Meat, infusion solids from 300g	2.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00
Final *p*H (at 25°C)	7.3±0.1

Nutrient Agar:

Composition	g L^{-1}
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.0
Final *p*H (at 25°C)	7.4±0.2

Nutrient Broth:

Composition	g L^{-1}
Peptone	10.00
Beef extract	10.00
Sodium chloride	5.00
*p*H after sterilization	7.3±0.1

Skim Milk Agar:

Composition	g L^{-1}
Skim milk powder	28.00
Casein enzymic hydrolysate	5.00
Yeast extract	2.50
Dextrose	1.00
Agar	15.00
Final *p*H (at 25°C)	7.0±0.2

Tryptone Soya Broth:

Composition	g L^{-1}
Pancreatic digest of casein	17.00
Papaic digest of soyabean meal	3.00
Sodium chloride	5.00
Dextrose	2.50
Dibasic potassium phosphate	2.50
Final *p*H (at 25°C)	7.3±0.2

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Research Article

Comparative analysis of antioxidant activities and phytochemical properties of some culinary herbs

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Abstract

The present work aimed to evaluate the antioxidant activities as well as phytochemical analysis of leaf extracts of some commonly used leafy spices such as *Murraya koenigii (Mk), Coriandrum sativum (Cs), Trigonella foenum-graecum (Tfg)* and *Mentha* x *piperita (Mp)*. Lyophilised plant extracts (LPEs) were obtained by hot water extraction (HWE) process followed by rotavap and lyophilisation. Among the herbs tested, *Mk* showed the highest antioxidant activity in DPPH scavenging (77.35 % mg^{-1} of LPE), superoxide anion radical scavenging (60.21 % mg^{-1} of LPE) and hydrogen peroxide scavenging (57.21 % mg^{-1} of LPE) model. *Tfg* showed least activity in DPPH scavenging (33.15 % mg^{-1} of LPE) and superoxide anion radical scavenging (25.36 % mg^{-1} of LPE) assay while *Cs* had the least activity in hydrogen peroxide scavenging (43.70 % mg^{-1} of LPE) system. Phytochemical investigations revealed the presence of major primary and secondary metabolites. *Mk* possessed highest amount of phenolics (5.70 mg GAE g^{-1} of LPE), soluble sugars (68.18 mg GLE g^{-1} of FTW) and proteins (69.84 mg BSAE g^{-1} of FTW) and plant pigments (total chlorophyll 6.22 mg g^{-1} of FTW and total carotenoid 0.19 µg g^{-1} of FTW) among the herbs. SDS-PAGE and HPLC finger printing had been performed for analysis of protein patterns and phenolic compounds respectively. In conclusion, addition of culinary herbs and leafy spices that show high to moderate antioxidant activity with an excellent amount of phytochemicals in dietary items would go a long way in assuring human health and wellness as well as enhancement of the disease fighting capacity against oxidative stress related disorders.

Key words: Culinary herbs, Leafy spices, Antioxidant activity, SDS-PAGE, HPLC, Phytochemicals, Human health, Oxidative stress related disorders.

Introduction

Currently, one of the most numerously pronounced keyword related to food-health-disease concept is ROS i.e., Reactive Oxygen Species. ROS chemically includes all those oxygenated free radical (OFR) species exemplified as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$) as well as some non-radical forms (hydrogen peroxide, H_2O_2; hypochlorous acid, HOCl), frequently generated in biological systems by endogenous or exogenous factors. ROS, surprisingly besides playing super roles in energy production, phagocytosis, regulation of cell growth and intercellular signalling, or synthesis of biologically important compounds (Halliwell, 1997), hammer the living systems when produced in excess, resulting in oxidative stress and causing oxidation of cellular biomolecules *viz*. carbohydrates, proteins, lipids and nucleic acids; membrane damage, decreasing membrane fluidity. Such damage in turn leads to several ROS-mediated diseases or disorders such as cancer, acquired immunodeficiency syndrome, malaria, cardiovascular disease, stroke, gastric ulcer, diabetes, malignant tumours, rheumatic joint inflammation, arthritis, cataracts, Parkinson's and Alzheimer's disease, old-age symptoms and aging etc. (Halliwell and Gutteridge, 1984; Maxwell, 1995; Halliwell, 2000; Young and Woodside, 2001; Moskovitz *et al*., 2002; Heinecke, 2003). Scientific reports advocate that these diseases or disorders can be controlled or cured by regular intake of dietary antioxidants (Atoui *et al*., 2005; Alasalvar *et al*., 2005) as antioxidant molecules scavenge free radicals by inhibiting initiation and breaking chain propagation or suppressing the formation of free radicals by binding to the metal ions, quenching hydrogen peroxide and

