CHAPTER 2: REVIEW OF LITERATURE

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. More than 80% of world population uses herbal medicines to deal with their daily medicinal issues. India has been considered as treasure house of a large number of valuable medicinal and aromatic plant species (Chopra et al., 1956; Kirtikar and Basu 2005; Anonymous 1976; 2001). The Ministry of Environment and Forests has identified and documented over 9500 plant species considering their importance in the pharmaceutical industry. An analysis of distribution of medicinal and aromatic plants in natural habitat showed that about 70% of them are found in tropical forests of Western and Eastern Ghats, Vindhyas, Chotta Nagpur plateau, Aravalis and the Himalayas.

The aromatic grasses belonging to the family Poaceae are a group of perennial herbs comprising of few genera such as Hierochloe, Elionurus, Cymbopogon, Bothriochloa and Vetiveria or Chrysopogon, widely distributed in the tropics and subtropics of the world. Cymbopogon and Vetiveria are well known for their essential oils of immense commercial significance in flavors, fragrances, cosmetics, perfumery, soaps, detergents and pharmaceuticals (Ganjewala 2009; Mishra et al., 2013).

Palmarosa oil extracted from Cymbopogon martini, well known as Indian ‘rosha’ and ‘motia’ has sweet floral with a hint of rose smell (Lawrence et al., 2012). Another important essential oils extracted from aromatic grasses is citronella oil obtained from citronella grass. Citronella oil is classified into two types- Ceylon citronella oil (inferior type), obtained from Cymbopogon nardus, while Java type citronella oil (superior type) is obtained from Cymbopogon winterianus (Wany et al., 2013). Lemongrass Cymbopogon citratus is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlini et al., 1986). The plant is a native herb from India and is cultivated in other tropical and subtropical countries (Figueirinha et al., 2008). Vetiveria zizanioides (Linn.) Nash, a member of the family Poaceae commonly known as the Khus grass in India,
is a perennial grass with thick fibrous adventitious roots which are aromatic and highly valued. Over 150 compounds have been isolated and characterized from vetiver oil so far. A major portion of oil consists of sesquiterpenoide, hydrocarbons and their oxygenated derivatives. Phytochemical screening of the powdered leaves shows the presence of alkaloids, flavonoids, tannins, phenols, terpenoids and saponins. The roots are aromatic with antifungal, antiemetic, diaphoretic, haemostatic and diuretic properties. It is used for insomnia, skin diseases, asthma, amnetia, amenorrhoea, and in kidney problems (Chomchalow and Chapman 2003; Rao and Suseela 1998).

The aromatic plant yields essential oils as secondary metabolites that are volatile, natural, complex compounds characterized by a strong odor. They are usually obtained by steam or hydro-distillation first developed in the middle Ages by Arabs. Known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and for anesthetic remedies. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia. In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favor the dispersion of pollens and seeds, or repel undesirable others. They are liquid, volatile, limpid and rarely colored; lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermal cells or glandular trichomes.

These oils are complex mixtures of numerous molecules, and their biological effects are the result of a synergism of all molecules or may reflect only those of the main molecules present at the highest levels according to gas chromatographical analysis. The major components like terpineol, eugenol, thymol, carvacrol, carvone, citral, geraniol, linalool, citronellol, nerol, safrole, eucalyptol, limonene, cinnamaldehyde reflect the
biophysical and biological features of the essential oils from which they were isolated (Ipek et al., 2005). The amplitude of their effects is dependent on their concentration when they were tested alone or comprised in essential oils. It is likely that several components of the essential oils play a role in defining the fragrance, the density, the texture, the color and above all, cell penetration (Cal, 2006), lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution. For biological purposes, it is more informative to study the entire oil rather than some of its components because the concept of synergism appears to be more meaningful.

At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. For example, d-limonene, geranyl acetate or d-carvone are employed in perfumes, creams, soaps, as flavor additives for food, as fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder (Hajhashemi et al., 2003; Perry et al., 2003; Silva et al., 2003;). Owing to the new attraction for natural products like essential oils, familiar to us as fragrances, it is important to develop a better understanding of their mode of biological action for newer applications in human health, agriculture and the environment. Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the same secondary effects (Carson and Riley, 2003).
Biological effects

Anti-bacterial activity

For years, food borne illness resulting from consumption of food contaminated with pathogenic bacteria and/or their toxins has been of vital concern to public health. Controlling pathogens could reduce food borne outbreaks and assure consumers a safe, wholesome, and nutritious food supply. Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit food borne bacteria and extend the shelf life of processed food. Rapidly developing resistance of pathogenic microorganisms against currently available drugs/treatment is another major concern. Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens (Deans and Ritchie, 1987; Janssen et al., 1985, Shelef 1984).

