CHAPTER V
DISCUSSION

In recent times, there has been a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines. However, these diseases still remain a leading cause of global disease burden with high morbidity and mortality, especially in the developing world. Furthermore, there have been threats of new diseases during the past three decades due to the evolution and adaptation of microbes and the re-emergence of old diseases due to the development of antimicrobial resistance and the capacity to spread to new geographic areas. The impact of the emerging and re-emerging diseases in India has been tremendous at the socioeconomic and public health levels. Their control requires continuous surveillance, research and training, better diagnostic facilities and improved public health system. Emerging and re-emerging zoonotic diseases, food and water-borne diseases and diseases caused by multiresistant organisms constitute the major threats in India (Abu-Shanab et al., 2004; Chugh, 2008).

More than two-thirds of the antibiotics used to treat human diseases are natural products or semisynthetic derivatives of these molecules. Advances in genetics, biochemistry and bioinformatics have transformed the study of antibiotics and other natural products, not just by revealing how they are synthesized but also by casting them as phenotypes encoded by genes. The World Health Organization (WHO) has estimated that approximately 80% of the world’s inhabitants rely mainly on traditional medicines for their primary healthcare (Akinnibosun et al., 2009).
The use of data on traditional medicine provides a very valuable short cut by indicating plants with specific folk medicinal uses, which might be likely sources of biologically active compounds. Recent investigations of medicinal plants used in traditional medicine have led to the discovery of many new drugs and hundreds of pharmacologically active substances for synthetic modifications (Wang, 2008). With the advent of modern tools and experimental methodologies, plant products are subjected to exhaustive screening by using various models to find out their potential under various conditions (Balick and Cox, 1996; Akobundu and Agyakwa, 1998).

*Hyptis suaveolens* locally known as ‘Kattu Tulsi’ was selected to screen its phytochemical compounds and to investigate its antioxidant, antihaemolytic, antidiabetic, anticancer, larvicidal and antimicrobial activity. The study experimentally confirms the potent bioactive compounds present in the plant.

5.1. Phytochemistry
5.1.1. Preliminary phytochemical screening

To explore the importance of any medicinal plant the initial step is to screen for its phytochemicals, as it gives a broad idea regarding the nature of compounds present in it. Phytochemical screening aids as an initial step for future determination of its activity like antioxidant, anticancer, anti-inflammatory, antimutagenic, etc. Different phytochemicals possess various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The medicinal value of plants depends on the chemical substances that have a definite physiological action on the human body. The most important of the bioactive constituents of plants are alkaloids, tannins, saponins, terpenoids, steroids, glycosides, flavonoids and phenolic
compounds (Hill, 1952). In the present investigation, qualitative analysis of five different extracts (aqueous, petroleum ether, chloroform, ethanol and acetone) of *H. suaveolens* leaves were analysed for phytoconstituents (Table 4.1). Different solvents have various degrees of solubility for different phytochemicals (Majorie, 1999). Among the various solvents tested, Irudayaraj *et al.* (2010) observed maximum separation of phytochemicals with the ethanolic extract. This is because ethanol is much polar than chloroform and acetone, hence extracting many of the active ingredients (Harborne and Baxter, 1995). There are more reports available indicating the maximum extraction of phytochemicals in the ethanolic extract (Ahmad *et al*., 1998; Panda *et al*., 2009). The results obtained by Agarwal and Varma (2013) regarding the methanolic extract of *H. suaveolens* revealed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycosides, tannins, phenolic compounds, proteins, amino acids, terpenoids and steroids, which is in accordance with the present result. Plants are considered as sources of antioxidants due to the presence of polyphenols and flavonoids which possess wide biological properties (Durga *et al*., 2000). Luo *et al.* (2002) related the presence of polyphenols to the antioxidant and antidiabetic activity of many plants. Pachkore *et al.* (2011) who analysed *H. suaveolens* by phytochemical screening revealed the presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides in leaves, and the absence of saponins in stem and root of the plants.

Phenols, flavonoids, alkaloids, terpenoids, and essential oil have proved to be responsible for the antimicrobial activity of plants (Raja *et al*., 2010; Teixeira *et al*., 2013). These secondary metabolites are not essential for the plant itself; however they play an important role in plants’ defense system and give them protection against
pathogens and herbivores (Harborne, 1988). Many reports suggest that secondary metabolites possess antimicrobial activity (Huang et al., 2001; Avato et al., 2005; Al-Dabbas et al., 2005; Eldeen et al., 2006). Phenol and polyphenol group of compounds consist of thousands of diverse molecules with heterogenous structures, with the common feature of having one or more phenol ring. Phenolic compounds are synthesized in plants by the shikimic acid pathway. The sites and numbers of hydroxyl groups on the phenol ring generally determine their toxicity to microorganisms; hence increased hydroxylation results in increased toxicity (Geissman, 1963). Phenolic compounds such as gallic acid, coumarins, polyphenols, caffeic acid, cinnamic acid, pyrogallol, eugenol, etc. show activity against virus, bacteria and fungi (Hoult and Paya, 1996; Thones, 1997; Chang et al., 2003; Saify et al., 2005).

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economically important materials as tannins, oils, gums, flavonoids, saponins and essential oil precursors for the synthesis of complex chemical substances (Akrout et al., 2010).

5.1.2. Quantitative Analysis of Phytochemicals

Tannins and phenolic compounds are the major plant secondary metabolites responsible for the antioxidant activity. This activity is believed to be mainly due to their oxidation/reduction properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Long et al., 2006). In the present investigation, the concentration of
tannins and flavonoids in the ethanolic leaf extract of *H. suaveolens* was 0.015 and 0.081µg/µl, respectively. Total phenolic and flavonoid contents of *H. suaveolens* methanolic extract was studied by Ghaffari *et al.* (2014). Studies done by Koche *et al.* (2010) revealed that the crude extracts of *H. suaveolens* contained the highest percentage of alkaloids and flavonoids (14.30 ± 0.36% and 12.60 ± 0.48%), respectively.

Phenolic compounds and tannins are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Singh *et al.*, 2007). Tannins are widely found in the secondary products of medicinal plants, as well as in many edible plants (Hagerman *et al.*, 1998). A number of studies have focussed on the biological activities of phenolic compounds, which are potential antioxidants and free-radical scavengers (Rice-Evans *et al.*, 1996; Cespedes *et al.*, 2008; Chanda and Dave, 2009; Annegowda *et al.*, 2010). Tannins are responsible for the differences in the antioxidant activity of different plants (Cai *et al.*, 2004).

Flavonoids are phenolic acids which serve as an important source of antioxidants in different medicinal plants and related phytomedicines (Pietta, 1998). Flavonoids contain several phenolic hydroxyl groups in their ring structure. Many flavonoids are found to be strong antioxidants capable of effectively scavenging reactive oxygen species because of their phenolic hydroxyl groups (Cao *et al.*, 1997). The data obtained from this study indicate that tannins and flavonoids are better extracted with ethanol than with other solvents. Tsao and Deng (2004) also found out that phenolic acids and flavonoids are generally better extracted using alcohols, water
or a mixture of water and alcohols. The differences between the tannin and flavonoid concentrations in extracts from our study and those of earlier studies are likely due to genotypic differences within species, the differences in their locations, choice of parts tested, time of taking samples and various determination methods. In plants, polyphenol synthesis and accumulation are generally stimulated in response to biotic/abiotic stresses (Shan et al., 2005). Lisiewska et al. (2006) have shown that the distribution of secondary metabolites might fluctuate between different plant organs.