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superoxide and singlet oxygen (Shi *et al*., 2001). Natural antioxidants present in dietary food-adjuncts or foodstuffs have only recently attracted attention because of their presumed safety concern as well as high therapeutic attributes. Different parts and products of plant such as fruits, vegetables, culinary herbs and spice etc. contain a wide variety of phytochemicals and provide a storehouse of natural antioxidants. Phytochemicals such as carotenoids, phenolic compounds, flavonoids, terpenoids, alkaloids, nitrogen compounds, vitamins, and some enzymes show remarkable antioxidant potentiality.

In most of the contemporary cuisine culinary herbs and leafy spices have been employed to impart diverse flavor, aroma, color and taste to various foods and drinks around the world. Foods and drinks in combination with culinary herbs are full of pharmacological agents; they act as drugs in the body and strengthen body's defense system. Ineffective defense system may lead to a high-risk of various disease developments. Majority of the present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic conditions in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of sedentary lifestyle, or due to the poor scavenging system in the body caused by the depletion of the dietary antioxidants (Schulz *et al.,* 2000; Dringen, 2000). In diet-based therapies, research investigations have been carried out to evaluate the medicinal importance of various culinary herbs and leafy spices. Hence the present study attempts to assess the healing power of culinary herbs and leafy spices in terms of antioxidant potentiality and explore their use in daily lives for the benefits of human health and management of diseases.

Materials and methods

Plant materials and sample collection

Fresh leaves or aerial parts of curry tree, coriander, fenugreek and peppermint were collected from local vegetable super market, Shivmandir (26º42'29.63" N and 88º21'40.52" E) Siliguri, Darjeeling. Plant specimens were identified and authenticated taxonomically by Prof. A.P. Das, Department of Botany, University of North Bengal, Siliguri, WB and Herbarium specimens were prepared (Table 1).

Extraction and preparation of lyophilized extract

For HWE, a modified method after Aliakbarlu and Tajik (2012) was followed. A 10 g of freshly washed and finely chopped leaf samples was extracted for 30 min under darkness by refluxing with HPLC grade deoxygenated water (1:10, w/v) at 100 °C in a temperature controlled water bath shaker with gentle agitation. After cooling, each sample was filtered through Whatman filter paper (Grade 1) and the solid residues obtained were further treated with same procedure twice. The filtrate fractions from every extraction process were pooled and concentrated under reduced pressure at 40 °C in a rotary evaporator equipped with chiller, followed by lyophilisation in a vacuum freeze-dryer to obtain the lyophilized crude extracts. The lyophilised extracts were weighed and re-dissolved in same fluid to prepare stock solutions of desired concentrations and subsequently stored in air tight vials at -20°C until use for analyses.

Determination of water soluble extractive value

The water soluble extractive (WSE) value was expressed in percentage (%) and was determined using the formula: % WSE = ($Weight_{lyophilised\ crude\ extract}$ / $Weight_{initial\ plant\ material}$) X 100. Water-soluble extractive value plays an important role in evaluation of crude plant extracts. It was observed that highest WSE value was in *M. koenigii* (7.73 %), followed by *M. piperita* (5.75 %) and *T. foenum-graecum* (4.96 %), and lowest in *C. sativum* (2.05 %).

