CHAPTER II
REVIEW OF LITERATURE

Plants represent the reservoir of secondary metabolites, responsible for its medicinal and aromatic properties. Last few decades have witnessed the growth of inter-disciplinary fields variously termed as ethnomotany, ethnopharmacology, ethnopharmacognosy or phytochemistry. It is basically concerned with the biochemistry of plants and microbial interactions in correlation to their pharmacological effect. Of late there has been a renewed interest in studying the bioactive constituents of plants, especially the antioxidant, antidiabetic and antimicrobial constituents in terms of its potential health functionality in pharmaceutical and food industry (Menichini et al., 2009). The knowledge of the phytochemical constituents provide an insight into its biological functions beyond nutrition. Recent research shows that in developing countries the majority of rural dwellers do not have access to modern healthcare, so they mostly depend on medicinal plants to prevent or curing diseases (Ikenebomeh and Metitiri, 1988; Rojas et al., 2003). Hyptis suaveolens L. Poit. is one such common weed with enormous medicinal qualities. The focus of this chapter is to provide information on the morphology, active constituents and pharmacological activities of H. suaveolens.
2.1. Common and Local Names

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2.2. Botanical Description

*Hyptis suaveolens* is an erect annual or short-lived perennial herb, subshrub or vine commonly growing to about 1.5 m tall, rarely to 3 m, and strongly aromatic. Its stems are green or reddish-green, extremely hairy and square in cross-section. Leaves are oppositely arranged and hairy; the leaf blade is usually ovate (egg-shaped), elliptic (oval) or slightly cordate (heart-shaped) in outline, about 2 to 10 cm long and 1 to 7 cm wide, quite hairy and with shallow teeth along the margins. The pinkish or lavender-blue flowers are about 5 to 7 mm long and are arranged singly or in clusters.
of up to 5 in the axils of the upper leaves. Though the corolla withers, the tubular calyx persists and turns brown and is very distinctive, with each of the five lobes topped by a bristle about 5 mm long. The fruit held within the calyx is a lobed capsule that divides into two 'nutlets', each of which contains a single seed. The 'nutlets' are dark brown to black in colour with whitish markings at one end and are flattened and shield-shaped and 3 to 4 mm long and 2.5 to 3 mm wide (Parsons and Cuthbertson, 2001).

2.3. Origin and Distribution

*Hyptis suaveolens* a member of the family Lamiaceae or Labiatae is a common weed of roadsides and waste grounds, native to tropical America. The plant is generally described as annual, perennial herb or subshrub or vine. *H. suaveolens* is widespread in Australia (northern territory and Queensland), China, India, Indonesia, Papua New Guinea, Solomon Islands, French Polynesia, Federated States of Micronesia (Chuuk and Yap Islands), Niue Islands, and Guam and the Hawaiian Islands in the USA. It is widespread in West and Central Africa where it is considered an insidious species in some countries. In India *H. suaveolens* occupies roadsides, rail tracks, wastelands, watercourses, pastures and open forests (Sastri, 1959; Yoganarasimhan, 2000).

2.4. Earlier works on the experimental plant

2.4.1. Traditional Medicinal Uses

Traditionally all parts of *H. suaveolens* are being used to treat various diseases all over the world. The leaves of this herb are utilized as a stimulant, carminative, sudorific, for wounds, catarrhal condition, and infection of uterus, galactogogue and
as a cure for parasitic cutaneous diseases (Anonymous, 1964, 2001). In Niger, Nassarawa and Kaduna States a decoction of the leaf is used by traditional healers for the treatment of diabetes mellitus and fever associated with cold (Abdullahi et al., 2003). This herb holds a reputed position among the traditional healers who are experts in the treatment of different types of cancers in India (Masum et al., 2013). Different parts of the plant are used both internally and externally for dermatitis and eczema (Pankaj, 2005). Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi, 1997). Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains (Mandal et al., 2007).

The roots are used for the treatment of rheumatism, tuberculosis, leprosy, skin disease, dyspepsia, pruritis, flatulence, laxative, aphrodisiac, antipyretic and leaves are used in biliousness, gonorrhea, opthalmia, sexual debility, leucorrhoea and neuralgia (Warrier et al., 2005). Leaf paste is used in the treatment of cancer and tumors in Bangladesh (Masum et al., 2013).

