CHAPTER 5

DISCUSSION

5. Discussion

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

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

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

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

As an important source of antioxidants, herbs and spices are considered to have a great potential as food preservatives (Hinneburg *et al*., 2006; Szabo *et al*., 2010). For example, the extract obtained from a mixture of culinary herbs namely basil, lovage, milfoil, oregano, rosemary, marjoram and thyme has proven to be potential food preservative which may replace synthetic antioxidants in some food products (Szabo *et al*., 2010). Unfortunately, strong aroma of natural herb-derived antioxidants may restrict their use to some extent and duration ofantioxidant activity may be limited to less than two years (Szabo *et al*., 2010).

In the present investigation effort has been made to evaluate twelve commonly used herb and spice samples collected from various areas of North Bengal region, for the presence of diversified phytochemicals along with their biological activities like antioxidant, antimicrobial and antidiabetic through *in vitro* and *in vivo* test models. In the present study, plant samples were cleaned, dried and powdered and extracted with hot water and methanol, followed by lyophilization to obtain lyophilized aqueous and methanolic extracts respectively. The phytochemical screening of the sample extracts of *M. piperita*, *T. foenum-graecum*, *C. sativum*, *M. koenigii*, *G. oppositifolius, F. vulgare, I. verum*, *M. fragrans*, *C. pentandra*, *C. annuum*, *P. perlata* and *D. volubilis* revealed the presence of different bioactive phytochemicals. In most of the cases, the presence of different phytochemicals was more prominent in methanolic extract than in the hot aqueous extract. The crude plant extracts contain a mixture of various active phytocompounds which may act synergistically and their overall biological activity is supposed to be usually superiortothe individual compounds.Preliminary phytochemical study of the herb and spiceextracts from *M. koenigii*, *C. sativum, T. foenum-graecum, M. piperita, G. oppositifolius*,*F. vulgare*,*I. verum*, *M. fragrans*, *C. pentandra*, *C. annuum*, *P. perlata* and *D. volubilis*showed the presence of major plant secondary metabolites *viz*. reducing sugars, phenolic compounds, flavonoids and cardiac glycosides, etc. but tannins, alkaloids, saponins, steroids and anthraquinones occurred in some of them. Some of the phytochemicals were present in both hot aquous and methanolic extracts. The present study revealed the presence of secondary metabolites considered as medicinally active phytochemical constituents. Most important phytochemicals such as reducing sugars, phenols, flavonoids and free amino acids were present in all the plant samples. The result of the phytochemical analysis showed that *T. foenum-graecum*, *C. sativum*, *M. koenigii*, *G. oppositifolius, F. vulgare* are rich in at least one of alkaloids, flavonoids, terpenoids, reducing sugars and phlobatannins. Saponins and cardiac glycosides were absent in the extracts of *C. sativum* and *T. foenum-graecum* respectively. Phlobatannins were absent in all the hot aqueous extracts of herb samples, except *G. oppositifolius*, but phlobatannins were present in methanolic extracts of *T. foenum-graecum*, *G. oppositifolius* and *F. vulgare*. In case of cardenolides, hot aqueous extracts of all the herb species did not show the positive result, except methanolic extracts of *G. oppositifolius* and *F. vulgare*. On the other hand, Resins was absent in the extracts of *I. verum* and *P. perlata*. In *I. verum*, *C. pentandra* and *P. perlata* anthraquinone was not ditected. Triterpenoid was absent in *M. fragrans* among the spice samples. Cardiac glycoside was also in some spice extracts such as *I. verum* and *C. pentandra*. Further in case of spice extracts, cardenolides and phlobatannins were not detected in all the samples, except *D. volubilis*. Methanolic extracts of *P. perlata* showed positive result of phlobatannin test.Such secondary metabolites contribute significantly towards the biological activities of plant extracts such as antioxidant, antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory activities etc. (Negi *et al*., 2011). Studies by Sohail *et al*., 2016 revealed the presence of reducing sugars in *Syzygium cumini, Mentha arvensis, Trigonella foecum-graecum* and *Ocimum tenuiflorum*.In addition, Singh *et al*., 2015alsoreported that leaf extracts of *Mentha piperita* which contained phenols, flavonoids, terpenoids, steroids and tannins showed remarkable antioxidant potential.