Determination of total moisture content

Moisture content of leaf samples was determined using a laboratory oven kept at 105 ± 3 ºC for 24 h. The moisture content (%) was calculated according to AOAC (1975), using the following formula: Total moisture

Table 1. List of the herbs used in the present study along with their reported uses

English common name	Bengali vernacular name	Scientific name	Taxonomic family	Culinary use	Medicinal use
Curry tree	Kari pata	*Murraya koenigii* (L.) Spreng.	Rutaceae	As spice in different food preparations, curry powder, pickle, chutney, sausages and seasonings.	For curing dysentery, vomiting; essential oil from leaves exhibited a strong antibacterial and antifungal activity.
Coriander	Dhonay	*Coriandrum sativum* L.	Apiaceae	As seasoning in curries, salads and soup; a garnish on cooked dishes; in gravies and as green curry paste.	An appetite stimulant; leaves have antibacterial and antifungal properties; useful for headaches, muscle pain, stiffness and arthritis.
Fenugreek	Methi	*Trigonella foenum-graecum* L.	Fabaceae	In spicy soups and stews; as garnish; seasoning in curries, salads and sauses; microgreens used in salads.	Leaf showed anticholesterolemic, anti-inflammatory, antitumor, carminative, expectorant, febrifuge, hypoglycaemic, parasiticide.
Peppermint	Pudina	*Mentha* x *piperita* L.	Lamiaceae	In foods and drinks preparation, confectioneries and sweet liquors, sauces and salads; as garnish and stuffing.	Pain reliever, stimulating, stomachic, carminative, anti-spasmodic, treatment of cholera and diarrhoea.

content (%) = [(Weight$_{initial}$ - Weight$_{final}$)/ Weight$_{initial}$] x 100. The experiment was performed in triplicates (n = 3).

Analysis of antioxidant activities

Determination of total polyphenol content

The total polyphenol content was assayed spectrophotometrically at λ_{750} with FCR using gallic acid as the standard (Taga *et al*., 1984). The total polyphenol content (TPC) was calculated as gallic acid equivalents (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of GAE g^{-1} of LPE. The experiment was performed in triplicates (n = 3).

Determination of total flavonoid content

Total flavonoid content was estimated spectrophotometrically at λ_{420} using the method described by Ordon ez *et al*. (2006). Total flavonoid content (TFC) was calculated as catechin equivalents (CAE) from a calibration curve of (+)-catechin standard solutions and expressed as mg of CAE g^{-1} of LPE. The experiment was performed in triplicates (n = 3).

DPPH• free radical scavenging activity

The DPPH• scavenging activity was monitored at λ_{517} using the method of Yen & Duh (1994), with slight changes. Free radical scavenging (FRS) activity expressed as percentage

inhibition (% I) of the DPPH• radical was calculated according to the formula given by Viuda-Martos *et al*. (2010): FRS activity (% I) = $[(A_c - A_s)/ A_c] \times 100$, where A_c refers to the absorbance of control (t = 0 min) and A_s is the absorbance of sample plus DPPH• (t = 30 min). The experiment was performed in triplicates ($n = 3$).

Superoxide anion scavenging activity

The superoxide anion radicals ($O_2^{\cdot -}$) scavenging activity was determined according to the method described by Nishikimi *et al.* (1972). Percentage of $O_2^{\cdot -}$ scavenged at λ_{560} was measured using the formula: Superoxide anion scavenging (SAS) activity (% I) = $[(A_0 - A_s)/A_0] \times 100$, where A_0 was the absorbance of control, and A_s was the absorbance of sample extract at λ_{560}. The experiment was performed in triplicates ($n = 3$).

