In the Name of *Allah*,

the supremely Merciful, the most Kind.

CHEMICAL COMPOSITION AND BIOLOGICAL (ANTIOXIDANT AND ANTIMICROBIAL) ACTIVITIES OF ESSENTIAL OILS FROM SELECTED PLANTS OF POTHOHAR PLATEAU

By

MOIN UD DIN

M.Phil. (UAF)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

DEPARTMENT OF CHEMISTRY,
FACULTY OF SCIENCES,
UNIVERSITY OF AGRICULTURE, FAISALABAD
PAKISTAN

2015

To

The Controller of Examinations,

University of Agriculture,

Faisalabad.

"We, the Supervisory Committee, certify that the contents and form of thesis submitted by ***MR. MOIN UD DIN,*** Regd. No.2003-ag-281, have been found satisfactory and recommend that it be processed for evaluation, by the External Examiner(s) for the award of degree".

Supervisory Committee

1. *Supervisor* _____
   (Dr. Raja Adil Sarfraz)

2. *Co-Supervisor* _____
   (Dr. Abdullah Ijaz Hussain)

3. *Member* _____
   (Dr. Ijaz Ahmad Bhatti)

4. *Member* _____
   (Dr. Muhammad Shahid)

DEDICATED

To My

Mother (Late)
For her deep love and
Affection to me
And it is to say that
"All that I am or hope to be, I
Owe to my Angel Mother"

&

Brother
Islam ud Din
For his keen interest,
Creative suggestions and
Inexhaustible cooperation

ACKNOWLEDGEMENTS

In the name of Almighty Allah, the most beneficent, most merciful, the Lord of the lords, Who guides us in difficulties and blessed me with courage and power to complete this thesis. And all praise for Prophet Hazrat Muhammad (Sallallahhu-Alaih-e-Wasallam), for enlightening our conscious with the essence of faith in Allah and Who is a torch of guidance for the entire humanity, forever.

It is a great honor for me to express my profound and cordial gratitude to my kind, respected and worthy supervisor Dr. Raja Adil Sarfraz, Assistant professor, Department of Chemistry & Biochemistry, University of Agriculture, Faisalabad for his valuable and skilled guidance, ever encouraging and constructive criticism, which always helped me to carry in with my research work. I thank with core of my heart of my Co-Supervisor Dr. Abdullah Ijaz Hussain, Assistant professor, Department of Chemistry, Government College University, Faisalabad for his erudite suggestions, sympathetic and inspiring attitude, patronizing supervision and technical guidance during my studies. I express my deep gratitude to Dr. Ijaz Ahmad Bhatti and Dr. Muhammad Shahid Department of Chemistry & Biochemistry, University of Agriculture, Faisalabad for their expert guidance, generous assistance, cooperation and friendly affection during the course of my research and writing of this manuscript. Thanks to Dr Mansoor and Dr Faiz ul Hasan for helping in selection of plant material.

I ardently extend my special thanks to Dr. Lyle E. Craker for his warm hospitality, dedicated cooperation and encouragement during the part of my Ph.D. in Medicinal and Aromatic Plants Laboratory, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA. I'm also thankful to Dr. Masood Sadiq Butt, DG NIFSAT to be very much cooperative and friendly to me during my stay in USA.

I am also grateful to my friends Muhammad Yasir and Shazia Nourin to be very much Cooperative and their moral support, meanwhile thanks to other laboratory fellows Nadia, Huma, Aisha and Arfaa for their cooperation. A lot of thanks to my Hitech friends Khalid sb, Fazil Shah sb and other members of lab to be very much caring of me during my whole tenure.

I'm thankful to my family for keeping me spare for this study which requires a lot of patience. All credits go to my affectionate and dearest father whose prayers are accompaniment in the journey of my life. I ardently extend thanks to my respectable and dearest Sisters for their help and prayers which has proved courage, goodness and good luck for me. My feelings will not be completed without mention of my dearest brother Nizam for his love, encouragement and good wishes that inspired me to accomplish this humble effort. Thanks to my bhabies and all of my nephews and nieces for their love and mellifluous affections, which hearten me to achieve success in every sphere of life with special thanks to Sara for giving me nice pieces of suggestions and helping me a lot in thesis write up. May Allah give all of them long and happy life (Ameen)!

Finally, Thanks to all those who taught me ever one word in my life.

Moin ud Din

C O N T E N T S

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Essential oils of different medicinal and aromatic plants have been potential candidates as source of food preservation, pharmaceuticals, alternative medicines and natural therapies in addition to their pharmacological properties like hepatoprotective, carminative, anticarcinogenic and antiviral effects.

In present study some selected plants from Pothohar plateau were collected and subjected to essential oil extraction by hydrodistillation followed by assessment of their physical parameters like color, specific gravity, % yield etc and their biological activities including antioxidant, antimicrobial and cytotoxic attributes. The oils were then characterized by using GC and GC-MS techniques to find the chemical constituents. Overall the examined oils exhibited good antioxidant and antimicrobial behavior. To the best of our knowledge the essential oil of *Parthenium hysterophorus* has never been reported with regard to its composition as well as biological activities before present study. In general, *Trachyspermum ammi* and *Cuminum cyminum* essential oils showed significant activity in most of the assays performed in this study. Both showed remarkable potential for scavenging of DPPH free radical with IC_{50} values 2.61and 16.86 µg/mL, respectively and inhibited linoleic acid by 80.73 and 79.36% respectively. For *T. ammi* most sensitive bacterium was *Streptococcus mutans* (Inhibition zone=19.7mm; MIC=1.41 mg/mL) and fungus was *Ganoderma lucidum* (Inhibition zone=17.0mm; MIC=1.72 mg/mL) while for *C. cyminum* most sensitive bacterium was *Bacillus subtilis* (Inhibition zone=27.3mm; MIC=1.10 mg/mL) and fungus was *Ganoderma lucidum* (Inhibition zone=27.0mm; MIC=1.40 mg/mL). Both *T. ammi* and *C. cyminum* exhibited minimum hemolysis of human erythrocytes i.e. 7.98 and 4.51% respectively at 0.5 mg/mL concentration. GC-MS analysis revealed that thymol (37.75%) was the major component of *T. ammi* essential oil while cumaldehyde (24.10%) in *C. cyminum* essential oil. All the experiments were done in triplicate and mean ± S.D. was calculated.

CHAPTER 1

INTRODUCTION

The history of medicinal plants for the treatment of different diseases is as ancient as that of humans. The use of these plants have provided a source of basic health care in the whole world, especially in South American countries (Maciel *et al.*, 2002). According to the WHO, more than 80% of the world's total population depends upon indigenous forms of medicine which are mostly obtained from plants to fulfill the basic health care needs (Holley and Cherla, 1998). It has been estimated that total number of plant species which are present on earth range from 250 to 500 thousand and among them only 1 to 10% are being consumed as food by animals and human beings (Cowan, 1999). There are considerable evidences that plants contain certain classes of compounds e.g. flavonoids, phenolic acids, vitamins and terpenes etc. which play an important role in maintainance of health and also show preventive role against some common diseases like cancer, neurodegenerative disorders and cardiovascular diseases etc (Dorman *et al.*, 2003; Fan *et al.*, 2007)

Reactive oxygen and nitrogen species are the dangerous byproducts of normal cellular metabolism, under normal conditions. The harmful effect of free radicals towards biological system involve overproduction of ROS/RNS and is termed as oxidative and nitrosative stress respectively (valko *et al.*, 2001). The overproduction of free radicals in living systems or deficiency of enzymatic or non-enzymatic antioxidants is responsible for this stress. The excess ROS can damage proteins, lipids or DNA by inhibiting their normal functions and changing their structures. The oxidative stress, in fact, is responsible for a number of human diseases. Within the biological system the primary ROS is the superoxide radical ($O_2^{\bullet -}$) which is created by premature electron leak to oxygen in electron transport phase of aerobic metabolism. The presence of unpaired electron in valence shell make this radical reactive, due to which it reacts with other molecules to give secondary radicals such as hydroxyl radical ($OH^{\bullet}$), peroxynitrate ($ONOO^{\bullet}$), hydrogen peroxide H_2O_2 and peroxyl radical ($LOO^{\bullet}$), it can also split to give singlet oxygen ($O^{\bullet}$).

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^{\bullet} + OH^{-}$$

$$O_2^{\bullet -} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^{-}$$

$$NO^{\bullet} + O_2^{\bullet -} \longrightarrow ONOO^{-}$$

By nature a large number of food products decay and it is necessary to take serious steps to prevent them from spoilage at the stage of their preparation, storage and distribution in order to check their proper shelf life. Normally the area where food is prepared is far away from where it is to be sold. So properly expanded shelf life is much desired in such food items. Although certain advancements have been done in cold distribution to perform trade of such perishable food internationally, but only refrigeration cannot guarantee the complete safety and quality of all such kind of food. There are large number of commonly used preservatives which have shown much effectiveness but their safety is the matter of great concern (Branen, 1983). Some other alternatives for preservation of food include pulsed light, high pressure, pulsed electric and magnetic fields, inert gases, antimicrobial agents and different radiations are being practiced for their use in food industry (Butz and Tauscher, 2002; Lado and Yousef, 2002)

Poisoning of food has been a great problem for both industrialists and common users although there are several classical methods for their preservation but due to the immunity which microbes develop against antimicrobial agents resulting in various intestinal disorders, diarrhea and vomiting (Friedman *et al.*, 2002). Lipid peroxidation and microbial contamination in foods not only reduce shelf life and deterioration of eatables but also causes several diseases and ultimately the economic and health losses. To prevent or slow down this autoxidation process, various traditional synthetic antioxidants for example bulylated hydroxytoluene (BHT), bulylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) have been used for past 50 years. These artificial compounds are although much effective but have adverse health effects. Due to this problem, studies are focused to discover the substances of natural origin which can serve the purpose. In this regard antioxidants obtained from large number of different aromatic and medicinal plants have been studied. Among them a large number of plants have proved to be effective antioxidants and delayed the process of lipid peroxidation of fatty food and oils. So many research groups are showing much interest in such

aromatic and medicinal plants (Kulisic *et al.*, 2004). Due to this reason their demand and worth have increased rapidly throughout the world.

A lot of work is in progress in order to find out such plants including fruits, vegetables, leaves, bark, seeds, roots and herbs (Rababah *et al.*, 2004). Like extracts of weeds and herbs, spices have also shown much potential as antimicrobial agents and as food preservatives. In fact the crude extract and biologically active compounds can be obtained from different plant species that can play a very important role as medicines and in preservation of valuable food items (Fatimi *et al.*, 2007).

Essential oils

Essential oils (EOs) are also known as volatile oils (Guenther, 1948) and are composed of low molecular weight aromatic compounds with characteristic fragrances. These are extracted from either whole plant or from its different parts like flowers, leaves, buds, bark, wood and roots. Different methods are there in practice for their extraction which include fermentation, enfluerage, expression, solvent extraction etc but the most commonly used is hydro or steam distillation which is equally popular at both laboratory and industrial scale. About 3000 EOs have been reported so far, among of which 300 have got commercial importance as fragrances and flavors of different food items (Braak and Leijten, 1999).

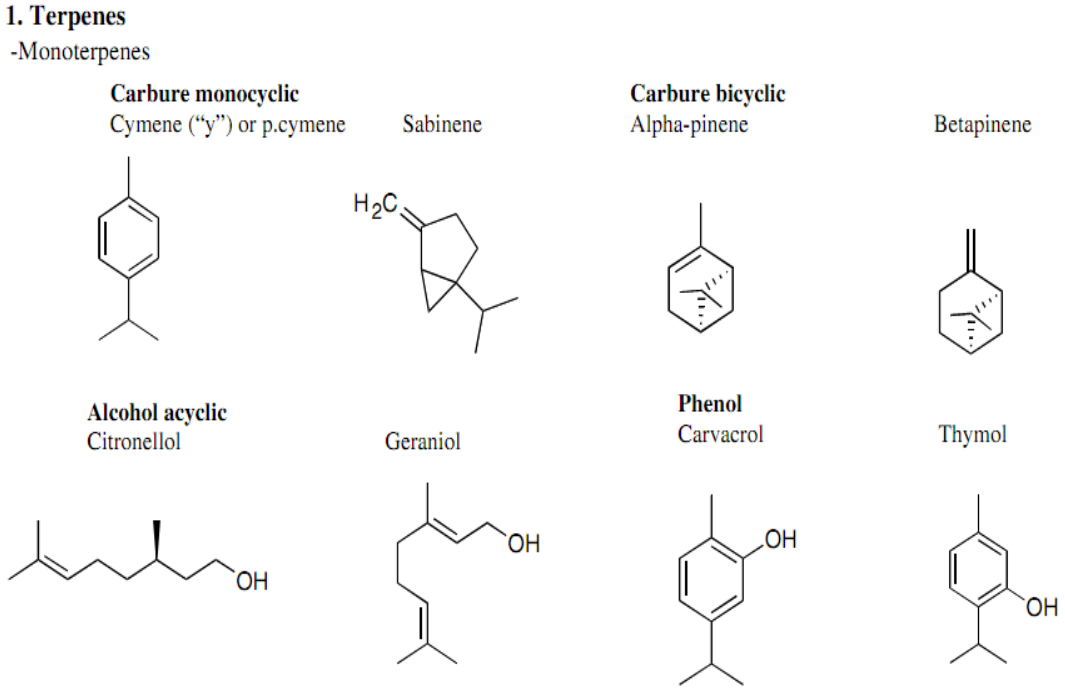
Essential oils are hydrophobic liquid which are concentrated in mixtures of volatile aromatic compounds produced by plants of known taxonomic origin. These complex compounds vary from plant to plant and are generated in them as secondary metabolites. A variety of these essential oils have many ecological roles e.g. they can perform action as internal messengers, they produce scents which can attract insects and can be helpful in pollination, on the other hand this scent could be noxious and help them as repellents for animals by causing irritation (Harrewijn *et al.*, 2001).

Essential oils are very complex mixtures mostly composed of 25-65 compounds in varying percentages. Every oil has 2-3 major components which may constitute 25-75% of total oil, while other are present in traces, for example, In essential oil of *Coriandrum sativum* linalool is the major component (68%) , whereas carvacrol and thymol are the major components in the essential oil of *Origanum compactum* (30% and 27%, respectively). Similarly in *Artemisia*

herba-alba major components are α- and β-thujone 57% and camphor 24%, whereas α -phellandrene and limonene are 36% and 31% of leaf and carvone and limonene 58% and 37% respectively in the seed essential oil of *Anethum graveolens*, 50% of the total composition of *Cinnamomum camphora* essential oil is occupied by 1,8-cineole and in *Mentha piperita*, menthol is present in 59%. Normally, the properties shown by the oils are due to presence of these major components. The components of essential oils can be divided into two groups; (i) terpene and terpenoids (ii) aliphatic and aromatic constituents (Croteau *et al.*, 2000; Betts, 2001; Bowles, 2003; Pichersky *et al.*, 2006)..

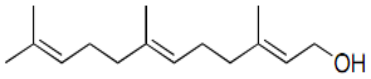
Terpenes

Terpenes are of various classes, different in structure and functions. A 5-carbon unit known as isoprene is the base of their structure. Actually they are hydrocarbons with both single and double covalent bonds. Terpenes containing 10 carbon atoms is called monoterpene, 10 C-atoms sesquiterpene, 20C-atom ditepene and 30 C-atom triterpene etc. If there is present O-atom in terpene, it is also called terpenoid. When two isoprene units join together they give monoterpenes; the major components of essential oils (about 90%).

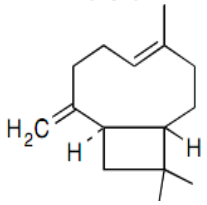


-Sesquerpitenes

Carbure
Farnesol

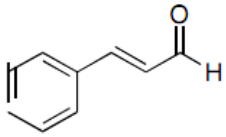


Alcohol
Caryophyllene

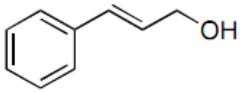


2. Aromatic compounds

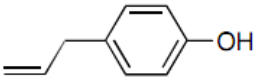
Aldehyde
Cinnamaldehyde



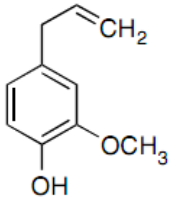
Alcohol
Cinnamyl alcohol



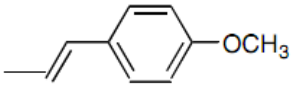
Phenol
Chavicol



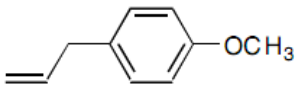
Phenol
Eugenol



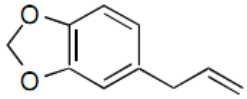
Methoxy derivative
Anethole



Methoxy derivative
Estragole

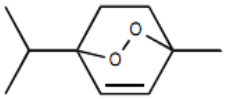


Methylene dioxy compound
Safrole

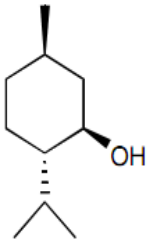


3. Terpenoides (Isoprenoides)

Ascaridole



Menthol



Extraction methods

Some of the methods of extraction are discussed here

Expression

This method was mostly applied for the extraction of orange peel oil. It does not involve any heat source, also known as cold pressing. Fruit is removed and peels are soaked into water

which are then pressed using stones or wooden tools which break down the oil bearing cells and oil is squeezed out.

Hydro / Steam Distillation

It is the process in which evaporation is done followed by condensation. These are most widely used methods for extraction of essential oils worldwide. The equipment for extraction may vary in size and design depending upon the requirement but the principle is same. Plant material from which oil to be extracted is dipped in water (Hydrodistillation) or suspended over boiling water (Steam distillation). High energy steam molecules break the oil glands in plant material and carry volatile molecules together with them and reach condenser, where they are collected as the mixture of oil and water.

Maceration

Maceration procedures utilize a variety of solvents to extract complex oils and fragrances from plant material. Traditional maceration procedures require whole or ground plant material to soak in a water, oil or alcohol-based solvent, inside a sealed glass container, at ambient temperature for days or weeks. This technique varies depending on the location, composition and temperature stability of the extractable oil. Oils stored near the surface of the plant part may diffuse in a solvent more quickly than oils deep within the plant.

Enfleurage

In this method we use glass plates in wooden frame. These plates are covered with animal fat. Freshly collected flowers are embedded into this fat for some days, then these flowers are replaced by other fresh petals. The process is continued till the saturation of fat. Then petals are removed and fat is washed with alcohol to remove essential oil from it and this fat can be used in synthesis of soap.

Solvent extraction

Another method for extraction of EO's is solvent extraction. In this technique different solvents are used like hexane, methanol, petroleum ether etc. this technique is normally used for extraction through delicate parts of plants like in case of jasmine, tuberose etc.

Supercritical carbon dioxide Extraction

Sometimes supercritical carbon dioxide is used as the solvent for extraction of EO's. this is the most safe and reliable method because in solvent extraction the solvent could be toxic and flammable but it is safe to use and can be easily separated too; its advantage over steam distillation is that it is operated on reduced temperature so other products can also be obtained like plant waxes.

EO's are mainly composed of mono and sesquiterpenes and their oxygenated derivatives. Besides these there could be present compounds of other classes like esters, alcohols and aldehydes (Croteau *et al.*, 2000). Since they are composed of large number of molecular species, so their chemical characterization is performed using modern analytical techniques. Chromatography is the basic technique which is used in most of the analytical methods used for EO's characterization. But for reliable identification of compounds, further confirmatory sources are required.

Gas liquid chromatography (GLC) was first described by James and Martin, also known as GC (James and Martin, 1952), a milestone in the evolution of instrumental chromatographic methods. In mass spectrometry (MS) substances are identified by converting them into gaseous ions by the bombardment of high energy electron beam, following their characterization according to their mass to charge ratios (m/z) and relative abundances (Todd, 1995). The combined technique of gas chromatography-mass spectrometry (GC-MS) has shown a great potential for analysis of volatile components, which are present in flavors and fragrances. The identity of compounds is further confirmed when retention indices are used together with the information supplied by the GC-MS. It means retention indices when merged with MS libraries, act as a filter and thus make it easy to confirm compounds by matching and thus increasing the quality of MS identification (Costa *et al.*, 2007).

There are many factors defining the yield and the quality of the EO's in addition to the methods of processing and storage (Viljoen, 2006; Vuuren *et al.*, 2007). Actually EO's inhere in special oil glands present in the cellular structure of the plants. Although EO's may be extracted from the same plant population, there could be variation in their chemical composition and thus quality. There are different factors like genetic, environmental, physiological and even

processing methods which directs their phytochemistry and chemical composition (Masotti *et al*., 2003; Angioni *et al*., 2006). Moreover, the effect of different environmental factors like climate, soil quality, water stress, location of oil cells, timing of harvest and method of extraction have also been reported in literature together with physic-chemical variation of EO's of various plants (Novak, 2005 ; Hussain *et al*., 2008; Anwar *et al*., 2009a)

EO's are well known for their action to kill germs, harmful bacteria, fungi and viruses. These medicinal properties and their pleasant smell make them useful for preservation of dead bodies, food preservative, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic medicines. These characteristics are not much changed, even today, but there is emergence of much more of their mechanism of action and properties specially antimicrobial. They are frequently being used especially in large number of industries like food, sanitary, pharmaceutical and agronomic. Some of EO's components or they as a whole are used in perfumes and cosmetics , in dentistry, in agriculture, as food preservatives and additives, and as natural remedies. For example, in creams, soaps and perfumes geranyl acetate , d-limonene, or d-carvone are used, whereas these are also used in household cleaning products as fragrances and in food as flavors. EO's massages have become very much popular where there are applied together with any carrier oil because its molecules are smaller with low molecular weights so they can easily enter the body giving soothing effect. One more application is aromatherapy. Due to their medicinal potential they are also used to cure different diseases in systemic order (Silva *et al*., 2003; Hajhashemi *et al*., 2003; Perry *et al*.,2003).

Pothohar Plateau

The Pothohar Plateau is situated between 32° 32' and 34° north latitude and 70° 17' and 73° 5' east longitude, north-eastern Pakistan, forming the northern part of Punjab. It borders the western parts of Azad Kashmir and the southern parts of Khyber Pakhtunkhwa (KPK). It covers an extensive area of 1.5 million hectares. The area was the home of the Soanian culture, which is evidenced by the discovery of fossils, tools, coins, and remains of ancient archaeological sites. The Pothohari Punjabi language is a major language, while Hindko Punjabi is also spoken by much of the population. It is bounded on the east by the Jhelum river, on the west by the Indus river, on the north by the Kala Chitta Range and the Margalla Hills, and on the south by the Salt Range (Dasti *et al.,* 2007). The Kāla Chitta Range

thrusts eastward across the plateau toward Rawalpindi; the valleys of the Haro and Soan rivers cross the plateau from the eastern foothills to the Indus. The ramparts of the Salt Range stretching from east to west in the south separate Pothohar from the Punjab Plain. The Pothohar Plateau includes the current four districts of Jhelum, Chakwal, Rawalpindi and Attock. The terrain is undulating. The Kala Chitta Range rises to an average height of 450-900 metres (3,000 ft) and extends for about 72 kilometres (45 miles). The Swaan River starts from nearby Murree and ends near Kalabagh in the Indus river. Sakesar is the highest mountain of this region.