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

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

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

Extraction of different phytochemicals from plant species and biological activities of the extracts aresignificantly influenced by various factors including solvent types, solvent concentration, extraction methods used, extracting time, geographical origin of plant materials, harvest time, drying methods, storage conditions and rout extract (Cervenka *et al*., 2006, Bernard *et al*., 2014).

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

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

In vitro antioxidant activity, principally,can be determined by hydrogen atom transfer (HAT) method and single electron transfer (SET) or electron transfer (ET) method (Joon and Shibamoto, 2009).HAT based methods measure the ability of an antioxidant to scavenge free radical by hydrogen donation to form a stable compound. SET based methods detect the ability of the antioxidant to transfer one electron to reduce compound including metals, carbonyls and radicals (Prior *et al*., 2005, Huang *et al*., 2005). Superoxide anion scavenging (SAS) assay, hydrogen peroxide scavenging (HPS) assay, etc. involve HAT method, and the assays of total polyphenolic content (TPC), total flavonoid content (TFC) etc. are of ET method, while DPPH• assay include both the method predominantly via SET method (Karadag *et al*., 2009; *Badarinath et al*., 2010). The relatively stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, medicinal herb and fruit extracts and food materials (Purohit *et al*., 2005). Superoxide anion appears as a weak oxidant that generates powerful and harmful hydroxyl radicals as well as dangerous singlet oxygen, both of which contribute to the oxidative stress.Superoxide anion scavenging activity is correlated to the total flavonoids (Thaipong *et al*., 2006). Hydrogen Peroxide radical scavenging activity is correlated to the presence of phenolic compounds. Generally, extracts that contain a high amount of phenolic compounds exhibit high antioxidant activity.

Antioxidant activity in terms of scavenging potentiality of hot water extracts of *M. koenigii*, *C. sativum*, *T. foenum-graecum* and *M. piperita* have been evaluated. All of them could act as potential radical scavengers in a concentration oriented fashion. Interestingly *M. koenigii* showed the highest antioxidant activities scoring 77.354 % mg^{-1} of lyophilized extract in DPPH scavenging, 60.205 % mg^{-1} of lyophilized extract in superoxide anion radical scavenging and 57.209 % mg^{-1} of lyophilized extract in hydrogen peroxide scavenging assay, followed by *M. piperita.* Extract from *T. foenum-graecum*showed least scavenging activity in DPPH scavenging (33.145 % mg^{-1} of lyophilized extract) and superoxide anion radical scavenging (25.364 % mg^{-1} of lyophilized extract) system while *C. sativum*had the least activity in hydrogen peroxide scavenging assay (43.695 % mg^{-1} of lyophilized extract).The DPPH· activity of *D. volubilis* stem bark extracts was estimated as 70.70% and 61.35% for methanolic extract and hot water extract respectively. Superoxide anion radical scavenging activity of methanolic extract of *D. volubilis* stem bark was observed 59.63% and that of hot water extract was 56.62%. Hydroxyl radical scavenging potentiality was measured as 63.41% for methanolic extract and 53.50% for hot water extract, whereas hydrogen peroxide scavenging efficiency was found to 61.48% for methanolic extract and 56.84% for hot water extract. The results showed that the scavenging activity of methanolic extract showed significantly higher than the hot water extract. It may be of the reason that methanol have higher polarity than water that enable it to extract greater amount of phytochemicals. However, the radical scavenging potentiality for both the extracts at different concentrations was quite low as compared to standard reference, L-ascorbic acid (AsA). A study on methanolic fruit extract of *D. volubilis* showed scavenging efficiency of 84.81% and 89.92% in DPPH· and superoxide anion scavenging assays (Biswas *et al*., 2010). Results revealed that stem showed comparatively less efficiency than fruits of the plant due to accumulation of lesser amount of secondary metabolites in that part of plants.In a research by Dragland *et al*. (2003) dried greenhouse herbs containing the highest levels of antioxidants were arranged in higher to lower concentrations as oregano > sage > peppermint > thyme > lemon balm. But commercially dried spices showed a different order of sequence: rosemary and thyme having higher antioxidant content than oregano, and total antioxidant concentrations were mostly lower than those of dried culinary herbs due to different varieties used as well as different drying methods and storage conditions.