Hydrogen peroxide scavenging activity

The hydrogen peroxide (H_2O_2) scavenging activity was carried out following the procedure of Ruch *et al*. (1989). The percentage of H_2O_2 scavenging at λ_{230} by the extracts and standard were calculated using the following equation: H_2O_2 scavenging (HPS) activity (%) = $[(A_c - A_s) / A_c] \times 100$, where A_c was the absorbance of control and A_s was the absorbance of test sample at λ_{230}. The experiment was performed in triplicates ($n = 3$).

Phytochemical screening of extracts

Qualitative analysis of phytochemicals

Phytochemical screening to detect the presence or absence of some significant phytochemicals *viz.* phenols, flavonoids, tannins, alkaloids, cardiac glycosides, saponins, terpenes, steroid, etc. were performed according to standard methods (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993). The tests were based on the visual observation of colour change, chomophor formation or formation of a precipitation after addition of specific reagents or treatments.

Quantitative analysis of phytochemicals

Estimation of total soluble sugar content

For the estimation of total soluble sugar content, Anthrone's method as described by Plummer (1978) was followed. Total soluble sugar (TSS) content was calculated from a D-glucose calibration curve and results were expressed as mg of glucose equivalents (GLE) g^{-1} of fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total soluble protein content

Total soluble protein was extracted using the standard protocol given by Chakraborty *et al.* (1995) and quantification was done according to Lowry *et al.* (1951) using BSA as standard. Total soluble protein (TSP) content was calculated as BSA equivalents (BSAE) from a calibration curve of BSA and expressed as mg of BSAE g^{-1} of fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total carotenoid content

Carotenoids were extracted and estimated according to the method given by Lichtenthaler (1987). Absorbances of the sample were observed spectrophotometrically at λ_{645}, λ_{663} and λ_{480} and the total carotenoid content (TCR) was calculated by using the formula: TCR = $A_{480} - (0.114\ A_{663} - 0.638\ A_{645})$ µg g^{-1} fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

Estimation of total chlorophyll content

Chlorophyll was extracted according to the method of Harbone (1998). Total chlorophyll content was estimated by observing the absorbance at 645 nm and 663 nm and calculated by the formula: total chlorophyll content (TCL) = $(20.2\ A_{645} + 8.02\ A_{663})$ mg g^{-1} fresh tissue weight (FTW). The experiment was performed in triplicates ($n = 3$).

SDS-PAGE analysis for protein pattern

To analyze the protein patterns of the samples, SDS-PAGE was performed on 10% resolving gels, as described by Sambrook *et al*. (1989).

HPLC fingerprint analysis for Phenolic compounds

For HPLC fingerprint analysis of phenolic compounds present in leaf extracts, a Shimadzu system (Shimadzu Corp., Kyoto Japan) was used. A flow rate of 1 ml min^{-1}, and gradient elution of acetonitrile-water-acetic acid (10:86:4, v/v/v) (solvent A) and of acetonitrile-water-acetic acid (80:16:4, v/v/v) (solvent B). 0-50 min solvent B from 0-100%; and injection volume of 20 µl were applied; whereas the separation of compounds was monitored at 280 nm and 320 nm (Pari and Latha, 2004).

Table 2. Qualitative detection of phytochemicals

Phytochemical category	*M. koenigii*	*C. sativum*	*T. foenum-graecum*	*M. piperita*
Reducing sugars	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	-	+
Alkaloids	+	+	+	-
Cardiac glycosides	+	+	+	+
Saponins	-	-	+	+
Terpenes	-	-	-	-
Steroids	+	+	+	-
Anthraquinones	+	-	+	-
Vitamin C	+	+	+	+

+ = present, - = absent

Statistical analysis of data

Experimental analyses were carried out in triplicate (n=3) and data ware expressed as mean ± standard deviation (SD). Statistical analysis was carried out by SPSS software (IBM SPSS, USA). One-way analysis of variance was performed by ANOVA procedures.