Most of the hills and rivers are bordered by dissected ravine belts. Agriculture is dependent largely on rainfall, which averages 15 to 20 in. (380 to 510 mm) annually; rainfall is greatest in the northwest and declines to arid conditions in the southwest. The chief crops are wheat, barley, sorghum and legumes while onions, melons, and tobacco are grown in the more fertile areas near the Indus.

The diverse wildlife includes urial, chinkara, chukar, hare, mongoose, wild boar, and Yellow-throated Marten. Due to low rain fall, extensive deforestation, coal mining, oil and gas exploration, the area is becoming devoid of vegetation. The underwater areas of lakes (Uchali, Khabeki, Jhallar and Kallar Kahar) have been reduced to much smaller areas than in the past. The plateau is the location of major Pakistani oil fields. The major cities of Islamabad, Rawalpindi and the smaller cities of Chakwal, Jhelum and Attock are situated on the plateau.

The climate of the area is of an extreme nature. The western portion of tract is hotter and drier than the eastern and northern parts. The winter is bitterly cold while the summer is unbearably hot. The temperature rises first in April, than remains almost steady, due to windstorms from Baluchistan, up to the middle of May when it shoots up again. June and July are the hottest months (average maximum temperature 42°C), while December and January are the coldest months (average minimum temperature 1.7°C). The monsoon starts by the third week of July and continues till the beginning of September when the nights get cooler. The cold weather sets in by the middle of October. During winter the days are bright, and the nights are clear. Early spring frosts are common and sufficiently severe to cause wide spread injury to plants, even to the indigenous tree growth in a fairly advanced stage of development. Preliminary analyses of weather data indicate that mean temperature decreases in a linear fashion with

altitude (Champion *et al.*, 1965). Frost may occur especially at the upper altitudinal limits and in valley bottoms. Rainfall is scanty and uncertain, and its annual distribution is very uneven. The annual rainfall varies from 250 to 750 mm. Monsoons start late in July, and most of the annual rain is before September. Spring and fall rains are rare and uncertain. Winter rains start by the end of December. They stop by the end of February when the windstorms set in. Winter rains generally extend over a shorter period than the monsoons, followed by a prolonged period of dry weather. Humidity is also generally low, falling to about 15% in the summer afternoons, the annual mean being about 50% (Hussain & Ilahi 1991). The ground water resources are limited. The sources of water for humans and livestock are wells and dugout ponds. The drainage is very satisfactory. The entire area is drained off to the west into the Indus River, largely through Soan River. Springs are mostly seasonal and flow only during the rains. Perennial springs are very few and are found at only 14 places.

Aims and Objectives of the Present Study

Although plenty of studies have been carried out and a lot of results has been obtained regarding chemical characterization and biological activities of spices and herbs (Muthamma *et al.*, 2008; Rota *et al.*, 2008), however, to the best of our knowledge there are no detailed findings on chemical composition and biological activities of spices and herbs native to Pothohar region of Pakistan. The present study was actually designed to explore the aromatic plants of Pothohar plateau and to check their medicinal value.

The present project was designed with the following principal objectives:

1) Exploration of essential oil potential of selected plants indigenous to Pothohar Plateau.
2) Investigation of antioxidant and biological (antibacterial and antifungal) activities of essential oils.
3) Studying the profiles of bioactive constituents of essential oils using modern chromatographic/spectroscopic techniques.
4) Evaluation of essential oils for the potential uses as food additives/ food preservatives.

CHAPTER 2

REVIEW OF LITERATURE

Medicinal Plant's revival of learning is happening all over the globe. Safety is highly concerned with the herbal products when compared with the synthetic medicinal products which are considered unsafe for both human and environment. The synthetic products although have remarkable importance due to their effective and quick mode of action yet herbs are considered important due to their medicinal abilities, flavoring and aromatic qualities. Despite the distinctive properties of the synthetic drugs, herbal products due to their security and safety are turning the people to trust on theses, blindly. Over three quarters of the world population is entangled by the plants and plants extracts for their health. About 3-% or more of botanical species are being used all over the world for medicinal intentions. The consumption of the plant drugs was estimated to be 25% of the total drugs consumed in the developed countries such as United States whereas the consumption was 80% in the developing countries including China and India. Thus, the medicinal plants are economically important to the countries such as India when compared to the rest of the world. Two third of the plants is reported to be used in modern medicinal and health care system by these countries where local medicinal system are used by the rural population (Joy, *et al.,* 1998).

2.1. Essential Oils

Essential oils (EOs) are considered as the secondary metabolites produced by the aromatic plants. The strong odor is a main characteristic of these volatile oils. Complex natural compounds are used in order to concentrate these Essential Oils (Bakkali *et al.,* 2008; Guenther, 1948). Variety of chemically pure and volatile (under normal conditions) aromatic substances combine to give these essential oils having a characteristic odor thus making them to be used in many ways by the society (Gunther, 1952).

2.2. Sources:

A plant of a known origin or a part of it produce essential oils when subjected to physical means of isolation like pressing and distillation. Principally, volatile compounds are produced by all the plants quite often or in traces. By definition, "Essential oil plants" are those plant species which are known to produce an essential oil of commercial interest (Harrewijn *et al.,* 2001). Approximately 300 out of 3000 known essential oils are being used commercially in pharmaceuticals, food, sanitary, cosmetics agronomics and perfume industries these days (Braak and Leijten, 1999). The families of Lamiaceae, Umbelliferae and Asteraceae contain most of the plants which produce essential oils (Burt, 2004; Celiktas *et al.,* 2006; Hussain *et al.,* 2008). Different parts of the plants are used as a source of different aromatic oils such as flowers of rose, jasmine etc, clove's buds, seeds of carrot and caraway, curry leaves and basil leaves, twigs of pine, clove's stem, cinnamon's bark, wood of sandalwood, fruits of citrus and fennel, lemon grass and ginger roots (Shahat *et al.,* 2011; Burt, 2004; Hassine *et al.,* 2012).

2.2.1. *Parthenium hysterophorus*

Parthenium hysterophorus (carrot weed) belongs to a large and widespread family of angiosperms called Asteraceae; the largest family of flowering plants distributed worldwide (except Antarctica) with 1,620 genus and above 23,600 species (Stevens, 2001). This family is generally found in Brazil, southern Africa, central Asia and in the tropical and subtropical regions of North America. Asteraceae is the family of herbs, shrubs and even trees but principally in the tropical regions, herbs are of primary importance. All the disturbed land, including farms, pastures, and roadsides are invaded by *P. hysterophorus* (Shabbir and Bajwa, 2006).

An annual herb is approximately 2 m high with trichomes covering the branched stem. Leaves are up to 30 cm long, closed to the soil, lobed, hairy (small hair on both the sides), with a pale green color, alternate, irregularly dissected and bipinnate. About 6-55 leaves are present per plant. Five seeds (2 mm long) with scales are present in each flower. Up to 100,000 seeds in the lifecycle of a single plant are produced. 22 to 25°C is the optimum temperature required by Parthenium seeds to germinate but the seeds can also germinate at 8 to 30° C. The outbreaks of epidemic proportions, in some areas also affect agriculture, livestock and human health.

P. hysterophorus after their extraction of water-soluble drug substances, has shown activity to treat various diseases like fever, diarrhea, neurologic disorders, infections in urinary tract, dysentery, in malaria, inflammation, eczema, skin rashes, herpes, rheumatism, cold, heart pain and gynecological disorders (Surib-Fakim *et al.*, 1996). It has also shown potential pharmacologically as pain reliever in muscular rheumatism, therapeutic for neuralgia and as vermifuge and in hepatic disorders (Maishi *et al.*, 1998). The major element of this plant is Parthenin, which has anticancer properties (Venkataiah *et al.*, 2003). Significant antitumor activity was shown by the methanol extract of its flowers (Das *et al.*, 2007). Formerly, the *in vivo* and *in vitro* antitumor potential of *P. hysterophorus* extracts were established by Ramos *et al.* (2002) showing positive results with reduced tumor size and overall survival of cell lines. When the aqueous extracts were tested on alloxan-induced diabetic rats, *P. hysterophorus* showed hypoglycemic activity against them (Patel *et al.*, 2008). So drugs can be developed for diabetes mellitus by using flower extracts of this weed. Silver nanoparticles synthesized from the plant's extract have shown bactericidal, wound healing and other medical properties (Parashar *et al.*, 2009).

2.2.2. *Eucalyptus camaldulensis*

It belongs to Myrtaceae; a family of flowering trees and shrubs. Dicotyledonous plants occupy the major space in this family. All the species included in this genus are woody, with essential oils (Wilson *et al.*, 2001) with evergreen simple leaves, alternate to opposite, usually with an entire margin which is not toothed. Recently, it is estimated that over 5650 species are included in some 130-150 genera of Myrtaceae (Cheewangkoon *et al.*, 2009). Species of some Corymbia, Eucalyptus and Syzygium genera (within the Myrtaceae) are widespread in tropical and temperate regions of the Southern Hemisphere (Wilson *et al.* 2001; Ball, 1995).

Eucalyptus spp. being more abundant than other myrtaceous genera is widely distributed due to their frequent growth as exotics in commercial plantations. The tree flora of Australia is dominated by the members of this genus. Only 15 species are present in the regions other than Australia, out of which, 6 are present in Australia as well. Though most of the species are unable to tolerate the frost, yet the wide cultivation is observed in the America, Europe, Africa, the Middle East, India, Pakistan and China. Some eucalyptus species have characteristic properties including fast growth of wood, producing oil which is being used as cleaning, insecticidal purpose, or in drain swamps reducing the risk of malaria. The total impact of eucalyptus is controversial as they are laudable for their economically beneficial impact for poor populations on one hand while they are criticized for being invasive water-suckers on the other hand (Ball, 1995).

E. camaldulensis, native to Australia is distributed worldwide, especially in Africa (Francisco *et al.*, 2001). Eucalyptus trees are also distributed over a considerable extent in all Mediterranean basins (Ergin *et al.*, 2004). *E. camaldulensis* is a perennial, single-stemmed, size ranging from medium to tall tree with a height of up to 30 m (Bren and Gibbs, 1986), although the heights up to 45 m and 0.8m in diameter are also reported. February to April is the flowering time followed by fruiting in May until August. 15-30 cm long leaves with 2-5 cm width and of yellow-green in color (Baytop, 1999). Many species of eucalyptus produce three types of volatile oils; medicinal oils, perfumery oils and industrial oils (Toloza *et al.*, 2008). Both plant extract and essential oil from some of the eucalyptus species have been reported to exhibit antifungal, antibacterial, analgesic and anti-inflammatory properties. (Falahati *et al.*, 2005; Hasegawa *et al.*, 2008). The essential oil extracted from leaves of eucalyptus is widely used as a mosquito repellant, externally and as an insecticide in India. In Spain, colds and catarrh are being treated by the inhalation therapy for which the essential oil of fruits and leaves is used (Ross and Totowa, 2001). Leaf extract obtained by hot water has shown hypoglycemic abilities if given orally (Ross and Totowa, 2001). The camphor smell of eucalyptus oil affects the nervous system and provides a feeling of alleviation. Moreover, it is useful to stop bleeding, migraine headaches (when applied by massaging), malaria, inflammations and wounds. Its chewing is helpful in treatment of congestion in respiratory tract, bronchitis, cough, tuberculosis, gum and mouth diseases (Baytop, 2000).

2.2.3. *Eucalyptus citriodora*

It is a tall tree with a height of 35 m (or more sometimes), mostly found in temperate and tropical north eastern Australia natively but its commercial cultivation was introduced in other parts of the world including Asian countries (Grieve, 1997).

Eucalyptus citriodora has blatantly narrow-leaves with a strong smell of lemon. The tree is covered by the smooth, powdery, pale bark which is completely or slightly blotched and sheds in thin curly flakes throughout the height of the tree. Corner of the leaf and the stem junction is occupied by the pear-shaped buds present in clusters of three whereas fruit (capsule) is in urn-shape. Flowering of this plant takes place in January, April, May-October and December.

The tree is highly enriched in citronellal essential oil which is extracted from its dried leaves with a highest percentage of essential oil (0.4%) greater than the other species (Elaissi *et al.*, 2011). It potentially acts as insects repellent (Olivero-Verbel *et al.*, 2010) to control poultry red mites (George *et al.*, 2009). The essential oil extracted from leaves shows some other properties such as it acts as an acaricidal (Clemente *et al.*, 2010), anti-inflammatory (Silva *et al.*, 2003), antibacterial (Low *et al.*, 1974), and to treat influenza, skin rashes and chest problems (Khalil and Dababneth, 2007). Antifungal activity is significantly associated with the oil extracted from bark, flowers and fruits (Musyimi and Ogur, 2008). Besides essential oils, eucalyptin, β-sitosterol and triterpenoids are some other active compounds extracted from leaves (Asolkar *et al.*, 1992). *In vitro* cytokine's production and arachidonic acid metabolism in human blood monocytes is inhibited by eucalyptol (1, 8-cineole), an active element of the essential oil (Jeurgens *et al.*, 1998).

2.2.4. *Eucalyptus tereticornis*

Eucalyptus tereticornis is a tall tree with a height of 30-45 m with a faster growth rate. The specie occupies its space along streams, in open forests or as scattered in alluvial plains. They show better growth in light textured, neutral, or slightly acid. The species tolerates temperatures of -7°C in the South of China and Pakistan. Long periods of drought are not tolerable by the specie. The areas with annual precipitation of 800 mm to 1500 mm are the best for its vigorous growth, but the areas with less rainfall such as India, Israel and Zimbabwe and the areas with considerably more rainfall (Colombia and Papua New Guinea) are also suitable for its growth.

The earlier reports support the presence of α-pinene and 1,8-cineole as major active components of *E. tereticornis* essential oil (Coelho *et al.*, 2005; Ogunwande *et al.*, 2003; Pino *et al.*, 2001). The antioxidant activity was associated with essential oil extracted from fresh and decaying leaves of *E. tereticornis* (Singh *et al.*, 2009). A strong fungitoxic activity against *Fusarium oxysporum* and *Heminthosporium oryzae* was also found in essential oil of *E. tereticornis* (Kaur *et al.*, 2011).

2.2.5. *Syzygium aromaticum*

Syzygium aromaticum (common name: clove) from the family Myrtaceae, is an important aromatic spice. Commercial cultivation of clove is exercised in India, Sri Lanka, Indonesia and South China. Clove oil is widely used as a flavoring agent to flavor pastry, special sauces and condiments. It is also used in medicines, especially in those prepared for gum and teeth. The tinctures, extracts and oleoresins of Clove are also used (Atal and Kapur, 1982). Clove bud oil has antioxidant property and is effective against bacteria, fungi and insects. It is used as flavoring agent and antimicrobial material in food (Huang *et al.*, 2002; Lee and Shibamoto, 2001; Velluti *et al.*, 2003). The strong biological and anti-microbial activity of the Clove essential oil is due to the presence of eugenol; a phenolic compound which denatures proteins and changes the permeability by reacting with membrane phospholipids (Briozzo, 1989; Deans & Ritchie, 1987).

2.2.6. *Myristica fragrans*

Myristica fragrans (Nutmeg) is a member of Myristicaceae family of flowering plants present in Europe, Asia and America. This family after the name of nutmeg plant is also called Nutmeg family. About 20 genera along with approximately 440 species of trees and shrubs of this family are found in tropical areas. The large trees are valuable for the timber industries.

Myristica fragrans is a 12 m high evergreen plant imported to the Europe in 12th century by the Arab traders (Barceloux, 2008). Two important species of the family; Nutmeg (nut) and Mace (dried scarlet) are processed separately when dried. The composition and organoleptic properties of nutmeg and mace oils has been evaluated in the last century (Choo, 1999). Meat dishes, pastry, liqueurs, cola drinks and perfumes are flavored by using nutmeg and mace oils. They were found useful against stomachache, rheumatism and vomiting during pregnancies. α - pinene being a dominant component of *Myristica fragrans* oils was 25.07% along with sabinene

(21.38%), 4-terpinol (13.92%) and myristicin (13.57%). when analyzed chemically (Olajide *et al*., 1999; Sonavane *et al.*, 2002).

Studies (both *in vivo* and *in vitro*) show pharmacological activities associated with nutmeg (El-Alfy *et al*., 2009) for example hepatoprotective activity against some chemicals damaging liver (Morita *et al*., 2003). The volatile oil of *M. fragrans* showed antibacterial activities against some bacteria (Dorman and Deans, 2000). When compared with antioxidants, ascorbic acid and alpha tocopherol, the essential oil acted as a scavenger. About 30% in the extract is myristicin in the essential oil acting as an anticancer agent (Chirathaworn *et al*., 2007; Stefano *et al*., 2011). The presence of this compound in the nutmeg essential oil contributes to its anti-tumoral properties.

2.2.7. *Citrus reticulata*

It is a member of citrus family called Rutaceae. *Citrus* is one of the most economically important genus including 17 species such as *C. sinensis* (orange), *C. limon* (lemon), *C. paradisi* (grapefruit), and *C. aurantifolia* (lime) etc. (Davies and Albrigo, 1994; Shaw, 1977). Generally found in tropical and temperate regions and in the hilly states of northern India with 1200m altitude (Davies and Albrigo, 1994). It is one of the ancient crops cultivated by the humans 1200 BC (Moore, 2001). They are traditionally used as jams, juices and desserts. A considerable amount (50%) raw processed food as a byproduct from seeds, peels and pulps is produced by food and agro food processing industries (Anwar *et al*., 2008). Flavonoids, dietary fibers and essential oils are produced by these by products as functional ingredients (Senevirathne *et al*,. 2009). Lime is used to flavor beverages, cookies and desserts (Buchel, 1989; Dharmawan *et al*; 2007).

Essential oil is extracted from fruits and leaves and contains d-limonene, β-myrcene, α-pinene, sabinene, 3-carene, α-terpinolene and others (Stashenko *et al*., 1996; Sharma and Tripathi, 2008). Limonene and β-pinene were the major components of the essential oil extracted from *C. jambhiri* found in Florida, USA (Shaw and Wilson, 1976). When oil extracted from the same specie collected from France, 91.4% of limonene and 2% sabinene were present (Lota *et al*,. 2002). The percentages were different in the essential oils of the species from Jeju island and Korea (Baik *et al*,. 2008). The variation in quantity of limonene directly influences the odor and

quality of the oil extracted from the species of different climatic conditions (Dharmawan *et al*., 2007).

A wide spectrum of biological activities of Citrus essential oils makes them safe to use. They showed antimicrobial, antioxidant and anti-inflammatory properties (Fisher and Phillips, 2008; Rehman, 2006). Essential oils of *Citrus limon* and *Citrus aurantifolia* showed more inhibitory actions against fungus growth than *Citrus paradisi* and *Citrus sinensis* (Kaute *et al*., 2006). *C. limettioides* oil has linalool, α-pinene, α-terpinol (Myoatasyoh *et al.,* 2007) along with citral which showed fungicidal and bactericidal activities by forming a charge transfer complex to fungal cell with an electron donor (Kurita *et al*., 1981). A significant antioxidant activity was found associated with EO of *C. pyriformis* when compared with ascorbic acid (Hamdan *et al*., 2010).

2.2.8. *Murraya koenigii*

Murraya koenigii with a common name: "curry leaf" or "Kari patta" is a member of Rutaceae family including more than 150 genera with 1600 species (Satyavati *et al*., 1987). It is generally found in India, Sri Lanka and South Asia. It is a 6 m high deciduous shrub with a diameter of 15-40 cm; characterized by short trunk, smooth grey or brown bark and thick shadowy crown (Mhaskar *et al*., 2000). Essential oils extracted from fresh or dried leaves powder both are used as flavors in soups, curries, fish, meat and egg dishes. Different soaps and cosmetic aromatherapy industries also utilize its essential oil (Rao *et al*., 2011). It has been used traditionally as antiemetic, anti-diarrheal, antipyretic, blood purifier, antifungal, antidepressant, anti-inflammatory, in body aches and kidney pains (Rana *et al*., 2004; Ningappaa *et al*., 2010; Khuntia *et al*., 2011).

Highest antioxidant activity was shown by 1:1 ratio alcohol-water extract of curry leaves (Ningappaa *et al*., 2008). Carbazole alkaloids and carotenoids being the most described components in essential oil contribute to remarkable antioxidant activity. *In vitro* antioxidant activity of different extracts of *Marraya koengii* was found to be as followed; seeds aqueous > leaf aqueous > leaf $CHCl_2$: MeOH > seed $CHCl_2$: MeOH (Minakshi and Minali, 2004).

A strong antifungal activity against human pathogenic fungi was shown by Murrayanine, girnimbine and mahanimbine, the components present in stem bark (Das *et al*., 1965). The presence of β-caryophyllene and gurjunene in the essential oil makes it effective against *Rhizoctania batiticola* and *Helminthosporium oryza* (Iyer and mani, 1990). Oils and aqueous

extracts of leaves are active against some staphylococcus and streptococcus bacterial species. A promising antibacterial activity was shown by the crude extracts, chloroform soluble and petroleum ether soluble fractions (Akerel and Ayinde, 1998; Sanjay and Singh, 2001).

2.2.9. *Piper nigrum*

Piper nigrum is famous for its pungent quality and is called as "spice king" belonging to the family Piperaceae (Srinivasan, 2007; Ahmad *et al*., 2010; Abbasi *et al*., 2010). The group includes 13 genera with almost 1920 species estimated roughly. *P. nigrum*, *P. longum* and *P. betle* are the most famous species in tropical and subtropical regions of Asia (Khan *et al*., 2010).

Black pepper is generally used in dietary items and medicines. They are also used as preservatives (Hussain *et al*., 2011). Piperine; an active component of the plant activates pancreatic enzymes and the enzymes of intestines and thus increases the bile secretion when given orally (Tiwari and Singh, 2008). It is used to treat digestive and respiratory disorders such as cold, fever and asthma (Dhanya *et al*., 2007; Parganiha *et al*., 2011). *P. guineense* is beneficial to treat bronchitis, gastric ulcer, rheumatism and some viruses (Parmar *et al*., 1997). Piperine can also influence mood and cognitive disorders (Wattanathorn *et al*., 2008).