5.1.3. Fourier Transform Infrared (FT-IR) spectroscopy

Spectroscopic technique has become a powerful tool for the qualitative and quantitative analysis of biological and pharmaceutical materials. FT-IR spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants (Kogel-Knaber, 2000). Plant constituents involved in the reduction and capping of nanoparticles can be identified by the FT-IR technique (Li et al., 2007). The region between 4000 and 400 cm\(^{-1}\) is of greatest practical use to the organic chemist (Udhayakala et al., 2011).

In the present study FT-IR analysis was carried out to identify the functional group of the compounds present in the powdered leaf samples of *H. suaveolens*. The absorption at 3313 cm\(^{-1}\) is due to the stretching of alkyne groups that are present in the extract. The bands at 3193 and 2364 cm\(^{-1}\) are due to ammonium ion and aliphatic cyanide or nitrite groups, respectively. The peaks at 1668, 1454, 1400, 1334 and 1195 cm\(^{-1}\) are due to open-chain imino (-C=N-) stretch, methyl C–H asymmetric or symmetric bend saturated aliphatic alkane or alkyl group frequencies, phenol or tertiary alcohol OH bend, primary or secondary alcohol OH in plane bend and
aromatic C–H in plane bend, respectively. Similar studies conducted by Okoye and Chukwu (2014) on the crude alkaloid extract of *H. suaveolens* showed the presence of the following functional groups: O–H stretch, C–H stretch, C=O stretch, C–O stretch, and C–O deformation.

Alkanes are found in the powdered leaf samples of *H. suaveolens*. Generally alkanes are found in the plant cuticle and epicuticular wax of many plants. They protect the plant against water loss; prevent leaching of important minerals by rain; and protect against bacteria, fungi, and harmful insects (Baker, 1982). Alkynes are highly bioactive and act as nematocides (Jørgen, 1988). The presence of alkynes suggests that this plant can be used as a nematocidal agent in the near future.

An infrared spectrum (IR) represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same IR spectrum. Therefore, an IR spectrum is a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of the material present. With modern software algorithms, IR is an excellent tool for quantitative analysis. Because all the frequencies are measured simultaneously, most measurements by FT-IR are made in a matter of seconds rather than several minutes (Ali *et al.*, 2011; Vahabi *et al.*, 2011).
5.1.4. Gas Chromatographic-Mass Spectrometric Analysis

5.1.4.1. GC-MS Analysis of ethanolic leaf extract

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractions of the ethanolic extract of *H. suaveolens*. These compounds were identified through mass spectrometry attached with GC. The results of the present study are tabulated in Table 4.3. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peaks indicate the relative concentrations of the components present in the leaves. The mass spectrometer analyzes the compounds eluted at different times to identify their nature and structure. The large compound fragments into small compounds giving rise to peaks at different *m/z* ratios. Each mass spectrum is a fingerprint of a certain compound which can be identified from the data library. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The results revealed that 5,5-dimethylimidazolidin-2,4-diamine (20.35%) was the major compound followed by 1-hexadecene (8.43%), 1-nonadecanol (6.68%), 5-chloro-2-furancarbaldehyde oxime (5.7%), 1-tetradecanol (5.55%), 1-octadecanol (6.51%), (E)-4-methoxy-2,2-dimethyl-5-phenylhex-4-en-3-ol (5.06%), α-elemene (3.78%), ethyl N-cyano-N-pentylcarbamate (3.77%), 1,8-cineole (3.21%), (1rs, 2rs)-2-(di-O-tolylphosphinoyl)-1-phenyl-1-pentanol (3.19%), 2-propenoic acid, 2-ethylhexyl ester (3.14%), bis(3-formyl-4-hydroxyphenyl) disulphide (2.74%), 3-isopropylisoxazole (2.32%), 1-heptacosanol (2.13%), (+)-(1aR, 2R, 5S, 5As)-2,5-Diisopropylperhydroxireno[2,3-d][1,2]dioxine (2%), (+)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3-dimethyl-1H-naphtho[2,3-c]pyran-6,9-dione (1.84%), 4-tert-butyl-3,5,5-trimethyl-3-hexane
(1.74%), 3-trifluoroacetyl-4,5-dihydrofuran (1.25%), phytol acetate (1.23%), 2-iodo-2-phenylethanol (1.16%), 1-(benzothiazol-2-yl)-3-phenylpenta-3,4-dien-2-ol (1.2%), bicyclo(2.2.1)heptan-1-ol (1%), neroloxide (0.98%), cis 3-hexenyl tiglate (0.94%), 2-isopropyl 4b,8,8-trimethyl-4b,5,6,7,8a,9,10-heptahydro-phenanthrene (0.9%), 1,4-bis-(p-tolylsulphinyl) piperazine (0.85%), heptyl 2-hydroperfluoroheptanoate (0.81%), isopropyl(trans-2-methylcyclopentyl) isoproxyborane (0.82%) and 5-bromo-1,2-dimethyl-4-nitroimidazole (0.71%). The GC-MS spectrum showed the presence of more long-chain hydrocarbons, which hence have complex chemical compositions. When the number of carbon atoms increases in the molecule, hydrophilicity is reduced and lipophilicity increased. Higher the lipophilicity of a drug higher is its distribution; because once the drug is in systemic circulation, it is distributed to all the tissues at a particular rate depending on its physicochemical characteristics such as lipophilicity and charge (Wils et al., 1994; Parasuraman et al., 2009).

*Hyptis suaveolens* has been the subject of some previous studies regarding its chemical nature. From the petroleum ether extract of *H. suaveolens* aerial parts, a pentacyclic triterpene was isolated (Mukherjee et al., 1984); the A-ring contracted triterpene obtained is the first example of a compound presenting skeletal type outside the lupine series (Rao et al., 1990). The compounds hentiracontane, friedelin, netriacontanone, lupeol acetate and lupeol were isolated from the benzene extract of air-dried powdered leaves and floral parts of *H. suaveolens* (Saluja and Santani, 1984). Recently, Mary et al. (2014) isolated 11 compounds including allyloctadecanoate and octadec-9-enoic acid from the aqueous extract of *H. suaveolens*. The Gas Chromatography-Flame Ionization Detector (GC-FID)
analysis of the extracted oil revealed 36 chemical compounds (99.99%), of which 72.54% are monoterpenoids, 21.96% are sesquiterpenoids and 5.49% are non-terpenoid constituents. The major constituents of the oil are sabinene (25.0%), α-terpinolene (13.64%), β-caryophyllene (12.75%), 1,8-cineole (9.11%), β-pinene (5.65%), bicyclogermacrene (5.61%) and limonene (5.40%) (Azeez et al., 2014).