Result and Discussion

Because of their enchanting flavoral and toptastic qualities, culinary herbs and leafy spices have always been prized to Indian cuisine. Besides, these botanicals are an excellent source of versatile phytochemicals which have been reported to show good antioxidant activity. Natural antioxidants present in culinary herbs and leafy spices are responsible for inhibiting or preventing the deadly effects of oxidative stress. Herbs and spices contain free radical scavengers like polyphenols, phenolic acids and flavonoids. Polyphenols form a complex group of molecules associated with the cell walls of most plant species. This group of compounds ranges from simple phenolic acids (e.g., caffeic acid) to high-molecular-weight tannins. Polyphenols have various applications, such as in the production of paints, paper, cosmetics, as tanning agents, and as natural colorants and preservatives in the food industry. In addition, some phenolic compounds are antibiotics and anti-diarrheal, antiulcer, and anti-inflammatory agents and can be used in the treatment of diseases such as hypertension, vascular fragility, allergies, and hypercholesterolemia (Bravo, 1998; Higdon and Frei, 2003).

Preliminary phytochemical study of the extracts from *M. koenigii, C. sativum, T. foenum-graecum* and *M. piperita* showed the presence of major plant secondary metabolites *viz*. reducing sugars, phenolic compounds, flavonoids, cardiac glycosides and vitamin C, etc. but tannins, alkaloids, saponins, steroids and anthraquinones occurred in some of them (Table 2). Such secondary metabolites contribute significantly towards the biological activities of plant extracts such as antioxidant, antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory activities etc. (Negi *et al*., 2011).

In vitro antioxidant activity, principally, can be determined by hydrogen atom transfer (HAT) method and single electron transfer (SET) or electron transfer (ET) method (Joon & Shibamoto, 2009). HAT based methods measure the ability of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET based methods detect the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals (Prior *et al*., 2005; Huang *et al*., 2005). Superoxide anion scavenging (SAS) assay, hydrogen peroxide

scavenging (HPS) assay, etc. involve HAT method, and the assays of total polyphenolic content (TPC), total flavonoid content (TFC) etc. are of ET method, while DPPH• assay include both the method predominantly via SET method (Karadag *et al*., 2009; Badarinath *et al*., 2010). The relatively stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, medicinal plants and fruit extracts and food materials (Purohit *et al*., 2005). Superoxide anion is a weak oxidant that gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Superoxide anion scavenging activity is correlated to the total flavonoids (Thaipong *et al*., 2006). Hydrogen Peroxide radical scavenging activity is correlated to the presence of phenolic compounds. Generally, extracts that contain a high amount of phenolic compounds exhibit high antioxidant activity.

Antioxidant activity in terms of scavenging potentiality of hot water extracts of *M. koenigii*, *C. sativum*, *T. foenum-graecum* and *M. piperita* have been evaluated. All of them could act as potential radical scavengers in a concentration oriented fashion (Fig. 1). Interestingly *M. koenigii* showed the highest antioxidant activities scoring 77.354 % mg^{-1} of LPE in DPPH scavenging, 60.205 % mg^{-1} of LPE in superoxide anion radical scavenging and 57.209 % mg^{-1} of LPE in hydrogen peroxide scavenging assay, followed by *M. piperita.* Extract from *T. foenum-graecum* showed least scavenging activity in DPPH scavenging (33.145 % mg^{-1} of LPE) and superoxide anion radical scavenging (25.364 % mg^{-1} of LPE) system while *C. sativum* had the least activity in hydrogen peroxide scavenging assay (43.695 % mg^{-1} of LPE) .

