CHAPTER - 1

INTRODUCTION

1.1. PLANTS IN HUMAN WELFARE

Plants and herbs have played an overwhelming role in human life and welfare from time immemorial. They have significantly contributed to human welfare and are continued to be valued as industrial, economic, commercial and medicinal resources (Sahu et al., 2013). Plants have been linked to the physical, emotional, and spiritual wellbeing of man (Pepin, 2002). The remarkable role of plants as a source of medicine, food, shelter and clothing is an undeniable fact. Millions of population across the globe particularly in developing countries derive significant income for survival from gathered herbal products. Collection of potentially worthy products such as mushrooms, medicinal and aromatic plants for cultural and economic reasons in many developed countries is noteworthy (Schippmann et al., 2002). Indian epics have described about the various uses of plants and herbs; according to the great Hindu epic Srimad Bhagavatam 10.22.34 “Trees will fulfil one’s desires with their leaves, flowers, fruits, their shade, roots, bark, wood, fragrance, sap, ashes, pulp and shoots”. Traditional worship of plants in India clearly signifies the symbiotic relationship between humans and plants.

1.2. PLANTS AS A SOURCE OF MEDICINE ACROSS THE GLOBE

Paleontologic records clearly demonstrated the usage of plants by Neanderthal man as medicine as early as Middle Paleolithic which dates back to 60,000 years (Cowan, 1999; Fabricant and Norman, 2001). Over 5000 years ago, textual evidences support the usage of herbal medicine as found in the many ancient Indian, Chinese, Egyptian, Greek, Roman and Syrian texts (Pal and Shukla, 2003). There are many ancient traditional therapeutic systems like Ayurveda, Siddha, Unani, Chinese and Japanese which are known to be successful in eliminating human ailments and these treatment systems are still being practiced by many physicians across the globe. As per World Health Organization (WHO) records more than 80% of the world’s population depend on herbal medicines for their basic healthcare needs (Hassan et al., 2009). Until the nineteenth century plants and herbs were only major source of therapeutics to sustain
health and combat infectious diseases (Mendonca-Filho, 2006). Over 21,000 plant species have been identified and tagged as plants for medicinal purpose across the globe by WHO (Sharma et al., 2007). Although 15000 plants have been recorded in India as medicinal plants; only 7,000 - 7,500 plant species are being used by traditional communities for treating different ailments (Meena et al., 2009). In the Vedic literature of India, there is a mention of about 700 different herbal substances which includes; cinnamon, coriander, ginger, myrrh, sandalwood and spikenard (Lawless, 2013). Importance of plants is significantly increasing day by day and even in allopathic (or western) medicine.

Herbal therapeutic molecules such as aspirin, digitalis, quinine, and opium are known to have a long history of usage (Elumalai and Eswaraiah, 2012). The advent of modern scientific research in the field of herbal medicine has led to isolation of a large numbers of therapeutic molecules from herbal sources, like Vinblastine (Anticancer) from Catharanthus roseus, Podophyllin (Anticancer) from Podophyllum emodi, Rescinnamine (Tranquilizer) from Rauvolfia serpentine, Pilocarpine (Antiglaucoma) from Pilocarpus jaborandi, Codeine (Anticough) from Papaver somniferum, Artemisinin (Antimalarial) from Artemesia annua, Berberine (For leishmaniasis) from Berberis sp. Plumbagin (antibacterial, antifungal) from Plumbago indica, Gossypol (Antispermatogenic) from Gossypium sp. Nerrifolin (Cardiotonic) from Thevetia sp., Morphine (pain killer) from Papaver somniferum, Ephedrine (stimulant) from Ephedra vulgaris, and Atropine (spasmolytic) from Atropa belladonna and etc (Joy et al., 1998; Prakash and Gupta, 2005). Further a large number of plant extracted natural chemicals such as calanolides, castanospermine and prostratin are promisingly useful in the treatment of Human Acquired Immune Deficiency Syndrome (AIDS) (Dias et al., 2012). Last two decades of 20th century have witnessed a tremendous upsurge of novel pharmaceutical drugs from natural products which include 60% of antimicrobials (Rates, 2001).