Essential oils of Cymbopogon species have strong antimicrobial properties and thus could produce alternative therapeutics to current antibiotic drugs. The antimicrobial properties of C. flexuosus, C. citratus, C. martinii, C. winterianus, C. nardus, and C. parkeri have been well documented. Additionally, citronella oil demonstrated pronounced antibacterial activity against Propionibacterium acnes which is a major cause of acne (Duarte et al., 2007; Lertsatitthanakorn et al., 2006; Oussalah et al., 2007). The inhibitory effect of citronella oil against major species of spoilage bacteria was demonstrated against Staphylococcus aureus, Klebsiella spp. and Pseudomonas spp. (Matan et al., 2011). According to Onawunmi et al., (1984) geranial and neral, the major compounds in lemongrass oil also presented positive antimicrobial effect. Recently, antimicrobial activities of Cymbopogon citratus and Cymbopogon giganteus essential oils alone and in combination have been reported by Bassole et al., (2011).

The antibacterial activity of vetiver was measured by zone of inhibition (mm) test on four bacterial strains (two gram positive Staphylococcus aureus, Bacillus subtilis and two gram negative bacteria Pseudomonas aeruginosa, Escherichia coli). Ethanolic extract of Vetiveria zizanioides is known to posses flavonoids, alkaloids, terpenoids, saponins, tannins and phenols which either individually or combination exert antimicrobial activity. The study
showed that the extract inhibited gram negative bacteria than gram positive bacteria. Flavonoids are effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to form complex with extracellular and soluble proteins and bacterial cell wall, disrupting microbial membranes. The presence of tannins in the roots of Vetiveria zizanioides implied that tannin may be the active compound which may be responsible for in vitro antibacterial activity in their study. Tannin in the plant extract was found to possess antibacterial activity (Subhadradevi et al., 2010a). Nantachit et al., (2010) reported antimicrobial property of the alkaloids isolated from the vetiver roots. They also observed significant cytotoxicity by brine shrimp bioassay. Ratha et al., (2012) reported that ethanol and methanol extract of Vetiveria zizanioides exhibited inhibition of growth against Escherichia coli, Klebseilla pneumoniae, Salmonella typhimurium, Staphylococcus aureus and Vibrio cholera.

Anti-fungal activity

Food-spoiling organisms include some filamentous fungi that grow on all kinds of foods products. The fungal growth may result in several kinds of food spoilage such as off-flavors, toxins, discoloration, mycolytic enzymes, rotting and formation of pathogenic or allergenic propagules. The production of mycotoxins, in particular, has a major negative impact of fungal growth in foods.

Geraniol, which accounts for 90 % of palmarosa essential oil in Cymbopogon martinii, was found to inhibit growth of yeast Saccharomyces cerevisiae either by causing excessive K+ ion leakage from yeast cells or alterations in the cell membrane composition (Prashar et al., 2003). The oil has inhibitory effects on the growth of dermatophytes and filamentous fungi (Prasad et al., 2009).

Nakahara et al., (2003) studied the chemical composition of citronella oil and its antifungal activity. The crude essential oil markedly suppressed the growth of several species of Aspergillus, Penicillium and Eurotium. Among the 16 volatiles examined, the most active compounds, consisting of 6 major constituents of the essential oil and 10 other related monoterpenes, were citronellal and linalool. Antifungal activity (Harris 2002;
Nakahara et al., 2003), including anti-Candida action (Duarte et al., 2005; Hammer et al., 1998; Lertsatitthanakorn et al., 2005) were exhibited by the essential oil of citronella.

De Billerbeck et al., (2001) have studied the effect of the essential oil of *Cymbopogon nardus* (lemongrass) on the growth of *Aspergillus niger*. The study revealed that the essential oil (800 mg/ml) had strong inhibitory action causing cytological modifications on growth of the mycelium. It was suggested that the essential oil damaged the plasma membrane and mitochondrial structural organization. Subsequently, Helal et al., (2006) described a very similar antifungal property of *C. citratus* essential oil with negative effects on the growth, lipid content and morphogenesis in *A. niger* ML2-strain. The MIC of the essential oil was 1.5-2.0 μ/ml. Observation of *A. niger* hyphae treated with essential oils under the light, scanning electron and transmission microscope demonstrated the ultrastructural alterations in the hyphae, which might be developed during treatment with essential oil. Fumigation with essential oil also caused great loss in Ca^{2+}, K^{+} and Mg^{2+} ions from the mycelium. Moreover, *C. citratus* essential oil is reported to block aflatoxin B production in *A. niger* (Helal et al., 2006; Helal et al., 2007).