About 64.05% of the total *P. nigrum* oil was comprised of monoterpene compounds and relatively lower amount of oxygenated sesquiterpene (13.06 %). The major compounds present in the fruits of black pepper were 35.06% limonene, 12.95% β-pinene and 9.55% linalool (Fan *et al*., 2011). β-caryophyllene; a sesquiterpene in *P. nigrum* was reported to be the major component as 24.2% (Singh *et al*., 2004). The essential oil extracted from dried fruits of black pepper from Cameron contained D-germacrene, limonene, β-pinene, β-caryophyllene, α-phellandrene (Jirovetz *et al*., 2002). The essential oil from the leaves was found to be enriched with limonene, β-pinene and β-caryophyllene (Sasidharan and Menon, 2010).

When compared with tetracyclin (anti bacterial) and nystatin (anti fungal), fresh pepper berry oil was found to be more active against *Bacillus subtilus* and *Pseudomonas aerugenosa*. It was also active against *Penicillium spp.*, *Candida albicans*, *Saccharomyces cerevisiae* (yeast) and *Trichoderma spp*. Pepper leaf oil showed less activity against all the reference organisms studied (Sasidharan and Menon, 2010).

2.2.10. *Elettaria cardamomum*

Elettaria cardamomum is a perennial herb also known as cardamom (local name), small cardamom and elaichi etc. it is a 4 m tall plant commonly found in Pakistan, India, Burma and

Sri Lanka (Rahman *et al*., 2000; Nadkarni, 1976). It is the member of ginger family called Zingiberaceae; a family of flowering plants consisting of 52 genera and 1300 species of aromatic herbs. Once the teguments are opened, fragrance is lost so the premature green-colored seeds are collected which are then used as a spice. A large number of beauty products uses its essential oil. It is widely used in flatulence, acts as diuretic, abortifacient, antibacterial, antiviral and antifungal and also treats constipation, colic, diarrhea, vomiting, headache, epilepsy and cardiovascular disorders (Khan and Rahman, 1992; Duke *et al*., 2002).

Multiple chemical compounds were reported when analyzed such as α-terpineol, heptanes, linalool, α-pinene, β-pinene, limonene, phytol, borneol, geranyl acetate, terpinene and others (Shaban *et al*., 1987; Gopalakrishnan *et al*., 1990; Duke, 1992). Antibacterial and anticancer activities were also shown by cardamom essential oils (Gilani *et al*., 2008; Jamal *et al*., 2006).

2.2.11. *Amomum subulatum*

It is also a perennial herb called "large cardamom (elaichi)" commonly found in Asia, Himalya, Nepal and Bengal. It belongs to the family Zingiberaceae (approx. 150 species); the second largest genus (Thomas *et al*., 2009). It is a 2-6 m tall plant with long, dark green leaves and a pod with green color carrying 10-15 seeds (Hussain *et al*., 2009) which is used as a flavoring agent and spice (Naik *et al*., 2006). Its seeds are used s appetizer, diuretics, liver tonic to treat vomiting, indigestion, gastric and rectal diseases (Jafri *et al*., 2001). Biological, hepatoprotective, and anti-inflammatory activities are associated with essential oil of cardamom (Bisht *et al*., 2011; Alam *et al*., 2011; Parmar *et al*., 2009).

α-pinene, β-pinene, terpinene-4-ol, 1-8-cineole, α-terpineol, D,L-limonene and nerolidol were the major components reported in the essential oils when analyzed by GC-MS (Satyal, *et al*., 2012; Joshi *et al*., 2013). These oils were active against some Gram-positive bacteria (*Bacillus cereus*), *Staphylococcus aureus*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, fungus (*Aspergillus niger*) and yeast (*Sacharomyces cerevisiae*) (Agnihotri *et al*., 2012).

2.2.12. *Trachyspermum ammi*

Ajowain (common name) is a member of a well known family of aromatic plants Apiaceae including 434 genera and 3700 species such as cumin, dill, fennel, celery and coriander

etc. generally found in Pakistan, Iraq, Iran, Afghanistan and India (Zargari, 1989). It is a 60-90 m tall herb grows in waterless soil with high salt concentrations (Joshi, 2000).

The essential oil is chemically composed of thymol, *p*-cymene, β-pinene and γ-terpinene (Gandomi *et al.*, 2013; Singh *et al.*, 2004). Variations in thymol concentration may be due to geographical location changes, time taken by the plant to grow, preparation procedures etc. (Dehkordi *et al.*, 2010). *T. ammi* is one of those plants showing potent antimicrobial activities. Ethanolic extracts of *T. ammi* inhibited the mycelia growth and spore germination of a fungus named A*spergillus ochraceus* which produces toxins (Murthy *et al.*, 2009). The presence of a phenolic group (–OH group) in the thymol is responsible for its high microbicidal and anti-aflatoxigenic property (Farag *et al.* 1989).

The antioxidant activity increases as the concentration of *T. ammi*'s essential oil increases. The activity of alcohol extract of the oil was lower than its water extract (Khanum *et al.* 2011).

2.2.13. *Foeniculum vulgare*

Foeniculum vulgare (saunf) is a 2.5 m tall plant with yellow flowers, feathery leaves (40 cm long) and hollow stems. It is an aromatic plant whose seeds are used to flavor bakery products, ice cream, alcoholic beverages and some dishes (Maroto *et al.*, 2006). Fennel's essential oil is used to flavor bread, pickles and cheese. It is an active component in cosmetic and pharmaceutical products due to their medicinal activity (Piccaglia and Marotti, 2001). Its herbal drugs were reported to have hepatoprotective, antispasmodic effects (Ozbek *et al.*, 2003; Reynolds, 1982) along with anti-inflammatory, analgesic and antioixidant activities (Choi and Hwang, 2004). The anticancer activity was also associated with the fennel seeds oils (Anand *et al.*, 2008).

Renjie *et al.* (2010) reported phenylpropanoid derivatives and monoterpenoids as the major components along with anethole, limonene, β-thujaplicin (in leaf, having antifungal and antibacterial activities), pinene, limonene and fenchone (Maroto *et al.*, 2006; Arima *et al.* 2003; Morita *et al.* 2004). The percentage of anethole and limonene was different in the essential oils of leaves and seeds. Anethole was 51.08% in leaves oil and 58.54% in seeds whereas limonene was 22.9% in leaves and 19.6% in seeds as the major constituents (Chowdhry *et al.*, 2009).

β-myrcene (0.19%), limonene (5.76%) and trans-anethole (78.39%) in *Foeniculum vulgare* essential oil were reported to be major components by Yu *et al*. (2013) and they were found Trans-anethole (65.4%), fenchone (8.26%), estragole (5.2%) and limonene (4.2%) (Roby *et al*., 2013).

The quenching ability of *Foeniculum vulgare* of DPPH radical is higher (0.35mg/ml) when compared with ascorbic acid or BHT (0.44mg/ml) (Shahat *et al*., 2011). High antibacterial effect of fennel oil on *Candida albicans* was reported by Khaldun (2006). Ozcan *et al*. (2006) reported bactericidal action of *Foeniculum vulgare* on *Salmonella typhimurium* and *Salmonella dysenteriae*. Scavenging activity by methanol extract was high as compared to ethanol extracts. *B. cereus* and *A. flavus* showed highest sensitivity through largest inhibition zones whereas *E. coli* showed the smallest inhibition zone with highest MIC value (Cantore *et al.,* 2004). Fennel essential oils inhibit inhibit a wide range of bacillus species and aspergillus species (Ozcan *et al*., 2006; Mimica-Dukic *et al*., 2003).

2.2.14. *Cuminum Cyminum*

Cumin (*Cuminum cyminum*) with a common name "jeera: (in idia) and "zira" (in Pakistan and Iran) is a flowering plant of Apiaceae family with a distinctive aroma of the seeds and is found in India and Mediterranean (Nadeem and Riaz, 2012). *Cuminum cyminum* is herb with a 20-30 cm tall having branched stem and 5-10 cm long leaves or leaflets which are pinnate or bipinnate. A single seed is present in 4-5 mm long lateral cone-shaped fruit. The spice is regularly used as a flavoring agent (2nd most popular; the first being black pepper), in Indian and Roman cuisines. It is used on meat along with other common seasonings (Thippeswamy and Naidu, 2005; Raghavan, 2007). Cumin is a famous spice in Pakistan, India, North Africa, Middle East, Sri Lanka, Cuba, Northern Mexico and in some parts of Western China (Daniel and Maria, 2000).

The GC-MS analysis inferred that *C. cyminum* essential oil was enriched with *α*-pinene (29.1%), limonene (21.5%), eucalyptol (17.9%), and linalool (10.4%) as the main components contributing the well characterized flavor and strong aroma (Allahghadri *et al*., 2010). Supercritical carbon dioxide extraction method reported the presence of *β*-pinene and *γ* -terpinene as the chief components of cumin oil (Eikani *et al*., 1999). About 37 major components isolated through hydrodistillation of the essential oil were *γ* -terpinene, cuminal, safranal, cuminic alcohol, *p*-cymene, and *β*-pinene (Rong and Zi-Tao, 2004). GC-MS of the essential oil

showed p-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene, and t-anethole as main components as biologically active ingredients (Sultan *et al.*, 2009).

C. cyminum L. exhibited potential activity against various microbes including bacteria and fungi (Iacobellis *et al.*, 2005). In fact the oil was found to be more effective than antibiotics even when used in its very low concentration (Singh *et al.*, 2002). The scavenging activity for DPPH radical (presented as IC50 values) was 31 µg/mL for *C. cyminum* oil and 11.5 µg/mL for BHT (Hajlaoui *et al.*, 2010). β-carotene bleaching was also inhibited by the *C. cyminum* essential oil and BHT. The IC_{50} values were found to be 20 and 75 µg/mL, respectively (Gachkar *et al.*, 2007). In another study, IC_{50} value was found to be 54.7 g/mL (Milan *et al.*, 2008). The oxidation of β-carotene and linoleic acid and DPPH was monitored in coupled form using black cumin essential oil as antioxidant and profound reduction in lipid peroxidation was observed (Sultan *et al.*, 2009).

2.2.15. *Cinnamomum zelenicum*

Cinnamomum zelenicum is a small evergreen tree with a height of 10-15 meters falls in family Lauraceae. It is the family of flowering plants including 3000 species in 50 genera, present in tropical and temperate areas of Asia and America. It is indigenous to Sri Lanka and tropical parts of Asia. Cinnamon bark is efficient in medicines acting as an antiemetic, anti-diarrheal and anti-flatulent (Hsieh, 2000). It is a spice and is used in perfumes, as a flavoring agent and by pharmaceutical industries in various medicines (Raina *et al.*, 2001; Simic *et al.*, 2004).

Eugenol was found to be the main component in the volatile oil of leaf (87.3%) when analyzed by GC-MS and GC. Other components were bicyclogermacrene, α-phellandrene, and β-caryophyllene, benzyl benzoate, linalool and eugenyl acetate in *Cinnamomum zeylanicum* leaf essential oil collected from Sri Lanka (Singh *et al.*, 2007; Schmidt *et al.*, 2006). The concentration of these compounds in the leaf essential oil may vary as the variation in origin of the plant (Patel *et al.*, 2007). There were 23 different chemical compounds detected with eugenol (being the major one) from the essential oils extracted through hydro-distillation of leaves and twigs of *C. zeylanicum* (Lima *et al.*, 2005).

Ooi *et al.* (2006) found that the growth of all the microbes studied was inhibited by cinnamon oil with the MIC range between 75 - 600 µg/mL with the best activity for *Vibrio parahaemolyticus* and least for *Pseudomonas aeruginosa* and *E.coil*. Leaf volatile essential oil at

6 µL was found to be 100% active against all fungus except *A. ochraceus* and *A. terreus.* The growth of these fungal species was completely inhibited at 2 µL. The remarkable activity was against *A. flavus* with complete inhibition at only 2 µL concentration whereas the other studied species were completely inhibited by bark oil at 6 µL (Singh *et al.*, 2007).

The methanolic extracts obtained from bark of *C. verum* showed the better antioxidant activity in comparison with other reference compounds. Bark extract exhibited more ability to donate hydrogen atom (reducing power) and also the scavenging ability towards free radicals and chelate formation with metals (Mathew and Abraham, 2006). Significant activity (more than 95%) with high (420 mg/g) gallic acid equivalent was associated with cinnamon leaf oil. The antiradical activity of leaf oil was reported to be higher when compared with butylated hydroxyl toluene (IC_{50} = 4.5 mg/L for the former and IC_{50} = 7 mg/L for the later) (Dongmo *et al.*, 2007). The radical activities were very strong with IC50 (18.4 μg/mL) (Chericoni *et al.*, 2005).

2.3. History of Essential oils

French chemist M. J. Dumas was the first to inspect the components of volatile oils systematically in 1800-1884. In his results published in 1884, he analyzed the components containing hydrocarbons, oxygen, sulfur and nitrogen. However, the most significant work was performed by O. Wallach, (1914) who found that the terpenes (naming based on their botanical sources) were chemically identical. An individual component of essential oil was isolated through distillation techniques and the basic characterization was performed by treating with a number of inorganic reagents. The hydrocarbons with the molecular formula $C_{10}H_{16}$ at that time were named "terpenes" by Kekule as they were present in turpentine oil. The components with the molecular formula $C_{10}H_{16}O$ and $C_{10}H_{18}O$ were also referred to as terpenes.

In 1891, various terpenes including pinene, limonene, phellandrene, terpinolene, sylvestrene, and fenchene were reported by Wallach in one of his book named Terpene and Campher consisting of 180 articles (Wallach, 1914). He got Nobel Prize in 1910 due to his proposal that isoprenes are the building subunits of terpenes. F. W. Semmler and G. Wagner, in 1899, analyzed geraniol, linalool, citral, and many more as those were occurring most frequently.

The structure of a bicyclic sesquiterpene, caryophyllene, has been the matter of doubt for several years for chemists. W. Treibs in 1952 isolated caryophyllene epoxide from the oxidation products of clove essential oil and then infrared (IR) studies showed that caryophyllene had 4

and 9 rings (Sorm *et al.*, 1950). Later on, the Nobel Prize in Chemistry was awarded to Barton (Barton and Lindsay, 1951), upon confirming Sorm's suggestion in 1969.

The chemical structures of various terpenes and products of natural origin were interpreted and explained by Woodward using UV-Vis spectroscopy during the early 40s. Maximum absorption of UV and the substitution pattern of a diene or unsaturated ketone (either α or β) were correlated to figure out the structure of new natural compound. The series of these rules were later called as Woodward rules (Woodward, 1941). Subsequently, separation methods of chromatography and NMR (nuclear magnetic resonance) spectroscopy were introduced to organic chemistry to clarify and explain the further structure of terpenes.

2.4. Chemistry of Essential oils

Essential oils are the complex mixtures of volatile components synthesized biologically by the living organisms. Hydro distillation, steam distillation and expression (for citrus fruit) can be used in order to extract the oil from their matrix (Baser, 1995). Volatile hydrocarbons and oxygen derivatives of terpenoids and non-terpenoid compounds are present in essential oils. Nitrogen or sulfur derivatives of alcohols, acids, esters, epoxide, aldehydes, ketones, amines, sulphides may also be present in essential oils. Monoterpenes, sesquiterpenes, diterpenes, phenylpropanoids, fatty acids, esters and their decomposed products may also contribute in the composition of essential oils (Breitmaier, 2005). Essential oils are generally extracted from the aromatic plants. Some are obtained from the animal sources such as musk, civet or sperm and microorganisms (Baser, 2005).

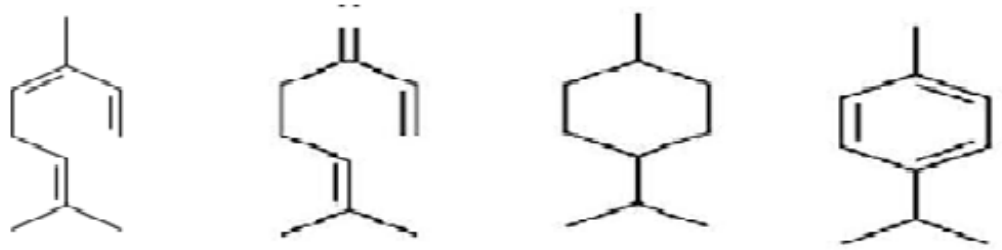
The components of essential oil are divided into terpenoids and non-terpenoid hydrocarbons.

2.4.1. Terpenoids

Terpenes being an important component of essential oil are one of the largest chemicals found naturally. They are also called isoprenoids. Two or more isoprene molecules in a head-to-tail fashion rearrange themselves to form a terpene molecule. A large number of terpenoids (>30,000) has been isolated from plants, animals and microorganisms (Dewick, 2002). Hemiterpenes have molecular formula of C_5H_8. Two molecules of isoprene join to form Monoterpenes with a molecular formula $C_{10}H_{16}$ and joining of three forms Sesquiterpenes with the formula $C_{15}H_{24}$. Four isoprene molecules form Diterpenes ($C_{20}H_{32}$) (Dewick, 2002).

2.4.2. Monoterpenes

Two isoprene units when join together, they form monoterpenes. They can be either cyclic or acyclic. *β*-myrcene, *β*-Ocimene are acyclic whereas *p*-menthane or *p*-cymene are monocyclic. *δ*-3-carene is a bicyclic whereas tricyclene is a tricyclic monoterpene found in essential oils (Wise and Croteau, 1999).

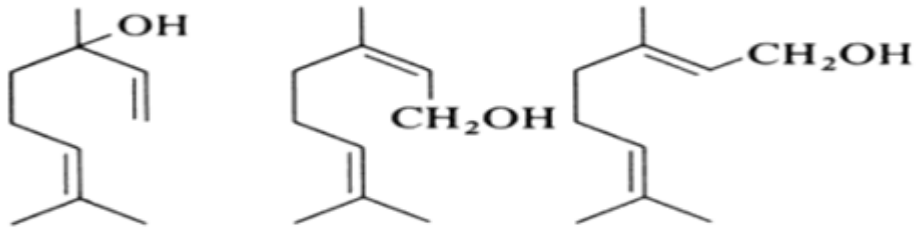


β-ocimene β-myrcene *p*-menthane *p*-cymene

Essential oils of many species contain aromatic monoterpenes like *p*-cymene, carvacrol, and thymol etc. are found in oregano, thyme, savory and rose essential oils.

2.4.3. Acyclic Monoterpenes

It is a small class of monoterpenes in which the trienes are included such as myrcene, Ocimene and alcohols like geraniol, nerol, linalool, etc.



Linalool Nerol Geraniol

2.4.4. Cyclic Monoterpenes

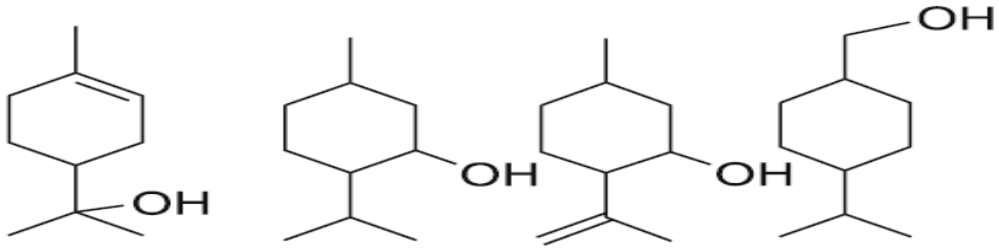
Based on the size of the ring, cyclic monoterpenes are divided in three subgroups:

I. Monocyclic monoterpenes
II. Bicyclic monoterpenes
III. Tricyclic monoterpenes

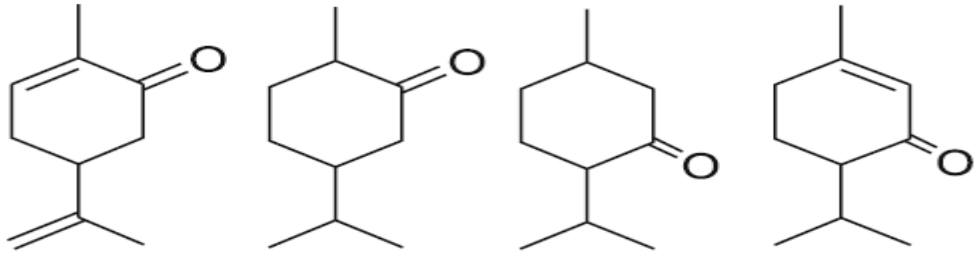
2.4.5. Monocyclic Monoterpenes

A single ring is present in these monoterpenes. They are also classified in monoterpene hydrocarbon's group. Limonene, (*α,β,γ)*-terpinene, terpinolene, *α*-phellandrene and *β*-phellandrene are some of the compounds included in this group. Aromatic monoterpenes include

p-cymene is an aromatic monoterpene which forms a thymol derivative when hydroxylated and carvacrol when isomerized. *α*-terpineol, menthol, isopulegol and *cis*-hexahydrocuminyl alcohol are the oxygenated derivatives also called as monoterpene alcohols whereas carvone, dihydrocarvone, isomenthone, piperitone etc are the ketones included in the group.



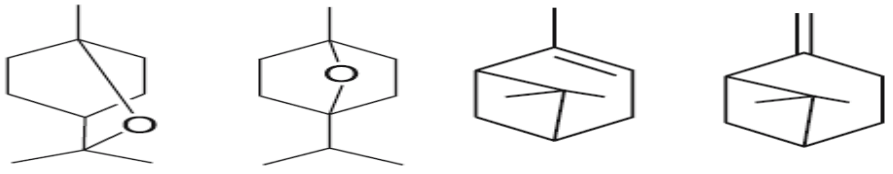
α-terpineol Menthol isopulegol *cis*-hexahydrocuminyl



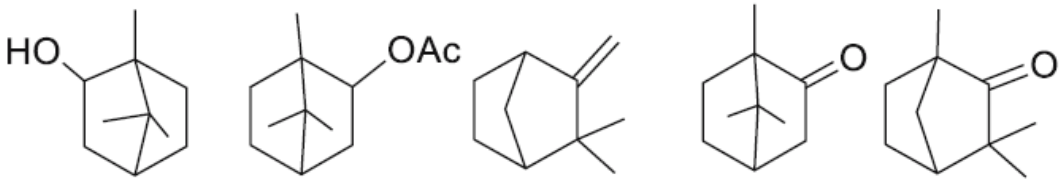
Carvone dihydrocarvone isomenthone piperitone

2.4.6. Bicyclic Monoterpenes

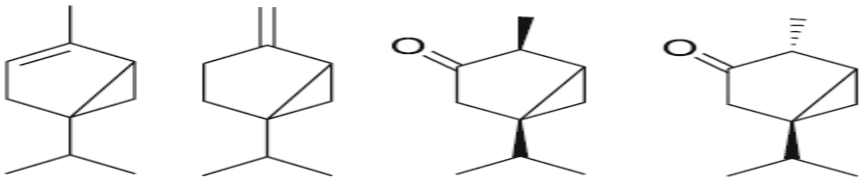
1, 8-Cineole, 1, 4-cineole**,** *α*-Pinene and *β*-pinene are the bicyclic monoterpenes**.** The bicyclic skeleton is present in borneol, isobornyl acetate, camphene, camphor, fenchone. An unusual group of monoterpenes called thujane type monoterpenes have a cyclopropane ring in the bicyclic skeleton. They include *α*-thujene, sabinene, *α*-thujone, *β*-thujone etc.