In the past efforts have been directed towards studying the non-volatile components of *H. suaveolens*, and a number of di- and triterpenoids (Manchand *et al.*, 1974; Misra *et al.*, 1983b) and steroids (Saluja *et al.*, 1981) have been identified. Volatile oil isolated from *H. suaveolens* was reported to contain sabinene and 1,8-cineole (Syamasundar *et al.*, 2012). The essential oil obtained after hydrodistillation of the leaves of *H. suaveolens* showed 1,8-cineole (32%) and β-caryophyllene (29%) as the major constituents (Peerzada, 1997). Similarly in the present study the ethanolic extract of *H. suaveolens* showed the presence of the compound 1,8-cineole (3.21%). The concentration of this compound is normally the highest in *Hyptis* and has been reported to be up to 47.64% in a sample from Brazil (Moreira *et al.*, 2010). The presence of these phytochemicals has been attributed to the bioactive principles responsible for ethnopharmacological activities of most medicinal plants (Edeoga *et al.*, 2005; Omoyeni *et al.*, 2012). The presence of various bioactive compounds confirms the application of *H. suaveolens* for various ailments by traditional medical practitioners. Moreover, isolation of individual phytochemical constituents may lead to the development of novel drugs.
5.1.4.2. GC-MS analysis of essential oil

The concentration and composition of the major components in essential oils produced from *H. suaveolens* varys, depending on the geographical location in which the plant grows (van Hac, 1996; Malele *et al.*, 2003) and hence it is termed a ‘chemotype’. In addition, the extraction method can also influence the type and amount of molecules extracted (Bakkali *et al.*, 2008; Meiri *et al.*, 2010). In the present study essential oil of the leaves of *H. suaveolens* analyzed by GC-MS resulted in the detection of 13 compounds. The results revealed the presence of ethyloleate, hexadecanoic acid ethyl ester, linoleic acid ethyl ester, ethyl 9-hexadecenoate, octadecanoic acid ethyl ester, 1,2-benzenedicarboxylic acid diethyl ester, 3,8-dioxaocta-2,9-disiladecane 2,2,9,9-tetramethyl-5,6-bis[(trimethylsilyl)oxy], 9-octadecenoic acid (Z)-methyl ester, ethyl 9-hexadecenoate, ethyl (9Z,12Z)-9,12-octadecadienoate, 1,2-benzenedicarboxylic acid, benzene, 1,2,3-trimethoxy-5-(2-propenyl)benzene and trimethyl[(1-methyldodecyl)oxy]silane as the minor compounds. The chemical components of essential oil analyzed in this work have not been reported so far in *Hyptis*.

Sharma *et al.* (2007) have found out that the major components of essential oils from *H. suaveolens* plants collected in India were 1,8-cineole (44.4%), β-caryophyllene, β-pinene and camphene. Essential oils obtained by hydrodistillation from *H. suaveolens* have been investigated by GC-MS analysis (Asekum and Ekundayo, 2000; Azevedo *et al.*, 2001). Sabinene, limonene, bicyclogermacrene, beta-phellandrene, and 1,8-cineole were found to be the major constituents; whereas, eugenol, beta-caryophyllene, beta-pinene, terpinolene and 4-terpino were also isolated
(Fun and Svendsen, 1990; Sidibe et al., 2001; Kossouoh et al., 2010; McNeil et al., 2011; Benelli et al., 2012; Kodakandla et al., 2012; Uzama et al., 2013).


Recent studies conducted by Chatri et al. (2014) resulted in the identification of 50 components from the essential oil of *H. suaveolens* leaves. The major components were β-caryophyllene (34.65%), germacrene-D (10.32%), α-bergamotene (6.56%), rimuene (6.46%) and α-copaene (5.94%). Chemical analyses showed that in the essential oil of *H. suaveolens* monoterpane hydrocarbons were the most represented class of volatiles (64.1%), followed by sesquiterpene hydrocarbons (24.0%), oxygenated monoterpenes (8.1%) and oxygenated sesquiterpenes (2.4%) (Conti et al., 2010).

Two structurally similar diterpenes, namely suavelol and methyl suaveolate were isolated from the leaves of *H. suaveolens* (Grassi et al., 2006). The essential oils from *H. suaveolens* have been extensively investigated and are mainly composed of monoterpenes and sesquiterpenes, although significant diterpene proportions have been reported. A great variation in quantity and chemical composition of the volatile oils from *H. suaveolens* has been reported previously, but a few researchers have investigated in detail the reasons for such variability (Martins et al., 2006, 2007). In a few cases, inquiries about environmental influences on the chemical composition
are made, but in most of the studies the authors attribute the volatile oil chemical variability to the geographic location of the plants investigated (Barbosa et al., 2013).

Sabinene (7.3-31.3%), eucalyptol (14.0-24.6%), β-caryophyllene (6.9-12.7%), 1,8-cineole (11.5%), β-phellandrene (10.2%), terpinolene (8.7-9.6%), β-pinene (4.9-7.4%) and terpinen-4-ol (5.4-5.9%) were the predominant compounds of essential oils analyzed from *H. suaveolens* (Noudogbessi et al., 2013).

Trans-beta-caryophyllene has been reported to occur abundantly in the essential oil present in *H. suaveolens* leaves (Din et al., 1988; Malele et al., 2003; Sharma and Tripathi, 2008). Compounds which have been frequently reported to be found in the essential oil such as 1,8-cineole (Din et al., 1988; Ahmed et al., 1994; Azevedo et al., 2001), germacrene D and germacrene B (Fun and Svendsen, 1990; Pant et al., 1992) were not found in the same in this study, suggesting that the assayed essential oil could correspond to a new chemotype.

Among the identified phytochemicals, hexadecanoic acid was reported to contain antioxidant, hypocholesterolamic, nematicidal, pesticidal, antiandrogenic and hemolytic properties and 9-octadecadienoic acid was reported to possess anti-inflammatory, anticancer and hepatoprotective properties (Rajeswari et al., 2013). Linoleic acid found in the essential oil of *H. suaveolens*, previously reported in *H. spicigera* seed, could be used as a dietary supplement to increase the production of anti-inflammatory 1-series prostaglandins (Wretenjo and Karlberg, 2002). The above-said components found in the essential oil of *H. suaveolens* can be used for further pharmacological investigations.
5.2. Bioactivity Studies

5.2.1. Antioxidant activity

Several mechanisms have been proposed to be involved in the antioxidant activity of compounds such as hydrogen donation, termination of free radical-mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides (Gordon, 1990). Antioxidant activity is system-dependent and the characteristic of a particular system could influence the outcome of analysis. Hence, a single assay would not be representative of the antioxidant potential of plant extracts. In the present study different models of antioxidant assays were employed, which could provide a more consistent approach to assess the antioxidant and radical-scavenging potential of the leaves of *H. suaveolens*.

