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 19^{th} 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-20^{th} 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).

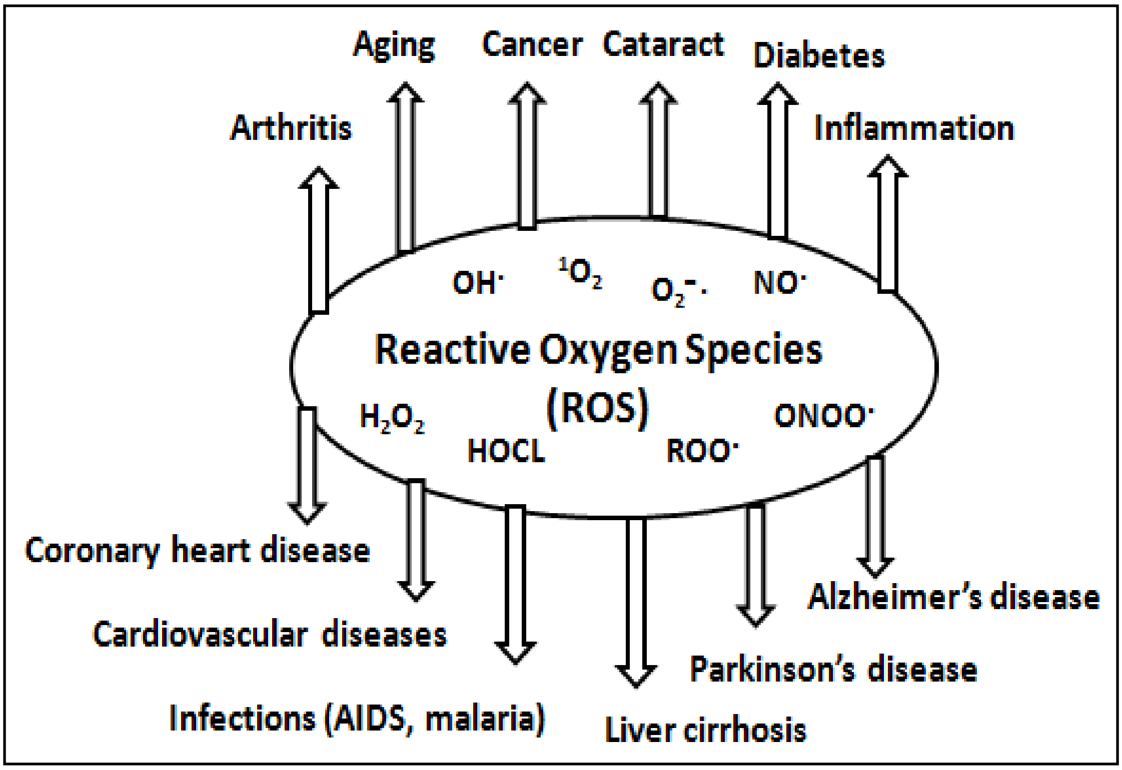

Figure 1. Diseases and disorders caused by reactive oxygen species, ROS. Here, hydroxyl free radical ($OH^•$), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{-•}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), peroxyl radicals ($ROO^•$), peroxynitrite ($ONOO^•$), andnitric oxide radical ($NO^•$). 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).

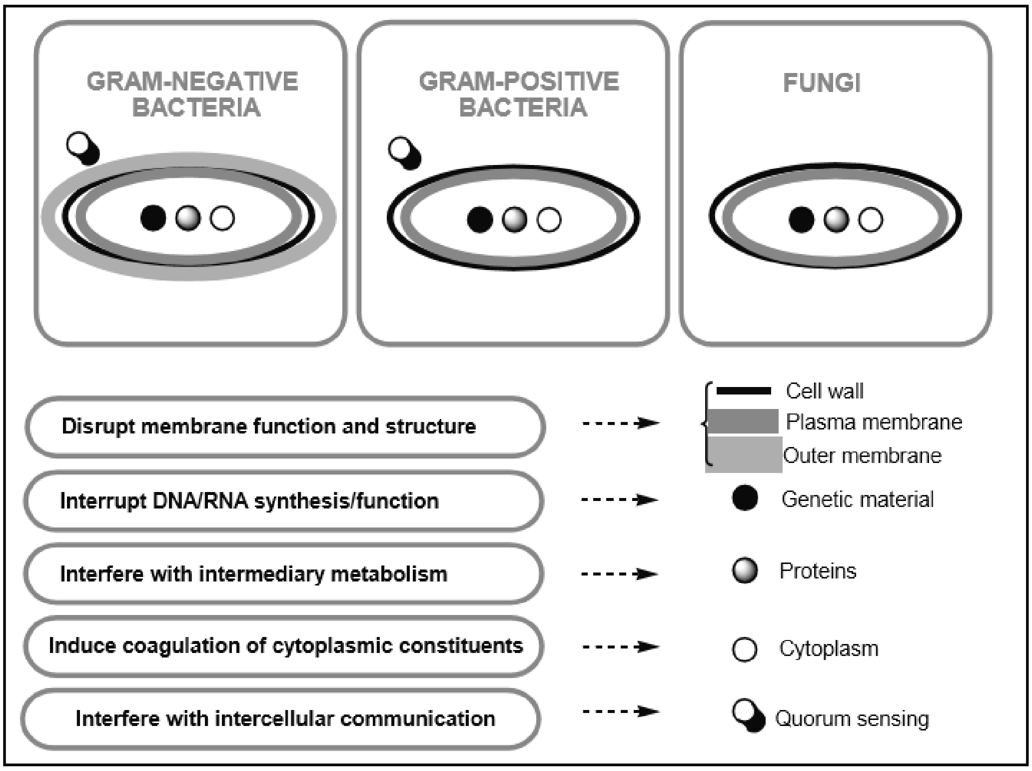

Figure 2. Mechanism of action of plant-derived antimicrobials on possible targets (Adapted from Radulović *et al.*, 2013).

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 ≤ 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.