Traditionally, the leaf infusion is used to cure uterus infections; leaf juice is taken in cases of colic and stomachache (Sastri, 1959). The shoot tips of the plant are edible and also used for flavouring purpose. Leaves are used in the preparation of mint flavoured beverages. Roots are chewed with betel nuts as a stomachic and its decoction is used as an appetizer, while some parts of the plant are used for the treatment of headache (Ambasta, 1986). The very strong aromatic mint/thyme-like
smell leads to the use of the plant as an insectifuge. In West Africa the infusion of *H. suaveolens* leaves is used as a substitute for tea. In Indonesia, the plant infusion is used to treat catarrhal (inflammation of mucous membranes, especially of the nose and throat) conditions, uterine disorders, parasitic cutaneous diseases and the leaves are used as stomachic. In Philippines, the leaves are used as antispasmodic, anti-rheumatic and antisoporific (Dalzial, 1937). In West Africa the leaves of *H. suaveolens* are used as antifertility agent (Sastri, 1959; Ambasta, 1986; Yoganarasimhan, 2000). In case of Dysuria (a burning sensation when passing urine) and other urinary complaints, dried seeds of *H. suaveolens* are soaked overnight in a glass of water and taken in the morning on an empty stomach along with small amount of sugar for about a week (Oliver-Bever, 1960). The plant has also been reported to possess anti-inflammatory and antiplasmodial properties (Chukwujekwu et al., 2005; Grassi et al., 2006).

2.5. Pharmacological Activities and Clinical Trials

2.5.1. Nutrition

Apart from medicinal uses the common weed *H. suaveolens* is reported to have nutritional benefits. Nutrition is the intake of food, considered in relation to the body’s dietary needs. Good nutrition combined with regular physical activities act as a cornerstone of good health. Poor nutrition can lead to reduced immunity, increased susceptibility to disease, impaired physical and mental development, and reduced productivity. Proximate analysis of *H. suaveolens* leaves showed that the plant contains appreciable amount of the basic food nutrients: protein (10.00-14.22%), carbohydrate (66.61-75.05%), fat (2.00-4.46%), and fibre (5.15-9.04%) (Edeoga et al., 2006; Aguirre et al., 2012). The high content of carbohydrate shows that it is a
good source of energy and can help in the oxidation of fats. A diet rich in fibre is desirable because fibre has a physiological effect on the gastrointestinal function. It also has a biochemical effect on the absorption and re-absorption of bile acids and consequently the absorption of dietary fats and cholesterol (Edeoga et al., 2006). Thus it can serve as a source of nutritional dietary supplements. Analysis of the protein composition of the seeds showed the presence of globulins (39%), glutelins (36%), albumins (24%) and prolamins (1%). The content of branched amino acids is higher in *H. suaveolens* than in maize and other cereals (Aguirre et al., 2012). Thus it could provide a good supply of almost all the essential amino acids for different age groups. Though there has not been any report on the extensive use of this plant as food, in Asian food recipes leaf and essential oil is used as an appetizer due to the presence of its essential oil (Witayapan et al., 2007; Aguirre et al., 2012). The herb *H. suaveolens* therefore serves as an edible aromatic flavoring agent. Leaves and seeds of *H. suaveolens* contain mineral elements which are very important in human nutrition. *H. suaveolens* has been shown to contain other metals like zinc, copper and iron. Zinc plays a vital role in growth, aids the catalytic and regulatory action of more than 300 enzymes and helps to maintain a healthy immune system. Copper plays an important role in a wide range of physiological processes in the body which include iron utilization, elimination of free radicals, development of bone, and production of the skin and hair pigment called melanin (Umedum et al., 2013). Iron is used at the active site of many redox enzymes associated with cellular respiration, oxidation and reduction in plants and animals, and also plays a vital role in forming complexes with molecular oxygen in haemoglobin and myoglobin. The seed oil of *H. suaveolens* is liquid at room temperature and has moisture content and yield of 7.93% and 17.44%
respectively. The low moisture content shows that the oil can be stored for a long time. The saponification value shows that it has less impurity. Fatty acid profile of the seeds revealed the presence of palmitic acid (8.09%), stearic acid (2.23%), oleic acid (13.59%), linoleic acid (76.08%), and absence of linolenic, palmitolic, and myristic acids. The polyunsaturated fatty acids found in the seed oil helps to reduce ‘bad’ cholesterol and thereby reduce the risk of atherosclerosis and other heart diseases (Rai et al., 2013).