The quantitative estimation of total moisture content, total soluble sugar, total soluble protein contents, total chlorophyll content, total carotenoid content present in the herb samples have been depicted in Table 3. Total moisture content among the herb samples was found in a range of 89% to 70%.The total soluble sugar content of was found highest in *M. koenigii* (68.18 mg GLE g^{-1} of FTW) and lowest in *T. foenum-graecum* (53.17 mg GLE g^{-1} of FTW), while content of

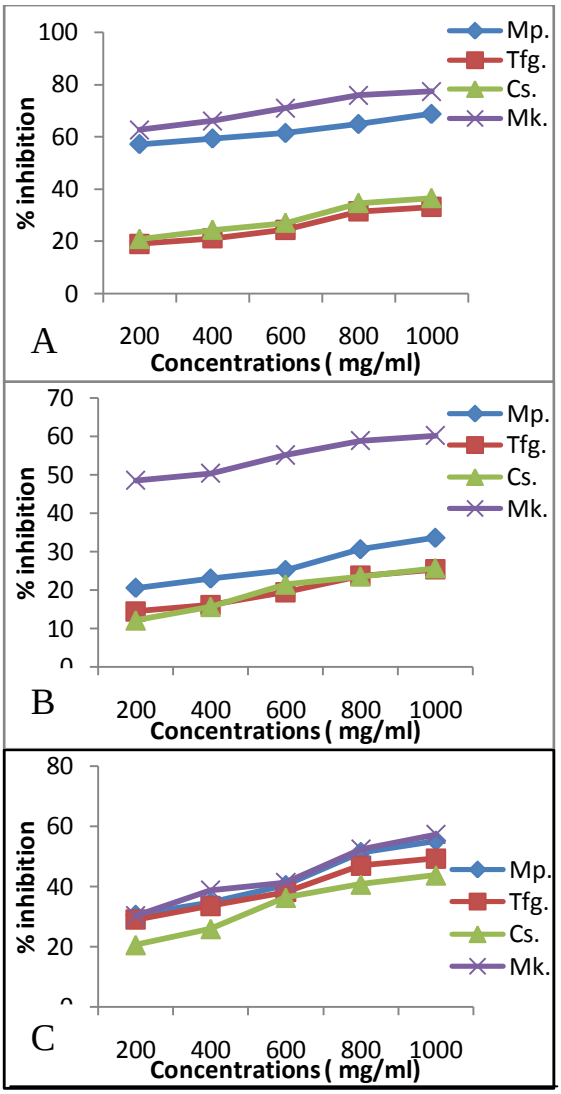

Fig. 1. Scavenging activity of the herb extracts. A. DPPH scavenging activity, B. Superoxide anion scavenging activity, and C. Hydrogen peroxide scavenging activity.

soluble protein was highest in *M. koenigii* (69.507 mg BSAE g^{-1} of FTW) and lowest in *C. sativum* (35.027 mg BSAE g^{-1} of FTW). Pigment analysis revealed that *M. koenigii* contained highest amount of total chlorophyll (6.223 mg g^{-1} of FTW) and carotenoid (0.190 µg g^{-1} of FTW) content. *T. foenum-graecum* and *M. piperita* showed the lowest chlorophyll content (1.639 mg g^{-1} of FTW) and lowest carotenoid content (0.063 µg g^{-1} of FTW) respectively. Garg *et al*., 2012 also reported that chlorophyll content was higher in curry

leaves due to the darker shade of green, then the coriander.

Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl (Khalaf *et al*., 2008). Carotenoids that include xanthophylls and carotenes have the ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. Dietary carotenoids are thought to provide health benefits due to their role as antioxidant molecules. SDS-PAGE analysis of proteins from the four herbs revealed the presence of a large number of bands in all cases, but not much difference was obtained among the herbs (Fig. 2).