Curiosity in herbal medicine has increased in the western world particularly in Europe and North America to uphold the wisdom of traditional herbal healing systems and many leading pharmaceutical companies across the globe, such as Merck, CIBA, Glaxo, Boehringer and Syntex have started Research and Development departments with a clear objective to figure out novel natural herbal therapeutic molecules (Rates,
Currently herbal products are being used under the names of alternative, complementary, holistic or integrative medical systems (IARC Monograph, Vol. 82 by WHO, 2002).

As per WHO traditional medicine strategy 2013-23 traditional medicine can be defined as “sum of the total knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether applicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness”. Since the recent past there has been increasing attention in alternative therapies and the therapeutics especially that originated from plants and herbs. This increased interest steamed from the fact that herbal drugs have better compatibility with human body, with no side effect unlike the conventional drugs (Kamboj, 2000; Rates, 2001).

1.2.1. Formulations of herbal medicine

In the herbal medicine crude plant material such as bark, flowers, fruit, leaves, roots, rhizomes, seed, stems and wood; herbal material like essential oils (EOs), fixed oils, fresh juices, gums, resins and powders; herbal preparations like extracts, fatty oils of herbal materials and tinctures are used (Guptha and Shaw, 2009). In Ayurvedic therapy various formulations ranging from simple basic viz. Avaleha (linctus), Churn (powder), Hima (cold infusion), Kalk (wet bolus), Kwath (decoction), Phant (hot infusion), Swaras (fresh juices), to complex ones like Arka (distillation), Pills of different sizes (Vati, Gutika, Modak) and Sneha (medicated oils), etc., are used (Verma et al., 2011). In many Indian traditional systems EOs are significant constituents of herbal remedies used for skin care, massage, pancha karma (rejuvenation program), chakra oils, and various other remedies that affect mind and body health.

1.3. ESSENTIAL OILS

EOs are an interesting group of plant natural products. Swiss German physician Philippus Aureolus Theophrastus Bombastus von Hohenheim coined the term essential oil (EO) in the 16th century (Edris, 2007 and Baser and Buchbauer 2015). EOs are generally composed of 20 to 60 distinct volatile constituents. However
cinnamon, jasmine, and lemon EOs have more than 100 constituents (Bakkali et al., 2008; Miguel, 2010; Abdelouaheb and Amadou, 2012). They usually dissolve in organic solvents which have lower density than water (Bakkali et al., 2008). They are also known as volatile oil, ethereal oil. In the expression of EO, the term “essential” represents the essence of the plant's characteristic fragrance from which it is derived (Andogan et al., 2002). These oils are generally considered as life force or the energy of the plants and produced as secondary metabolites in the aromatic plants (Bakkali et al., 2008).

1.3.1. Chronology of essential oils

Since ancient days the usage of EOs was evident in the form of perfumes, flavourings in food and beverages and also as therapeutics in healing of the ailments of both body and mind (Abdelouaheb and Amadou, 2012). The abilities and uses of the EOs were found in many ancient scriptures and depictions. In the Vedic literature of India over 700 aromatic substances have been listed; in the Indo-Aryan dialect ‘atar’ means odour, essence, smoke and wind. Rigveda clearly mentions the use of EO on ceremonial and curative occasions (Lawless, 2013). There is a clear mentioning of about EOs in the Bible (Apel, 2006). Pen Taso the first Chinese book of herbal medicine written by the Chinese emperor Shen Nung (of the Han Dynasty) listed 365 medicinal plants including Ephedra (Elumalai and Eswaraiah, 2012). Chinese ancient medicine clearly recorded in “yellow Emperor’s Classic of Internal Medicine” dates back to 2000 years clearly mentioned about many aromatic plants and their medicinal significance (Lawless, 2013). Very prominent evidences were traced out in support of the usage of EOs in many Ancient Indian, Egyptian, Roman and Chinese civilizations (Baser and Buchbauer, 2015). With reference to the prehistoric Egyptian pictorial evidences and Chinese scriptures, priests and alchemists had used EOs in healing of human ailments (Wilson, 2009). Evidences retrieved in 1922 from King Tut’s tomb, around 50 alabaster mason jars of EOs were excavated (Wilson, 2009).