1, 8-Cineole 1,4-cineole *α*-Pinene *β*-pinene



Borneol isobornyl acetate camphene camphor fenchone



α-thujene sabinene *α*-thujone *β*-thujone

2.4.7. Tricyclic Monoterpenes

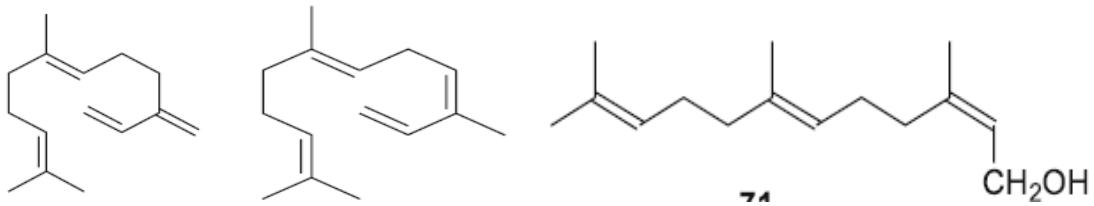
Various essential oils contain tricyclene or 1,7,7-trimethyltricyclo[2.2.1.02,6]heptanes which are good examples for this class of compounds.

2.4.8. Sesquiterpenes

These are formed when there is added an isoprene unit into monoterpene molecule. They could be linear, branched or cyclic unsaturated compounds with a molecular formula of $C_{15}H_{24}$.

2.4.9. Acyclic Sesquiterpenes

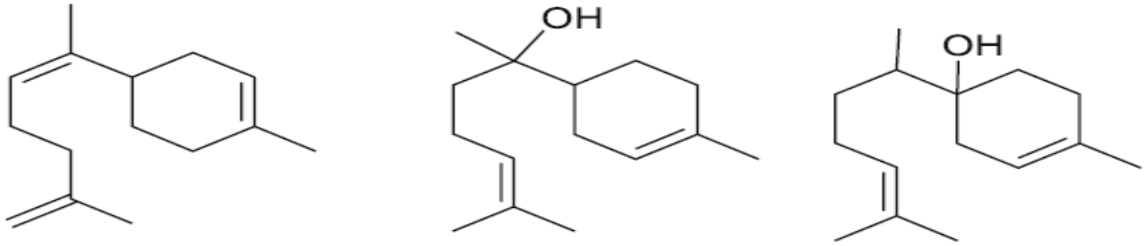
Hops oil and many others have *β*-farnesene as an essential component. It is the structural isomer of *α*-Farnesene**.** Flowers oils of rose, acacia and cyclamen are enriched in farnesol.



β-Farnesene *α*-Farnesene Farnesol

2.4.10. Monocyclic Sesquiterpenes

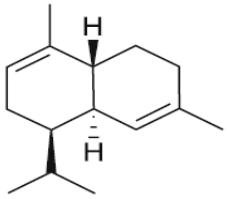
α-bisabolene is an example of monocyclic sesquiterpene which is distributed widely in nature. This sesquiterpene is an essential component of some oils including bergamot and myrrh. Chamomile is enriched in the oxygenated derivatives of this compound called *α*-bisabolol and *β*-bisabolol**.**



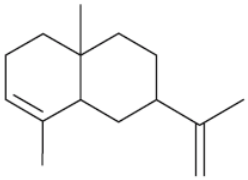
α-bisabolene *α*-bisabolol *β*-bisabolol

2.4.11. Bicyclic Sesquiterpenes

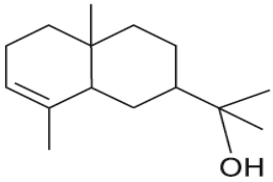
They are bi-ringed sesquiterpenes including *α*-cadinene, *α*-selinene, *α*-Eudesmol, α-Cyperone etc.



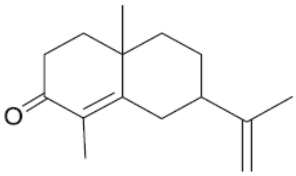
α-cadinene



α-Selinene



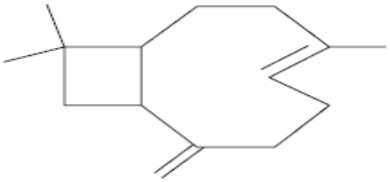
α-Eudesmol



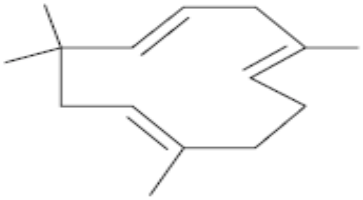
α-Cyperone

2.4.12. Miscellaneous Sesquiterpenes

Clove oil was the source from where caryophyllene was first time isolated which has become a common constituent of majority of essential oils. *β*-Caryophyllene is the widely found form of caryophyllenes. The later was first isolated in clove oil as a common and generally found component. Humulene is an isomer of caryophyllene.



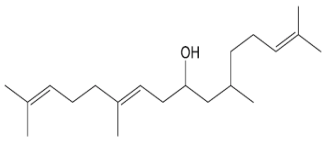
β-Caryophyllene



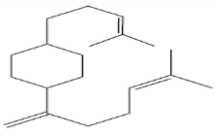
Humulene

2.4.13. Diterpenes

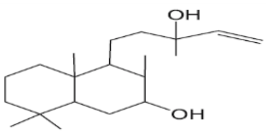
Four isoprene units rearrange themselves in an alternate fashion to give rise diterpenes ($C_{20}H_{32}$) which are generally found in pimaric acid. Phytol is a diterpene generally found in essential oils (MacMillan and Beale, 1999). More energy is required by the diterpenes to go to the vapor state. So a longer distillation time is required to recover them. Geranyl citronellol is an acyclic diterpene, camphorene is a cyclic, sclareol is a bicyclic and phyllocladene is a tricyclic diterpene.



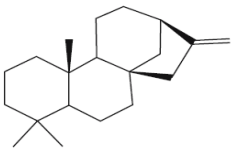
Geranylcitronellol



Camphorene



Sclareol



phyllocladene

2.5. Biological activities of essential oils

Essential oils extracted from different plants show variety of bioactivities including antioxidant, antitumor, antibacterial, antifungal and insecticidal properties (Burt, 2004).

2.5.1. Antioxidant activities:

Antioxidant:

The substances having a capability to delay or inhibit the oxidative processes and are less in concentration than the oxidation substrate are called antioxidants.

Antioxidant Assays

Various chemical reactions are involved when essential oils behave as antioxidants. Depending upon the chemistry of these reactions, the assays can be put into two categories: First assay is based on the principle that hydrogen atom is transferred (HAT) from antioxidant during reaction while in second electron is transferred (ET). There occur redox reactions in ET based assays in which an oxidant behaves as an indicator of end point. The radical scavenging capacity is basically measured by these assays rather than preventive antioxidant capacity of sample. In HAT-based assays, the competitive reaction kinetics is measured, in which the kinetic curves help to evaluate the quantitation from the reaction. A synthetic free radical generator, an oxidizable molecular probe, and an antioxidant are the major ingredients of HAT-based methods. Antioxidant reaction rates are relatively high against oxidants (especially peroxyl radicals), they act as standard parameters for antioxidant capacity (Huang *et al.*, 2005).

The DPPH assay due to its simplicity and high sensitivity is widely used nowadays to study natural antioxidants considering the antioxidants as hydrogen donors and DPPH• as hydrogen acceptor (Figure 1). DPPH• is a stable organic nitrogen radical available commercially (MacDonald-Wicks *et al.*, 2006). The higher the disappearance of DPPH in the sample, higher is the antioxidant potential of sample. ESR; electron spin resonance spectroscopy /plant powders (Calliste *et al.*, 2001), NMR / catechins (Sawai and Sakata, 2001), and UV spectrophotometry / polyphenols (Chaillou and Nazareno, 2006) are some methods used to monitor DPPH• amount in the sample among which UV spectrometer is widely used for its accuracy. A strong absorption of DPPH• is shown at 517 nm (purple). After the hydrogen donated by an antioxidant is absorbed, DPPH• is converted to DPPH which is indicated by change in the color from purple to yellow. IC_{50} is the amount of antioxidant required for reduction in concentration of DPPH• by 50% (Brand-Williams *et al.*, 2006).

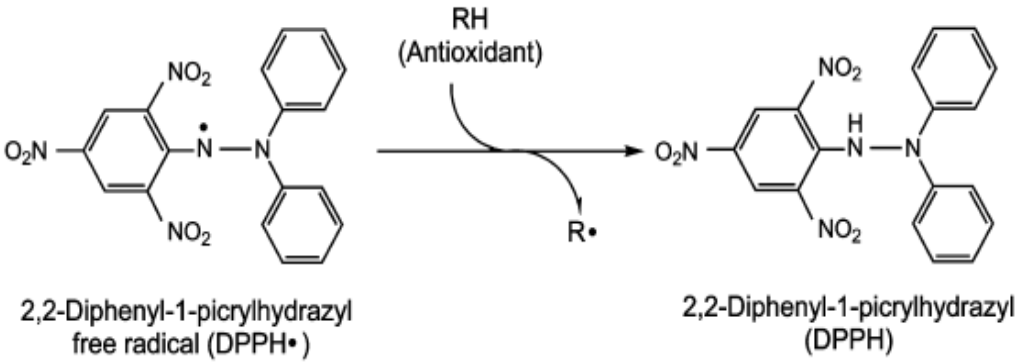


Figure 1. DPPH• / antioxidant reaction to form DPPH

DPPH method sometimes is the only method used to assess antioxidant activities of essential oils due to its sensitivity and simplicity. Mothana *et al*. (2010), studied antioxidant potential of essential oils of two species from Commiphora and found it to be very low even at high concentrations. Other methods were not used by the authors to evaluate the essential oils in which phenolic compounds were not present. Barra *et al*. (2010) reported that the essential oils of *E. camaldulensis* growing wild in locality of Sardinia had a high antioxidant activity.

β-carotene is also known to be an antioxidant or a scavenger generally reacts with the peroxyl radical to give *β*-carotene epoxides (Kennedy and Liebler, 1991). Lipids, such as linoleic acid, in presence of O_2 and ROS, form a peroxyl radical i.e. LOO• which has ability to react with *β*-carotene thus forming a stable *β*-carotene radical, reducing the concentration of *β*-carotene subsequently in a testing solution (Figure 2) (Tsuchihashi *et al.*, 1995). However, if there is present an antioxidant in the reaction media, it reacts competitively with the peroxyl radical (Takada *et al.*, 2006). So the antioxidant effect of *β*-carotene at 470nm using a spectrophotometer is monitored easily as the color of the test solution is bleached out.

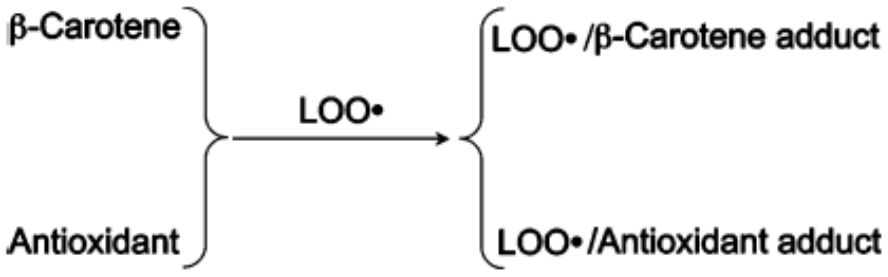


Fig 2; Adduct formation from *β*-carotene and antioxidant with a lipid peroxide radical

Essential oils of seven species belonging to Lauraceae family, enriched in sesquiterpenoids were subjected for evaluation of antioxidant potential using linoleic acid oxidation system. Both oil inhibited the oxidation of linoleic acid system. Furanodiene, germacrene D and curzerenone were the found to be the major constituents of the oils extracted (Joshi *et al*., 2010). Ahmadi *et al*. (2010) evaluated the antioxidant potential of Iranian *Hymenocrater longiflorus* in which α-pinene, 1, 8-cineole, β-eudesmol, spathulenol etc were found to be the chief constituents of the essential oil which inhibited the bleaching of β-carotene by 64%. The antioxidant activity of the oil extracted from *Artemisia herbaalba* Asso. collected from Tunisia was evaluated by Mighri *et al.* (2010) by using a number of methods especially the β-carotene bleaching test. The best percentage inhibition showed was 12.5% by the oil enriched in β-thujone despite it is much lower than BHA which was 89.2%. The results are credited to the absence of phenolic compounds in the samples.

2.5.2. Antimicrobial activities:

To treat the several infections, two main types of microbial agents were introduced by the Microbiologists: 1) naturally produced antibiotics by micro-organisms 2) chemically synthesized chemotherapeutic agents (Davidson and Harrison, 2002). Selective toxicity should be the first and foremost property when the host is considered. This property indicates that bacterial cells when are compared with the animal cells, impart different type of biochemical reaction. This difference between animal and bacterial cell is taken as benefit in chemotherapy. Generally, two modes of action are exhibited by the antibiotics. They are either bactericidal (killing bacteria) or bacteriostatic (inhibiting bacterial growth). The range of bacteria and other microorganisms at which they are influenced by specific types of drugs is called their spectrum of specificity (Burt, 2004).

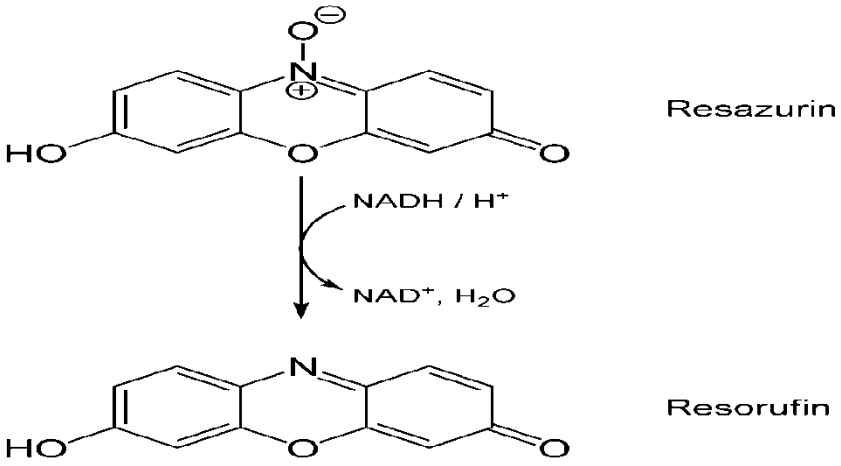
Antimicrobial assays

Antimicrobial activity can be checked by dilution and diffusion methods (Rios *et al*., 1988). The NCCLS method were used for antibacterial susceptibility, principally reasoned to test antibiotics which has been changed for testing EOs (Hammer *et al*., 1999; NCCLS, 2000). But still the results can be varied by certain factors like the method of extraction of essential oils from plant source, inoculum volume, phase of growth, type of culture medium used, pH of the media, incubation time and temperature (Rios *et al*., 1988). Many scientists have reviewed

methods used to study antibacterial potential of EOs (Nychas, 1995) and many researchers have cited the disc diffusion method and minimum inhibitory concentration (MIC) to check the performance of EOs. Often the previous one is used to screen the EOs for their antibacterial activity. In this method, a paper disk soaked with EO is placed in the middle of the agar plate. This is prelude checking of antibacterial activity over more studies. Some factors are very important to consider such as; volume of EO used, thickness of agar plate and the type of the solvent used. Hence it is proved by experiment that this method can be useful for selection of different EOs but when we compare the data with published papers then it is not proved to be possible. The agar first has to be tested very carefully in which EO is introduced. Many wells can be cut if EO or is to be tested against large number of isolates (Dorman and Deans, 2000). Dilution of EO in broth or agar can be useful to determine the strength of antimicrobial activity. Pear review of literature can be used to make a selection of different types of solvents in order to dilute EOs in medium (Pintore *et al*., 2002), variable volumes of inoculum ranging from 1–100 µl (Prudent *et al.,* 1995). End point determination in broth studies are carried out by optical density (OD) measurement and by viable counting of colonies which is very laborious. OD measurement is an automated method performed easily. Visual monitoring method to visualize the end points and conductivity is less used now-a-days. Minimum inhibitory concentration is determined by the new method called micro-dilution. Resazurin as a visual indicator is used in MIC of oil based compounds (Salvat *et al*., 2001). It is a redox indicator that helps to evaluate the cell growth, especially in many cytotoxicity assays (McNicholl *et al*., 2006). This indicator is blue in color, non-fluorescent and non-toxic dye. It gives pink fluorescence when converted into resorufin by the enzyme oxidoreductase present in the viable cells. This resorufin dye is further converted into hydroresorufin by further reduction which is non-colored and non flourescent. For decade, resazurin reduction has been used to detect bacterial and yeast contamination in milk (McNicholl *et al*., 2006).

This method varies because in many cases EOs whether or not is dissolved in emulsifier or water based solvents to stabilize the EO. Certain solvents have been used for this aim: ethanol (Packiyasothy and Kyle, 2002), methanol (Onawunmi, 1989), Tween-20 (Bassole *et al*., 2003), acetone with Tween-80 (Prudent *et al*., 1995), polyethylene glycol (Pintore *et al*., 2002), propylene glycol (Negi *et al*.,1999), n-hexane (Senatore *et al*., 2000), dimethyl sulfoxide (Firouzi

et al., 1998) and agar (Burt and Reinders, 2003). The use of additives was found unnecessary by some researchers (Cimanga *et al.*, 2002).



2.5.3. Cytotoxicity

The cytotoxicity of a drug is necessarily tested by testing its hemolytic activity. In pharmacological applications, the testing assists to determine the antioxidant and other biological activities of a specific drug (Kalaivani *et al.,* 2010). Physical and structural properties of the membrane of erythrocytes are tested in order to measure the cytotoxicity of various components. The mechanical stability of the membrane in this aspect acts as an indicator. *Plasmodium falciparum* a resident parasite along with other microbial organisms cause hemolysis on the membrane of red blood cells in case of infectious diseases (Mohan *et al.*, 1992). The erythrocytic membrane leads to the significant variations in its interaction; best instanced with detergents (Aki and Yamamoto, 1991) and well characterized drug-induced hemolysis due to its dynamic structure. The distinct actions of chloroquine, primaquine and quinine (antimalarial agents) on red blood cell membrane *in vitro* are determined based on the principle that membrane proteins have different electrophoretic patterns (Kotsifopoulos, 1975).

Erythrocytes of human, bovine and chicken when compared in the presence of essential oils, the highest sensitivity shown by bovine erythrocytes was at 158.3 µg/mL and it was 156.2 µg/mL for human erythrocytes (highest for both) and MCnH 583.7 µg/mL for chicken (showing less sensitivity) without causing hemolysis (Silva *et al.,* 2008). When hemolytic assay was applied, six different grasses were evaluated for their cytotoxicity, in which the extract of *Cymodocea rotundata* exhibited the haemolytic activity with 5.26 ± 1.63% at 1000 µg/mL

concentration (maximum activity) and *H. pinifolia* extract showed the haemolytic activity with 2.07 ± 0.63% at 1000 µg/mL concentration (minimum activity). The increase in hemolytic activity was reported as the concentration of the sample was increased (Kannan *et al*., 2013). The cytotoxic activity of essential oils of *Eugenia uniflora* at the highest concentration of 400μg/mL was 63.22% for the erythrocytes of O^+ human blood type. At a reduced concentration of 50μg/mL, it was found non toxic (Rodrigues *et al.* 2013).

Chapter 3

MATERIALS & METHODS

The research work presented in this dissertation was conducted in the Central Hi-Tech Lab, University of Agriculture, Faisalabad; Bioassay Section, Medicinal and Molecular Biology Lab (MMBL), Department of Biochemistry**,** University of Agriculture, Faisalabad, Pakistan and Lab of Medicinal and Aromatic Plants, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA

3.1. Materials:

3.1.1. Reagents:

Linoleic acid, 2, 2,-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite, aluminum chloride, ammonium thiocyanate, ferrous chloride, ferric chloride, potassium fericyanate, butylated hydroxytoluene BHT (99.0 %), Dimethylsulfoxide (DMSO), rifampicin, homologous series of C_9-C_{28} *n*-alkanes and various reference chemicals (α-pinene, camphene, β- pinene, β-myrcene, α-phellandrene, limonene, *p*-cymene, β-Ocimene, δ-terpinene, 1,8- cineol, γ-terpinene, linalool, menthone, borneol, menthol, terpinene-4-ol, α-terpineol, estragole, dihydrocarveol, dihydrocarvone, pulegone, carvone, pipretone, thymol, fenchone, fenchyl alcohol, fenchyl acetate, anethole, piperitenone oxide, *p*-anisaldehyde α-copaene, β-bourbonene, β-elemene, β-caryophyllene, β-cubebene, α-bergamotene, α- caryophyllene, γ-muurolene, germacrene D, γ-cadinene and caryophyllene oxide etc.) used to identify the constituents were obtained from Sigma Chemical Co. (St Louis, MO, USA). Sterile resazurin tablets were obtained from Sigma chemical Co. All other chemicals (analytical grade) i.e. anhydrous sodium sulphate, ferrous chloride, ammonium thiocyanate, chloroform and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise. All culture media and standard antibiotic discs were purchased from Oxoid Ltd., (Hampshire, UK).

3.1.2. Instruments

The instruments used for different analyses during the study along with their company identification are listed in Table 3.1.

Table 3.1. Instruments used with their model and company

Instrument	Manufacturing company
GC/MS	HP (5890) series II equipped with mass spectrophotometer, MSD 5972 system
GC	HP (5890)-series II with Flame ionization detectors (FID)
Double beam spectrophotometer	Lambda 25, Perkin Elmer, USA
Laminar air flow	Memmert, Germany
Analytical balance	AUY 220, SHIMADZU, Japan
Water Bath	Memmert, Germany
Orbital shaker	Yellowline, OS10 Basic
Magnet Stirrer	Yellowline, IKA, USA
Autoclave	JICA, Japan
Centrifuge	Eppendrof, 5804, Hamburg, Germany
Refractometer	R 3261, ATAGO Digital refractometer
Sonicator	Transsonic T 460/H Elma, Germany
Vortex mixer	Heidolph Reax Top D-91, Schwabach
ELISA microplate reader	Bio-Tek-USA
Incubator	Memmert, Germany
pH meter	WTW Inolab multi, 720, Germany
Commercial blender	(BL-335) Kenwood

3.1.3. Collection of Plant Material & Identification

Plant material was collected from different parts of Pothohar plateau including leaves, flowers fruits, buds etc. Cultivated species were collected from Kamal Laboratories present on Rawalpindi-Chakwal road. Whereas, the leaves/aerial parts of wild species were collected from wild populations in the periphery of Islamabad, Rawalpindi, Chakwal and Gojar khan region. Collections were made in the months of March-April. The plant materials were further identified

and authenticated by a Taxonomist, Dr. Mansoor Hameed, Associate Professor, Department of Botany, University of Agriculture, Faisalabad, Pakistan.

