INTRODUCTION

1.1 General introduction

“Medicinal and Aromatic Plants” (MAPs), occupy an important place in the socio-cultural, spiritual, perfumery and health care aspects all over the world. Due to their vast untapped economic potential, they continue to play a central role in the healthcare systems of large proportions of the world’s population (Koduru et al., 2007). The MAPs offer a wide range of safe and cost-effective, preventive and curative therapies, which are useful in achieving the goal of ‘health for all’. The basis for this use is that these plants contain so called fragrant (aromatic, ethereal) ingredients or active ingredients (biologically active principles) that affect physiological (metabolic) processes of living organisms, including human beings.

The genus *Cymbopogon* is one such major aromatic plant genera belonging to the tribe Andropogoneae of the family Poaceae, comprising about 180 species, subspecies, varieties and subvarieties (Bor, 1960; Chase & Niles, 1962; Soenarko, 1977). They are unique in possessing essential oil as a source of wide array of terpenes of enormous International potential in perfumery, flavouring and pharmaceutical preparations.

1.2 Origin and Distribution

<table>
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<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tr>
<td>Order</td>
<td>Poales</td>
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<tr>
<td>Family</td>
<td>Poaceae</td>
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<tr>
<td>Genus</td>
<td><em>Cymbopogon</em></td>
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<tr>
<td>Species</td>
<td><em>C. citratus</em></td>
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*Cymbopogon citratus* (DC.) Stapf is an important and oldest species of *Cymbopogon* commonly called ‘West Indian lemongrass’ or ‘lemongrass’. *Cymbopogon* is derived from the Greek word ‘kymbé’, meaning a boat, and ‘pogon’, meaning a beard, referring to the beardlike appearance of the
inflorescences, and the boat-shaped spathes; ‘citratus’ in Latin means ‘steeped in citrus-oil’. It is a tall tufted perennial grass with a short rhizome, which rarely flowers. Nowadays, C. citratus exist only as ‘Cultigen’ (not known in wild form and presumably has originated from cultivation). The origin of C. citratus is very vague as there are many causes which tend to obscure the history of this plant (Otto Stapf, 1906). The ancient history details on the use of this lemongrass mostly in South India by the natives of Madras (Browne, 1702) with subsequent popularity throughout the Carnatic and finally all over India.

These properties which recommended the grass to the native gardener of India also contributed to its early introduction into the colonies of those European powers which then had colonized in India. Further in the literature it is also presumed that the Indian lemongrass had similar features with that of the Malaysia ‘Sereh’ whose use dates back from the first half of the 17th century (Otto Stapf, 1906).

1.3 Habit

It is a perennial, tufted, aromatic grass with numerous erect culms arising from a short oblique, ring-shaped, sparingly branched rhizome (Fig 1.1-A&E). The culms (stem) grow up to 2-3m tall, smooth and glabrous (Fig1.1-D). Leaves sheathing (Fig 1.1-B,C&E); coriaceous, terete, embracing the culm, glabrous, striate; blade linear, 50-100cm x 0.5-2cm, long attenuate at both ends, apex acuminate, drooping, glabrous, glaucous-green, midrib prominent below and white above, top part and margins often scabrid as the membranes of epidermal cells accumulate silica. The plant is essentially non-blooming and flourishes in well-drained sandy soil. An annual rainfall of 80-100 in. and an average temperature of 75-80°F are reported to be favorable for its growth.
Figure 1.1: *Cymbopogon citratus* (DC.) Stapf. A- Tufted grass with numerous stiff stems arising from the rhizomatous rootstock; B- Stout culms bearing linear, drooping leaves (50-100cm long) tapering to long membranous acuminate tip; C- Natural habitat; D- Tufted culms; E- Rhizome and leaf blades.
1.4 Economic importance of the lemongrass oil

It has been grown principally for its qualities in perfumery and in medicine, qualities that derive from the fragrant oil that the grass yields commercially known as ‘West Indian lemongrass’ or ‘lemongrass’ oil. It is one of the commercial sources of Citral apart from East Indian lemongrass oil obtained from C. flexuosus. The oil has strong lemon-like odour, due to high percentage of Citral (over 70%) and for many years has occupied a prominent place in the perfume industry and as a starting material for manufacture of ionones, which produces vitamin-A. The freshening note of oil makes its use in scenting of soaps, cosmetics, detergents, insect repellent preparations as well as in beverage and non-beverage drinks.

1.5 Medicinal properties

Along with fragrance and flavouring agent, lemongrass oil has also been used for a wide variety of ailments in folk medicine. In India it has a long history (nearly 2000 yrs) of application against fever in Ayurvedic medicine. The lemongrass plant extract and its essential oil are approved for food use by the USFDA as ‘generally recognized as safe’ GRAS No.2624 and possesses the International Standardization Organization standard as ISO3217 since 1974 (Simon et.al., 1984).