1.3.2. Source of essential oils

The commercial aspects of EOs have been extensively reviewed by Verlet (1993). Wherein it was mentioned that two-thirds of the total quantity of EOs, produced in the world are extracted from the woody perennials; with more than half of this volume being from Citrus species; the remaining comes from cultivated herbaceous plants.
and the contribution of wild plants is not more than 1-2% (Verlet, 1993). The main habitat of EO producing plants is distributed among the countries of Mediterranean and tropical regions (Bakkali, 2008; Hussain, 2009). EOs are also produced by certain plants and mosses (bryophytes, particularly liverworts) (Sadgrove and Graham, 2015). These oils were generally accumulated in oil cells, secretory ducts, secretory cavities or resin ducts, or in glandular hair of the plants (Bernath, 2009; Abdelouaheb and Amadou, 2012). Several plants have the ability to produce EOs, however different parts of the plants acts as potential source of EO vary including bark, flowers, fruits, leaves, peels, rhizome, roots and seeds (Tongnuanchan and Soottawat, 2014).

1.3.3. Modern use of essential oils

Interest in EOs is increasing in the general public and entrepreneur in view of their diversified uses and variety of biological capabilities (Hussain, 2009). Hence a large number of plants, herbs and EOs have been screened for their probable biological activities for possible trading (Burt, 2004; De Sousa et al., 2004; Busatta et al., 2008; Maksimovic et al., 2008; Hussain, 2009). EOs are widely applicable as bactericidal, insecticidal, antiparasitical, viricidal, fungicidal, therapeutic and cosmetic. The use of EOs in food, agriculture and phrama industry is increasing (Bakkali et al., 2008). EOs like anise, verbena, fennel, eucalyptus and sage contain estrogens, and hence are used in cosmetics that helps the skin collagen, tone and regulates sebaceous gland production which effect oil skin, oily hair and acne. EOs also stimulates endocrine glands and influence the secretion of the body’s own hormones (Keller, 1999). Eco Smart Technologies of United States have developed and marketing Insecticides comprising rosemary, clove and thyme Eos. Australia has approved use of citrus oil, Mexico and Brazil has approved clove oil, Denmark approved lemon grass, clove and eucalyptus oils as insecticides (Isman, 2006).

1.3.3.1. Essential oils as flavours and fragrances

Sense of smell and taste are the most sensitive and selective of the human senses. From the beginning of mankind, fragrance has been appreciated by human very profusely. Jasmine oil is considered as the "King of Oils" and Rose the "Queen". Flavour and fragrances are everlasting features of Indian life and use of aromatics has been generously mentioned in Ayurveda “Gandha Sastra” the science of odour which deals with the cosmetics and fragrances (Gode, 1956). Because of the pleasant odour
and sensible aroma EOs also were generously used in the preparations of perfumes and perfumed products, as well as for the flavouring of foods and beverages (Baser and Buchbauer 2015). Mankind has a basic knowledge of approximately 3000 different EOs, of which 300 oils are commercially significant and particularly used as flavours and fragrances (Burt, 2004). Individual compounds from EOs, used as food flavourings, such as d-carvone, d-limonene or geranyl acetate; preparation of creams, perfumes, soaps; flavour additives for food; as fragrances for domestic cleaning products and also as industrial solvents (Bakkali, 2008). Essential oil components such as carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol have been approved by European commission for use as flavourings in food stuffs (Burt, 2004; Surburg and Panten, 2006).