The fundamental antioxidant property of plant extracts is their ability to scavenge free radicals, which are believed to contribute significantly to etiology and pathogenesis of various chronic diseases. The free radical-mediated chain reaction is widely accepted as a common mechanism of lipid peroxidation. The model of free-radical scavenging is used to assess chain-breaking activity in the propagation phase of lipid and protein oxidation (Manzocco et al., 1998). Radical scavengers may directly react with and quench reactive oxygen and nitrogen radicals to terminate peroxidation chain reaction, which is thought to be due to their hydrogen-donating ability (Gülçin et al., 2004). Polyphenolics have been shown to exert antioxidant activity through this mechanism (Soobrattee et al., 2005).
5.2.1.1. DPPH-radical scavenging activity

The free radical-scavenging activity of the ethanolic extract of the leaves of *H. suaveolens* was evaluated based on its ability to scavenge the synthetic 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour) (Husain *et al.*, 1987; Visioli *et al.*, 2000; Parr and Bolwell, 2000; Solai *et al.*, 2010). As the electron is paired during free-radical scavenging, the absorption vanishes and the resulting discoloration stochiometrically coincides with respect to the number of electrons taken up. (Montalleb *et al.*, 2005; Arokiyaraj *et al.*, 2008; Muthukumaran *et al.*, 2011).

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases, and extensive lysis. One of the solutions to the problem is to consume natural antioxidants from food supplements and traditional medicine. Recently, many natural antioxidants have been isolated from different plant materials. An antioxidant works in stopping oxidation by neutralizing the free radicals produced. In order to neutralize free radicals, the antioxidant itself undergoes oxidation. DPPH is the commonly used reagent to evaluate the free radical-scavenging activity of antioxidants. Table 4.5 and Figure 4.7 show the dose-dependent DPPH radical-scavenging activity of *H. suaveolens*. This increased scavenging capacity of the ethanolic extract of *H. suaveolens* may be due to the presence of high levels of phytochemical constituents in this extract. The percentage of inhibition for the ethanolic leaf extract increased from 20 to 100 µg/mL concentration, with a scavenging activity of 16.09±0.95 to 75.50±1.04%. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance
decreases (Koleva et al., 2002; Vinayakak et al., 2010). Though the DPPH radical-scavenging ability of the extract was less effective than a commercially available synthetic like quercetin, the study showed that the leaf extract has the ability to donate a proton and hence could serve as free radical inhibitor or scavenger, acting as a primary antioxidant. The IC\textsubscript{50} values of the test extract and the standard were found to be 25.35±0.25 and 4.42±0.04 µg/mL respectively. From the results, it may be postulated that the plant extract has hydrogen donors, enabling the scavenging of the free radical DPPH. The plant extracts are quite safe and their toxicity is not a problem of concern. Therefore the plant \textit{H. suaveolens} could be exploited as an antioxidant additive.

Studies of the methanolic extract of \textit{H. suaveolens} using gallic acid and Butylated hydroxyanisole (BHA) standards by Gavani and Paarakh (2008) proved that the extract exhibited strong DPPH radical-scavenging activity with IC\textsubscript{50} values of 0.4, 1.15 and 14.04 µg/mL for gallic acid, BHA and \textit{H. suaveolens}, respectively. The antioxidant activity of the methanolic extract could be due to the presence of flavonoids which correlates with the recent study conducted on the leaf by Ghaffari \textit{et al.} (2014) and root by Ahmad \textit{et al.} (2013) on the methanolic extract.

\textbf{5.2.1.2. Superoxide radical-scavenging activity}

Superoxides are produced from molecular oxygen due to oxidative enzymes (Pratt, 1992) of the body as well as through non-enzymatic reactions such as autooxidation by catecholamines (Sainani \textit{et al.}, 1997). Superoxide anion is a reduced form of molecular oxygen that is generated during normal metabolic processes mainly in mitochondria. It is known to be destructive to cellular components as a precursor of
other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Stief, 2003), contributing to tissue damage and various chronic diseases (Halliwell, 1991). Therefore, studying the scavenging activity of plant extracts on superoxide radical is one of the most important ways of clarifying the mechanism of antioxidant activity.

In the present study, the ethanolic extract of \textit{H. suaveolens} leaf produced dose-dependent inhibition of superoxide radicals. The IC$_{50}$ values of the leaf extract and the standard were found to be $123.69\pm4.70$ and $9.03\pm0.07$ µg/mL respectively. From the results it appears that the superoxide scavenging activity of \textit{H. suaveolens} leaf extract is negligible compared to the standard quercetin but positive correlation between polyphenol and flavonoid contents and superoxide scavenging activity of the extract has been previously recorded by Policegoudra \textit{et al.} (2007). Robak and Gryglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions.

The scavenging activity of \textit{H. suaveolens} leaf extracts on superoxide radicals is shown in Figure 4.8. Extracts displayed concentration-dependent protective activity against superoxide radicals. Earlier reports on the hexane, ethylacetate, acetone and methanolic extract of \textit{H. suaveolens} showed considerable scavenging activity (Priyadharshini and Sujatha, 2013). No studies have so far been done to analyse the scavenging ability of the ethanolic leaf extract of \textit{H. suaveolens} on superoxide radicals.
5.2.1.3. Hydroxyl radical-scavenging activity

Hydroxyl radical is an extremely reactive oxidizing free radical formed in biological systems and has been implicated as a highly damaging species in free-radical pathology (Wang et al., 2007). It has an extremely short half-life but is capable of causing damage within a small radius of its site of production. A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function, and lead to cell death.

The extracts inhibited hydroxyl radical-mediated deoxyribose degradation in a concentration-dependent manner. The ethanolic extract had significant scavenging effects on the hydroxyl radical, which increased with increase in its concentration from 20 to 100 µg/mL. The IC₅₀ value of the test extract and the standard quercetin were 23.28±0.52 and 6.26±0.18 µg/mL respectively. Thambiraj and Paulsamy (2012) observed similar type of hydroxyl radical scavenging activity for Acacia caesia. The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Adewusi and Steenkamp, 2011; Oyedemi and Afolayan, 2011; Sajeesh et al., 2011).

5.2.1.4. ABTS radical-scavenging activity

The free radical-scavenging activity of H. suaveolens extracts was also studied using the ABTS (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate) cation. ABTS is a stable free radical, bluish-green in colour and the antioxidant assay is based on the reduction of ABTS by plant extracts. This assay is based on the inhibition of the absorbance of the cation, ABTS⁺, which has a characteristic wavelength at 734 nm, by
antioxidants. In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS (Sreejayan and Rao, 1996). In the present study the ethanolic leaf extracts of *H. suaveolens* were found to be fast and effective scavengers of the ABTS radical with an IC\textsubscript{50} value of 25.59±0.58 µg/mL. The percentage activity was compared with the standard quercetin which showed IC\textsubscript{50} value of 9.39±0.15 µg/mL. The scavenging effect of the extract was lesser than that of the standard quercetin. Higher the IC\textsubscript{50} lower will be the scavenging ability. Ghaffari *et al.* (2014) revealed that the methanolic leaf extract of *H. suaveolens* showed good ABTS scavenging activity. Similar scavenging studies conducted on the essential oil of *H. suaveolens* showed time-dependent activity. The IC\textsubscript{50} of the oil extracted from *H. suaveolens* was found to be 3.72 mg/mL (Nantitanon *et al*., 2007).