2.5.2. Phytochemistry

Phytochemicals are compounds with chemical nature that occur naturally in plants (Sathishkumar and Paulsamy, 2009). The medicinal values of plants depend on the bioactive compounds it possesses. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds. Petroleum ether, chloroform, methanol, ethanol, n-hexane, and water extraction of various parts of *H. suaveolens* shows the presence of phytochemicals such as alkaloids, flavonoids, terpenoids and tannins (Harborne, 1988; Hang and Lautsch, 1983; AOAC, 1990; Edeoga et al., 2006; Mbatchou et al., 2010; Prasanna and Koppula, 2012; Sulta et al., 2013). Very few researchers reported the presence of saponins in the leaves (6.10±0.42%) and stems (10.50±0.79 %) of *H. suaveolens* (Ijeh et al., 2007; Prasanna and Koppula, 2012), but were absent in the root (Prasanna and Koppula, 2012). In a study conducted by Okoye and Chukwu (2014) C_{24}H_{40}O_{3}, C_{15}H_{32}O_{2} and C_{20}H_{30}O_{2} were the alkaloid fragments identified by GC-MS while, the three major flavonoid fragments identified were C_{24}H_{40}O_{3}, C_{20}H_{30}O and C_{18}H_{34}O_{3}. Studies conducted by Pachkore and his co-workers (2011) on the aqueous and ethanolic leaf extracts showed the availability of volatile oil, starch, proteins, tannins, saponins, fats,
alkaloids, glycosides, etc. Leaves contain alkaloids (2.80±0.28 %), flavonoids (1.90±0.14 %), and tannins (5.50±0.074 %), while stems had alkaloids (1.60±0.00 %), flavonoids (0.30±0.14 %), and tannins (0.23±0.07 %) (Prasanna and Koppula, 2012). The presence of these phytochemicals has been attributed to the bioactive principles responsible for ethnopharmacological activities of medicinal plants (Edeoga et al., 2005; Omoyeni et al., 2012). Essential oils obtained by hydrodistillation from *H. suaveolens* was investigated by GC-MS analysis (Asekun and Ekundayo, 2000; Azevedo et al., 2001). The results showed that sabinene, limonene, bicyclogermacrene, beta-phellandrene, 1,8-cineole were the major constituents, others include eugenol, beta-caryophyllene, beta-pinene, and terpinolene (Fun and Svendsen, 1990; Sidibe et al., 2001; Kossouoh et al., 2010; McNeil et al., 2011; Kodakandla et al., 2012; Benelli et al., 2012; Uzama et al., 2013).

Satish et al. (2010) isolated hyptadienic acid (Rao et al., 1990), suaveolic acid, suaveolol, methyl suaveolate, β-sitosterol, oleanolic acid, ursolic acid, rosamarinic acid, dehydroabietinol (Ziegler et al., 2002), 3β-hydroxy lup-12-en-28-oic acid (Misra et al., 1983a), 3β-hydroxyl lup-20(29)-en-27-oic acid (Misra et al., 1983b) and essential oil (Peerzada, 1997). Recent studies conducted by Azeez et al. (2014) determined oil recovery potential of *H. suaveolens* which resulted in the extraction of 36 chemical components of which 72.54% were mono-terpenoids, 21.96% sesquiterpenoids and 5.49% non-terpenoid constituents.
Figure 2.1. Structures of phytoconstituents of *Hyptis suaveolens* L. Poit.

- α-copaene
- Eugenol
- Fenchol
- Sabinene
- 4-terpinenol
- α-Bergamotene
- limonene
- α-phellandrene
- Thujane
- 3,7-dimethyl-1,6-octadien-3-ol
- elemene
- β-pinene
- γ-terpinene
- fenchone
- bicycogermacrene
- β-caryophyllene
- 1, 8-cineole
- β-sitosterol
- ursolic acid
- suaveolic acid
2.5.3. Antioxidant activity

Production of Reactive Oxygen Species (ROS) causes various diseases and cellular anomalies in human beings (Sun et al., 2002; Bimal et al., 2011). Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. *H. suaveolens* has potent antioxidant ability of 69.46% at 100 μg/mL concentration and IC$_{50}$ value at 40.91 μg/mL concentration and a good correlation was found to exist between concentration of extract and % inhibition (Agarwal and Varma, 2013). The antioxidant activity of the methanol extract of the leaves of *H. suaveolens* has been evaluated in vitro by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity using gallic acid; a potent free radical scavenger and butylated hydroxyanisole (BHA); a known antioxidant, as reference standards (Gavani and Paarakh, 2008).