1.3.3.2. Essential oils as preservatives

Generally EOs containing eugenol, cinnamic aldehyde and citral are strong antimicrobials (Smith, 2001). The antimicrobial potentialities of EOs can be capitalized to contend with microbes responsible for food spoilage or for pre treatment of fruits and vegetables at the harvesting stage, for increasing shelf life (Sadgrove and Graham, 2015). Many researchers have reported the antimicrobial efficacies of EOs on food borne pathogens and their possible application as natural food preservatives (Bajpai et al., 2012). Some tooth pastes and hygienic products are formulated with EOs as one of the key ingredients. Some tooth pastes are prepared by addition of bioactive components of oils such as limonene, geranyl acetate or carvone. (Hussain, 2009). “DMC Base Natural” a food preservative, developed by DOMCA S.A of Spain comprises of 50% of EOs of rosemary, sage, citrus and 50% of Glycerol. Protecta – I and Protecta – II are blended herbal extracts produced by Bavaria Corp., Apopka, FL, USA and are classed as generally recognized as safe (GRAS) food additives in the US (Burt, 2004).

1.3.3.3. Essential oils as therapeutics

The French physician, Joseph Du Chesne has mentioned that preparations of 15 – 20 different EOs were generally maintained as stock in pharmacies in the 17th century (Guenther, 1948; Burt, 2004). EOs, alone or in combination, were widely used for the healing of wounds, swellings, cystitis, joint pains, skin lesions, bleeding, mycotic
infections, burns, pharyngitis, syphilis, and leprosy (Boire, 2013). Many research studies confirmed that EOs have the ability to diffuse quickly through skin, oral route or nostril administration; can cross the blood-brain barrier and interact with receptors in the central nervous system, where it can affect the appropriate biological functions such as relaxation, sleep, digestion etc (Abdelouaheb and Amadou, 2012). Many EOs have the capacity to enhance the penetration thereby can decrease skin barrier resistance; these have the capacity of improving of transdermal drug delivery (Adorjan and Bauchbauer, 2010). In recent times wide usage of some EOs is gaining prominence in aromatherapy as they are believed to possess certain therapeutic properties which help even in curing of organ dysfunction or systemic disorder (Perry et al., 1999; Hajhashemi et al., 2003; Hussain, 2009).

Attention of the many researchers has been drawn on EOs for their anticancer properties as they have high therapeutic potentials. Unlike the other chemotherapeutic agents their mode of action on tumour cells is different and early research reports indicated that EO components, especially monoterpenes, have multiple pharmacological effects on mevalonate metabolism which could account for the tumour suppressive activity (Elson, 1995; Rajesh, 2003; Edris, 2007). EOs rich in phenolic constituents such as eugenol and thymol can inhibit LDL oxidation and thereby minimizes the risk of atherosclerosis (Naderi, 2004; Edris, 2007). EO of *Lavandula hybrida* is known to inhibit platelet aggregation and hence it is used in the treatment of cardio-circulatory disorder (Edris, 2007).

Essential oil usage in the aromatherapy is clearly evident nevertheless no EOs has been formulated into therapeutics for human usage; however in the last decade certain commercial formulations are made with EOs. For example Mentofin® made of eucalyptus and peppermint has a great potential in controlling respiratory diseases in poultry (Carli et al., 2008). Gloves off® made with 0.18% thymol and 0.02% carvacrol used for cleaning and disinfection purpose is given a drug identification number (0222775) by Canadian health departments (Adebayo, et al., 2013). SporanTM is a agricultural fungicide available in the US market formulated with rosemary oil (Koul et al., 2008).
1.4. COMMERCIAL POTENTIALITIES OF HERBAL INDUSTRY

According to the reports of World Bank the business of medicinal and aromatic plants, herbal based raw materials and natural drug products across the globe is increasing tremendously at the rate of 5-15% (Garodia *et al.*, 2007; Cravotto, 2010). As per an estimate in the year 2010 the herbal industry annual turnover is approximately 62 billion US dollars and it is expected to increase phenomenally (Cravotto, 2010). Around 2.2 billion US dollars worth of global herbal exports are being primarily used in pharmaceutical industries (Shararrock, 2014). The production of herbal and aromatic raw materials in India is expected to grow from 200 crores in 1998 to 5 trillion US dollars by 2050 (Joy *et al.*, 1998).