It is soothing to the nerves while also being invigorating. The essential oil and infusions of this aromatic grass is used to treat a number of human diseases like digestive disorders, menstrual disorder, inflammation, nervous disorder, rheumatism, sprain, cough, cold, fever and various other health problems (Kirtika and Basu, 1935; Anonymous 1950; Nadkarni, 1954; Bor, 1960; Chopra & Handa, 1961; Soenarko, 1977; Suri et.al., 1978; Simon et al., 1984). It is also known to be a good laxative and anthelmintic. The oil is considered a useful application in ringworm (Ravinder et al., 2010). Limited studies have demonstrated antifungal and insecticide efficacy, as well as potential anticarcinogenic activity, while suggested hypotensive and hypoglycemic actions have not been confirmed.
1.6 Significance of the present study

Although claims of the lemongrass essential oil therapeutic value are rife and as such demand internationally is spiraling, but more information on functionality and efficacy of aroma compounds, which is more clinically studied to authenticate results, is needed to make health claims. Also in present scenario, the lemongrass oil it is not being adequately explored and feasible business opportunities in terms of essential oil industries are ignored as it is considered inferior to its competent East Indian lemongrass oil because the oil has tendency to polymerize due to high monoterpane content, and at the same time natural lemongrass market is facing tough competition from the synthetic market (Akhila, 2010). In contrast to this, unsustainable resource management due to complex environment and economic factors in essential oil trade has led to a decline of lemon grass cultivation and in turn oil production.

In view of this, approaches directed towards enhancing the quality of *C. citratus* essential oil for its upgradation to be used at commercial level, thereby restoring back its national and international market is the need of the hour. For sustainable development of this species, conventional method (cultivation by suckering) and biotechnological methods (*in vitro* culture technique by tissue and cell culture) are required to harness essential oil and its isolates for economic usage.
1.7 Objectives of the research work

The main objectives framed under this study are,

➢ To collect and maintain the germplasm of *C. citratus* from various locations of South India.

➢ To extract, analyse and fractionate *C. citratus* essential oil.

➢ To study the biological activities of essential oil

➢ To evaluate the stability of essential oil.

➢ To standardize the protocol for in vitro mass propagation and production of secondary metabolites.
REVIEW OF LITERATURE

The investigations on grasses of Poaceae can make a better understanding of plant evolution in general. Wild Cymbopogon species have already been characterized as highly polymorphic taxa because of their variable essential oil composition owing to different environmental and genetical factors (Mártonfi et al., 1994; Pluhár et al., 2005, 2007; Loziené et al., 2005; Dajić-Stevanovic et al., 2008). In India, native Cymbopogon species have been described from various habitat conditions (Pluhár et al., 2005, 2007).

C. citratus is considered to be the most highly specialized grass tribe by many workers (Bews, 1929; Hartley, 1950). It is a well-defined monophyletic tribe, which exhibit greater morphological and adaptive variations due to hybridization, polyploidy etc. Introgression of various traits, intermittent mutations, and selection through human intervention may also have led to variation in chemotypic characters across geographical distributions (Kuriakose, 1995). It is reported to be present in both hexaploid (2n=60) and tetraploid (2n=40) state (Gupta, 1965; Babu, 1936). The rich species diversity and high degree of endemism also indicate that the group is actively evolving in the Western Ghats region.

Wide scale GC screening of C. citratus essential has been carried out in past 15 to 20 years. According to a recent review a total of 158 compounds were identified in this oil by several research groups from different countries (Rajeswara, 2013). The studies on essential oil composition of C. citratus reports Citral, β-Myrcene (characteristic and active ingredient of lemongrass oil), Geraniol, geranyl acetate, piperitone, limonene, elemecin, monoterpenes alcohols and sesquiterpenes as major constituents (Menut et al., 2000; Kasali et al., 2001; Sidibe et al., 2001; Mohammad et al., 2004; Negrelle and Gomes, 2007; Owolabi et al., 2008; Andrade et al., 2009; Singh et al., 2011, Tajidin et al., 2012; Quintanilla et al., 2012).
Many studies have reported the antimicrobial activity of lemongrass oil originating from different parts of the globe against a diverse range of microorganisms comprising Gram positive and Gram negative bacteria, yeast and fungi (Chalchat et al., 1997; Mahanta et al., 2007; Naik et al., 2010; Bassole et al., 2011; Falcão et al., 2012). The oil shows fungicidal and anti-aflatoxigenic effects against Aspergillus flavus Link. (Paranagama et al., 2003). The major components of oil as α-Citral and β-Citral have been shown to possess antibacterial activity (Onawunmi et al., 1984). In another study, the antibacterial properties of three main components of the essential oil have been recorded. The α-Citral (geranial) and β-Citral (neral) components individually elicit antibacterial action, the third component, Myrcene, did not show observable antibacterial activity on its own. However, Myrcene provided enhanced activities when mixed with either of the other two main components identified (Grace et al., 1984).