The essential oil from *C. citratus* has been found to be useful in treatment of oral and vaginal candidiasis (Abe et al., 2003), with citral exhibiting powerful inhibitory effects on growth of the yeast (Abe et al., 2003). Other yeasts affected include *Candida oleophila, Hansenula anomala, Saccharomyces cerevisiae, S. uvarum, Schizosaccharomyces pombe* and *Metschnikowia fructicola*. The growth of number of filamentous fungi, namely *Alternaria alternata, Aspergillus niger, Fusarium oxysporum* and *Penicillium roquefortii* were inhibited (Irkin and Korikluoglu 2009). The essential oil and powder of *C. citratus* is used to control storage deterioration and aflatoxin contamination of melon seeds caused by *Aspergillus flavus, A. niger, A. tamarii* and *Penicillium citrinum* (Bankole et al., 2005). The advantages of this treatment are that the essential oil does not affect the biochemical composition of the seeds and has strong effects comparable to that of a commercial fungicide iprodione and is a better and safe natural fungicide.

The essential oil presented significant antifungal activity against *Candida albicans* (Hammer et al., 1998; Syed et al., 1990); *Candida pseudotropicalis* and *Mycosporum*
gypseum, Aspergillus niger and Beauveria bassiana (Raghavaiah and Jayaramaiah, 1987). It inhibited the growth of Aspergillus flavus, a fungus that is a common originator of alimentary deterioration and it totally inhibited the mycelial growth and the germination of the Didymella bryoniae spores, which damages the fruit quality and can lead to plant death (Mishra and Dubey, 1994). The aqueous extract of the plant leaves inhibited in vitro and in vivo the vegetal pathogenic fungi (Bankole and Adebamjo, 1995). Studies by Valarini et al., (1996) demonstrated that the essential oil of lemongrass totally inhibited the mycelial growth of Fusarium solani f. sp. phaseoli, Sclerotinia sclerotiorum and Rhizoctonia solani that affected bean, soy and potato cultures, among many others. The plant (oil) was also active against several dermatophyte fungi among them Trichophyton rubrum, Microsporum gypseum, Aspergillus fumigatus and Cladosporium trichoides (Kishore et al., 1993). The plant oil presented inhibition activity of the fungi growth also associated to cereals storage as Aspergillus flavus, A. fumigatus, Microphomina phaseoli and Penicillium chrysogenum (Adegoke and Odesola, 1996). Wright et al., (2009) have reported that lemon juice and Cymbopogon citratus infusion is safe and highly effective in the treatment of oral thrush in HIV/AIDS patients in comparison to group using gentian violet aqueous solution (0.5%). This randomized controlled trial validated the efficacy of lemon juice and lemongrass for the treatment of oral candidiasis in an HIV population in South Africa.

The antifungal activity of ethanol and aqueous extracts of Vetiveria zizanioides were exhibited on Aspergillus niger, Aspergillus clavatus and Candida albicanus etc (Dikshit and Hussain 1984; Sridhar et al., 2003).

Antiprotozoal activity

In the present scenario of protozoal infections, new drugs are urgently needed to treat and control infections such as malaria, sleeping sickness, chagas disease, leishmaniasis and intestinal infections, which affect millions of people each year. The essential oils could be promising antiprotozoal agents, opening perspectives to the discovery of more effective drugs of vegetal origin for the treatment of diseases caused by protozoa. Various essential oils show inhibitory action against diverse human parasites such as Leishmania major and L. amazonensis, and Trypanosoma cruzi (Calixto 2000; Camacho et al., 2003; Santoro et al.
Treatment of axenic promastigotes and amastigotes, and intracellular amastigotes of *L. amazonensis* with essential oil from *Cymbopogon citratus* or citral showed a dose-dependent inhibition effect on the proliferation of *L. amazonensis*. Moreover, both the essential oil and citral demonstrated a strong effect against the intracellular amastigote, as revealed by the survival index (Santin et al., 2009). Similar results have been described in assays with other protozoa. Pedroso et al., (2006) studied the *in vitro* anti-protozoan activity of *C. citratus* essential oil against *Crithidia deanei*, and found that the mean IC50 for symbiont-bearing and symbiont-free strains were 120 and 60 μg/ml, respectively. Studies on *L. amazonensis* and *T. cruzi* with various hydroalcoholic extracts of the plants, including *C. citratus* extract, demonstrated that extracts showed activity against both protozoa, reaching high levels of growth inhibition (over 90 %) at 100 μg/ml (Luize et al. 2005). More recently, it was demonstrated that the essential oil of *C. citratus* was effective in killing *T. cruzi* with low IC50/48 h (5μg/ml) for intracellular amastigotes and IC50/24 h (15μg/ml) for bloodstream trypomastigotes (Santoro et al. 2007)