1.5. COMMERCIAL POTENTIALITIES OF ESSENTIAL OILS

Approximately 1000 million US dollars world trade has been taking place with respect to EOs extracted from farmed and wild plant material (Shararrock, 2014). Top five exporters of EOs are Brazil, India, America, China and Argentina respectively (Figure 1). In India, between 2000 and 2005 the EO export raised from 62.634 million US dollars to 166.548 million dollars with the export growth percentage of 21.6% (Trade Information, EOs, Australia). As evident in the Figure 2, the EO export in India has been increased three times during 2006 to 2013. In its vision document Indian Council of Agriculture Research (ICAR) estimated that the EO export is expected to raise 5 trillion US dollars by the year 2050 (Satyabrata, 2013); and the major consumers are America 40%, West Europe 30% and Japan with 7%. From the statistical data it is evident that India is one of the premier producers of EOs but not the prime consumer.
Source: Baser and Buchbauer 2015

Figure 1: Production of essential oils worldwide (2008)

Source: www.tradingeconomics.com, Ministry of Commerce and Industry, India.

Figure 2: India Exports/Imports of Essential oils and Resinoids
1.6. FUTURE RESEARCH SCOPE

The use of EOs will elicit various applications in a wider range for industries such as pharmaceutical, agricultural, cosmetic and perfumery, food and beverages, etc. (Guenther, 1985; Burt, 2004). In apparent to the multi dimensional uses of EOs, it is necessary to build up the better understanding of their mechanism of action and biological activities for novel applications in various industrial and environmental fields (Gustafson et al., 1998; Carson and Riley, 2003). Recently scientific focus is gaining day by day on antioxidant and biological properties of EOs (Yuenyongsawad and Tewtrakul, 2005; Skocibusic et al., 2006; Tepe et al., 2007; Hussain, 2009; Anwar et al., 2009). The use of natural antioxidants is gaining significance in food and preventive medicine as they are claimed to have disease preventing and health promoting attributes and also safe to use. Herbal extracts and EOs of some spices such as oregano, sage, satureja and thyme etc., have shown their antioxidant properties (Ruberto and Baratta, 2000; Rota et al., 2004; Rota et al., 2008), so that they can be used as natural antioxidants for the protection of fats/oils and related products (Burt, 2004; Sacchetti et al., 2005; Bozin et al., 2006). Most scrupulously examine, the antimicrobial properties of EOs are better than the conventional antibiotics because of their broader spectrum of activity. Focus should keep on the possibility of use of EOs as air disinfection agents as they are volatile in nature and also for fungus-free storage of food material (Burt, 2004; Devlieghere et al., 2004; Holley and Patel, 2005). Research is now going on across the globe to explore the herbal medicine and EOs for their therapeutic benefits, to promote the use EOs as alternative to standard drugs remedies for management of infectious diseases (Bozin et al., 2006; Sokovic and Van Griensven, 2006; Celiktas et al., 2007; Politeo et al., 2007; Kelen and Tepe, 2008). At this juncture there is a great need to identify individual bioactive compounds in EOs and their possible application for the better and wide use.