### 5.2.2. Antihaemolytic activity

Erythrocytes are considered major targets for free radicals because of the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and oxygen transport associated with redox-active haemoglobin molecules, which are potent promoters of activated oxygen species (Ebrahimzadeh *et al*., 2009). The extent of haemolysis was found to be much greater when red blood cells were treated with hydrogen peroxide (toxicant). This could be attributed to the oxidizing nature of hydrogen peroxide leading to the destruction of the cell membrane and the subsequent liberation of haemoglobin from the cells. Mobilization of Fe\textsuperscript{2+} by Ca\textsuperscript{2+} via Fenton reaction is also caused due to hydrogen peroxide which further leads to the production of OH radicals (Kupier-Goodman and Scott, 1989). All these factors, in unison, cause the deterioration of cell membrane, which may, perhaps, be the key episode in the lysis of the cell (Devjani and Verma, 2010). Nevertheless, the antihaemolytic activity
is the expression of collaborative action of the various antioxidant mechanisms which function in nature.

It is clear from the present analysis that the ethanolic extract of the leaves of *H. suaveolens* and the standard quercetin at 250 µg/mL presents the best profile of haemolysis inhibition with percentage activity of about 22.54±6.45 and 62.74±4.46% respectively. The effective antihaemolytic activity of the ethanolic leaf extract of *H. suaveolens* is because of the ability of phenolic compounds including flavanoids in neutralizing the free radicals generated by H$_2$O$_2$ and thereby protecting the erythrocyte membrane from destruction and lysis. Insignificant haemolysis was observed when erythrocytes were treated only with the plant extract, indicating the nontoxic nature of the extract. Thus the extract can be justified as harmless for the cells. Membrane lipid peroxidation is regarded as a key factor for cell lysis. Malondialdehyde (MDA), a low-molecular-weight end product of lipid hydroperoxide decomposition is the most often measured parameter of membrane destruction. Lesser the cell lysis lesser will be the MDA content. Earlier reports have suggested the potent antihaemolytic activity of the bioactive components namely flavonoids and phenols obtained from plant extracts (Niki, 1982; Chakraborty and Shah, 2011).

Flavonols and glycosides are efficient antioxidants which can guard human red blood cells from free radical-mediated oxidative haemolysis (Dai *et al.*, 2006). Moreover, binding of flavonoids to red blood cell membranes significantly inhibits lipid peroxidation and at the same time, enhances their integrity against lysis (Chaudhuri *et al.*, 2007).
There are no previous reports on the antihaemolytic activity of *H. suaveolens* leaf extracts. Several other plants have been previously studied for this quality. *In vitro* evaluation of the haemolysis inhibitory activity of aqueous extracts of *Morinda lucida, Uvaria chamae, Lonchocarpus cyanescens, Croton zambesicus, Raphiostylis beninensis* and *Xylopia aethiopica* at different doses showed significant antihaemolytic effect (Avaligbe *et al.*, 2012). Similarly a study conducted by Nabavi *et al.* (2012) proved that the stem (23.58±0.7 μg/mL) and leaf (26.21±1 μg/mL) extracts of *Hyssopus angustifolius* showed higher antihaemolytic activity than the floral extract.

5.2.3. *In vitro* antidiabetic activity

5.2.3.1. α-amylase-inhibiting activity

Alpha-amylase is one of the key enzymes in the human body, which breaks down starch to more simple sugars and increases the absorption rate of glucose. As a consequence of the activity of the enzyme, postprandial blood glucose level is increased (Ranilla *et al.*, 2010; El-Kaissi and Sherbeeni, 2011). Slowing the digestion and breakdown of starch may have promising effects on insulin resistance and glycemic index control in people with diabetes mellitus (Ghavami *et al.*, 2001; Notkins, 2002; Russell *et al.*, 2013). The ethanolic leaf extract of *H. suaveolens* was found to moderately inhibit α-amylase. At 500 μg concentration the percentage activity was found to be 36.96±0.54 for *H. suaveolens* extract. From preliminary phytochemical screening, phytoconstituents like glycosides, flavonoids, tannins, phytosterols, terpenoids, coumarins and quinones were reported. This activity may be attributed to these natural compounds. Natural polyphenols have been described to have the potential to hinder the activity of carbohydrate -hydrolyzing enzymes like
α-amylase and α-glucosidase (Mai et al., 2007). Flavonoids have been recognized as potent candidates for inhibition of amylase activity. Several types of flavonoids like flavones, amentoflavone, isoflavone, flavanonol and luteolin have been identified and tested for amylase inhibitory property (Kim et al., 2000; Xiao et al., 2013). Studies of the administration of methanolic extracts of *H. suaveolens* to alloxan-induced diabetic rats showed significant reduction in the blood glucose concentration, of the animals implying the potent antidiabetic activity of these extracts (Danmalam et al., 2009). Many types of terpenoids, e.g., lupeol, ursolic acid and oleanolic acid have been found to exhibit α-amylase inhibitory activity (Sales et al., 2012). The significant α-amylase inhibitory activity of tannins is due to their capability to strongly bind to carbohydrates and proteins (Eom et al., 2012), but studies have revealed that tannins are not always an effective inhibitor of α-amylase (Kandra et al., 2004; Kunyanga et al., 2011; Barrett et al., 2013). Inhibitory activities of cyanides and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase have been proven successfully (Akkarachiyasit et al., 2010). The ethanolic leaf extract of *H. suaveolens* might have exerted this activity on the carbohydrate-binding regions of α-amylase that catalyze hydrolysis of the internal α-1,4 glucosidic linkages in starch. Natural inhibitors from this herb have α-amylase inhibitory activity and could be used as effective treatment for the management of postprandial hyperglycaemia. A drug-development programme should be undertaken to develop modern drugs with the compounds isolated from this plant.

5.2.3.2. α-glucosidase inhibition effect of the ethanolic extract of *H. suaveolens*

Diabetes mellitus is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of organs to secreted
insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body’s systems, including blood vessels and nerves (Matsui et al., 2007). Extracts of select natural products retard the absorption of glucose by inhibiting carbohydrate-hydrolyzing enzymes. Several α-amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but their prices are high and clinical side effects occur (Scott and Spencer, 2000). In contrast to acarbose, plant-derived α-amylase and α-glucosidase inhibitors have lower inhibitory effect against α-amylase activity and stronger inhibitory activity against α-glucosidase (Kwon et al., 2008), an indication that plant extracts and their constituents may be effective therapeutic agents for the management and control of postprandial hyperglycaemia with lesser side effects than acarbose (Mogale et al., 2011). One of the therapeutic approaches for treating diabetes is to decrease postprandial hyperglycaemia. This is performed by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzyme (α-glucosidase) in the digestive tract, delaying carbohydrate digestion (Chiasson et al., 1994). α-glucosidase is one of the glucosidases located in the brush border surface membrane of intestinal cells and is a key enzyme of carbohydrate metabolism (Puls et al., 1997). The extracts block the actions of α-glucosidase in the small intestine which is the rate-limiting step in the conversion of oligosaccharide and disaccharide to monosaccharide, necessary for gastrointestinal absorption, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level (Davis and Granner, 2001). Many α-glucosidase inhibitors such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides and phenolic compounds have been isolated from plants. In this context, the α-glucosidase inhibitory effect of the
ethanolic extract of *H. suaveolens* was investigated at various concentrations (100, 200, 300, 400 and 500 μg/mL). There was a progressive increase in the percentage of inhibition with the increase in the concentration of the extract (Table 4.11). The presence of secondary metabolites may be one of the possible reasons for the antidiabetic activity of this common weed in *in vitro* models. Alloxan-induced diabetic rats were administered *H. suaveolens* leaf extracts which was found to inhibit glucose level (Nayak *et al*., 2013). Several reports on extracts of plants such as *Cuscuta reflexa* (Anis *et al*., 2002), *Alstonia scholaris* (Anurakkun *et al*., 2007), *Andrographis paniculata* (Edwin *et al*., 2008) and *Asystasia dalzelliana* (Satish *et al*., 2011) have corroborated the findings of this study.