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

Phenolic compounds are ubiquitously present in all the plants and plant parts at varying concentrations and contribute immensely towards the medicinal properties of the plants. Plant phenolics are effective vasodilators, help in reducing inflammation and serve as anticancerous, antioxidant, antidiabetic and antimutagenic agents. Further, its preventive role in various neurodegenerative diseases are encouraging and exploring (Kusirisin *et al*., 2009; Zhang *et al*., 2011; Mohanlal *et al*., 2013). Despite the beneficial health effects of phenolics, it is only in the recent years that enormous attention has been paid towards the role of phenolics (Manach *et al.,* 2004). Currently, structures of more than 8000 plant phenolics have been elucidated.

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

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

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

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

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

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

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

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

Differences in the phenolic content between the intraspecific and/or interspecific samples studied by different workers may be attributed to the method used for determination. Presence of diverse natural phenolics in the plant material and other oxidized substrates prevalent in the extract may interfere in the measurement of total phenols either by inhibiting, enhancing or adding the content (Singleton and Rossi, 1965; Singleton *et al.*, 1999).

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

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

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

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

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

The antibacterial activity of different herb and spice extracts was studied against two Gram-positive and two Gram-negative bacterial strains. The Gram-positive organisms include *B. cereu* and *B. pumilus* whereas Gram-negative strains are *S. marcescens* field isolate and *Ps. aeruginosa*. Different concentrations or dose of lyophilized methanolic extracts *i.e*, 4-10 mg disc^{-1}were used to screen the antimicrobial activity. Extract of different plant samples investigated so far inhibited the growth of the microorganisms in a dose dependent manner for each extract and was evident with the formation varying diameter of inhibition zones. Parekh and Chanda (2007) reported that methanol was a better solvent for the extraction of antimicrobial compounds from medicinal and aromatic plants compared to other solvents such as water, ethanol and hexane. Our investigation also supported this conclusion. Therefore methanol was used for phytochemical extraction in our study as well as antimicrobial and anti-quorum sensing activities were quantitatively assessed by the presence or absence of zone of inhibition. Among the herb extracts, *M. piperita* and *T. foenum-graecum* was found in inhibiting *B. cereus* and *B. pumilus.* Rest of the samples (*C. sativum*, *M. koenigii*, *G. oppositifolius* and *F. vulgare*) did not show any antimicrobial activity. Chloramphenicol (C_{25} µg), Kanamycin (K_{30} µg) and Ampicillin (A_{25} µg) exhibit inhibitory activity against specific organisms, not all the strains of microbial species. *B. cereus* and *B. pumilus* were inhibited only by the *M. piperita* and *T. foenum-graecum* extracts at higher doses. No inhibition was observed by the other herbal extracts and against other test organisms. The MID value of *M. piperita* extract against *B. cereus* and *B. pumilus* was found to be 8.5 and 5.5 mg lyophilized methanolic extract disc^{-1} respectively. The MID value of *T. foenum-graecum* extracts against *B. cereus* and *B. pumilus* was determined as 3.5 and 7.5 mg lyophilized methanolic extract disc^{-1} respectively. The MID values of other herb extracts against respective organisms were found to be >10 mg lyophilized methanolic extract disc^{-1}. Among the spice extracts, extract of *I. verum* was found to be most potent showing highest zone of inhibition against all test organisms, whereas *D. volubilis* did not show antibacterial activity against microorganisms except *B. cereus*. Several studies have been executed to confirm the antimicrobial activity of the major groups of chemical compounds identified from different medicinal plants (Jamuna Bai *et al.*, 2011; Bibi *et al.*, 2011). Chouksey *et al*. (2010) and Huang *et al.* (2010) also reported the antimicrobial properties of *I. verum*. Methanolic extract of dry fruit of *I. verum* had antibacterial activity against *E. coli, B. cereus* and *S. aureus* (Shan *et al*., 2007). The present study showed that methanolic extract of fruits of *I. verum* inhibited the growth of *B. cereu,B. pumilus, S. marcescens* and *Ps. aeruginosa*. The MID value of *I. verum* extract against *B. cereus, B. pumilus, S. marcescens* and *Ps. aeruginosa* was found to be 1.25, 2.5, 3.5 and 1.5 mg lyophilized methanolic extract disc^{-1} respectively.