1.7. MOTIVATION FOR STUDY

In spite of the therapeutic significance of EOs from centuries, not adequately significant research has been taking place across the world. This has initiated the acceleration of more scientific studies on EOs around the globe in recent times (Megha et al., 2014). Each EO produced from a plant is unique in its compositional
chemistry and never to be exactly the same (Megha et al., 2014), on the other hand synthetic pharmaceutical drugs are always produced identical every time. On account of this discrepancy; EOs are not intended as direct therapeutic agents. The inconsistency in the composition of EOs by seasons, area and environmental factors and other conditions is a greater challenge for the conduct of a convincing research.

Despite the great interest of herbal medicine and gold mines of well-recorded and well practiced traditional knowledge, India has not been benefited commercially by promoting it to the rest of the world; nevertheless neighbouring country China is far ahead in this regard by introducing its herbal knowledge in the western world (Kamboj, 2000). In order to uphold India’s own herbal formulations and EOs in the global markets, there is a potential urge for analysing the therapeutic values of herbal drugs as per different pharmacopeia and WHO guidelines; moreover many herbal products of India are not produced in standardized form, which is the prerequisite for introduction to western markets (Dubey, 2004).

Many EOs possess multifarious utilization possibilities like anti-cancer, antimicrobial, food flavouring and preservation, aromatherapy and as rich source bioactive compounds (Prabuseenivasan, 2006). From the mid 20th century EO usage has been nearly reduced to level of preparation of perfumes, cosmetics and food flavourings than therapeutic formulations in pharmaceutical industries, as it was declining day by day (Edris, 2007).

It is pertinent to mention that research on EOs has been much less in South India and hardly any in the forest tracts of Eastern Ghats. The researchers have hence focussed on biologically rich Vizianagaram district of Andhra Pradesh which comprises substantial forest cover and herbal medicinal practice by tribals of the region. Preliminary studies have led to an observation that foliage contains as much EO as fruit peel in members of Citrus spp. Similarly zingiberaceae foliage also contain significant amount of EOs and in which its rhizome is used more than the foliage. There are several such examples found in this region. There is a paucity of research and newer probing into the availability of EOs in hitherto unexamined parts of plants. Further exploration of these materials for monetary benefits by local farmers is insufficient.
Keeping in view these facts and the challenges and opportunities in the field of herbal remedies and EOs; the present work has been designed to determine antimicrobial properties of EOs from eighteen different plants belonging to six different families (Rutaceae, Lamiaceae, Myrtaceae, Zingiberaceae, Poaceae and Asteraceae) against bacteria and fungi (Yeast). This work also focussed on the identification of bioactive compounds of the EOs using TLC – Bioautography; comparison of the activities of the individual oils based on the number and type of bioactive compounds; and finally affirmation of antimicrobial activities using selected standard compounds (reference compounds).

1.8. STUDY HYPOTHESIS

Generally EOs are very rich source of bioactive compounds belonging to different groups like hydrocarbons, esters, oxides, lactones, alcohols, phenols, aldehydes and ketones (Djilani and Dicko, 2012). Essentially, each of the individual oil components contributes to the beneficial or adverse effect; identification of bioactivity of individual compounds of oils permits better and specially directed application.

We hypothesized that certain combinations of compounds are as potential as or more potential than the oil itself. Hence, this study focuses on identification of potential combination/s of the components of EO that possibly enhance its activity. This combinatorial study would help in formulation of EO components with greater precision and potency.
1.9. AIM AND OBJECTIVES

**Aim:**

The present study is designed to identify antimicrobial compounds of EOs extracted from selected plants of Vizianagram area, AP, India., using TLC-bioautography and the affirmation of the activities of oils using selected standard compound.

**Objectives:**

- Extraction of EO by Hydro-distillation method using Clevenger type apparatus.
- Determination of antimicrobial activity by Kirby-Bauer disc diffusion method and broth dilution method (MIC).
- Identification of antimicrobial compounds by TLC-bioautography and GC – HRMS analysis.
- Affirmation of the activity of EOs by comparing with selected standard compounds (reference compounds).
- Identification of combination of compounds with potential antimicrobial activity through combinatorial study.