**Anthelmintic activity**

Gastrointestinal nematodes in livestock are usually controlled by commercial anthelmintics. However, few commercial anthelmintics are available for veterinary use due to reduced effectiveness caused by emerging drug-resistant parasite strains (Molan et al., 2002). *Haemonchus contortus* is the most prevalent and pathogenic nematode found in small ruminants in the tropics. Many alternative strategies to control nematodes have been studied such as adequate nutrition, selection of resistant animals, integrated pasture management, use of nematophagus fungus, and new anthelmintic compounds derived from plants. There is increased evidence indicating that some bioactive plants might possess anthelmintic properties and, thus, represent a promising alternative to commercially available drugs (Brunet and Hoste, 2006). Palmarosa oil demonstrated significant anthelmintic activity against the nematode *Caenorhabditis elegans* (Kumaran et al., 2003) and earthworm *Pheretima posthuma* (Nirmal et al., 2007).
Anti-tubercular activity

Tuberculosis, a fatal infectious disease, is the leading cause of mortality worldwide, infecting about 9 million people and kills approximately 2 million people annually (World Health Organization, 2004). Since 1980s, the number of tuberculosis cases throughout the world has been increasing rapidly due to the emergence of multi-drug resistant *Mycobacterium tuberculosis* (Chan and Iseman, 2002). The situation has recently been complicated by the association of tuberculosis with human immunodeficiency virus (HIV) in Africa and many developing countries of the World (Corbett et al., 2003; Lurie et al., 2004). Therefore, for the development of new drugs, the essential oils have been screened for effective antitubercular activities.

The essential oil of *Vetiveria zizanioides* showed significant antimycobacterial activity against the drug-resistant strains of *Mycobacterium smegmatis* (Gupta et al., 2012). Purification of the bioactive fractions resulted in the isolation and characterization of six compounds: 5, 10-pentadecadiyn-1-ol, a-curcumene, hydroxy junipene, (+) cycloisosativene, valencine and selino 3,7(11)-dien. All these compounds showed significant antimycobacterial activity against the drug-resistant strains (MDR-R and MDR-40) of *M. smegmatis* and their minimum inhibitory concentration (MIC) was in the range of 31.25–62.5 µg/ml.

In another study, *Vetiveria zizanioides* L. Nash root (intact and spent) extracts and fractions were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* H(37)Rv and H(37)Ra strains using radiometric BACTEC 460 tuberculosis system. The ethanolic extract of intact as well as spent root was showed potent antituberculosis activity at a minimum concentration 500 µg/ml. The hexane fraction also showed antibacterial action by recording continuous decline in growth index of *Mycobacterium tuberculosis* at 50 µg/ml. It was furthermore observed that root extract and hexane fraction showed activity even after the extraction of essential oil by hydro-distillation (Saikia et al., 2012).
Insect repellent property and pest control

Currently, there are plant-based insect repellents on the market that contain essential oils such as citronella, cedar, eucalyptus, geranium, lemongrass, peppermint, neem and soybean. Most of these essential oil-based repellents tend to give short-lasting protection for less than 2 h (Choochote et al., 2007). Palmarosa oil is safe for human use and is thus recommended for protection from malaria due to its potent repellent action against mosquitoes. *C. martini* var. *sofia* essential oil exhibited repellent action against *Anopheles sundaicus* (Das and Ansari 2003). Beside mosquito repellent activity, palmarosa essential oil also showed strong pesticidal activity against insect infestation and is used to protect stored wheat and grain from the beetles *Callosobruchus chenesis* and *Tribolium castaneum* (Kumar et al., 2007).