Apart from antimicrobial and antifungal properties, antioxidant and radical-scavenging properties are of great interest to health and food science researchers. The antioxidant activity of essential oil is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Essential oil from C. citratus and their components are known for their antioxidant activities from earlier reports (Melo et al., 2001; Sacchetti et al., 2005; Cheel et al., 2005; Maestri et al., 2006; Leratsithlhanakori et al., 2006; Mahmoud et al., 2010; Koh et al., 2012). C. citratus also shows cytoprotective and anti-inflammatory property by reducing the oxidative stress (Tiwari et al. 2010). The lemongrass essential oil protects DNA against chemically-induced damage and also exhibits anticarcinogenic activity against chemically induced mammary carcinogenesis in female Balb/C mice (Bidinotto et al., 2011) and shown to be beneficial in reducing the blood cholesterol level (Costa et al., 2011).

Literature also shows that there is very good evidence for the efficacy of C. citratus either as hypoglycemic agents or as useful agents in the management of diabetic complications. In Eastern Nigeria, this plant has been utilized for treating diabetes, obesity and coronary disease (Adeneye and Agbaje, 2007). Recent research investigations have provided a pharmacological evidence of C.
citrus essential oil as antihyperglycemic mediated by various phytoconstituents interaction with multiple targets operating in diabetes mellitus (Bharti et al., 2013). This preliminary information warrants further in vitro and in vivo studies involving this plant oil samples.

Thus based on the available literature it is noted that, the C. citratus essential oil and their components (majorly monoterpenes) have many uses, both in pharmacology and in food. In addition, they are endowed with interesting biological activities and have a therapeutic potential and it seems that it can be used as a suitable therapy for many pathologies. In the cosmetic and in the food industry, essential oils and its components uses is an integral part, as they may play different roles (Table 1.1). Therefore, economic importance of C. citratus essential oil is indisputable. It appears therefore imperative to preserve its natural, diverse flora and support its protection in order to keep this inexhaustible source of molecules destined for multiple targets.

Though extensive studies have been conducted on grasses all over the world (Myers, 1947; Tateoka, 1960; Watson et al., 1985; Soderstrom et al., 1986), there is practically little work on the C. citratus species from South India. Revisionary work on this plant is required for a better understanding of the tribe (Clayton & Renovoize, 1986). Hence, for the present study, different accessions of C. citratus grown in various locations of South India were collected, its traits were assessed, chemotypes were screened based on the chemical composition for possible exploration of the essential oil towards commercial usage.
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<th>USES</th>
<th>PART USED</th>
<th>TREATMENT/ACTIVITY</th>
<th>REFERENCES</th>
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<tbody>
<tr>
<td>Traditional/ Folk medicine</td>
<td>Leaves/root/ entire plant/ hot water extract/ infusion or essential oil</td>
<td>Headaches, fever, elephantiasis flu, abdominal swelling, stomach pains, diarrhea, coughs, sore throat, gingivitis, leprosy, malaria, ophthalmia, pneumonia, vascular disorders, hypertension, catarrah, diabetes, rheumatism, wounds, nervous, gastrointestinal disturbances, diuretic, sedative, antispasmodic, anti-inflammatory, emmengogue, carminative</td>
<td>Lorenzetti et al., 1991; Husain, 1994; Farooqi &amp; Sreeramu, 2001; Owolabi et al., 2008; Oloyede, 2009; Ravinder et al., 2010</td>
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<tr>
<td>Pharmaceutical Industry</td>
<td>Essential oil</td>
<td>Vaporizer, effective panacea against bacteria, flu and colds, headaches, fever, toothaches, in baths, fomentations; diuretic insects/ mosquito repellent.</td>
<td>Shah G. et al., 2011</td>
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<td>Food Industry</td>
<td>Whole plant/ Infusion or essential oil/Citral</td>
<td>Condiment, flavouring soups, wines, sauces, beverages, confectionery, liquors and curries, herbal teas</td>
<td>Lorenzetti et al., 1991; Husain, 1994; Farooqi &amp; Sreeramu, 2001; Owolabi et al., 2008; Oloyede, 2009; Ravinder et al., 2010</td>
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<td>Cosmetics, Toiletries and Perfumery Industries</td>
<td>Essential oil/Citral</td>
<td>Soaps, hair oils, scents, detergents, washing soaps. Citral converted to ionones (a &amp; b) required for synthetic violet perfumes and for the synthesis of vitamins A, E and carotenoids, detergents, perfumes and toiletries, mosquito repellent, household fumigant</td>
<td>Husain, 1994; Purnima et al., 1999; Rajeswara, 1999; Farooqi &amp; Sreeramu, 2001</td>
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<td>Other Uses</td>
<td>Spent grass/ grass powder/ essential oil</td>
<td>Cattle feed, fuel for distillation unit, manure after composting/vermicomposting and as mulch, making paper. Lemongrass powder as supplement with cattle feed. Distillation water finds application in aromatherapy and can be employed as herbal pesticide</td>
<td>Rajeswara, 1999; Wanapat et al., 2008</td>
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