Inhibition of the enzymes α-amylase and α-glucosidase reduced the high postprandial blood glucose levels in diabetics (Conforti *et al*., 2005). Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes. The results suggest that ethanolic extract of *H. suaveolens* efficiently inhibits α-glucosidase enzymes *in vitro*, thus reducing the rate of digestion and absorption of carbohydrates. Thus the anti-diabetic activity of *H. suaveolens* extracts can also be attributed to the intestinal α-glucosidase inhibitory activity.

**5.2.4. Anticancer activity**

Many plants have been investigated for their anti-tumour or anticancer activity. The *H. suaveolens* extracts investigated in this study showed reasonably good response against *in vitro*-tested MCF-7 breast cancer cell lines. Hence the cytotoxicity of the isolate was further studied. The viability of the human breast cancer cells MCF-7 was estimated. As shown in Figure 4.14 there was a steep
decrease in the percent viability as the concentration (7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 μg/mL) of the plant extract increased. At the highest concentration of 1000 μg/mL, the plant extract exhibited a viability of 2.3%, on the cancer cells, though the percent cell viability observed was moderate, this study has proved that the ethanolic extract of *H. suaveolens* has cytotoxic effects on human breast cancer cell lines.

Reports on the antitumour mechanism of the essential oils show that the cytotoxic activity could be due to the liposolubility of the extracts which could affect membrane fluidity, leading to dissolution or destruction of the plasma membrane (Wink, 2008). Nowadays attention has been focused towards natural antioxidants because findings indicate that the use of synthetic antioxidants may lead to the induction of cancer (Jose and Radhamany, 2013).

Cytotoxicity (MTT 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide assay) tests of the two bioactive compounds menthol and linalool isolated from the crude ethyl acetate extract of *H. suaveolens* showed moderate cell viability with respect to its concentration using cisplatin as positive control (Priyadharshini and Sujatha, 2013). Studies by Dalziel (1937), Oliver-Bever (1986) and Mabberley (1990), showed that the leaves of *H. suaveolens* possessed anticancer virtues. Gurunagarajan and Pemaiah (2011) who investigated the anticancer potential of the ethanolic extracts of *H. suaveolens* against Ehrlich ascites carcinoma cell line showed that the cytotoxicity of the extracts is due to the activation of the apoptotic pathway. In a study conducted by Ximenes and his coworkers (2012) both water and hot water extracts of *H. mutabilis* showed promising antitumour activity against
sarcoma 180 murine tumour. Recent studies of the ethanolic and aqueous extracts of *H. suaveolens* inhibited the growth and induced the apoptotic death of the human T-cell leukaemia cell line, Jurkat cells (Musika and Indrapichate, 2014). There are no previous reports available on the anticancer activity of the leaves of *H. suaveolens* against MCF-7 human breast cancer cell lines. Therefore the present study contributes towards identification of the crucial metabolites of *H. suaveolens* which can be potential anticancer agents.

5.2.5. Antibacterial activity studies

5.2.5.1. Antibacterial activity of *H. suaveolens* leaf extracts

Medicinal plants have been used as remedies for human diseases for centuries; this is because they contain components with therapeutic properties in their parts. The antibacterial effect of several plant extracts has been proved (Habbal *et al*., 2011; Masoud and Gouda, 2012; Yaouba *et al*., 2012). Although a variety of solvents have been employed in the extraction of bioactive compounds, it is still uncertain as to what kind of solvent is the most effective and suitable for extraction. In the present study, the aqueous, petroleum ether, chloroform, ethanol and acetone leaf extracts of *H. suaveolens* inhibited the growth of all the bacterial pathogens tested, but their effectiveness varied (Table 4.13). The variation in the antimicrobial activity of extracts prepared using different solvents can be due to the polarity of the solvents used, polarity of the compounds being extracted by each solvent and, in addition to their extrinsic bioactivity and by their ability to dissolve or diffuse in the media used in the assay (Anjana *et al*., 2009). The other possibility may be the loss of some active compounds during extraction of the sample, lack of solubility of active constituents in the solvent (Kumar *et al*., 2008) or the presence of active compounds in insufficient
quantities in the crude extracts to show their activity with the dose levels employed (Taylor et al., 2001). Alternatively, if the active principle is present in high enough quantities there could be other constituents exerting antagonistic effects (Jäger et al., 1996). Extracts which have lesser antibacterial activity may be active against other untested bacterial species (Shale et al., 1999).

Researchers have tried using different solvents for screening the antibacterial activity of plant extracts and made evaluations. Raj et al. (2011) indicated that acetone was the best solution for extracting the effective antimicrobial compounds from the epidermal glands of Christella parasitica. Acetone extract of H. suaveolens in the present study showed a maximum of 7 mm zone against Staphylococcus aureus. Nair and Sumitra (2007) and Malar et al. (2011) reported the usage of ethanol as a solvent for the preparation of plant extract for antibacterial studies. Johnson et al. (2011) determined the anti-bacterial efficacy of chloroform, ethanol, ethyl acetate and aqueous extracts of Mentha arvensis against Salmonella typhi, Streptococcus pyogenes, Proteus vulgaris and Bacillus subtilis. Irudayaraj et al. (2010) used five different extracts (petroleum ether, benzene, chloroform, ethanol and distilled water) of the spike-moss Selaginella inaequalifolia to examine their antibacterial activity against select pathogens of which petroleum ether extract showed the maximum zone of inhibition. Raja et al. (2011) used methanolic extract of Cyclea peltata and found that it exhibited strong antibacterial activity. Sastry and Rao (1994) have revealed that extracts prepared using solvents like ethanol, hexane and methanol are capable of inhibiting both Gram-positive and Gram-negative bacteria. The antimicrobial activity of plant extracts might be due to the presence of lipophilic compounds that might bind within the cytoplasmic membrane (Jabeen et al., 2008).
Although ethanolic extract exhibited more pronounced inhibition than aqueous extracts, the effectiveness of the aqueous extract to inhibit the growth of the pathogens studied could not be contemned. Ethanolic extract of *H. suaveolens* showed maximum antibacterial activity (11 mm) and so this extract can be used in the treatment of infectious diseases caused by resistant microorganisms. Novel bioactive compounds can be discovered from this plant, which can be used for the development of new pharmaceuticals. Such screening of various natural organic compounds and identifying active agents is the need of the hour. The data obtained from the present are concordant with those of previous studies, which implies that the leaf extracts of *H. suaveolens* are effective against certain bacterial strains such as *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterobacter* sp., *Proteus mirabilis* and *Salmonella typhi* (Samrot et al., 2010; Prasanna and Koppula, 2012). Results from these researches indicated that whole-plant extracts exhibited the highest antimicrobial activity in comparison with stems and roots in chloroform and methanolic extracts. Similar studies were conducted by Pachkore and his coworkers (2011) on the aqueous and ethanolic extracts of *H. suaveolens* using *K. pneumoniae, S. aureus, E. coli* and *P. aeruginosa*. The aqueous extract did not show any inhibition zone against microbes like *S. aureus* and *P. aeruginosa*; but these bacteria were found to be susceptible to the ethanolic extract, with the inhibition zone ranging from 12 to 29 mm in diameter. Studies conducted by Satish *et al.* (2010) showed that petroleum ether extracts of *H. suaveolens* were found to be more effective against *E. coli* but in the present study ethanolic extract showed a maximum zone of inhibition of 11mm diameter against *E. coli*, while the petroleum ether extract showed a maximum zone of inhibition of
4.66 mm diameter zone against *Streptococcus pyogenes*. The findings of Rajarajan *et al.* (2014) on the methanolic extract of *H. suaveolens* were concordant with the results obtained in the present study. Determination of the MIC is necessary for prescribing its appropriate dose.