Citronella oil has demonstrated good efficacy against 44 mosquito strains in concentrations ranging from 0.05 % to 15 % (w/v) alone or in combination with other natural or commercial insect repellent products (Fradin 1998; Sakulku et al., 2009). Larvicidal effect has been demonstrated against *Aedes aegypti* (De Mendonca et al., 2005). Murugan et al., (2012) demonstrated that microencapsulation based on coacervation method, gave the best mosquito repellency by microencapsulated citronella oil. Trongtokit et al., (2005) compared the repellent efficiency of 38 essential oils against mosquito bites, including the species *Aedes aegypti*. Citronella oil was the most effective among other essential oils. The essential oil of *Cymbopogon* species (*C. citratus*, *C. nardus*, *C. martini*) were very effective against anopheline mosquitoes, *Anopheles culicifacies* and *Anopheles quinquefasciatus* (Ansari and Razdan 1995), and it also inhibited certain development phases of the mosquito *Aedes aegypti* – host vector of the yellow fever and dengue (Osmani and Sighamony 1980). Wong et al., (2005) studied five commercial plant extracts, including citronella, and found it effective in deterring the infestation of cartons containing muesli and wheat germ by red flour beetles. Moreover, Olivo et al., (2008) proved that citronella oil has other effects, such as the control of cattle ticks. The plant oil and powder showed efficiency in protecting stored seeds against *Callosobruchus maculatus* – bean woodworm, resulting in reduction or inhibition of eggs laid and its emergency (Adebayo and Gbolade 1994; Gbolade
and Adebayo 1993). This insecticide activity was also confirmed by Rajapake and Vamemden (1997) including Callosobruchus chinensis and C. rhodesianus. The lemongrass oil acted as ovicide and larvicide of Spodoptera exigua (Sharaby 1988). This plant oil showed excellent results both in direct and indirect application, against the Diptera species that causes cutaneous mycosis (Subramanian and Mohanan 1980). The aketonic extract of C. citratus caused significant insect repellent activity against Aphis craccivora (Ofuya and Okuku, 1994) greenfly considered pest in pea and bean cultures. According to Onabanjo et al., (1993), the aqueous extract of the plant of Nigerian origin was effective in fighting malaria in mice by Plasmodium yoelii nigeriensis.

Singh et al., (1978) advocated that vetiver oil could be utilized as anti-bacterial and anti-fungal agents to combat agricultural pests. Juliard (2001) reported that vetiver is used in traditional grain storage as a way to preserve rice crops. Maistrello and Henderson (2001) found a group of compounds, such as nootkatone, in vetiver roots, which were able to disrupt termite behavior and physiology as a consequence of direct physical contact, ingestion, or exposure to the vapors. They also found that ingestion of wood treated with vetiver oil or nootkatone causes the progressive death of the protozoa living inside the termite gut, ultimately results in a progressive decline of its colony through starvation, as these termites rely on the protozoa for the digestion of their wooden food. The powdered root, used in sachets, protected Indian muslin from moths and insects (Sellar 1992). The two tricyclic sesquiterpenoids – zizanal and epizizanal – isolated from vetiver oil show insect repelling activity (Jain et al., 1982). Nootkatone from vetiver oil and its derivatives, tetrahydronootkatone and 1, 10-dihydronootkatone have been patented as repellent against mosquitoes, cockroaches, termites and ants (Henderson et al., 2005 a, b; Zhu et al., 2005). The nanoemulsions composed of citronella oil, hairy basil oil, and vetiver oil with mean droplet sizes ranging from 150 to 220 nm were prepared and investigated both in vitro and in vivo. Larger emulsion droplets (195-220 nm) shifted toward a smaller size (150-160 nm) after high-pressure homogenization and resulted in higher release rate. It was proposed that thin films obtained from the nanoemulsions with smaller droplet size would have higher integrity, thus increasing the vaporization of essential oils and subsequently prolonging the mosquito repellent activity. In the laboratory oviposition deterrent test, the root extract of
*Vetiveria zizanioides* at each concentration greatly reduced the number of eggs deposited by the gravid *Anopheles stephens* (Arthi and Murgan 2012).

**Anti-nociceptive property**

The anti-nociceptive effect is a reduction in pain sensitivity made within neurons when endorphin or a similar opium-containing substance combines with a receptor. Numerous herbal medicines are recognized as active in the central nervous system (CNS), and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy that do not respond well to conventional treatments (Carlini, 2003). Epilepsy is a neurological disorder awaiting safer drugs with improved anticonvulsant and anti-epileptogenic properties. Currently available drugs fail to provide adequate control of epileptic seizures in about one-third of patients and do not prevent progressive epileptogenic changes (Cockerell, 1996). The essential oils are known to exhibit a variety of biological properties, such as analgesic anticonvulsant and anxiolytic (De Almeida et al., 2004). There are a few controlled experimental studies on their CNS activity, with some discrepant results. Lemongrass oil produced marked depression on the CNS of mice Seth et al., (1976) and prolonged the sleep time of mice (Ferreira & Fonteles 1985). On the other hand, studies on mice and rats using tea obtained from fresh and dry leaves did not confirm the sedative effect of this plant (Carlini et al., 1986). The lemongrass dry leaf tea, administered to healthy volunteers did not show any hypnotic effect (Leite et al., 1986). However, antinociceptive activity of the essential oil of *C. citratus* was reported by Viana et al., (2000). As pointed out by Viana et al., (2000) negative results obtained in rodents (Carlini et al., 1986; Souza-Formigoni et al., 1986) and in human beings (Leite et al., 1986) could be due to different chemotypes of lemongrass evaluated, as there are at least two varieties: East Indian (containing equal amounts of myrcene and citral) and the West Indian type (containing little myrcene but high amount of citral). *Cymbopogon winterianus* essential oil demonstrated depressant effect on the central nervous system with anticonvulsant effect (Blanco et al., 2009; Quitans et al., 2008) and antinociceptive properties in rodents (Leite et al., 2010). Antidepressant activity of the ethanolic extract of
**Vetiveria zizanioides** was observed in rats with depressive behavior (Glory Josephine et al., 2012).