List of Plants studied in present research

- *Parthenium hysterophorus* (Gajar Ghass)
- *Eucalyptus camaldulensis*
- *Eucalyptus citriodora*
- *Eucalyptus tereticornis*
- *Citrus reticulata* (Kinnow)
- *Syzygium aromaticum* (clove, long)
- *Myristica fragrans (N)* (Nutmeg, Jaifal)
- *Myristica fragrans (M)* (Mace, Jawatri)
- *Murraya koenigii* (Curry leaves, curry patta)
- *Piper nigrum* (Black pepper, Syah mirch)
- *Elettaria cardamomum* (Green cardamom, Choti Ilaichi)
- *Amomum subulatum* (Black cardamom, Bari ilaichi)
- *Trachyspermum ammi* (Ajowain)
- *Foeniculum vulgare* (fennel, Saunf)
- *Cuminum cyminum* (Cumin, Zeera)
- *Cinnamomum zeylanicum* (Cinnamon, Dar Chini)

3.1.4. Strains of microorganisms utilized to access the antimicrobial activity of essential oils

Bacterial Strains

- *Bacillus subtilis* (Gram positive)
- *Lactobacillus rhamnosus* (Gram positive)
- *Staphylococcus aureus* (Gram positive)
- *Streptococcus mutans* (Gram positive)
- *Escherichia coli* (Gram negative)
- *Pasteurella multocida* (Gram negative)

Fungal Strains

- *Alternaria alternata*

- *Aspergillus niger*
- *Aspergillus flavus*
- *Ganoderma lucidum*

The pure cultures of bacterial and fungal strains were obtained from Bioassay Section, Medicinal and Molecular Biology Lab (MMBL), Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan, except two of bacterial strains including *Lactobacillus rhamnosus and Streptococcus mutans* which were obtained from Medicinal and Aromatic plants laboratory, Stockbridge School of Agriculture, University of Massachusetts, USA. These bacterial and fungal strains were used to evaluate the antimicrobial activity of selected plant's essential oils.

3.2. Experimental Protocol

3.2.1. Isolation of Essential Oils

The shade-dried and finely ground plant materials were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4 °C until analyzed.

3.2.2. Analysis of Essential Oil

3.2.2.1. Physical analyses

The refractive index (30°C) and density (30°C) of essential oils were determined following standard methods (Guenther, 1960). A digital refractometer R 3261, ATAGO Digital refractometer, was used for the determination of refractive index of the essential oils tested. Other physical parameters which were noted include color, specific gravity and percent yield.

3.2.2.2 Chromatographic analysis

3.2.2.3. Gas chromatography analysis

The gas chromatograph, HP 5890-series II equipped with Flame ionization detectors (FID), was used for the analysis of the volatile oils using a Phenomenex ZB-5MS column (30 m length x 0.25 mm ID x 0.25 μm film thickness). Nitrogen was used as carrier gas (0.7 mL/min). The programming of oven temperature was 1 min isothermal at 40°C, then 40–240°C @ 8°C/min and held isothermal for 2 min, then 240-300°C @ 10°C/min. The temperature for injection port was 250°C while for detector it was 275°C. Volume injected was 1μL of 1% solution (diluted in hexane). The quantitative analysis of the components in the form of percentages was done by electronic integration of FID peak areas.

3.2.2.4. Gas chromatography/mass spectrometry (GC-MS) analysis

The gas chromatograph, HP 5890-series II equipped with mass spectrophotometer, MSD 5972 system, was used for the analyses of the volatile samples. Phenomenex ZB-5MS column (30 m length x 0.25 mm ID x 0.25 µm film thickness) was directly coupled to the MS. Helium was used as carrier gas, with a flow rate of 0.7mL/min. The programming of oven temperature was as follows; 1 min isothermal at 40°C, then 40–240°C @ 8°C/min and held isothermal for 2 min, then 240-300°C @ 10°C/min. The injection port temperature was 250°C, detector 275°C. Volume injected was 1µL of 1% solution (diluted in hexane); HP 5972 recording at 70 eV; mass range 50–550 amu. Software used to handle mass spectra and chromatograms was Chem Station.

3.2.2.5. Compounds identification

The components of the essential oils were identified by comparison of their mass spectra with those in NIST 98 NIST/EPA/NIH mass spectral library, as well as by comparison of their retention indices with literature data (Adams, 1995; Sibanda *et al.*, 2004). Retention indices of the components were determined relative to the retention times of a series of n-alkanes (relative to C_9– C_{28} on the same column).

3.3. Biological Activities of Essential oils

3.3.1. Evaluation of antioxidant activity of essential oils

Following antioxidant assays were employed for the assessment of antioxidant activity of essential oils.

3.3.1.1. DPPH radical scavenging assay

The ability of essential oils to scavenge 2, 2-diphenyl-1-picrylhydrazyl stable radical was used as a test to check their antioxidant activity (Hussain, *et al.*, 2008) with slight modifications. The essential oils were diluted in methanol to get different concentrations from which 2 mL were added to equal quantity of a 90 µM methanolic solution of DPPH. The mixture was shaken firmly and left in dark at room temperature, standing. The absorbance was measured after 30 min at 515 nm using double beam spectrophotometer (Lambda 25, Perkin Elmer, USA) and percent (%) scavenging of free radicals by DPPH in percent (%) was calculated as follows:

$$Scavenging\ (\%) = 100 \times \left(A_c \times \frac{A_{sam}}{A_c}\right)$$

Where A_c and A_{sample} are the absorbances of control and test compound respectively. The antioxidant activity was expressed as IC_{50} (µg/mL), which is defined as the dose which creates 50% scavenging of free radical. Lesser the IC_{50} value, higher the potential of that essential oil to show antioxidant activity (Hajlaoui *et al.*, 2010).

3.3.1.2. Percent inhibition in linoleic acid system

Inhibition of linoleic oxidation system was also used to test the antioxidant activity of essential oils (Iqbal and Bhanger, 2005) with modifications. The test samples (50, 30 and 10 µL) were dissolved to a 1 mL of ethanol, linoleic acid (2.5% v/v), 4mL of 99.5% ethanol and 4 mL of 0.05 M sodium phosphate buffer having pH=7. The entire composition was incubated for 168 hrs at 40°C and oxidation was measured by peroxide value using thiocyanate method (Yen *et al.*, 2000). In 10 mL of 75% ethanol, 0.2 mL of 30% aqueous solution of ammonium thiocyanate, 0.2 mL of sample solution and 0.2 mL of (20 mM in 3.5% HCl) ferrous chloride ($FeCl_2$) solution were added, sequentially. Absorption was calculated at 500nm as peroxide contents after 3 min of stirring, using spectrophotometer (Lambda 25, Perkin Elmer, USA). Linoleic acid without essential oil was taken as control. Butylated hydroxytoluene (BHT) was taken as positive control. Percent (%) Inhibition of linoleic acid oxidation was calculated as follows:

$$Inhibition\ (\%) of\ linoleic\ acid\ oxidation = 100 - \left[\frac{Abs.Increase\ of\ sample\ at\ 168h}{Abs.increase\ of\ control\ at\ 168h} \times 100\right]$$

3.3.1.3. β-Carotene / linoleic acid bleaching assay

Antioxidant potential of the essential oils was assessed by bleaching of β-carotene/ linoleic acid emulsion system (Cao *et al.*, 2009) with slight modification. β-carotene and linoleic acid mixture was prepared by adding 1 mg β-carotene in 1 mL chloroform (HPLC grade). In a boiling flask containing 20 mg of linoleic acid, 0.2 mL of carotene-chloroform solution was added to 200 mg of tween 40. Chloroform was evaporated at 40°C for 5 min and 50 mL distilled water was added with vigorous agitation to form an emulsion. The different concentrations of essential oil samples were prepared in methanol. BHT concentration was prepared as positive control. Then, 0.2 mL of these solutions were added individually to 5 mL of the above emulsion in test tubes and mixed gently. The absorbance was taken immediately at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed at 50°C in water bath and

oxidation of the emulsion was checked spectrophotometrically for every 30 min until 180 min, at 470 nm. Control samples contained 0.2 mL of methanol instead. The evaluation of antioxidant activity (%) of the essential oils in terms of the bleaching of the β-carotene was performed according to following equation:

$$Inhibition\ \% = \frac{(A_t - C_t)}{(C_o - C_t)} \times 100$$

where At and Ct are the absorbance values of the test sample and control, respectively, after a certain time (t) of incubation and Co is the absorbance value for the control, measured at the beginning of the experiments. All tests were carried out in triplicate.

3.3.2. Evaluation of antimicrobial activities of essential oils

The essential oils were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA) while the fungal strains cultured overnight at 30 ºC using potato dextrose agar (PDA). Following antimicrobial assays were employed for the determination of antimicrobial potential of essential oils.

3.3.2.1. **Disc Diffusion Assay**

Antibacterial and antifungal activities of essential oils were evaluated by using selected fungal and bacterial microbial strains using microbiological assay of disc diffusion assay by following the methodology of National Committee for Clinical Laboratory Standards (NCCLS, 2001). 100 µL of tested microorganism suspension, containing 10^8 CFU/mL of bacteria cells and 10^4 CFU/mL spores of fungal strains were spread on NA and PDA medium, respectively. The essential oils (10 µL) were separately applied to filter discs (6 mm in diameter) and placed in the agar medium which had previously been inoculated with the tested microorganisms. Negative control was a disc without samples while Rifampicin (30µg/disc) and Terbinafine (30 µg/disc) applied discs were used as positive control for bacteria and fungi, respectively, to compare sensitivity of strain. The plates were incubated at 37ºC for 24 h for bacteria and at 30ºC for 48 h for fungal strains. By measuring the diameter of the growth inhibition zones (mm) for the test organisms and comparing it with controls, antimicrobial activity was evaluated.

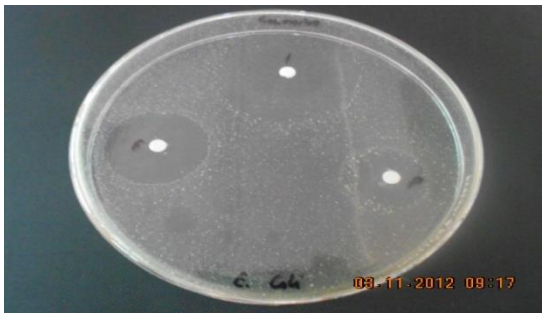


Figure 3.1. A typical agar plate showing the inhibition zones exhibited by essential oils

3.3.2.2. Determination of minimum inhibitory concentration (MIC) by Resazurin microtitre-plate assay

The minimum inhibitory concentration (MIC) was determined by following the methodology of Sarker *et al*. (2007). The medium used for all tests was Nutrient broth (NB) and Sabouraud dextrose broth (SDB) for bacteria and fungi, respectively. 100 µL of 10 mg/mL test sample (using 10% Tween 80 in distilled sterile water) was taken in the first column of plate. To all other wells 50 µL of broth medium was added. Dilutions were performed serially, following the addition of 30 µL of 3.3× strength broth in each well. Finally, 10 µL of microbial suspension (5×10^6 cfu/mL) was added. Plates were wrapped into para film and incubated at 37°C for 24 h for bacteria, and at 30°C for 48 h for fungi.

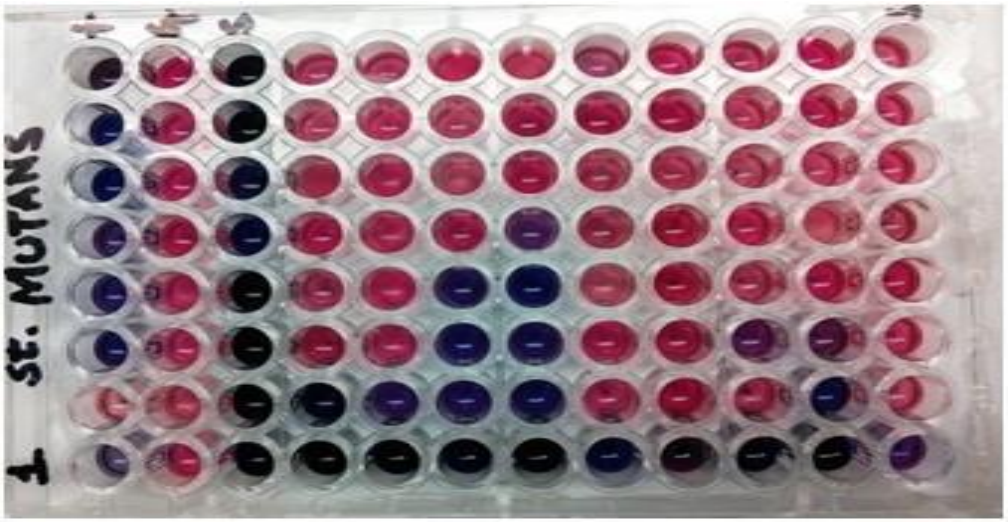


Figure 3.2. A typical plate in resazurin microtitre-plate assay showing the color change due to antibacterial effect of essential oils

Each plate had a set of controls: a row with broad spectrum antibiotic (Rifampicin for bacteria and Terbinafine for fungi) as positive control, a row with all solutions except test compound and a row with all solutions except microbe. After incubation, the absorbance of plates was measured using an automatic ELISA micro plate reader (Bio-Tek-USA) adjusted to 620 nm. These absorbance values were used to detect an increase or decrease in microbial growth. The values were plotted against control and the lowest concentration of sample causing 50% inhibition in microbial growth was recorded as MIC.

3.4. Essential oils as food preservatives:

3.4.1. Stabilization of Edible Oil Samples

The essential oils were added to RBD canola (70%) and Rapeseed (30%) oil blend. The mixtures were stirred for 30 min. at 40 °C for uniform dispersion. Control samples (without the addition of any antioxidant) were also prepared under the same conditions.

3.4.2. Ambient Storage Test and Measurement of Oxidation

Three replicates of edible oil treatments along with controls were carried out. In an ambient storage test the samples were stored for three months at 40 °C temperature. Analysis was done periodically after every 15 days. The oxidative deterioration level was followed by the measurement of peroxide value (PV), % FFA and *p*- anisidine value (Chatha *et al*., 2011).

3.4.3. % FFA

The %FFA was determined according to the IUPAC standard method (IUPAC, 1987). 25mL of spirit was taken in a flask and heated slightly. Few drops of phenolphthalein were added and titrated against 0.1N NaOH until it became light pink. This flask was put on weighing balance and reading was made auto zero. 4-5 g of sample as added and reading was noted on balance. Mixture was shake well and was titrated against 0.1N NaOH and noted the volume used. % FFA was calculated as

$$\% \text{ FFA} = \frac{Vol\ Used \times Normality\ (0.1) \times Eq.weight\ (282) \times 100}{Weight\ of\ Sample \times 1000}$$

3.4.4. Peroxide value (PV)

The determination of PV was made according to the IUPAC standard method (IUPAC, 1987). 2-5 g of sample was taken in iodine flask, to which 30 mL mixture of acetic acid and chloroform (3:2) was added. To this solution 1 mL of saturated solution of KI was added and

was covered with lid for 1 minute. Then 30 mL distilled water was added together with few drops of starch solution and was titrated against 0.01N sodium thiosulphate. Also reading of blank (without sample) was taken.

$$PV = \frac{Vol\ Used\ (Sample) \times N \times 1000}{Weight\ of\ Sample}$$

3.4.5. *Para*-Anisidine Value

The *p*-anisidine value was calculated according to the IUPAC standard method (IUPAC, 1987). In 2g sample, 25 mL iso-octane was added and absorbance wad taken at 350nm. 5mL was taken from this solution to which there was added 1mL of 0.25% para-Anisidine in acetic acid (w/v) and absorbance was taken at 350nm after 10 min.

$$P - Anisidine\ Value = \frac{25 \times (1.2A_{sam} \times \mathrm{A_{blnk}})}{m}$$

A_{sam} = absorbance after reaction with p-anisidine

A_{blnk} = absorbance of blank

m = mass of oil sample

3.5. Cytotoxicity:

3.5.1. *In vitro* Hemolytic assay

The hemolytic activity was investigated using the method given by Sharma and Sharma, 2001. 5 mL of 2% (B^-) RBC suspension was taken (Silva *et al*., 2008) to which was added 1 mL of essential oil at three different concentrations 0.5 mg/mL, 5 mg/mL and 50 mg/mL in DMSO. The mixture was kept in an incubator at 37°C for 30 minute. After that tubes were centrifuged at 2500 rpm for 15 minute in centrifuge (eppendrof 5804), to allow broken membranes and unbroken cells to settle at the bottom. For 100% hemolysis or positive control, 1 mL of 0.2% Triton X-100 (in PBS) was added to 5 mL of 10% RBC suspension. The supernatant was removed and the liberated hemoglobin in the supernatant was measured spectrophotometrically as absorbance (ABS) at 541 nm in a double-beam Spectrophotometer (Lambda 25, Perkin Elmer, USA). The experiment was done in triplicate and mean±S.D. was calculated.

$$\%\ Hemolysis = \frac{Hb_{Abs}}{Hb_{100\%\ Abs}} \times 100$$

CHAPTER 4

RESULTS & DISCUSSION

4.1. Parthenium hysterophorus

Table 4.1.1

Physical properties of *Parthenium hysterophorus* essential oil

Parameter	***Parthenium hysterophorus***
Colour	Orange
Yield (%)	0.02±0.001
Refractive Index	1.335±0.004
Specific Gravity	0.64±0.02

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

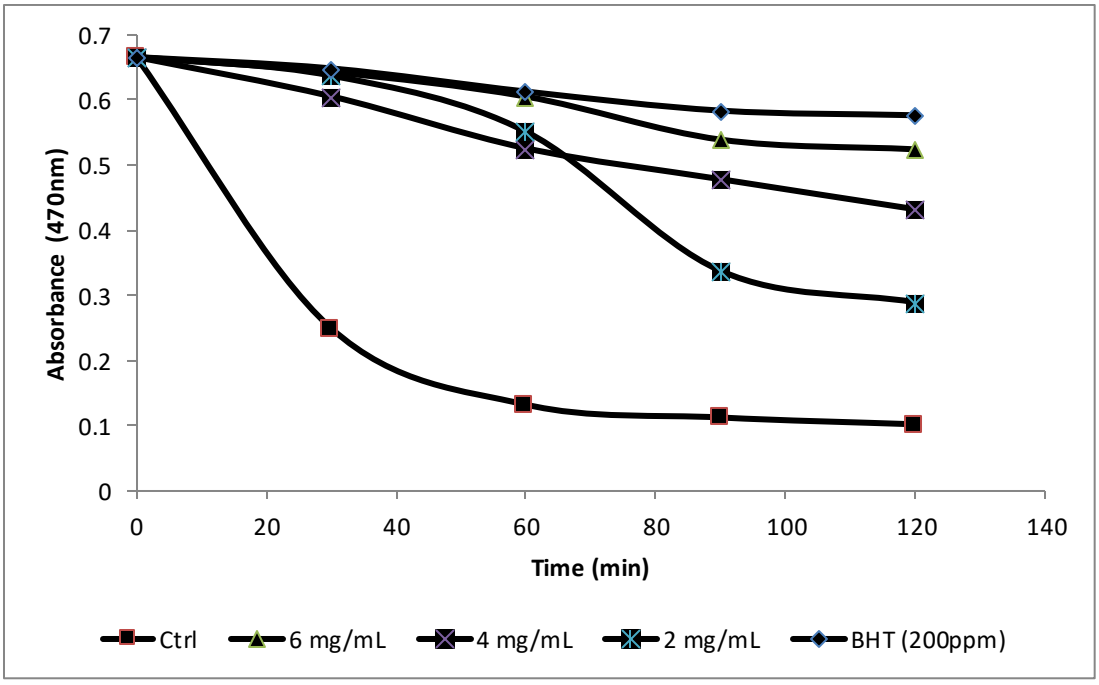
Table 4.1.2

Antioxidant activity of *Parthenium hysterophorus* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*P. hysterophorus*			BHT
IC_{50} (µg/mL)	21.95±1.9			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	81.80±2.7	65.23±2.1	48.03±1.4	85.10±2.8

Values are mean ± standard deviation of *Parthenium hysterophorus*, analyzed individually in triplicate.

Fig. 4.1.1



Antioxidant activity of *Parthenium hysterophorus* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.1.3

Antimicrobial activity of *Parthenium hysterophorus* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Parthenium hysterophorus		
Inhibition zone (mm)			
B. subtilis	10.2±0.5	10.8±2.1	-
L. rhamnosus	**20.9±0.3**	**15.5±0.8**	-
S. aureus	9.4±0.4	15.1±1.0	-
S. mutans	6.4±0.2	13.4±0.7	-
E. coli	9.2±0.4	9.6±1.3	-
P. multocida	9.7±0.5	11.6±1.5	-
A. alternata	14.6±0.5	-	14.2±0.8
A. flavus	**12.3±0.5**	-	**11.4±1.4**
A. niger	20.7±2.2	-	21.7±1.2

G. lucidum	**11.4±0.2**	**-**	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	2.5±0.3	1.72±0.2	-
L. rhamnosus	**1.17±0.1**	**2.81±0.3**	-
S. aureus	2.81±0.2	2.19±0.2	-
S. mutans	4.37±0.3	2.03±0.1	-
E. coli	3.12±0.2	2.34±0.2	-
P. multocida	2.5±0.2	2.03±0.1	-
A. alternata	2.03±0.2	-	2.34±0.2
A. flavus	**2.19±0.1**	**-**	**2.81±0.2**
A. niger	1.25±0.1	-	0.94±0.1
G. lucidum	**2.81±0.2**	**-**	**1.88±0.1**

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.1.4

FFA of edible oil stabilized by *Parthenium hysterophorus* essential oil

IPD Induction Period in days	% FFA			
	P. hysterophorus ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.1±0.011	0.08±0.01	0.08±0.01	0.16±0.014
30	0.13±0.014	0.1±0.02	0.09±0.015	0.22±0.016
45	0.18±0.02	0.13±0.02	0.11±0.012	0.34±0.03
60	0.25±0.021	0.16±0.016	0.14±0.014	0.48±0.02
75	0.29±0.024	0.2±0.03	0.18±0.018	0.57±0.03
90	0.33±0.027	0.24±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

Table 4.1.5 PV of edible oil stabilized by *Parthenium hysterophorus* essential oil

IPD Induction Period in days	PV meq/kg			
	P. hysterophorus ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.6±0.011	0.4±0.01	0.4±0.01	1.0±0.03
30	1.2±0.02	0.7±0.03	0.6±0.16	1.81±0.12
45	1.6±0.022	1.1±0.02	0.9±0.21	2.5±0.24
60	2.2±0.11	1.7±0.16	1.4±0.29	3.4±0.36
75	3.0±0.16	2.3±0.21	1.9±0.24	4.7±0.21
90	3.4±0.21	2.7±0.24	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

Table 4.1.6

***para*-Anisidine Value of edible oil stabilized by *Parthenium hysterophorus* essential oil**

IPD Induction Period in days	*para*-Anisidine Value			
	P. hysterophorus ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	4.7±0.18	4.3±0.27	3.4±0.27	10.2±0.52
30	8.3±0.23	6.9±0.36	5.8±0.36	17.5±0.74
45	11.4±0.29	9.5±0.27	8.5±0.27	24.1±0.81
60	15.6±0.51	13.1±0.31	10.9±0.55	29.6±0.63
75	21.4±0.83	17.4±0.47	13.6±0.47	37.4±1.2
90	25.6±1.2	22.2±0.55	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

Table 4.1.7

Cytotoxicity (% hemolysis) of *Parthenium hysterophorus* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.52%	0.73%
5mg/mL	1.46%	2.31%
10mg/mL	3.26%	4.42%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Parthenium hysterophorus*, analyzed individually in triplicate.