The antioxidant activity of *H. suaveolens* oil determined by the DPPH method expressed as IC$_{50}$ was 3.75mg/mL$^{-1}$ whereas, the TEAC value (Trolox Equivalent Antioxidant Capacity) as obtained by comparing the absorbance change at 750nm in a reaction mixture containing an oil sample with that containing Trolox) determined by the ABTS assay was 65.02 mM/mg. The results indicated that *H. suaveolens* oil possesses antioxidant activity (Nantitanon et al., 2007). Studies conducted by Priyadharshini and Sujatha (2013) on the ethyl acetate extracts of *H. suaveolens* leaves also showed good antioxidant activity showed excellent results (Priyadharshini and Sujatha, 2013). Recent studies on the methanolic extract of *H. suaveolens* leaves exhibited potent antioxidant activity as determined by ABTS, DPPH and ferric reducing antioxidant power assays (Ghaffari et al., 2014).
The antioxidant activity of the *H. suaveolens* essential oils determined by (DPPH) radical scavenging assay and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical decolourization assay indicated IC$_{50}$ (μg/mL) values of 3721±0.019 (Asekun and Ekundayo, 2000). The antioxidant activity of aqueous extract of *H. suaveolens* was determined by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test shows strong antioxidant radical scavenging activity with IC$_{50}$ value of 100 μg/mL (Mandwal *et al.*, 2009).

### 2.5.4. Antidiabetic activity

Diabetes mellitus is a leading metabolic disorder worldwide, caused by inherited or acquired deficiency in production of insulin in β cells of pancreas, or by the desensitization of insulin receptors for insulin. Such a deficiency results in the increased concentration of glucose in the blood which results in secondary complications affecting eyes, kidneys, nerves and arteries (Ismail, 2009). Danmalam *et al.* (2009) and Nayak *et al.* (2013) reported aerial parts of *H. suaveolens* have been reported to possesses antidiabetic activity, which might be related to the presence of tannins, terpenoids and flavonoids. Acute toxicity studies shows high LD$_{50}$ value (2154.1 mg/Kg body weight in rats) indicate that it can be considered as relatively safe (Danmalam *et al.*, 2009).

### 2.5.5. Anticancer activity

Cancer is one of the most dangerous diseases in humans and presently there is a considerable scientific discovery of new anti cancer agents from natural products. Cytotoxic studies conducted on compounds isolated from the leaves of *H. suaveolens* rendered potent effects on cancer cells (MCF-7). The growth of the cell lines was
inhibited in a concentration-dependent manner (Priyadharshini and Sujatha, 2013). Studies conducted by Musika and Indrapichate (2014) demonstrated that ethanolic and water extracts of *H. suaveolens* leaves possessed potent cytotoxic and apoptotic effects on T lymphocyte cancer cell line, Jurkat cells. The cytotoxic IC$_{50}$ values of ethanolic and aqueous extracts were 553.52±14.07 and 912.06±16.86 µg/mL, respectively. The apoptotic characteristics were detectable as cell nuclear blebbing, DNA fragmentation and up-regulation of Caspase-9, Bcl-2 and Bax proteins. The ethanolic extract of leaf was likely to enhance the growth of normal immunological cells.

2.5.6. Neuroprotective activity

Neurodegenerative diseases represent a large group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific subsets of neurons. Neurodegenerative disorders are a major cause of mortality and disability and as result of increasing life spans represent one of the key medical research challenges. The methanolic extract of *H. suaveolens* shows increased neuroprotective activity in mouse N$_2$A neuroblastoma cells. The methanolic extract also dose-dependently reduced LDH leakage and intracellular ROS production (p<0.05). Pretreatment with the extract promotes the up-regulation of tyrosine hydroxylase (2.41-fold, p<0.05), and brain-derived neurotrophic factor genes (2.15-fold, p<0.05) against H$_2$O$_2$-induced cytotoxicity in N$_2$A cells (Ghaffari *et al*., 2014).