### 5.2.5.2. Antibacterial activity of essential oil

Iwu *et al.* (1990) observed that essential oil of *H. suaveolens* displayed good antimicrobial activity against yeast and showed a mild inhibitory effect on *Candida albicans* and *Aspergillus niger*. Parichad and Krittaporn (1990) showed that 95% ethanolic extracts (2.39% w/w) of *H. suaveolens* had antifungal activity. Mandal *et al.* (2007) observed that the ethanolic extracts of *H. suaveolens* had lesser activity than that of steam distillated and petroleum ether extracts against both bacterial and fungal strains. In contrary to their observation, Malar *et al.* (2012) observed higher activity for extract against common fish bacterial pathogens. A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of monoterpenes which exert membrane-damaging effects on microbial strains (Sikkema *et al.*, 1994) and stimulate leakage of cellular potassium ions which provides evidence of a lethal action related to cytoplasmic membrane damage (Cox *et al.*, 1998). The results of the present study also concordant with previous observations. Many plants have been reported for antimicrobial properties across the world (Iwu *et al.*, 1990; Asekun *et al.*, 1999; Mandal *et al.*, 2007; Shenoy *et al.*, 2009; Satish *et al.*, 2010). The results of the present study confirm the previous observations and suggest that the components of the essential oil of *H. suaveolens* may be used against select pathogens.
5.2.6. Larvicidal activity

Various harmful insects including mosquitoes are continually developing resistance to the available insecticides. Vector control in the larval stage is the best available option as the larvae are confined to water bodies. Bioactive compounds present in *H. suaveolens* can be used as potential agents for control of mosquito larvae. Certain botanical derivatives have drawn the attention of researchers as they target only the larval stages of mosquitoes (Sukumar *et al*., 1991; Shaalan *et al*., 2005; Ghosh *et al*., 2012).

Although a large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vector, very few have been found to have practical utility in field conditions for mosquito control (Sun *et al*., 2006). In the present study the chloroform extract of *H. suaveolens* exhibited 100% mortality against mosquito larvae (species) at 70 ppm concentration. Extracts prepared using other solvents also showed mortality but at higher concentrations. The percentage mortality was dose dependent. The results of the present study are concordant with those of the previous studies conducted by Elumalai *et al*. (2013) on the ethanolic extracts of *Leucas aspera* and *H. suaveolens* against the 4\textsuperscript{th} instar larvae of *Culex quinquefasciatus*. The LC\textsubscript{50} and LC\textsubscript{90} values at 24 h were found to be 65.122 ppm and 288.397 ppm, respectively. The larvicidal property of the extracts of *H. suaveolens* may be attributed to the strong presence of terpenoids, alkaloids and phenols (Ghayal *et al*., 2010). Aqueous and ethanolic extracts of *H. suaveolens* besides possessing potent ovicidal qualities may equally be utilized as larvicides for purposes of controlling instars of *Anopheles gambiae* and possibly other dipterous arthropod vectors (Ivoke *et al*., 2009).
The potent larvicidal property of *H. suaveolens* may be due to the presence of secondary metabolites including phenolic compounds and terpenoids. Phenols are generally known to be important sources of potent insecticides, fungicides, bactericides and herbicides. Triterpenoids are generally credited with mosquito larvicidal activity (Gbolade, 2000). These hydrocarbons inhibit the developmental stages of mosquitoes (Peerzada, 1997; Azevedo *et al.*, 2001; Miller *et al.*, 2002).

Most studies report that the bioactive compounds derived from plants are freely soluble in both organic solvents and water, and they work by interacting with the cuticular membrane of the larvae. According to Islam *et al.* (2003) the failure in the development of larvae to ecdyse and then into perfect pupae may be due to the delayed lethal effects of phytocompounds which cause disturbances in the endocrine mechanism regulating moulting and metamorphosis (Zebitz, 1984). *H. suaveolens* leaf extracts have been found to possess larvicidal activity against *Aedes aegypti* (Cavalcanti *et al.*, 2004) and *Culex quinquefasciatus* (Okigbo *et al.*, 2010).

In this regard, it is interesting that *H. suaveolens* extracts showed mosquitocidal activity against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (Arivoli and Samuel, 2011).

### 5.3. Pharmacognostical studies

#### 5.3.1. Anatomy

The abaxial epidermis of *H. suaveolens* is thin and the cells are narrow and cylindrical. The palisade cells form a single vertical band of narrow less compact cells and abaxial zone of several lobed spongy mesophyll cells which form reticulate system of air chambers. Earlier reports on the lamina of *H. suaveolens* have shown
that epidermal cells are circular to oval shaped with thin cuticle and scandy content. Palisade cells are irregular in shape with large intercellular spaces (Jelani and Prabhakar, 1991). Glandular and non-glandular trichomes have been reported by Metcalfe and Chalk (1950) and Rudal (1980). However studies by Jelani and Prabhakar (1991) showed four types of trichomes. In the present investigation epidermal trichome, non-glandular trichome and glandular trichome (glandular trichome with one-celled stalk, glandular trichome with long thick and cylindrical stalk) were seen.

Werker (2006) suggested that there are glandular hairs on the vegetative and generative organs of the plants belonging to the Lamiaceae family. The species belonging to this family can be characterized by the presence of these secretion hairs. Tahir et al. (1995) examined the morphology of the leaf surface of 13 species belonging to Lamiaceae family and reported the presence of sessile glandular hairs but in the present study no sessile hairs were noticed. The leaves of *H. suaveolens* are often hairy which are derivative of epidermal and are known as trichomes (Pistelli, 2006). Sharma et al. (2007) reported that glandular trichomes usually produce essential oils. Essential oils secreted by glandular trichomes can protect the plants from insects’ infestation, herbivores and pathogens (Werker et al., 1993).