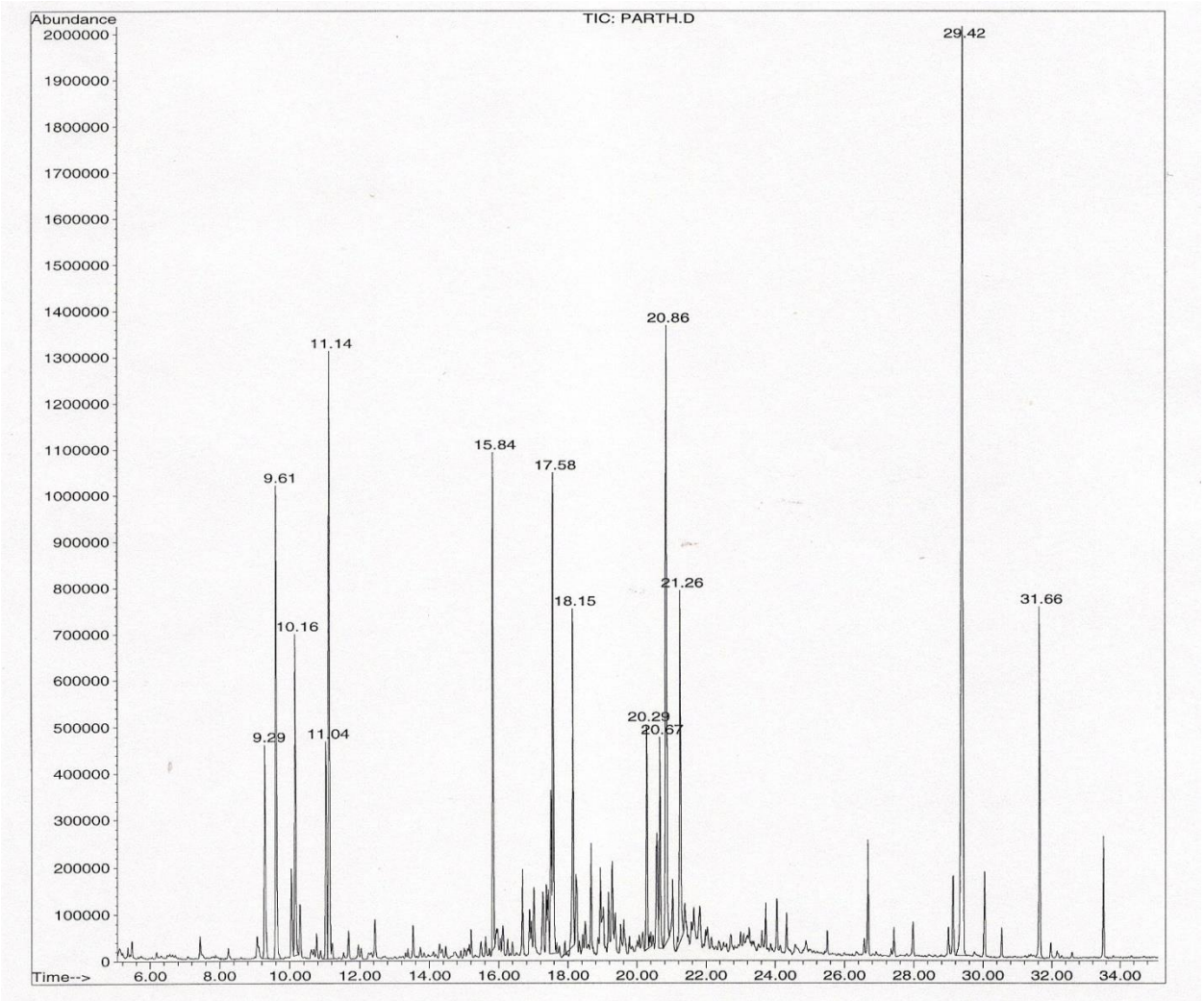


Figure 4.1.2. Typical GC-MS chromatogram of essential oil from *Parthenium hysterophorus*

Table 4.1.8. Chemical composition of *Parthenium hysterophorus* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
2,3-Dimethyl-1,3-butadiene	672	0.21	RI, MS
Methacrylic acid	711	0.25	RT, RI, MS
Octane, 2-methyl-	865	0.77	RI, MS
α-Thujene	926	0.48	RT, RI, MS
α-Fenchene	**952**	**7.14**	**RT, RI, MS**
Camphene	955	0.29	RT, RI, MS
Acetic acid, 2-ethylbutyl ester	957	0.39	RI, MS
Benzaldehyde, 4-methyl-	960	0.17	RT, RI, MS
1,9-Decadiene	965	0.20	RI, MS
Sabinene	972	0.36	RT, RI, MS
β-Pinene	**978**	**3.73**	**RT, RI, MS**
beta Myrcene	994	0.42	RT, RI, MS
Decane	999	0.49	RT, RI, MS
α – phellandrene	1005	0.56	RI, MS
cis-β-Ocimene	1044	0.46	RT, RI, MS
γ-Terpinen	1072	0.55	RT, RI, MS
m-Cymene	1081	2.72	RT, RI, MS
α-Terpinolene	**1089**	**5.96**	**RT, RI, MS**
Bicyclo[7.1.0]decane	1110	0.18	RI, MS
l-Menthone	1126	0.28	RT, RI, MS
1-Acetyl-2-methylcyclopentene	1137	0.23	RT, RI, MS
5-hydroxyheptanoic acid lactone	1145	0.32	RI, MS
p-Cymen-8-ol	1184	0.32	RT, RI, MS
Verbenone	1205	2.81	RI, MS
Cuminal	1240	0.34	RT, RI, MS
Methyl (Z)-3-hexenoate	1254	0.31	RI, MS
Bornyl acetate	**1285**	**5.16**	**RT, RI, MS**
3-Oxolene	1302	0.25	RI, MS
Piperitone	1342	3.11	RT, RI, MS
α-cubebene	1347	0.28	RT, RI, MS
Copaene	1366	0.18	RT, RI, MS
Patchoulane	1378	0.29	RT, RI, MS
Isocaryophyllene	1413	3.69	RT, RI, MS
Dihydro-β-ionone	1433	0.26	RI, MS
trans-α-Farnesene	1509	0.37	RT, RI, MS
7-epi-α-Cadinene	1522	0.32	RI, MS
Spathulenol	1574	0.33	RT, RI, MS

α-Limonene diepoxide	1724	0.24	RT, MS
Docosane	2200	0.41	RT, RI, MS
11-Tricosene	2287	0.58	RT, RI, MS
Tricosane	**2300**	**17.24**	**RT, RI, MS**
Heptacosane	2700	0.89	RT, RI, MS
Octacosane	2800	3.84	RI, MS
Total (43)		67.43	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.1.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Parthenium hysterophorus* is listed in Table 4.1.1. Yield was found to be 0.02% which was the minimum yield as compared to other plants taken in the present research work. The % yield shows that the plant is not enriched with essential oil which could be the reason that there is no reported work on essential oil of this plant. The color of oil was orange with strong aromatic smell and refractive index was 1.335. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.64.

The essential oil of *Parthenium hysterophorus* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple color) which reduced to form DPPH-H (yellow color) and this ability of essential oil was investigated. The DPPH radical scavenging by *P. hysterophorus* essential oil is represented in terms of 50% scavenging (IC_{50}) as shown in Table 4.1.2, which was found to be 21.95 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid is also being used to test the antioxidant capacity of essential oils. Due to unsaturation, it forms peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that forms complex with SCN^{-} present in reaction media. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher is the concentration of peroxides, higher will be value of absorbance and ultimately lower the antioxidant activity. Table 4.1.2 shows the % inhibition in linoleic acid system by *P. hysterophorus* essential oil taken at three different concentrations. It is clear that maximum inhibition was 81.80% at concentration 50µL/mL which decreased with the decrease in concentration. This decrease in inhibition could be attributed to decrease in concentration of bioactive compound with the decrease in concentration. The activity shown by essential oil was found to be much closer to that of synthetic antioxidant BHT (85.10% inhibition). β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction is inhibited if there is present some antioxidant in the reaction media that react with peroxyl radical

competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *P. hysterophorus* is shown in Figure 4.1.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there is less depletion of colour. *P. hysterophorus* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Pandey *et al.* (2012) studied the scavenging activity of ethanolic extract of *P. hysterophorus* leaves collected from Meerut (India) and calculated IC_{50} value as 52.02 µg/mL.

The antimicrobial activity of the essential oil of *P. hysterophorus* against various microbes including bacteria and fungi is shown in Table 4.1.3. It is clear that essential oil showed good activity against most of the fungi and some of the bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *P. hysterophorus* showed potential as an antimicrobial agent with larger inhibition zones (6.4-20.9 and 11.4-20.7 mm) and smaller MIC values (1.17-4.37 and 1.25-2.81 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was against *L. rhamnosus* (gram positive) with largest inhibition zone 20.9mm followed by minimum MIC value 1.17 mg/mL. These results were found much better than that of antibiotic reference standard, Rifampicin, which showed the inhibition zone 15.5mm and MIC 2.81mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 20.7mm followed by minimum MIC value 1.25 mg/mL which was comparable to that of antibiotic, Terbinafine, with inhibition zone 21.7mm and MIC 0.94mg/mL. Kumar *et al.* (2013) tested ethanolic extract of *P. hysterophorus* against various bacteria and fungi and found maximum inhibition zone against *Saccharomyces cerevisiae* with inhibition zone (15 mm) while minimum in case of *Candida albicans* (7 mm). Zaheer *et al.* (2012) also reported inhibitory potential of *P. hysterophorus* L. extracts against *Fusarium solani*.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for industrialists. Table 4.1.4 shows the effect of *P. hysterophorus* essential oil on formation of

FFAs from where it is clear that their concentration continuously increased with the increase in storage period. Highest FFA was exhibited by control, while maximum stabilization was experienced in case of *P. hysterophorus* at 1000ppm which was 0.24% even after induction period of 90 days, while with control and BHT it was 0.64% and 0.22% respectively. Peroxide value (PV) is presented in table 4.1.5 and it shows that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 2.7meg/kg after induction period of 90 days, which was 2.3 and 6.1meg/kg for BHT and control, respectively. *p*-anisidine value is an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.1.6 for *P. hysterophorus*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *P. hysterophorus* essential oil sample of 1000ppm with value 22.2 while in case of BHT it was found to be 17.9.

The effect of *P. hysterophorus* essential oil on the viability of human and bovine erythrocytes was evaluated to inspect the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis was calculated which is presented in Table 4.1.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *P. hysterophorus* exhibited the maximum hemolytic activity with 44.26% at 50 mg/mL concentration and the minimum hemolytic activity of 7.32% at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolytic activity with 32.63% at 50 mg/mL concentration and the minimum hemolytic activity of 5.20% at 0.5 mg/mL. Gupta *et al.* (2013) studied heat induced hemolysis effects of ethanolic and aqueous extracts of *P. hysterophorus* and found that maximum inhibition in hemolysis was 78.82 and 76.65 % for both ethanolic and aqueous extracts, respectively.

The data of chemical composition of the essential oil from *P. hysterophorus* is listed in Table 4.1.8. Among a lot of unidentified compounds, 43 compounds were identified representing 68.67% of the oil. The major constituents were determined to be tricosane (17.24%), α-fenchene (7.14%), α-terpinolene (5.96%), bornyl acetate (5.16%) and β-Pinene (3.73%). To our best knowledge there is no early work reported on chemical composition of essential oil of *P. hysterophorus*. Although there is some work reported on biological activities of different parts of extracts but no work is reported on essential oils.

4.2. *Eucalyptus camaldulensis*

Table 4.2.1

Physical properties of *Eucalyptus camaldulensis* essential oil

Parameter	***Eucalyptus camaldulensis***
Colour	Colourless
Yield (%)	1.68±0.02
Refractive Index	1.479±0.006
Specific Gravity	0.84±0.03

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

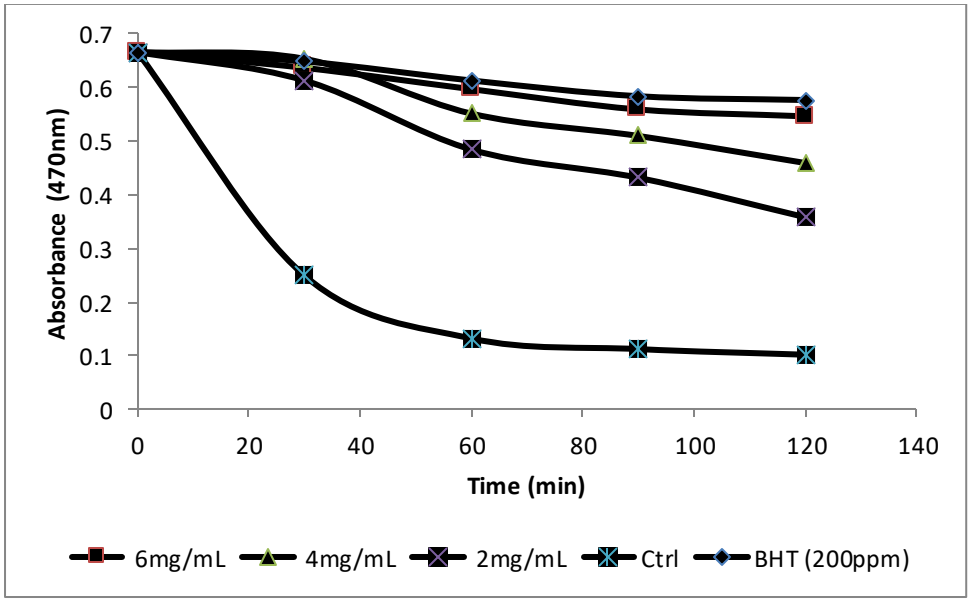
Table 4.2.2

Antioxidant activity of *Eucalyptus camaldulensis* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*E. camaldulensis*			BHT
IC_{50} (µg/mL)	28.78±1.62			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	76.06±2.4	55.73±1.8	38.71±1.2	85.10±2.8

Values are mean ± standard deviation of *Eucalyptus camaldulensis*, analyzed individually in triplicate.

Fig. 4.2.1



Antioxidant activity of *Eucalyptus camaldulensis* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.2.3

Antimicrobial activity of *Eucalyptus camaldulensis* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Eucalyptus camaldulensis		
Inhibition zone (mm)			
B. subtilis	7.3±0.2	10.8±2.1	-
L. rhamnosus	**16.2±0.2**	**15.5±0.8**	-
S. aureus	11.6±0.8	15.1±1.0	-
S. mutans	13.5±0.3	13.4±0.7	-
E. coli	**14.0±0.4**	**9.6±1.3**	-
P. multocida	**24.7±1.01**	**11.6±1.5**	-
A. alternata	7.6±0.4	-	14.2±0.8
A. flavus	**22.7±2.0**	-	**11.4±1.4**
A. niger	**22.4±2.0**	-	**21.7±1.2**

G. lucidum	5.3±0.5	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	2.19±0.3	1.72±0.2	-
L. rhamnosus	**1.72±0.2**	**2.81±0.3**	**-**
S. aureus	2.81±0.1	2.19±0.2	-
S. mutans	2.03± 0.2	2.03±0.1	-
E. coli	**1.25±0.2**	**2.34±0.2**	**-**
P. multocida	**1.17±0.1**	**2.03±0.1**	**-**
A. alternata	2.34±0.2	-	2.34±0.2
A. flavus	**1.17±0.1**	**-**	**2.81±0.2**
A. niger	1.41±0.2	-	0.94±0.1
G. lucidum	2.5±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.2.4 FFA of edible oil stabilized by *Eucalyptus camaldulensis* essential oil

IPD Induction Period in Days	% FFA			
	E. camaldulensis ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.1±0.01	0.09±0.015	0.08±0.01	0.16±0.014
30	0.15±0.011	0.12±0.012	0.09±0.015	0.22±0.016
45	0.23±0.02	0.15±0.014	0.11±0.012	0.34±0.03
60	0.28±0.01	0.19±0.014	0.14±0.014	0.48±0.02
75	0.34±0.02	0.22±0.03	0.18±0.018	0.57±0.03
90	0.38±0.04	0.27±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

Table 4.2.5 PV of edible oil stabilized by *Eucalyptus camaldulensis* essential oil

IPD Induction Period in Days	PV			
	E. camaldulensis ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.9±0.016	0.6±0.03	0.4±0.01	1.0±0.03
30	1.5±0.03	1±0.12	0.6±0.16	1.81±0.12
45	2.2±0.15	1.7±0.21	0.9±0.21	2.5±0.24
60	2.9±0.22	2.2±0.21	1.4±0.29	3.4±0.36
75	3.3±0.31	2.8±0.24	1.9±0.24	4.7±0.21
90	3.9±0.15	3.5±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

Table 4.2.6
***para*-Anisidine Value of edible oil stabilized by *Eucalyptus camaldulensis* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	E. camaldulensis ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	5.4±0.25	4.8±0.31	3.4±0.27	10.2±0.52
30	9.1±0.41	8.3±0.36	5.8±0.36	17.5±0.74
45	12.3±0.33	11.2±0.31	8.5±0.27	24.1±0.81
60	17.5±0.51	16.4±0.36	10.9±0.55	29.6±0.63
75	20.8±0.38	21.6±0.55	13.6±0.47	37.4±1.2
90	24.2±0.46	28.1±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

Table 4.2.7

Cytotoxicity (% hemolysis) of *Eucalyptus camaldulensis* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.27%	0.56%
5mg/mL	1.38%	2.34%
10mg/mL	8.92%	9.53%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Eucalyptus camaldulensis*, analyzed individually in triplicate.

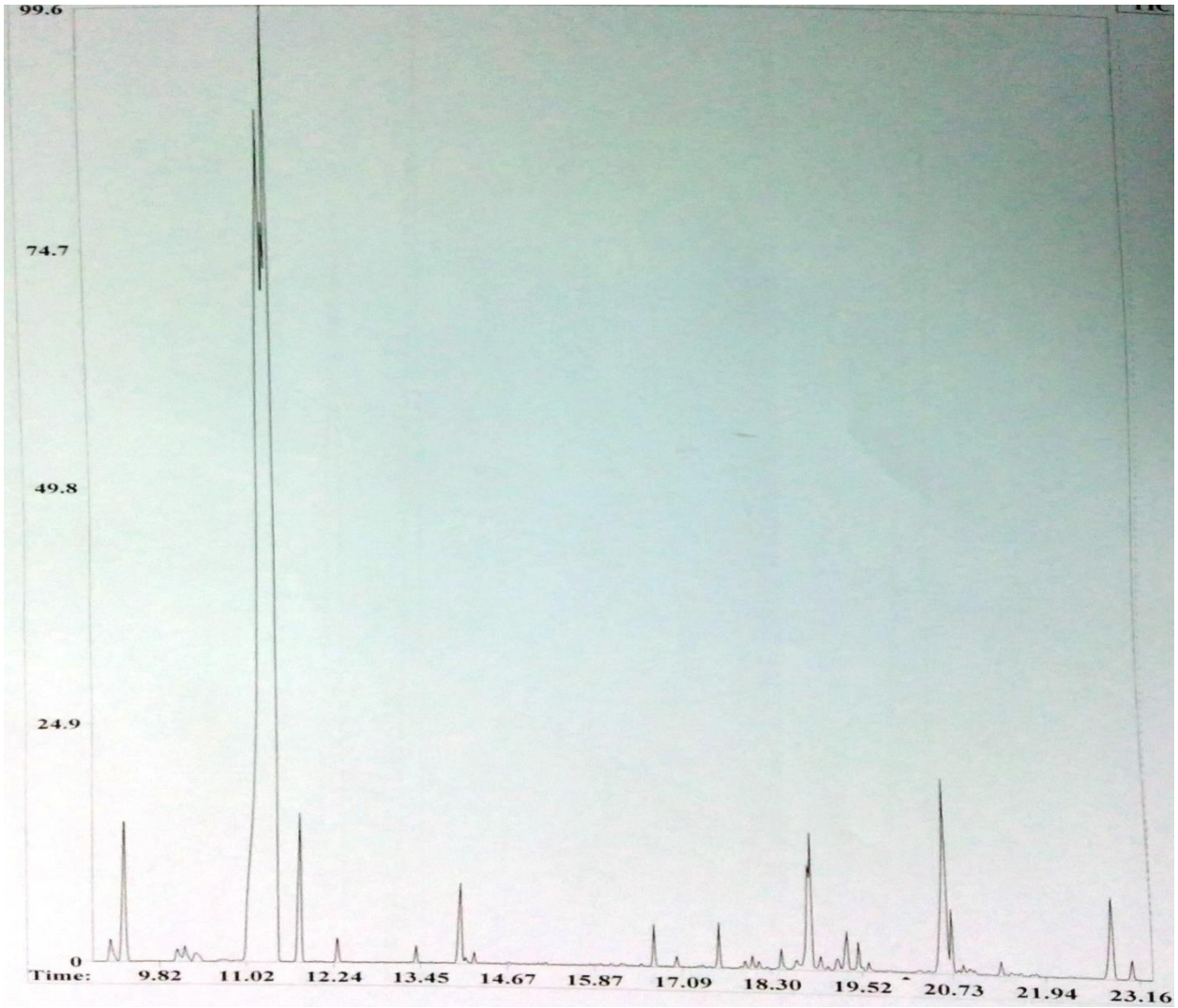


Figure 4.2.2. Typical GC-MS chromatogram of essential oil from *Eucalyptus camaldulensis*