2.5.7. Antimicrobial activity

Mbatchou *et al* (2010) reported that *H. suaveolens* possess phytochemicals which were more effective than antifungal drugs (griseofulvin) in inhibiting the
growth of fungal isolates such as *Aspergillus niger*, *Candida albicans*, *Rhizopus stolonifera*, *Cryptococcus* and *Fusarium* species. Chloroform and methanol extracts of *H. suaveolens* have antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter*, *Proteus mirabilis* and *Salmonella typhi* (Samrot *et al.*, 2010; Prasanna and Koppula, 2012). Similar studies were conducted by Pachkore and his co-workers (2011) on the aqueous and ethanol extracts of *H. suaveolens* using fungi like, *Candida albicans*, *Collectrotrichum capsici*, *Fusarium oxysporum*, *Fusarium* sp., *Lycopersici* sp. and four bacteria viz. *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The aqueous extract of plant material can not show any inhibition zone against microbes like *C. albicans*, *S. aureus* and *P. aeruginosa*. All the seven microbes tested were susceptible to ethanol extract with the inhibition zone range of 12-29 mm. The results of Nantitanon and his co-workers (2007) showed that the antifungal potential of *H. suaveolens* oil is more pronounced than its antibacterial properties. Its fungal growth inhibitory activity was dose dependent with a Minimal Inhibitory Dose (MID) value of 1:640. The 20% ethanolic solution of *H. suaveolens* oil had antifungal power similar to 6% boric acid, 2% benzoic acid or 5% salicylic acid but higher than 4% phenol. The activity decreased when the oil was stored at high temperature (>40˚C).

The antibacterial activity of *H. suaveolens* volatile oil was tested against various kinds of bacteria and fungi that caused dermatological diseases. It was reported that the volatile oil from *H. suaveolens* inhibits certain bacteria and fungi (Okonogi *et al.*, 2005). The essential oil of *H. suaveolens* leaves showed antibacterial activity at 5 mg/mL concentration against two gram-positive and four gram-negative
bacteria (Asekun et al., 1999). Steam distillation extract of *H. suaveolens* leaves exhibited broad spectrum antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus* and antifungal activity against *Fusarium oxysporum*, *Aspergillus niger* and *Helminthosporium oryzae*. (Mandal et al., 2007). Iwu et al. (1990) observed that essential oil of *H. suaveolens* displayed good antimicrobial activity against yeast, filamentous fungi and showed a mild inhibitory effect on *Candida albicans* and *A. niger*. The hydro distilled essential oil of fresh leaves of wild *H. suaveolens* exhibited significant antimicrobial activity against *Mucor* sp. when compared to ketoconazole (Malele et al., 2003). In another study antimicrobial activity of *H. suaveolens* was favor with MID values 1:160, 1:160, 1:80, 1:20, 1:20, 1:80 against *Staphylococcus aureus*, *Streptococcus suis*, *Erysipelothrix husiopathiace*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pasteurella multocida* and *Actinomyce pyogenes*, respectively (Tachakittirungrod and Chowwanapoonpoh, 2007). Antifungal studies of 95% ethanol extract of *H. suaveolens* leaves showed (2.39%) activity (Parichad and Krittaporn, 1990). It was also documented that the antifungal potential of *H. suaveolens* oil was more pronounced than its antibacterial properties. The study also reported that oil was less active against gram negative bacteria, particularly *P. aeruginosa* and *E. coli*, while it showed good results against gram positive bacteria.

**2.5.8. Antiplasmodial activity**

The effect of a substance in inhibiting the growth of *Plasmodium* is termed as antiplasmodial activity. *Hyptis suaveolens* is widely used in traditional medicine for malarial treatment and increased interest led to the identification of the constituent
responsible for this activity (Odugbemi et al., 2007). Dehydroabietinol isolated from *H. suaveolens* was found to inhibit the growth of chloroquine-sensitive as well as chloroquine-resistant strains of *Plasmodium falciparum* cultivated in erythrocytes *in vitro*. However, erythrocytes exposed to dehydroabietinol were transformed in a dose-dependent manner towards spherostomatoctytic forms with concomitant formation of endovesicles, as disclosed by transmission electron microscopy (Ziegler et al., 2002). Later, petroleum ether extract of the leaves of *H. suaveolens* was found to restrain an abietane-type diterpenoid endoperoxide known as 13α-epi-dioxiabiet-8(14)-en-18-ol which on further investigation displayed an elevated antiplasmodial activity with an IC\(_{50}\) of 0.1\(\mu\)g/mL. The antiplasmodial constituent dehydroabietinol of *H. suaveolens* showed its activity due to the transformation of discocytes into stomatocytes (Chukwujekwu et al., 2005).