**Anti-phlogistic activity**

Inflammation can be differentiated between two types (1) acute inflammation, an initial response of the body to harmful stimuli and (2) chronic inflammation, which lead to a number of diseases and needs to be treated by anti-inflammatory drugs. There are a number of inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-8, IL-10 and prostaglandin E2 (PGE2). The effects of lemongrass essential oil *Cymbopogon citratus* on IL-1β and IL-6 production by macrophages was analyzed by Sforcin et al., (2009). The *in vivo* and *in vitro* effects of water extracts of lemongrass were tested on proinflammatory cytokine (IL-1β and IL-6) production by macrophages of BALB/c mice. The results showed an inhibition of the production of IL-1β by macrophages, but the water extract induced IL-6 production. Furthermore, the essential oil of lemongrass led to an inhibition of the cytokine production *in vitro*. Based on these data, the authors suggest an anti-inflammatory activity of lemongrass. Citral, a chemical component of lemongrass oil was identified as a suppressor of COX-2 and an activator of PPARα and γ in *in vitro* studies by Katsukawa et al., (2010). They found that COX-2 promoter activity was suppressed by lemongrass oil in cell-based transfection assays. In human macrophage-like U937 cells, citral suppressed both LPS-induced COX-2 mRNA and protein expression, dose-dependently. Moreover, citral induced the mRNA expression of the PPARα-responsive carnitine palmitoyltransferase 1 gene and the PPARγ-responsive fatty acid binding protein 4 gene, suggesting that citral activates PPARα and γ, and regulates COX-2 expression.

Earlier studies by Carbajal et al., (1989) registered weak diuretic and anti-inflammatory action of the leaf decoction of lemongrass (*Cymbopogon citratus*) administered orally to mice.

Essential oil of vetiver exhibited potential antinociceptive and anti-inflammatory activities, which might involve a peripheral mechanism, such as, inhibiting the synthesis or action of prostaglandins (Lima et al., 2012).
Hepatoprotective activity

The aqueous leaf extracts of *Cymbopogon citratus* showed anti-hepatotoxic action against cisplatin induced hepatic toxicity in rats. Hence the extracts have the potential to be used for the management of hepatopathies and as a therapeutic adjuvant in cisplatin toxicity (Arhogro et al., 2012) Hepatoprotective activity of *V. zizanioides* Linn against ethanol-induced liver damage in rats was reported by Parmar et al., (2008). Parmar et al., (2013) also found that the methanolic extract of *Vetiveria zizanioides* roots had hepatoprotective effect against carbon tetrachloride-induced acute liver damage in rats.

Anti-hyperglycemic activity

The effect of root extract of *Vetiveria zizanioides* in rats after multiple doses showed significant antidiabetic activity at 2nd and 4th h after administration compared to diabetic control, results were comparable with standard glibenclamide. The study indicates the ethanolic extract of *Vetiveria zizanioides* roots possess better antihyperglycemic activity than any other extract, in both normal and allaxon induced diabetic rats (Karan et al., 2012). Rajeswari and Rajagopalan (2013) showed that *Crysopogon zizanioides* have hypolipidemic properties in streptozotocin induced diabetic Wistar rats.

Anti-oxidant activity

*Cymbopogon* species are considered as antioxidants. The antioxidant activity of palmarosa essential oil has been evaluated by Lawrence et al., (2012) using 2,2- Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) free radical assay, nitrogen oxide assay, reducing power assay, β-carotene bleaching assay and ferric reducing antioxidant power (FRAP) assay. Their results clearly indicated that palmarosa essential oil is effective in scavenging free radical and has the potential to be a powerful antioxidant.

Baratta et al., (1998) showed that the lemongrass oil exhibited anti-oxidant properties comparable to α-tocopherol and butylated hydroxytoluene (BHT). Cheah et al., (2001)
reported that the dichloromethanic and methanolic extracts of this plant showed powerful antioxidant activity. Methanol, methanol/water extracts, infusion, and decoction of *Cymbopogon citratus* (lemongrass) revealed free radical scavenging property measured by the bleaching of the DPPH radical, scavenging of the superoxide anion, inhibition of the enzyme xanthine oxidase (XO) and lipid peroxidation in human erythrocytes (Cheel et al., 2005).