Table 4.2.8. Chemical composition of *Eucalyptus camaldulensis* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
α-Thujene	926	0.38	RT, RI, MS
α-pinene	**934**	**2.23**	**RT, RI, MS**
Camphene	955	0.24	RI, MS
Sabinene	972	0.21	RT, RI, MS
β-Pinene	978	0.71	RT, RI, MS
β-Myrcene	994	0.18	RT, RI, MS
α-Phellandrene	1005	1.05	RI, MS
3-Carene	1011	1.89	RT, RI, MS
p-Cymene	**1026**	**46.12**	**RT, RI, MS**
Limonene	1033	1.36	RT, RI, MS
Eucalyptol	**1037**	**20.1**	**RT, RI, MS**
β-cis-Ocimene	1043	0.93	RI, MS
β-trans Ocimene	1051	0.17	RI, MS
γ-Terpinen	1072	1.97	RT, RI, MS
m-Cymene	1081	0.53	RT, RI, MS
Linalool oxide	1084	0.71	RT, RI, MS
α- Terpinolene	1089	0.27	RT, RI, MS
Linalool	1096	0.19	RT, RI, MS
Myrcenol	1117	0.08	RT, RI, MS
Fenchyl alcohol	1119	0.16	RI, MS
trans-Sabinol	1140	0.84	RT, RI, MS
Pinocarveol	1148	0.73	RT, RI, MS
Nerol oxide	1154	1.26	RT, RI, MS
Borneol	1169	0.43	RT, RI, MS
L-terpinen-4-ol	1178	1.04	RI, MS
p-Cymen-8-ol	1184	0.36	RT, RI, MS
Myrtenol	1196	0.31	RT, RI, MS
Carveol	1199	1.28	RT, RI, MS
Piperitol	1205	0.51	RT, RI, MS
Cis-carveol	1219	1.03	RT, RI, MS
Nerol	1230	0.16	RT, RI, MS
3-p-Menthene	1234	0.24	RI, MS
Cuminal	1240	0.37	RT, RI, MS
Geraniol	1258	1.24	RT, RI, MS
Cumic alcohol	1276	0.22	RT, RI, MS
Thymol	1290	0.73	RT, RI, MS
Terpinyl acetate	1350	0.95	RT, RI, MS
Patchoulane	1378	0.73	RT, RI, MS
Caryophyllene	1454	0.21	RT, RI, MS

Spathulenol	**1574**	**2.54**	**RT, RI, MS**
Globulol	1576	0.58	RT, RI, MS
Caryophyllene oxide	**1578**	**3.55**	**RT, RI, MS**
Guaiol	1594	0.56	RI, MS
α-Eudesmol	1664	0.27	RT, RI, MS
Total (44)		99.34	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.2.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Eucalyptus camaldulensis* was found to be 1.68 % as listed in Table 4.2.1 which shows that the plant is enriched with essential oil. The oil was colourless with strong aromatic smell and refractive index was 1.335. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.84. Ozel *et al.* (2008) collected five samples of *E. camaldulensis* from different places in Turkey and the maximum yield was obtained in case of Adrasan sample, 1.18 %.

The essential oil of *E. camaldulensis* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. The DPPH radical scavenging by *E. camaldulensis* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.2.2, which was found to be 28.78 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Table 4.2.2 shows the % inhibition in linoleic acid system by *E. camaldulensis* essential oil taken at three different concentrations. It is clear that maximum inhibition was 76.06 % at concentration 50 µL/mL which decreased with the decrease in concentration. This decrease in inhibition could be attributed to decrease in concentration of bioactive compounds. The activity shown by essential oil was found to be closer to that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *E. camaldulensis* is shown in Figure 4.2.1. The least antioxidant activity was

exhibited by negative control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *E. camaldulensis* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Our results are comparable to those of Basak and Candan (2010), who found *E. Camaldulensis* to be a potent antioxidant capable of scavenging DPPH free radicals and thus suppressed peroxidation of lipids interceded by free radicals with IC_{50} value 4.096 µL/mL. The phenolic compounds could be responsible for the antioxidant activity of essential oils (Lu and Foo, 2000). Singh and Marimuthu (2006) evaluated that essential oils efficiently inhibited the formation of peroxides in linoleic acid system during incubation. There are very few reports present in the literature showing the response of essential oils in β-carotene assay (Anwar *et al.*, 2009).

The antimicrobial activity of the essential oil of *E. camaldulensis* against various microbes including bacteria and fungi is shown in Table 4.2.3. It is clear that essential oil showed good activity against most of the fungi and some of the bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *E. camaldulensis* showed potential as an antimicrobial agent with larger inhibition zones (7.3-24.7 and 5.3-22.7 mm) and smaller MIC values (1.17-2.81 and 1.17-2.50 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was against *P. multocida* (Gram negative) with largest inhibition zone 24.7 mm followed by minimum MIC value 1.17 mg/mL. These results are much better than that of antibiotic, Rifampicin, which showed the inhibition zone 11.6 mm and MIC 2.03 mg/mL. In case of fungi, *A. flavus* showed to be most sensitive with largest inhibition zone 22.7mm followed by minimum MIC value 1.17 mg/mL which was much better than that of antibiotic, Terbinafine, with inhibition zone 11.4 mm and MIC 2.81 mg/mL. Barra *et al.* (2010) tested *E. camaldulensis* essential oil on different fungi and found *Fusarium oxysporum* to be the most sensitive one.

Table 4.2.4 shows the effect of *E. camaldulensis* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the increase in storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *E. camaldulensis* essential oil at 1000ppm which was 0.24 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.2.5

and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 3.5 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.2.6 for *E. camaldulensis*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *E. camaldulensis* essential oil sample of 1000 ppm with value 28.1 while in case of BHT it was found to be 17.9.

The effect of *E. camaldulensis* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.2.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *E. camaldulensis* exhibited the maximum hemolysis i.e. 9.53 % at 10 mg/mL concentration and the minimum of 0.56 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.92 % at 10 mg/mL concentration and the minimum of 0.27 % at 0.5 mg/mL. Agar *et al.* (1998) found hemolytic effect of monoterpenes and sesquiterpenes obtained from eucalyptus essential oil on human erythrocytes to be 14.2 and 23.2 %, respectively.

The data of chemical composition of the essential oil from *E. camaldulensis* is listed in Table 4.2.8. 44 compounds were identified representing 99.34 % of the oil. The major constituents were found to be p-cymene (46.12 %), eucalyptol (20.10 %), caryophyllene oxide (3.55%), spathulenol (2.54 %) and α-pinene (2.23 %). Our results got very well matched with those of Barra *et al.* (2010) who collected *E. camaldulensis* species from different parts of Sardinia (Italy). From the specie, collected from central west part, the major chemical constituents were found to be; p-cymene (42.7 %), cryptone (10.2 %), eucalyptol (9.8 %) and spathulenol (8.1 %). In another study Basak and Candan (2010) analysed essential oil extracted from leaves of *E. camaldulensis* collected from Adana (Turkey). p-cymene (68.43 %), eucalyptol (13.92 %), α-pinene (3.45 %) and limonene (2.84 %) were found to be the major components. Ozel *et al.* (2008) collected fruits of *E. camaldulensis* from Kuyucak, Izmir (Turkey), which was subjected to essential oil extraction. The major components were found to be eucalyptol (12.61 %), Terpinolene (8.39%), α-pinene (6.81 %) and p-cymene (68.43 %)

4.3. *Eucalyptus citriodora*

Table 4.3.1

Physical properties of *Eucalyptus citriodora* essential oil

Parameter	***Eucalyptus citriodora***
Colour	Colourless
Yield (%)	1.91±0.01
Refractive Index	1.453±0.004
Specific Gravity	0.85±0.02

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

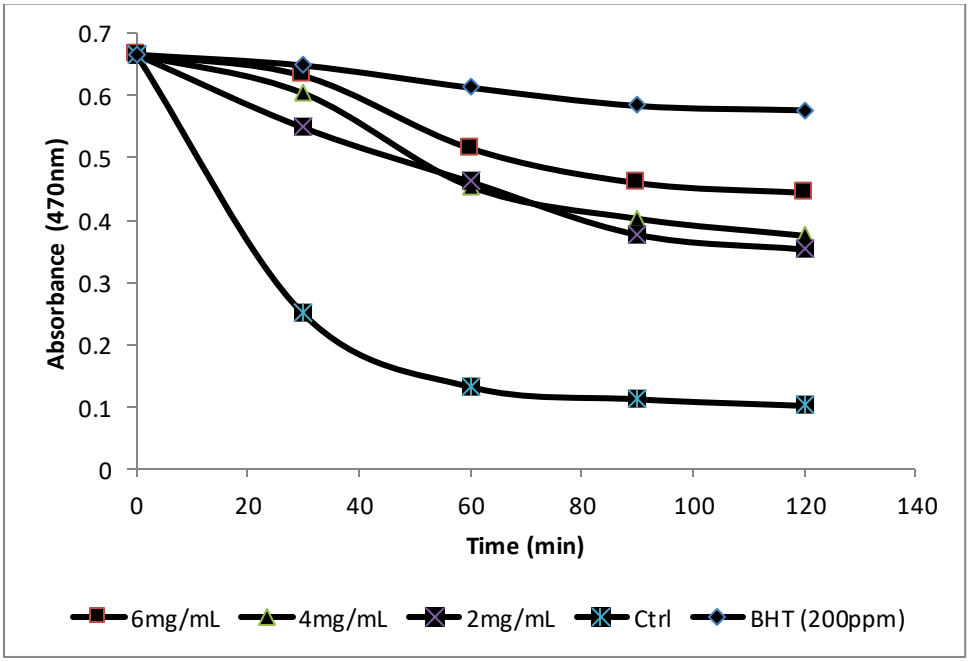
Table 4.3.2

Antioxidant activity of *Eucalyptus citriodora* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*E. citriodora*			BHT
IC_{50} (µg/mL)	15.95±1.10			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	68.63±1.6	58.50±1.5	27.38±1.6	85.10±2.8

Values are mean ± standard deviation of *Eucalyptus citriodora*, analyzed individually in triplicate.

Fig. 4.3.1



Antioxidant activity of *Eucalyptus citriodora* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.3.3

Antimicrobial activity of *Eucalyptus citriodora* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Eucalyptus citriodora		
Inhibition zone (mm)			
B. subtilis	4.9±0.6	10.8±2.1	-
L. rhamnosus	**21.4±0.1**	**15.5±0.8**	-
S. aureus	14.2±1.3	15.1±1.0	-
S. mutans	**15.7±0.6**	**13.4±0.7**	-
E. coli	8.1±0.3	9.6±1.3	-
P. multocida	9.3±1.08	11.6±1.5	-
A. alternata	**16.3±0.6**	-	**14.2±0.8**
A. flavus	**16.6±1.5**	-	**11.4±1.4**

A. niger	**25.3±1.5**	-	**21.7±1.2**
G. lucidum	8.7±0.3	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	4.3±0.5	1.72±0.2	-
L. rhamnosus	**1.17±0.1**	**2.81±0.3**	-
S. aureus	1.25±0.2	2.19±0.2	-
S. mutans	**1.56±0.3**	**2.03±0.1**	-
E. coli	3.12±0.3	2.34±0.2	-
P. multocida	2.19±0.2	2.03±0.1	-
A. alternata	2.81±0.1	-	2.34±0.2
A. flavus	**1.72±0.2**	-	**2.81±0.2**
A. niger	1.09±0.2	-	0.94±0.1
G. lucidum	2.03±0.1	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.3.4 FFA of edible oil stabilized by *Eucalyptus citriodora* essential oil

IPD Induction Period in Days	% FFA			
	E. citriodora ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.010	0.08±0.010	0.08±0.010
15	0.12±0.02	0.09±0.015	0.08±0.010	0.16±0.014
30	0.15±0.01	0.11±0.012	0.09±0.015	0.22±0.016
45	0.22±0.03	0.14±0.016	0.11±0.012	0.34±0.030
60	0.27±0.022	0.19±0.018	0.14±0.014	0.48±0.020
75	0.34±0.03	0.25±0.016	0.18±0.018	0.57±0.030
90	0.39±0.03	0.29±0.020	0.22±0.018	0.64±0.020

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

Table 4.3.5 PV of edible oil stabilized by *Eucalyptus citriodora* essential oil

IPD Induction Period in Days	PV			
	E. citriodora ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.8±0.02	0.6±0.02	0.4±0.01	1.0±0.03
30	1.5±0.11	1.1±0.21	0.6±0.16	1.81±0.12
45	2.1±0.21	1.6±0.24	0.9±0.21	2.5±0.24
60	2.6±0.20	2.1±0.12	1.4±0.29	3.4±0.36
75	3.2±0.23	2.9±0.24	1.9±0.24	4.7±0.21
90	3.8±0.31	3.3±0.24	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

Table 4.3.6

***para*-Anisidine Value of edible oil stabilized by *Eucalyptus citriodora* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	E. citriodora ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	5.3±0.25	4.5±0.36	3.4±0.27	10.2±0.52
30	8.5±0.29	7.4±0.31	5.8±0.36	17.5±0.74
45	11.0±0.35	9.7±0.47	8.5±0.27	24.1±0.81
60	16.6±0.42	15.4±0.31	10.9±0.55	29.6±0.63
75	20.4±0.21	18.6±0.47	13.6±0.47	37.4±1.2
90	23.7±0.36	21.4±0.55	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

Table 4.3.7

Cytotoxicity (% hemolysis) of *Eucalyptus citriodora* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	2.01%	1.25%
5mg/mL	3.47%	2.94%
10mg/mL	9.27%	9.67%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Eucalyptus citriodora*, analyzed individually in triplicate.

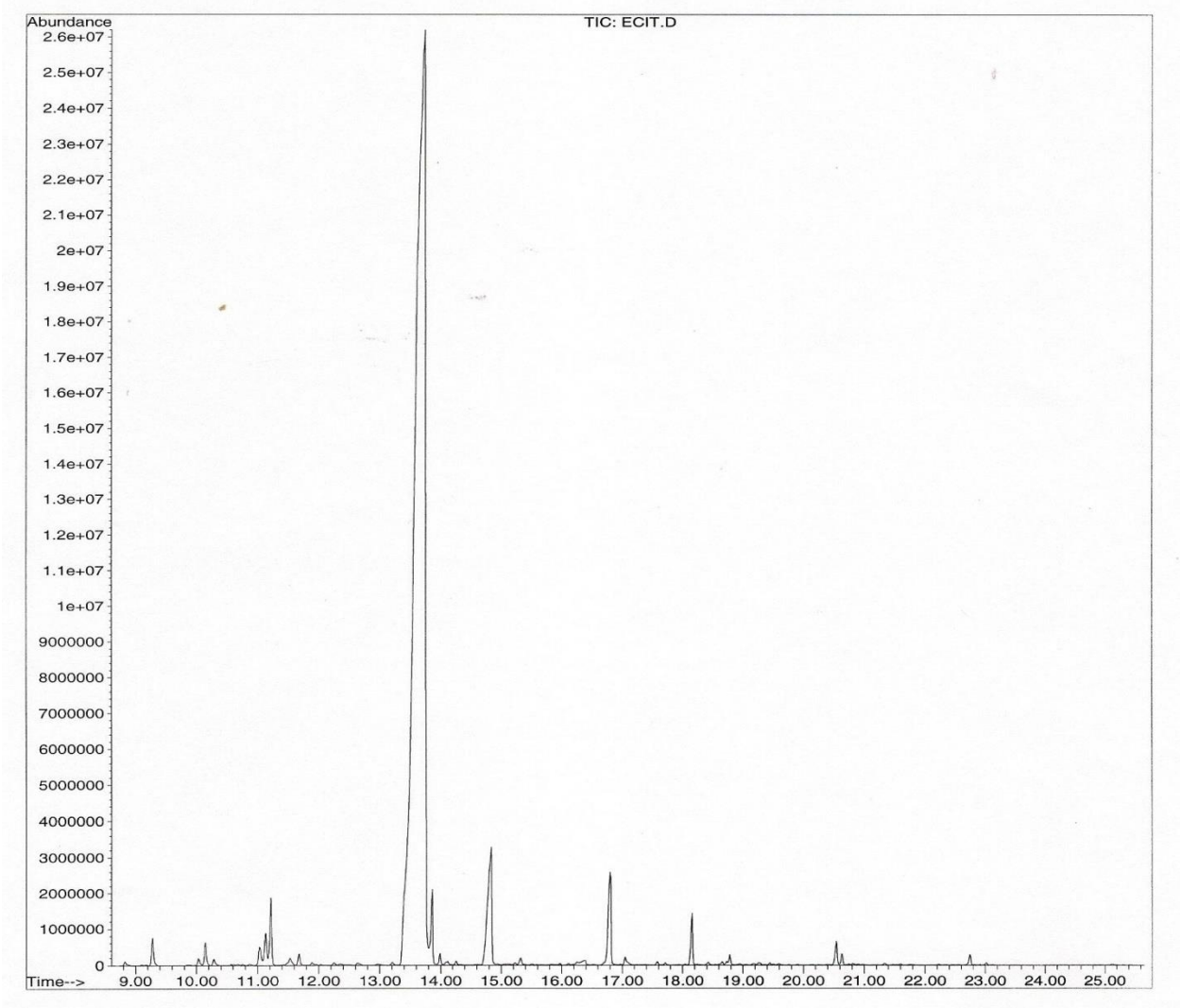


Figure 4.3.2. Typical GC-MS chromatogram of essential oil from *Eucalyptus citriodora*

Table 4.3.8. Chemical composition of *Eucalyptus citriodora* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
α-Thujene	926	0.78	RT, RI, MS
α-pinene	934	0.512	RT, RI, MS
α-Fenchene	952	0.28	RI, MS
Sabinene	972	0.49	RT, RI, MS
β-Pinene	978	0.40	RT, RI, MS
β-Myrcene	994	0.64	RT, RI, MS
p-Cymene	**1026**	**6.48**	**RT, RI, MS**
Limonene	1033	0.69	RT, RI, MS
Eucalyptol	1037	1.13	RT, RI, MS
β-cis-Ocimene	1043	1.12	RI, MS
p-Mentha-3,8-diene	1069	0.82	RT, RI, MS
γ-Terpinen	1072	0.21	RT, RI, MS
Linalool	1096	1.14	RT, RI, MS
Isopulegol	1146	1.34	RI, MS
β-Citronellal	**1162**	**66.16**	**RT, RI, MS**
β-Citronellol	**1228**	**4.40**	**RT, RI, MS**
Citral	1237	1.12	RT, RI, MS
Geraniol	1258	0.96	RT, RI, MS
Isopulegyl acetate	1268	0.48	RI, MS
Eugenol	1356	0.46	RT, RI, MS
Citronellyl acetate	**1357**	**2.43**	**RI, MS**
Geranyl formate	1384	0.26	RT, RI, MS
β-Elemene	1392	0.62	RT, RI, MS
Aromadendrene	1440	0.72	RI, MS
α-Humulene	1448	0.57	RT, RI, MS
Caryophyllene	1454	0.46	RT, RI, MS
α-Farnesene	1509	0.43	RT, RI, MS
Germacrene B	1559	0.63	RI, MS
Spathulenol	1574	0.74	RT, RI, MS
Globulol	1576	0.57	RI, MS
Caryophyllene oxide	1578	0.61	RT, RI, MS
Guaiol	1594	0.36	RT, RI, MS
β-Eudesmol	1652	0.73	RT, RI, MS
Total (34)		98.75	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS = identification based on comparison of mass spectra

4.3.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Eucalyptus citriodora* was found to be 1.91 % as listed in Table 4.3.1 which shows that the plant is enriched with essential oil. The oil was colourless with strong aromatic smell and refractive index was 1.453. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.85. Singh *et al.* (2012) found the yield of pale yellow coloured essential oil obtained from leaves of *E. citriodora* to be 1.2 % v/w.

Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *E. citriodora* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.3.2, which was found to be 15.95 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Table 4.3.2 shows the % inhibition in linoleic acid system by *E. citriodora* essential oil taken at three different concentrations. It is clear that maximum inhibition was 68.63 % at concentration 50 µL/mL which decreased linearly with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction is inhibited by some antioxidants if present in the reaction medium that react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *E. citriodora* is shown in Figure 4.3.1. The least antioxidant activity was exhibited by control with the maximum colour

depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *E. citriodora* was used at three different concentrations where minimum depletion was observed at 6mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. There are very few reports present in the literature showing the response of essential oils in β-carotene assay (Anwar *et al.*, 2009). Singh *et al.* (2012) evaluated the IC_{50} value for *E. citriodora* to be 425.4 µg/mL. The antioxidant activity of essential oils may be attributed to the presence of phenolic compounds (Lu and Foo, 2000). Singh and Marimuthu (2006) found that essential oils effectively suppress the peroxide formation in linoleic acid system during incubation.

The antimicrobial activity of the essential oil of *E. citriodora* against various microbes including bacteria and fungi is shown in Table 4.3.3. It is clear from the Table that essential oil showed good activity against most of the fungi and some of the bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *E. citriodora* showed potential as an antimicrobial agent with larger inhibition zones (4.9-21.4 and 8.7-25.3 mm) and smaller MIC values (1.17-4.3 and 1.09-2.81 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *L. rhamnosus* (Gram positive) with largest inhibition zone 21.4mm followed by minimum MIC value 1.17 mg/mL. These results are much better than that of antibiotic, Rifampicin, which showed the inhibition zone 15.5 mm and MIC 2.81 mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 25.3mm followed by minimum MIC value 1.09 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 21.7 mm and MIC 0.94 mg/mL. Hassine *et al.* (2012) tested extract and essential oil of *Eucalyptus gilii* against various bacterial and fungal strains and found the greatest inhibition in case of essential oil against *Listeria monocytogenes* (Gram positive bacteria) with MIC value 0.78 mg/mL. In another study antimicrobial activity of *E. globulus* essential oil was found to be (MIC = 0.9 mg/mL) against *E. coli* whereas (MIC = 0.36 mg/mL) against *Candida albicans* (Damjanovic-Vratnica *et al.*, 2011)

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.3.4 shows the effect of *E. citriodora* essential oil on

formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *E. citriodora* essential oil at 1000ppm which was 0.29 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.3.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.3 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.3.6 for *E. citriodora*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *E. citriodora* essential oil sample of 1000 ppm with value 21.4 while in case of BHT it was found to be 17.9.