### 2.5.9. Antidiarrhoeal activity

Diarrhoea is one of the main causes of high mortality rate in developing countries where over five million children under the age of five die annually from severe diarrhoeal diseases. Three to five billion cases occur annually (WHO, 1996), and approximately five million deaths are accountable to diarrhoea (Heinrich et al., 2005). It is most prevalent in area with high population coupled with poor hygiene; a major contributor to malnutrition and cause of rapid dehydration in infants and elderly people. It could, therefore, result in death if treatment is not given (WHO, 1995). Studies on the antidiarrhoeal activity of ethanol extract of *H. suaveolens* leaves against an experimental model of castor oil induced diarrhoea in mice has been reported (Zeshan et al., 2012). Oral administration of the ethanol extract of *H. suaveolens* (250 and 500 mg/kg) showed significant (P<0.01)
and dose-dependent inhibitory activity against castor oil induced diarrhoea. The onset of diarrhoea induced by castor oil was significantly delayed by the administration of the plant extract (Shaikat et al., 2014).

2.5.10. Antihelmintic activity

Helminths are among the most widespread infections in humans, distressing a huge population of the world. Especially in tropical region, helminths cause enormous hazard to health of humans and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia (Bundy, 1994). In vitro anthelmintic activity of ethanol and aqueous extracts of the whole plant of H. suaveolens extracts of the plant were investigated for activity against the Indian adult earthworm; Pheretima posthuma and Ascardia galli using piperazine citrate as positive and distilled water as negative control (Nayak et al., 2010). Different concentrations (25, 50 and 100 mg/mL) of each extract were studied for activity, based on the time of paralysis and the time of death of the worm. Extracts of H. suaveolens were found to exhibit significant anthelmintic activity at the highest concentration of 100 mg/mL (Ajaiyeoba et al., 2001).

2.5.11. Anti-inflammatory activity

Inflammation is the reactive state of hyperemia and exudation from blood vessels with consequent redness, heat, swelling and pain which a tissue manifests in response to physical or chemical injury or bacterial invasion (Mcdonald, 1988). The anti-inflammatory activity of two diterpenes, suaveolol and methyl suaveolate isolated from the leaves of H. suaveolens by column chromatography and repeated preparative thin layer chromatography has been
reported (Grassi et al., 2006). The anti-inflammatory activity of the compounds was tested as inhibition of croton oil-induced dermatitis of mouse ear. Doses ranging from 0.1 to 1μmol/cm³ were administered in comparison to those of the non-steroidal anti-inflammatory drug indomethacin. The anti-inflammatory activity was expressed as percentage of the oedema reduction in mice treated with the tested substances compared to control mice. ID₅₀ (dose giving 50% oedema inhibition) values of the tested compounds were calculated as an index of their anti-inflammatory activity. Results showed that suaveolol (ID₅₀=0.17μmol/cm²) and methyl suaveolate (ID₅₀=0.60μmol/cm²) were only two to three times less active than indomethacin (ID₅₀=0.26μmol/cm²). The anti-inflammatory properties of the diterpenes were considered to be contributors to the antiphlogistic activity of extracts of H. suaveolens, thus confirming its use in dermatological diseases (Grassi et al., 2006). The ethanolic extract of H. suaveolens was tested to study the effects on the inflammatory reaction, using the technique of Carageenan induced paw edema in albino rats. The extract showed significant anti-inflammatory activity comparable to the reference standard Ibuprofen. Antioxidant investigations of the ethanol extract along with its fraction using nitric oxide induced free radical assay methods showed good free radical scavenging activity thereby supporting its anti-inflammatory properties (Parichad and Krittaporn, 1990). The wound healing activity of H. suaveolens has been attributed to the presence of flavonoids and triterpenoids (Shenoy et al., 2009). These compounds possess astringent and antimicrobial properties which may be responsible for wound contraction and increased rate of pithelialisation. Shirwaikar et al. (2003) evaluated H. suaveolens for its wound healing activity in ether anaesthetized Wistar rats at doses of 400 and 800 mg/kg
using incision, excision, and dead space wound model. Significant increase in skin breaking strength, granuloma breaking strength, wound contraction, hydroxyproline content, dry granuloma weight and decrease in epithelization period was observed. However the enhanced wound healing activity may be due to free radical scavenging action of the plant and enhanced level of antioxidant enzymes in granuloma tissue.