The ethanolic extracts of the roots of *V. zizanioides* showed antioxidant activities such as reducing power ability, superoxide anion radical scavenging activity, deoxyribose degradation assay, total antioxidant capacity, total phenolics and total flavonoid composition (Subhadradevi et al., 2010b). Antioxidant capacities of vetiver (*Vetiveria zizanioides*) oil were evaluated by two different *in vitro* assays, the DPPH free radical scavenging assay and the Fe²⁺ metal chelating assay (Kim et al., 2005). Their results showed that the vetiver oil possessed a strong free radical scavenging activity when compared to standard antioxidants such as butylated hydroxytoluene (BHT) and alpha-tocopherol.

**Anti-carcinogenicity/anti-mutagenicity/anti-genotoxicity**

Cancer is a growing health problem around the world and is the second leading cause of death after heart disease (Reddy et al., 2003). According to a report by the International Agency for Research on Cancer (IARC), in 2008 there were 12.7 million new cancer cases throughout the world such as cancer of the liver, lung, stomach, colon, breast, etc. (Luk et al., 2007; Surh 2003) The most rational way to affect carcinogenesis is by interfering with the modulation steps (initiation, promotion and progression) as well as the associated signal transduction pathways (Fresco et al., 2006).

The cancer-protective effects elicited by dietary compounds are believed to be due to the induction of cellular defense systems, including detoxifying and antioxidant enzyme systems, as well as the inhibition of anti-inflammatory and anti-cell growth signaling pathways culminating in cell cycle arrest and/or cell death (Kwon et al., 2007). Many plant volatile constituents have been reported to possess potent antioxidant activity and to have anticancer or anticarcinogenic/ antimitagenic/ antiproliferative effects (Adorjan and
Buchbauer 2010; Bhalla et al., 2013). The essential oils under study are themselves devoid of mutagenicity and carcinogenicity.

Several studies have aimed at explaining the antitumor and anticancer activities of the lemongrass extract. According to Suayun et al., (1997), the ethanolic extract (80 %) of lemongrass promoted inhibition of colorectal neoplasia in mice. The study demonstrated that lemongrass extract inhibited the formation of axoxymethane (AOM)-induced DNA adducts and abberant crypt foci (ACF) in the rat colon. Although the exact mechanisms involved in the protective effects against ACF formation are not clearly understood at present, the results suggest that the inhibitory effects of the lemon grass extract depend partially on competitive inhibition of fecal β-glucuronidase and antioxidant activity of this plant.

The ethanolic extract of lemongrass was found to possess anti-mutagenic properties towards chemical induced mutation in Salmonella typhimurium strains TA98 and TA100 (Vinitketkumnuen et al., 1994).

Chromosomal aberration in human lymphocytes exposed to mitomycin C (Meevatee et al., 1993), and micronucleus formation in rats exposed to cyclophosphamide (Pinsaeng 1993) were reduced by lemongrass oil. It was responsible for retardation of tumor growth and lessening the degree of tumor metastasis in rats transplanted with fibrosarcoma (Puatanachokchai 1994). In addition, the lemon grass oil enhances glutathione-S-transferase activity in the mouse intestine (Lam and Zheng 1991). Puatanachokchai et al., (2002) evidenced inhibitory effects of lemongrass extract on the early phase hepatocarcinogenesis in male Fischer rats induced by diethylnitrosamine. Murakami et al., (1994) also registered antitumor properties of the lemongrass plant. Dubey et al., (1997) evidenced that citral, major component of lemongrass oil, exhibited cytotoxicity in P388 leukemic cells. Citral possesses many useful bioactivities and one of these is an anti-clastogenic effect in nickel chloride-treated mouse micronucleus system (Rabbani et al., 2006). The citral caused inhibition of micronuclei formation and enhanced the superoxide scavenging activity. Elevated superoxide scavenging activity was thought to be responsible for the anti-clastogenic effects of the citral (Rabbani et al., 2006).