There are no previous reports on the anatomy of stem of *H. suaveolens*. Young and fairly old stems of *H. suaveolens* were studied. The young stem is 4 angled with 4 thick ridges and shallow furrows in between the ridges. It consists of epidermal layer, cortex, thin vascular cylinder and wide central pith. The central core of the pith is occupied by narrow pith canal. Metcalfe and Chalk (1950) pointed out that the
stems of the family Lamiaceae species are rectangular and the collenchymatic tissue covers broad area at the corners, and a developed sclerenchymatic tissue surrounds the vascular tissue. Anatomical studies of some species of the family Lamiaceae showed that they had similar characteristics (Kaya et al., 2000; Kandemir, 2003).
SUMMARY

Plants are store houses of novel drugs, as plant derived medicines have made large contributions to human health and well-being. Almost all parts of the plant, like leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. There is an increasing trend of using natural products and the active plant extracts are frequently screened for new drug discoveries for the presence of antimicrobials, antioxidants, anticancer, antidiabetic, antihaemolytic and larvicidal agents. Higher plants have been shown to be a potential source for new antimicrobial compounds. As plants are commended as potent biochemists, man is able to obtain from them a wondrous assortment of industrial chemicals.

In the present study the common weed, Hyptis suaveolens was selected to evaluate its phytochemical, antioxidant, antihaemolytic, antidiabetic, anticancer, antibacterial, larvicidal and pharmacognostical properties. Almost all parts of this plant are being used in traditional medicine to treat various diseases. The leaves of H. suaveolens have been utilized as a stimulant, carminative, sudorific, curative for wounds, treating catarrhal condition, and infection of uterus, galactogogue and as a cure for parasitic cutaneous diseases. This herb holds a reputed position among the traditional healers in India, who are experts in the treatment of different types of cancers. The different parts of the plant are used both internally and externally for dermatitis and eczema. Crude leaf extract is also used as a relief to colic and stomach ache. Leaves and twigs are also applied as an antiseptic in burns, wounds, and various skin complaints.
Phytochemistry has been making a rapid progress and plant products have become increasingly popular in various traditional, complementary and alternative systems as they are pharmacologically potent and have low or no side effects. The medicinal properties of *H. suaveolens* are due to the presence of secondary metabolites in it. In the present study the medicinal properties of this plant are explored to identify its bioefficacy. Phytochemical screening revealed the presence of alkaloids, quinones, steroids, coumarins, flavonoids, saponins, phytosterols, glycosides, tannins and phenols in leaves of *H. suaveolens*. As per quantitative analyses tannin and flavonoids were found to be 0.015 µg/µL and 0.081 µg/µL respectively in the ethanolic extract. FTIR analysis showed the presence of alkynes, aliphatics, cyanide, nitrite, alkanes and aromatics. GC-MS analysis was carried out using ethanol leaf extract and essential oil isolated from *H. suaveolens* leaf. GC-MS spectra strongly indicated the presence of 30 bioactive phytochemical compounds in ethanol extract and 13 compounds in essential oil. These compounds could also be responsible for the antimicrobial, antioxidant and anticancer properties of this plant.

*In-vitro* antioxidant activity of ethanol extract of *H. suaveolens* leaves was analysed using DPPH, superoxide radical scavenging, hydroxyl radical scavenging activity and ABTS radical scavenging method which showed significant percentage of inhibition in a dose dependent manner with Quercetin as a standard reducing agent. It is evident from the results that the ethanolic extracts of *H. suaveolens* exhibited good antioxidant effect and strong free radical scavenging effects. The antioxidant activity could be attributed to the presence of alkaloids, steroids, flavonoids and saponins. Antihemolytic effect of the ethanolic extract of *H. suaveolens* leaves and
the standard Quercetin at 250 µg/mL presented the best profile of haemolysis inhibition with percentage activity of about 22.54±6.45 and 62.74±4.46 respectively.

Antidiabetic properties were evaluated in vitro by inhibition of α-amylase and α-glucosidase enzymes. The results of the present study point out the possibility of utilizing *H. suaveolens* as a potential antidiabetic agent.

The anticancer activity of *H. suaveolens* was tested using the ethanol extract on MCF7 breast cancer cell line and MTT assay showed cell viability of 55.2% at 7.8 µg/mL which decreased with increase in the concentration of leaf extract. The results of this study showed that the ethanol leaf extract of *H. suaveolens* at 1000 µg/mL was toxic against cancerous cell. This study points to the probable anticancer potentials of ethanol extract of *H. suaveolens* leaves. The leaf extract of *H. suaveolens* has been proved to contain glycosides, alkaloids, steroids and saponins which might be responsible for strong antioxidant and anticancer activity.

Susceptibility testing by disc diffusion assay exhibited the broad spectrum antimicrobial activity of the aqueous, petroleum ether, chloroform, ethanol and acetone extracts of the leaves of *H. suaveolens* against four Gram-positive (*Actinomyces howellii* MTCC-3048, *Bacillus circulans* MTCC-9720, *Staphylococcus aureus* MTCC-3160 and *Streptococcus pyogenes* MTCC-1927) and three Gram-negative (*Escherichia coli* MTCC-9721, *Pseudomonas aeruginosa* MTCC-1688 and *Proteus vulgaris* MTCC-7299) bacteria. The intensity of the antimicrobial action varied depending on the microorganism. All the solvent extracts exhibited appreciable antimicrobial activity against most of the pathogens tested. The aqueous extract of leaves showed less antimicrobial activity.
*H. suaveolens* is a potent, fast acting botanical in the field of mosquito control. Among the extracts tested chloroform extract had great impact on mortality of larval form of *A. aegypti* and *C. quinquefasciatus*.

Anatomical studies showed the presence of epidermal trichome, non glandular trichome and glandular trichome, which may be considered as distinguishing characters to evaluate the taxonomy of the plant.

*Hyptis suaveolens* is a unique plant containing a rich and rare collection of phytochemicals. It is unparalleled in curing multitude of disorders and has aroused great interest for its potential role in maintaining human health. Thus, the present study strongly establishes the medicinal properties of the common weed, *H. suaveolens*, and scientifically validates folkloric use of this plant as a remedy for various infections. The results obtained from phytochemical screening, antioxidant, antimicrobial and anticancer activity of *H. suaveolens*, indicate this plant as a ‘natural herbal medicinal source’ which can be used in pharmaceutical industry.

**Suggestions for Future Research**

The outcome of the present study has opened ways for addressing several other research problems in the current scenario. Some of the suggestions for the future research include the following:

- The phytochemicals may be purified further to isolate the single active component from the fractions and its structure can be elucidated.

- The active component can be subjected to clinical trials and developed into a novel drug.
There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle. The results of the study have to be confirmed using \textit{in vivo} models.

Further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition which will allow the rationale design for more effective molecules for the eventual use as chemopreventive, against malignancy.