2.5.12. Antiulcer activity and Gastroprotective Activity

Peptic ulcer is one of the most common gastrointestinal diseases (Dandiya and Kulkarni, 2005). The exact cause of peptic ulcer disease is not known but it may result from an imbalance between acid-pepsin secretion and mucosal defense factors (Udaykumar, 2005). Antiulcer activity of aqueous (500 mg/kg) and ethanolic extract (500 mg/kg) of the *H. suaveolens* was evaluated on cysteamine hydrochloride (450 mg/kg) induced gastric and duodenal ulceration. The aqueous extract of the plant showed potent activity than ethanolic extract, concluding that the plant increases the healing of duodenal ulceration and prevents the development of experimentally induced duodenal ulceration in rats (Das *et al.*, 2009). Vera-Arzave *et al.* (2012) reported that suaveolol isolated from hexane extract showed gastroprotective activity at doses between 10 and 100 mg/kg.

2.5.13. Antifertility activity

The magic of Indian plants in reducing fertility of mammalian species is well established. Fertility control is an issue of global and national public health concern. The anti-fertility effects of the petroleum ether, alcohol, and aqueous extracts of *H. suaveolens* were studied in pregnant rats. The alcoholic extracts of leaves showed
100% anti-fertility action at doses of 150 mg/kg and 125 mg/kg, respectively (Garg, 1976).

2.5.14. **Immunomodulatory activity**

Immunomodulatory agents of plant origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. The alcoholic extract of *H. suaveolens* possesses immunomodulatory as well as antioxidant property, and the latter property may be responsible for the amelioration of the immunosuppressant effect of pyrogallol (Bhagwat and Umathe, 2003). A recent investigation, by Jain *et al.* (2010) reported that the dried alcoholic (90%) extract of the aerial parts of *H. suaveolens* not only prevented the pyrogallol induced suppression of Humoral Immune Response (HIR) and Cell Mediated Immune Response (CMIR) but also prevented the rise in Lipid Peroxidase Enzyme (LPO) levels, when administered orally (75 mg/kg for 28 days), to the group of mice with artificially induced immune suppression and oxidative stress using pyrogallol (50 mg/kg for 05 days). However, the immunomodulatory activity of *H. suaveolens* has been attributed to their anti-oxidant properties confirmed by TBARS (Thiobarbiturate Acid Reactive Substance) method.

2.5.15. **Insecticidal activity**

Botanical insecticides have long been touted as alternatives to synthetic chemical insecticides for pest management because they pose little threat to the environment and human health. *H. suaveolens* has been reported to be effective against infestation by the pink stalk borer, *Sesamia calamistis* on maize. It has been used to control *Trogoderma granarium* (Coleoptera: Dermestidae) in stored
groundnuts (Adda et al., 2011; Musa et al., 2009). Other reports have shown that methanolic extracts of the plant were effective in the biological control of *Sitophilus oryzae* (rice weevil), *Sitophilus zeamais* (maize weevil), and *Callosobruchus maculatus* which are serious stored product pests that attack various economically important crops. The essential oil has also been reported to be effective against the adult granary weevil *Sitophilus granaries* (Benelli et al., 2012). A protease inhibitor isolated from the seeds of *H. suaveolens* has been reported to have a high activity against the intestinal trypsin-like proteases from different insect pests, particularly against the insect *Prostephanus truncatus*, a most important insect pest of maize. Research conducted on its use for protection against mosquito bites has shown that it is as effective as DEET (N, N-dimethyl-3-methyl benzamide), one of the well-known arthropod repellents (Aguirre et al., 2009; Abgali and Alavo, 2011). Another study conducted on *Meloidogyne javanica* showed that the application of *H. suaveolens* as soil amendment reduced the pathogenicity of *M. javanica* but at higher levels it became phytotoxic and highly nematicidal (Onyekw et al., 2014). The ability of *H. suaveolens* to act as an effective insecticide or pesticide has been attributed to its essential oils. However, it is advised that in cases where it has been employed by method of mixed cropping, caution should be applied since *H. suaveolens* is a fairly prolific plant and may compete with crops for space, water and nutrients.