Our findings suggest that Gram positive bacterial strains are susceptible to the plant extracts than Gram negative bacterial strains. These results were consistent with previous reports shown by other herbs and spices (Ceylan *et al.*, 2004; Lopez *et al.*, 2005). A possible explanation of the fact lies in the structural differences in Gram positive and Gram negative bacteria. Generally Gram negative bacteria have an outer membrane (LPS) and a periplasmic space not common in Gram positive bacteria (Duffy and Power, 2001). The drug resistance of the Gram negative bacteria towards commercial antimicrobials is related to the lipopolysaccharide outer membrane which is hydrophilic in nature and serving as a barrier to the cellular permeability of antibiotic molecules and is also associated with the enzymes present in the periplasmic space, which are capable of degrading antibacterial drugs directly introduced from outside (Gao *et al*., 1999). On the other hand, Gram positive bacteria do not possess such a lipopolysaccharide outer membrane and cell wall structure. Antimicrobial substances can easily disrupt the bacterial cell wall composition through enzymatic degradation and leakage of the cytoplasmic components and its coagulation (Kalemba and Kunicka, 2003).

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

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

In our study, preliminary screening of twelveplant species of herb and spice was performed to investigate their ability to inhibit synthesis of violacein production in *C. violaceum*. Studies based on the anti-QS activity of plants by inhibition of violacein production highlightedonly the ability to affect short acyl-HSLs in *C. violaceum* (Zahin *et al*. 2010). In this study, the focus was to screen the anti-QS activity and anti-biofilm ability of plant extracts to explore the possibility of finding new scope for preventing or treating biofilm-associated infectious diseases.

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

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

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

The present study also shows that methanolic extracts of *I. verum* and *G. oppositifolius* at the doses of 250 and 500 mg kg^{-1} BW are able to produce a consistent reduction in blood glucose, serum cholesterol and serum triglyceride. The extracts have also shown presence of active constituents responsible for various biological activities.From our findings, it is suggested that the methanolic extract of *I. verum* and *G. oppositifolius* can be chosen as primary antihyperglycemic, antihyperlipidemic and antioxidant supplement. Further, clinical evaluation will throw more light on clinical usefulness, safety, and efficacy of these plant extracts. Methanolic extract of *G. oppositifolius* whole plant at single oral doses 200 and 400 mg kg^{-1} have shown significant antidiabetic activity in glucose-overloaded hyperglycemic mice (Hoque *et al*., 2011) and rats (Behera *et al*., 2010; Panigrahi *et al*., 2012) compared to the standard antidiabetic drug metformin and glibenclamide treatment, respectively. Studies by Sahu *et al*. (2012) have exhibited that ethanolic extract at 200 and 400 mg kg^{-1}, *p.o*. of the aerial parts of *G. oppositifolius* produced significant reduction in the blood glucose level compared with the controls and glibenclamide treatment in alloxan-induced Wistar Albino diabetic rats. Aqueous and methanolic extracts of *G. oppositifolius* were found to have significant anti-hyperlipidemic activity in Streptozotocin-induced diabetic rats when treated with 200 and 400 mg kg^{-1} for 14 days and 28 days (Behera *et al*., 2010). The methanolic extract of *G. oppositifolius* was tested for anti-hyperlipidemic activity in Triton-induced hyperlipidemic rats at 200 and 400 mg kg^{-1} dose. Such a treatment exhibited a significant reduction in serum lipid profile like triglycerides, total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and increase in high-density lipoprotein (HDL) in hyperlipidemic rats compared to the controls (Panigrahi *et al*., 2012). Our results supported this observation. *G. oppositifolius* is reported to be a good source of carbohydrates, polysaccharides, phenols, flavonoids, alkaloids, saponins, steroids and several other aromatic compounds. In previous studies, triterpenoidal saponins were reported to act as hypoglycemic and hypolipidemic agents (Behera *et al*., 2010; Hoque *et al*., 2011; Panigrahi *et al*., 2012; Sahu *et al*., 2012). It is established that inhibition of α-glucosidase enzyme plays a vital role in the management of hyperglycaemic conditions, as it delays the digestion process of carbohydrates. Kumar *et al*., 2013 reported that chemicals like hopanoid triterpene saponins from *G. oppositifolius* were observed to exert α-glucosidase inhibitiory activity. The plausible mechanism, by which the plant extracts and/or phytochemicals lowered the blood glucose levels in experimentally induced diabetic rats, may be by inhibiting the absorption of glucose from the intestinal cells, inhibiting gluconeogenesis in the liver cells, increasing the glycogenesis process or stimulation of the insulin secretion (Mowla *et al*., 2009).

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

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