The study also revealed that among the herbs *M. koenigii* ranked highest and *C. sativum* was lowest in total phenolic content and total flavonoid content (Table 4).Total phenolic content and total flavonoid content in extracts of *M. koenigii* was found as 5.70 mg GAE g^{-1} of LPE and 1.68 mg CAE g^{-1} of LPE. Leaf extract from *C. sativum* contained 2.55 mg GAE g^{-1} of LPE as total phenolic content and 0.66 mg CAE g^{-1} of LPE as total flavonoid content. HPLC analysis of the phenols from all four herbs was carried out and results are shown in fig. 3. Among the four, maximum number of peaks was obtained in *M.koenigii* and the least in *M.piperita*. The health benefit of phenolics is directly linked to their antioxidant potentiality. Phenolics act as effective antioxidants is mainly due to their redox properties, which allow them to behave as reducing agents, hydrogen donors, and singlet oxygen quenchers. The potential hazard from oxidative stress in the body may be compensated through the consumption of a diet exclusively rich in antioxidant phenolics including polyphenols, phenolic acids and

Table 3. Nutritional and pigment contents of the herbs

List of herbs	Total moisture content[a]	Total soluble sugar content[b]	Total soluble protein content[c]	Total chlorophyll content[d]	Total carotenoid content[e]
M. koenigii	78.83 ± 0.16	68.18 ± 0.87	69.84 ± 0.39	6.22 ± 0.37	0.19 ± 0.01
C. sativum	82.11 ± 0.67	62.55 ± 0.65	35.00 ± 0.45	2.336 ± 0.10	0.12 ± 0.00
T. foenum-graecum	70.41 ± 0.72	54.17 ± 0.57	35.03 ± 0.53	1.639 ± 0.09	0.07 ± 0.00
M. piperita	89.56 ± 0.50	56.42 ± 0.07	45.53 ± 0.61	2.040 ± 0.02	0.06 ± 0.00

Content expressed as mean ± SD in a. percentage, b. mg GLE g^{-1} of FTW, c. mg BSAE g^{-1} of FTW, d. mg g^{-1} of FTW, e. µg g^{-1} of FTW. Fresh tissue weight is abbreviated as FTW.

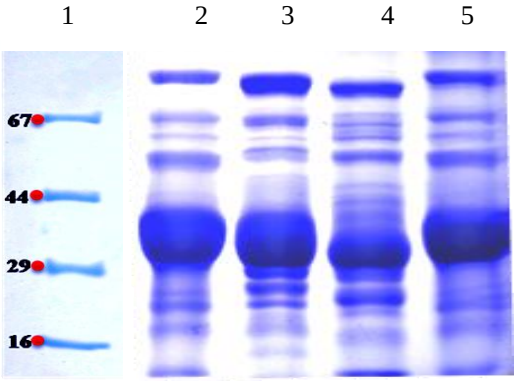

Fig. 2. SDS-PAGE analysis of protein of the herbs. Lane 1- Molecular weight markers in KDa; 2 - Tfg; 3- Mp; 4- Cs;5-Mk

Table 4. Total phenol and flavonoid contents[#].

Herb samples	TPC	TFC
M. koenigii	5.70 ± 0.20	1.68 ± 0.26
C. sativum	2.55 ± 0.05	0.66 ± 0.12
T. foenum-graecum	3.45 ± 0.10	1.33 ± 0.18
M. piperita	5.06 ± 0.01	1.75 ± 0.18

[#]Content expressed as mean ± SD, TPC as mg GAE g^{-1} of LPE and TFC as mg CAE g^{-1} of LPE.