**2.5.16. Pharmacognostical Studies**

Pharmacognostic evaluation of the leaves, stems and roots of *H. suaveolens* were studied by Pachkore and Dhale (2011) which showed the presence of glandular and non-glandular trichomes. Reports on the anatomy of the lamina of *H. suaveolens* have shown that epidermal cells are circular to oval shaped with thin cuticle and
scandy content whereas, 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 *Hyptis suaveolens*.

**2.5.17. Toxicity**

The ethanolic extract of *Hyptis suaveolens* was examined for its toxicity effect on the larvae of the yellow fever mosquito *Aedes aegypti*. Eight graded concentrations of: 0.9 ppm, 0.8 ppm, 0.7 ppm, 0.6 ppm, 0.5 ppm, 0.4 ppm, 0.3 ppm and 0.2 ppm of plant extract were tested on the larvae. The mean lethal dose LD$_{10}$ was 0.01 ppm while LD$_{50}$ was 0.60 ppm and LD$_{90}$ was 1.45 ppm. LD$_{10}$ for the control was 0.65 ppm, LD$_{50}$ 0.9 ppm and LD$_{90}$ 2.0 ppm. The extract caused high mortality rate on the larvae at concentrations of 0.9 ppm (80%) and 0.3 ppm (80%) (Bhagwat and Umathe, 2003). Ethanolic extract from the whole plants of *H. suaveolens* was screened using the brine shrimp lethality test. The extract was found to possess significant toxicity against brine shrimps with LD$_{50}$ value of 0.914 ppm at 99% confidence level. The result suggests the presence of highly active, bioactive compounds and requires further examination for detection of specific pharmacological properties. Repeated dose dermal toxicity studies of *H. suaveolens* cream for 28 days in various concentrations (3%, 10% and 30%) revealed that the cream in the concentrations of 3% and 10% produces no toxic effect. (Kumar *et al.*, 2006). Preliminary study showed no mortality during acute toxicity test with doses up to 5g/kg (Santos *et al.*, 2007).
2.6. Other Uses
2.6.1. Seed coat as a source of mucilage

The mucilage isolated from the seed coat of *Hyptis suaveolens* contains l-fucose, d-xylose, d-mannose, d-galactose, d-glucose and 4-\(O\)-methyl-d-glucuronic acid in the mol ratios 1.0:2.5:1.5:7.0:12.5:1.1. Fractionation of the mucilage with Fehling's solution gave a neutral and an acidic polysaccharide. The neutral polysaccharide appears to be homogeneous and is composed of d-mannose, d-galactose and d-glucose in the mol ratios 1.0:4.5:7.5. The acidic polysaccharide is composed of l-fucose, d-xylose and 4-\(O\)-methyl-d-glucuronic acid in the mol ratios 1.0:2.5:1.1. It is homogeneous on gel filtration, DEAE-cellulose chromatography, sedimentation analysis and electrophoresis (Gowda, 1984).

2.6.2. Effect of *H. suaveolens* on seed mycoflora

Recent studies conducted by Patil and Madane (2014) showed that aqueous leaf extracts of the common weed *H. suaveolens* were assessed on seeds of *Cicer arietinum* and *Cajanus cajan*. The percentage of infected seeds was proved to increase with concentration of plant extracts and the soaking period decreases seed mycoflora.

Need for the present study

Extensive review of literature has indicated an accumulation of voluminous literature on functional and pharmacological properties of the common weed *Hyptis suaveolens*. Even though the plant is widely distributed and cheaply available, there are gaps to be filled with respect to knowledge of the pharmacognosy and pharmacology of the plant. Hence efforts were taken to find the phytochemistry, antioxidant, antihaemolytic, antidiabetic, anticancer, antimicrobial and larvicidal activities of the select plant.