The most defined work on anticancer activity of Cymbopogon essential oil has been recently carried out by Kumar et al., (2008) and Sharma et al., (2009). The two reports
provided deeper insight of the anticancer principle of the essential oil and its mechanisms of action. Kumar et al., (2008) have investigated the anticancer activity of *C. flexuosus* (lemongrass) essential oil and its major constituent, isointermedeol in human leukaemia HL-60 cells. Their study revealed that *C. flexuosus* essential oil and isointermedeol induces apoptosis in human leukaemia HL-60 cells. The anticancer activity of these materials was correlated with down regulation of NF-κB expression and caspase activation. It was suggested that the increased levels of cytochrome c in mitochondria after essential oil treatment, played a role in triggering apoptosis. Sharma et al., (2009) have studied the anticancer activity of *C. flexuosus* essential oil in human cancer cell lines HL-60, murine Ehrlich and Sarcoma-180 tumors. Electron microscopy show morphological changes favoring induction of apoptosis in cancer cells lines. Chromatin condensation and induced apoptosis; fragmentation of the nuclei and apoptosis were apparent morphological changes in HL-60 and Sarcoma-180 cell lines, respectively (Sharma et al., 2009). DNA damage and carcinogenic activity induced by N-methyl-N-nitrosourea (MNU), 7,12-dimethylbenz(a)anthracene, 1,2-dimethylhydrazine and N-butyl-N-(4-hydroxibuthyl)nitrosamine in female Balb/c mice was reduced by administration of lemongrass oil (Bidinotto et al., 2011). Similarly, in female Balb/c mice lemongrass oil reduced the carcinogenic activity of N-methyl-N-nitrosourea (MNU) as reported by Bidinotto et al., (2012).

Most of the mutagenicity (and anti-mutagenicity) studies on essential oils were performed on bacteria (*Salmonella typhimurium* with Ames test, *Escherichia coli* with SOS Chromotest, *Bacillus subtilis* with DNA Repair test) or mammalian cells (MLA, human lymphocytes and hepatocytes) or on insect (*Drosophila melanogaster* SMART assay). In these test systems it is impossible to distinguish the mode of action of essential oils and their targets. Usually, cytotoxicity, mutagenicity or anti-mutagenicity are defects in energy metabolism and respiration as direct or indirect causes assessed without being able to take into account possible defects in energy metabolism and respiration as direct or indirect causes. It has been shown that exposure to essential oils could reduce mitochondrial damage involving mitochondrial membranes and DNA damage.
Antioxidants are believed to be directly antimutagenic (Clark, 2002) and anticarcinogenic due to their radical scavenging properties (Ames et al., 1993; Birt et al., 2001; Collins, 2005). Until now, most studies indicated that anti-mutagenic properties of the plant extracts may be due to the inhibition of infiltration of the mutagens into the cells. Inactivation of the mutagens may be by direct scavenging of free radicals by the antioxidant enzymes. It may be so that the plant extracts could interfere by activation of enzymatic detoxification of the mutagens (Ipek et al., 2005; Kada and Shimoi 1987; Premkumar et al., 2003). Less known is a possible antimutagenic interference with DNA repair systems after induction of genotoxic lesions. Some antimitogenic agents can either inhibit error-prone DNA repair or promote error-free DNA repair (De Flora et al., 1999; Vukovic-Gacic et al., 2006). The biochemistry of anti-mutagenic interference with promutagen metabolism to prevent mutagenesis is known and relatively well documented. During recent years, the role and reactions of ROS scavengers, such as glutathione, superoxide dismutase, catalase, N-acetylcysteine, provitamins like retinoids, carotenoids and tocopherols, flavonoids and other polyphenols, etc. are in focus (De Flora et al., 1999). However, Kada and Shimoi (1987) and Kuroda and Inoue (1988) has carried in details studies antimutagenicity on Escherichia coli involving interference of DNA repair via intracellular prooxidants generated by terpene and phenolic compounds isolated from aromatic plants. Several studies have shown antioxidant properties in vitro of many natural products including essential oils. It was inferred that they could be beneficial for human health in line with recent findings and common belief that many diseases are due to an overload of oxidative stress reactions following excessive consumption of fat, sugar, meat, etc. In contrast to the established notion that the antioxidant properties of natural compounds such as fruit and vegetable polyphenols or herb and essential oil phenols and terpenes determine their protective effect against mutagens. It has become clear that also the prooxidant properties of these compounds can play a significant “protective” role by removing damaged cells by apoptosis (Bakkali et al., 2006). An interesting new aspect of antioxidants would be their possible prooxidant activity in cells (Martin, 2006). In the latter case, protection is quite indirect.

The cytotoxic capacity of the essential oils based on a prooxidant activity can make them excellent antiseptic and antimicrobial agents for personal use, i.e. for purifying air,
personal hygiene, or even internal use via oral consumption, and for insecticidal use for the preservation of crops or food stocks. A big advantage of essential oils is the fact that they are usually devoid of long-term genotoxic risks. Moreover, some of them show a very clear antimutagenic capacity which could well be linked to an anticarcinogenic activity. Recent studies have demonstrated that the prooxidant activity of essential oils or some of their constituents, as also that of some polyphenols, is very efficient in reducing local tumor volume or tumor cell proliferation by apoptotic and/or necrotic effects.

Owing to the new attraction for natural products (essential oils) obtained from the aromatic grasses a comprehensive safety evaluation is urgently warranted for their future use.