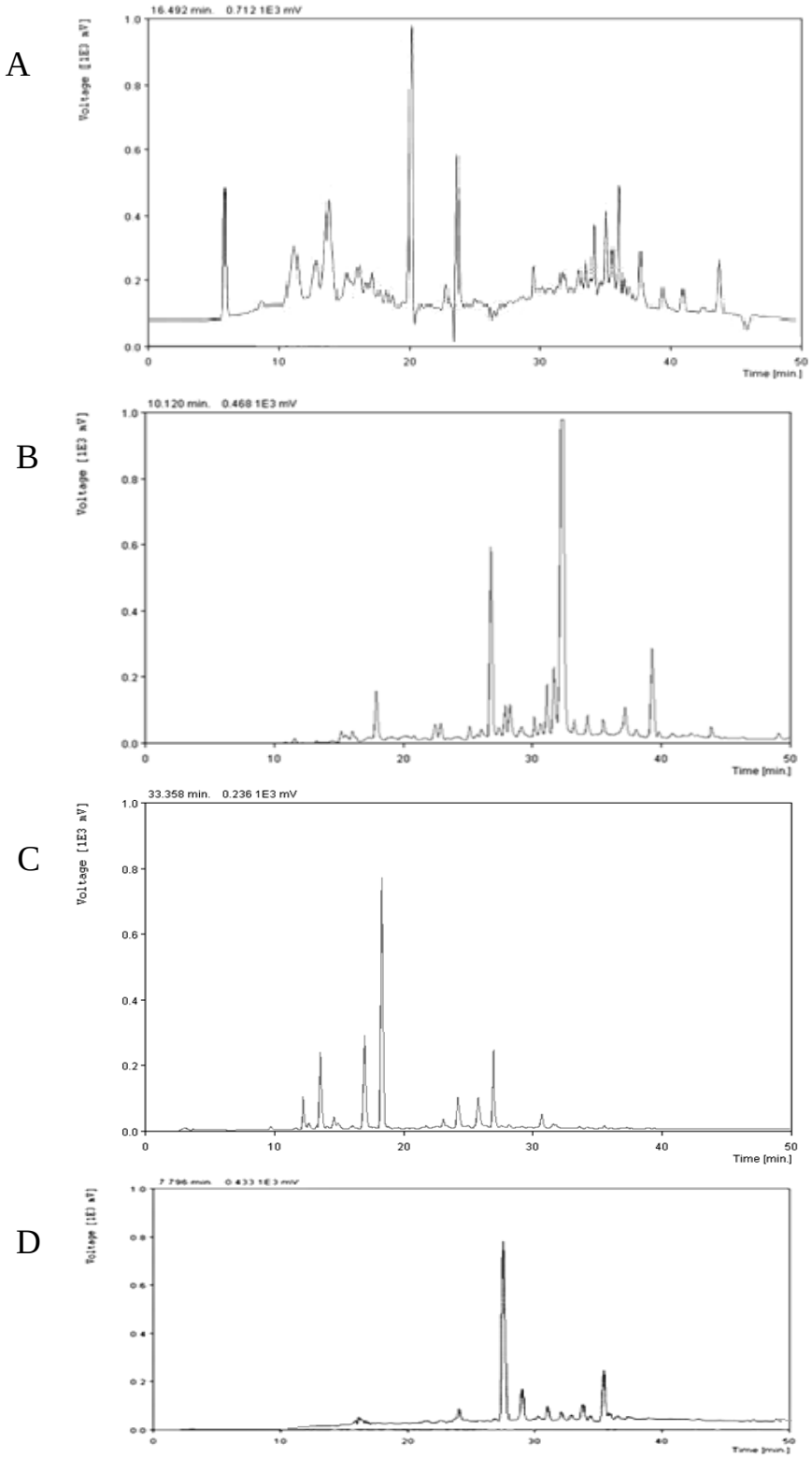

Fig. 3. HPLC chromatogram of phenolic compounds of herb extracts. A. *M. koenigii* B. *C. sativum* , C. *T. foenum-graecum* and D. *M. piperita*.

flavonoids. According to Scalbert and Williamson (2000) the amount of total human intake of phenolic compounds is about 1 g day^{-1} consisting two-thirds of flavonoids and one-thirds of phenolic acids.

Conclusion

Recently much attention has been focused on the analysis of dietary foods and food components. Food beyond its basic nutritional values has played some functional effects on prevention of diseases and maintenance of good health. Functional foods as the concept "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Thomas and Earl, 1994) are the centre of attraction for searching newer well-accepted functional foods from culinary herbs and leafy spices. As culinary herbs and leafy spices are full of variety in bioactive phytochemicals including antioxidant molecules and nutricomponents they can be incorporated as functional foods in our everyday's diets to rejuvenate ourselves.

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