The effect of *E. citriodora* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and results and % hemolysis were calculated which is presented in table 4.3.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *E. citriodora* exhibited the maximum hemolysis i.e. 9.67 % at 10 mg/mL concentration and the minimum of 1.25 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.27 % at 10 mg/mL concentration and the minimum of 2.01 % at 0.5 mg/mL. Agar *et al.* (1998) found hemolytic effect of monoterpenes and sesquiterpenes obtained from eucalyptus essential oil on human erythrocytes to be 14.2 and 23.2 %, respectively.

The data of chemical composition of the essential oil from *E. citriodora* was listed in Table 4.3.8. 34 compounds were identified representing 98.75 % of the oil. The major constituents were found to be β-Citronellal (66.16 %), p-Cymene (6.48 %), β-Citronellol (4.40 %) and Citronellyl acetate (2.43 %). Our results found to be well matched with those of Singh *et al.* (2012) who collected *E. citriodora* leaves from Punjab (India). The major chemical constituents were found to be; citronellal (60.66 %), β-Citronellol (12.58 %), isopulegol (8.19 %), p-Menthane-3,8-diol (2.87 %) and citronellyl acetate (2.38 %). In another study Batish (2006) analyzed essential oil extracted from leaves of *E. citriodora* collected from Chandigarh (India). citronellal (48.33 %), citronellol (21.87 %), isopulegol (12.69 %) and β-citronellene (4.81 %) were found to be the major components.

4.4. *Eucalyptus tereticornis*

Table 4.4.1

Physical properties of *Eucalyptus tereticornis* essential oil

Parameter	***Eucalyptus tereticornis***
Colour	Light green
Yield (%)	2.67±0.2
Refractive Index (30 °C)	1.417±0.006
Specific Gravity	0.84±0.01

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

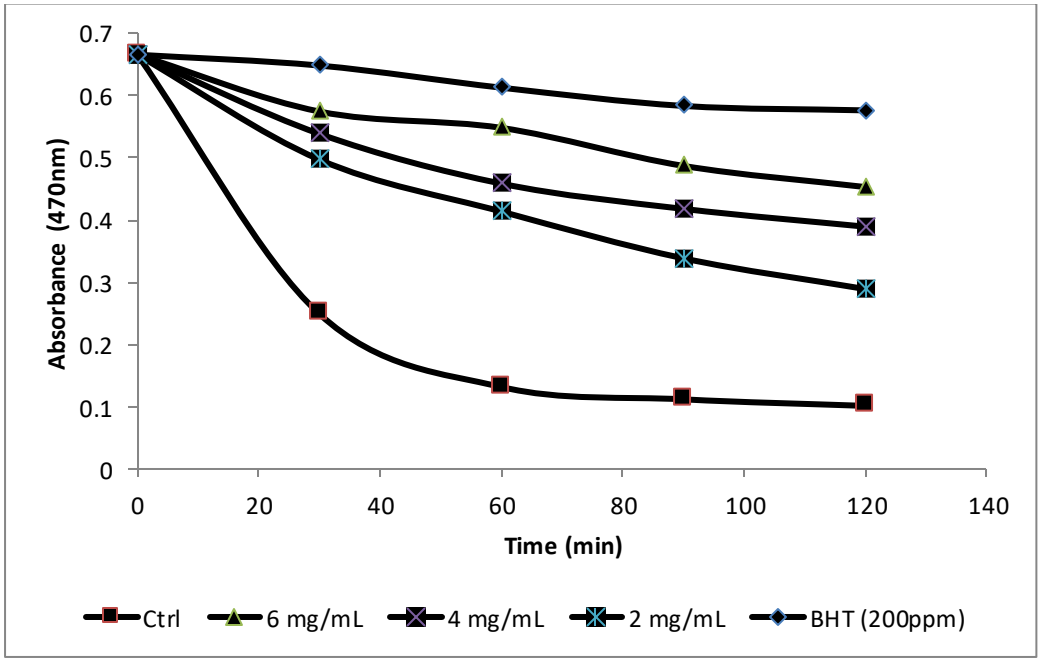
Table 4.4.2

Antioxidant activity of *Eucalyptus tereticornis* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*E. tereticornis*			BHT
IC_{50} (µg/mL)	42.71±2.37			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	57.90±1.9	42.94±1.4	32.94±2.1	85.10±2.8

Values are mean ± standard deviation of *Eucalyptus tereticornis*, analyzed individually in triplicate.

Fig. 4.4.1



Antioxidant activity of *Eucalyptus tereticornis* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.4.3

Antimicrobial activity of *Eucalyptus tereticornis* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Eucalyptus tereticornis		
Inhibition zone (mm)			
B. subtilis	6.7±0.3	10.8±2.1	-
L. rhamnosus	7.9±0.2	15.5±0.8	-
S. aureus	7.4±0.5	15.1±1.0	-
S. mutans	10.3±0.4	13.4±0.7	-
E. coli	8.5±0.7	9.6±1.3	-
P. multocida	**13.4±0.7**	**11.6±1.5**	-
A. alternata	8.6±0.4	-	14.2±0.8
A. flavus	9.3±1.5	-	11.4±1.4

A. niger	8.3±0.6	-	21.7±1.2
G. lucidum	**13.7±0.5**	**-**	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	4.37±0.3	1.72±0.2	-
L. rhamnosus	3.44±0.2	2.81±0.3	-
S. aureus	3.75±0.3	2.19±0.2	-
S. mutans	2.5±0.2	2.03±0.1	-
E. coli	3.12±0.2	2.34±0.2	-
P. multocida	2.34±0.2	2.03±0.1	-
A. alternata	3.12±0.2	-	2.34±0.2
A. flavus	2.81±0.1	-	2.81±0.2
A. niger	3.12±0.2	-	0.94±0.1
G. lucidum	2.03±0.1	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.4.4 FFA of edible oil stabilized by *Eucalyptus tereticornis* essential oil

IPD Induction Period in Days	% FFA			
	E. tereticornis ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.14±0.012	0.1±0.02	0.08±0.01	0.16±0.014
30	0.18±0.011	0.13±0.012	0.09±0.015	0.22±0.016
45	0.25±0.014	0.17±0.016	0.11±0.012	0.34±0.03
60	0.29±0.013	0.22±0.014	0.14±0.014	0.48±0.02
75	0.36±0.02	0.29±0.03	0.18±0.018	0.57±0.03
90	0.41±0.022	0.35±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

Table 4.4.5

PV of edible oil stabilized by *Eucalyptus tereticornis* essential oil

IPD Induction Period in Days	PV			
	E. tereticornis ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	1.1±0.02	0.8±0.03	0.4±0.01	1±0.03
30	1.9±0.10	1.5±0.12	0.6±0.16	1.81±0.12
45	2.5±0.24	2.2±0.21	0.9±0.21	2.5±0.24
60	3.2±0.25	2.8±0.16	1.4±0.29	3.4±0.36
75	3.6±0.16	3.4±0.24	1.9±0.24	4.7±0.21
90	4.2±0.28	3.9±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

Table 4.4.6

***para*-Anisidine Value of edible oil stabilized by *Eucalyptus tereticornis* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	E. tereticornis ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	5.9±0.28	4.5±0.36	3.4±0.27	10.2±0.52
30	8.8±0.33	7.4±0.31	5.8±0.36	17.5±0.74
45	11.5±0.27	9.7±0.47	8.5±0.27	24.1±0.81
60	16.1±0.43	15.4±0.31	10.9±0.55	29.6±0.63
75	22.2±0.78	22.4±0.47	13.6±0.47	37.4±1.2
90	33.3±0.93	27.6±0.55	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

Table 4.4.7
Cytotoxicity (% hemolysis) of *Eucalyptus tereticornis* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	3.18%	0.73%
5mg/mL	8.05%	5.54%
10mg/mL	9.09%	7.83%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Eucalyptus tereticornis*, analyzed individually in triplicate.

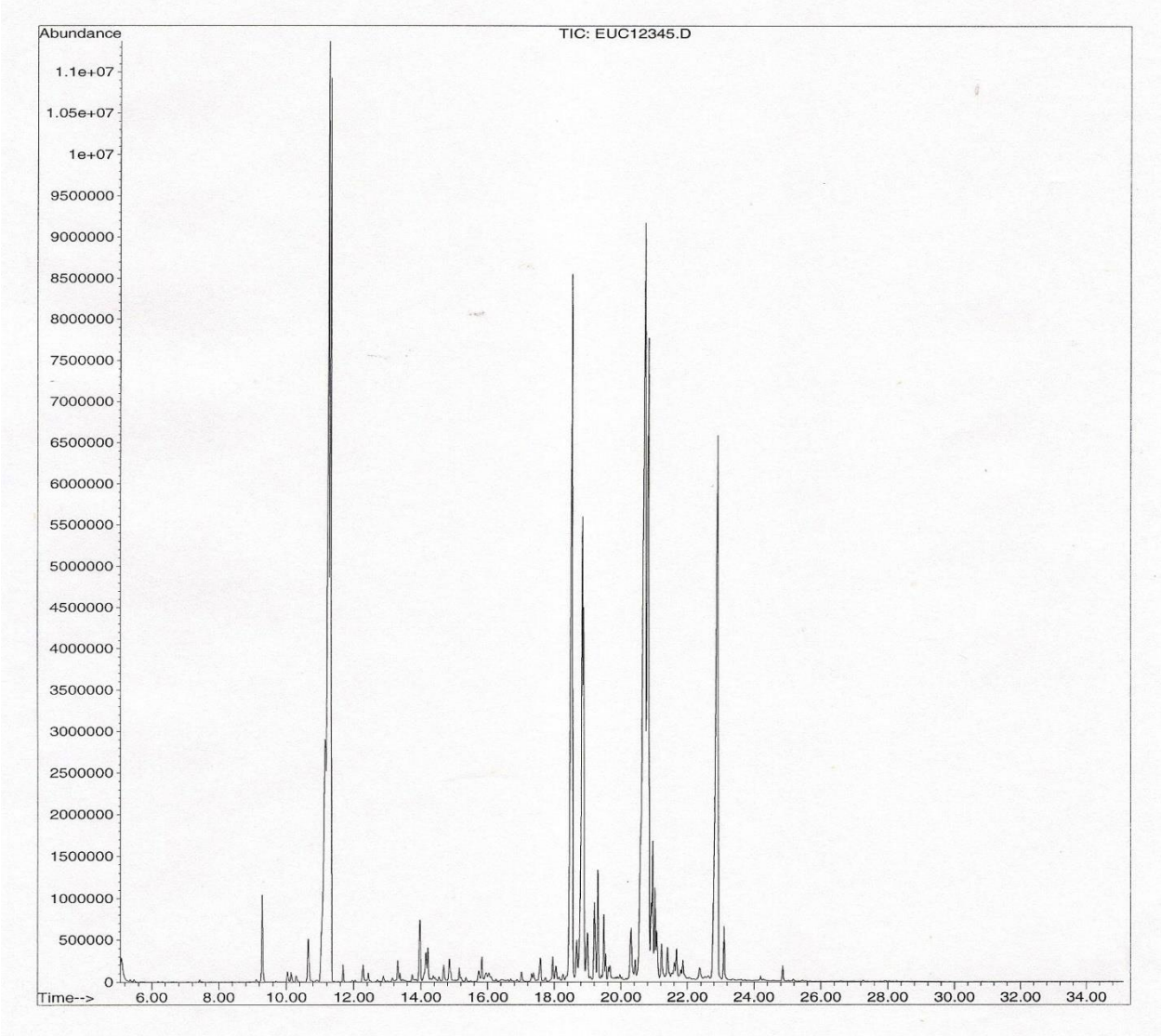


Figure 4.3.1. Typical GC-MS chromatogram of essential oil from *Eucalyptus tereticornis*

Table 4.4.8. Chemical composition of *Eucalyptus tereticornis* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
α-Thujene	926	0.54	RT, RI, MS
α-Pinene	934	3.19	RT, RI, MS
α-Fenchene	952	0.38	RI, MS
Camphene	955	0.31	RT, RI, MS
Verbenene	967	0.55	RT, RI, MS
Sabinene	972	0.22	RI, MS
β-Pinene	978	2.16	RT, RI, MS
β -Myrcene	994	0.32	RT, RI, MS
4-carene	1002	0.33	RT, RI, MS
α-phellandrene	1005	0.26	RT, RI, MS
α-Terpinene	1020	0.24	RT, RI, MS
p-Cymene	1026	0.27	RT, RI, MS
β-Phellandrene	1031	0.48	RI, MS
Limonene	**1033**	**19.97**	**RT, RI, MS**
Eucalyptol	**1037**	**7.46**	**RT, RI, MS**
β-Elemene	1040	0.16	RI, MS
β-cis-Ocimene	1043	0.61	RI, MS
γ-Terpinene	1062	0.42	RT, RI, MS
α-Terpinolene	1089	0.37	RT, RI, MS
Fenchone	1096	0.38	RT, RI, MS
trans.-Pinocarveol	1139	0.37	RI, MS
Isopulegol	1146	0.23	RI, MS
β-citronellal	1162	0.69	RT, RI, MS
Borneol	1169	0.80	RI, MS
Terpinen-4-ol	1178	0.19	RT, RI, MS
α-Terpineol	1188	1.17	RT, RI, MS
Myrtenol	1196	0.37	RI, MS
Piperitol	**1205**	**9.48**	**RI, MS**
Fenchyl acetate	1224	0.78	RI, MS
β-Citronellol	1228	0.29	RT, RI, MS
3-p-Menthene	1234	0.81	RT, RI, MS
Carvone	1242	0.20	RT, RI, MS
Geraniol	1258	0.24	RT, RI, MS
Anethole	1283	0.28	RI, MS
Bornyl acetate	1285	1.42	RT, RI, MS
Piperitenone	1342	0.46	RI, MS
α-Terpinyl acetate	**1350**	**5.22**	**RT, RI, MS**
Eugenol	1356	0.23	RT, RI, MS
Patchoulane	**1378**	**10.32**	**RI, MS**
β-Cubebene	1390	0.19	RT, RI, MS

trans-β-Caryophyllene	1418	0.35	RT, RI, MS
β-Gurjunene	1432	0.31	RT, RI, MS
Aromadendrene	1440	5.31	RI, MS
α-Humulene	1448	0.40	RT, RI, MS
α-Caryophyllene	1454	0.92	RT, RI, MS
Spathulenol	**1574**	**6.49**	**RT, RI, MS**
Globulol	1576	0.18	RT, RI, MS
Guaiol	1594	0.27	RI, MS
γ-Eudesmol	1623	0.21	RT, RI, MS
β-Eudesmol	**1652**	**9.98**	**RI, MS**
α-Eudesmol	1664	1.88	RT, RI, MS
Total (51)		98.67	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.4.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Eucalyptus tereticornis* was found to be 2.67 % as listed in Table 4.4.1. The oil was light green in colour with strong aromatic smell and refractive index 1.417. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.84. Kaur *et al.* (2011) found that hydrodistillation of leaves of *E. tereticornis* yielded yellow coloured oil (1.21 % v/w on fresh weight basis).

The essential oil of *E. tereticornis* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and DPPH radical scavenging by *E. tereticornis* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in 4.4.2, which was found to be 42.71 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.4.2 shows the % inhibition in linoleic acid system by *E. tereticornis* essential oil taken at three different concentrations. It is clear that maximum inhibition was 57.90% at concentration 50 µL/mL which decreased linearly with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *E. tereticornis* is shown in Figure 4.4.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion in colour. *E. tereticornis* was used at three

different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. The phenolic compounds could be responsible for the antioxidant activity of essential oils (Lu and Foo, 2000). Kaur *et al.* (2011) evaluated the antioxidant activity of *E. tereticornis* by measuring its scavenging effect towards DPPH and H_2O_2. The essential oil showed an IC_{50} value of 146 and 270 µg/mL for DPPH and H_2O_2. DPPH scavenging activity of oil was parallel to that of BHT (163 µg/mL) thereby indicating a strong antioxidant activity. Singh *et al.* (2009) reported the antioxidant activity of essential oil from fresh and decaying leaves of *E. tereticornis*.

The antimicrobial activity of the essential oil of *E. tereticornis* against various microbes including bacteria and fungi is shown in Table 4.4.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *E. tereticornis* showed potential as an antimicrobial agent with larger inhibition zones (6.7-13.4 and 8.3-13.7 mm) and smaller MIC values (2.34-4.37 and 2.03-3.12 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *P. multocida* (Gram positive) with largest inhibition zone 13.4mm followed by minimum MIC value 2.34 mg/mL. These results are much better than that of antibiotic, Rifampicin, which showed the inhibition zone 11.6 mm and MIC 2.03 mg/mL. In case of fungi, *G. lucidum* showed to be most sensitive with largest inhibition zone 13.7 mm followed by minimum MIC value 2.03 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 9.1mm and MIC 1.88 mg/mL. Hassine *et al.* (2012) tested extract and essential oil of *Eucalyptus gilii* against various bacterial and fungal strains and found the greatest inhibition in case of essential oil against *Listeria monocytogenes* (Gram positive bacteria) with MIC value 0.78 mg/mL. In another study antimicrobial activity of *E. globulus* essential oil was found to be (MIC = 0.9 mg/mL) against *E. coli* whereas (MIC = 0.36 mg/mL) against *Candida albicans* (Damjanovic-Vratnica *et al.*, 2011).

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.4.4 shows the effect of *E. tereticornis* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum

stabilization was exhibited by *E. tereticornis* essential oil at 1000ppm which was 0.35 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.4.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.9 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.4.6 for *E. tereticornis*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *E. tereticornis* essential oil sample of 1000 ppm with value 27.6 while in case of BHT it was found to be 17.9.

The effect of *E. tereticornis* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and results and % hemolysis were calculated which is presented in table 4.4.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *E. tereticornis* exhibited the maximum hemolysis i.e. 7.83 % at 10 mg/mL concentration and the minimum of 0.73 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.09% at 10 mg/mL concentration and the minimum of 3.18 % at 0.5 mg/mL. Agar *et al.* (1998) found hemolytic effect of monoterpenes and sesquiterpenes obtained from eucalyptus essential oil on human erythrocytes to be 14.2 and 23.2 %, respectively.

The data of chemical composition of the essential oil from *E. tereticornis* was listed in Table 4.4.8. Total 51 compounds were identified representing 98.67 % of the oil. The major constituents were found to be limonene (19.97 %), patchoulane (10.32 %), β-eudesmol (9.98 %), piperitol (9.48 %) and eucalyptol (7.46 %). Kaur *et al.* (2011) collected matured leaves of *E. citriodora* from Chandigarh (India). The major chemical constituents were found to be; α-pinene (30.10 %), eucalyptol (21.8 %), β-pinene (8.41 %), β-eudesmol (6.71 %) and α-eudesmol (5.77 %). In another study Yuan *et al.* (2006) analyzed essential oil extracted from leaves of *E. citriodora*. α-pinene (32.68 %), eucalyptol (13.64 %), limonene (8.31 %) and borneol (3.97 %) were found to be the major components.

4.5. *Citrus reticulata*

Table 4.5.1

Physical properties of *Citrus reticulata* essential oil

Parameter	***Citrus reticulata***
Colour	Colourless
Yield (%)	0.91±0.03
Refractive Index (30 °C)	1.355±0.003
Specific Gravity	0.81±0.04

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

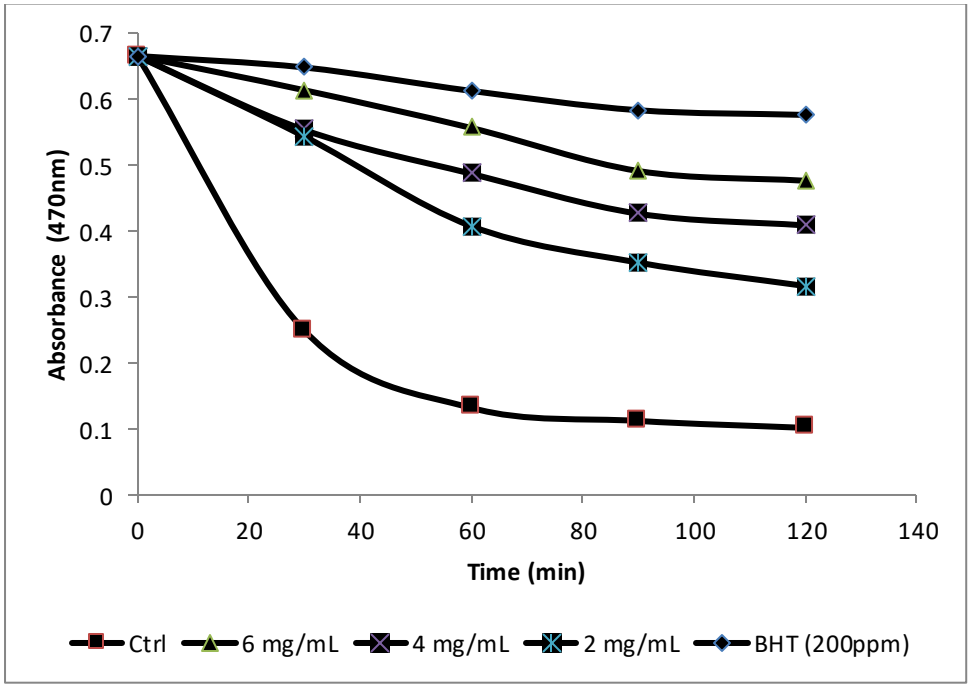
Table 4.5.2

Antioxidant activity of *Citrus reticulata* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*C. reticulata*			BHT
IC_{50} (µg/mL)	24.77±0.78			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	70.03±2.7	60.63±2.4	45.36±2.6	85.10±2.8

Values are mean ± standard deviation of *Citrus reticulata*, analyzed individually in triplicate.

Fig. 4.5.1



Antioxidant activity of *Citrus reticulata* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.5.3

Antimicrobial activity of *Citrus reticulata* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Citrus reticulata		
Inhibition zone (mm)			
B. subtilis	**19.0±1.6**	**10.8±2.1**	**-**
L. rhamnosus	14.3±0.1	15.5±0.8	-
S. aureus	6.1±1.0	15.1±1.0	-
S. mutans	6.8±0.4	13.4±0.7	-
E. coli	5.3±0.4	9.6±1.3	-
P. multocida	7.4±0.1	11.6±1.5	-
A. alternata	5.5±0.3	-	14.2±0.8
A. flavus	**20.1±1.4**	-	**11.4±1.4**
A. niger	19.6±2.1	-	21.7±1.2

G. lucidum	3.6±0.3	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	**1.41±0.1**	**1.72±0.2**	-
L. rhamnosus	2.03±0.1	2.81±0.3	-
S. aureus	3.75±0.3	2.19±0.2	-
S. mutans	4.06±0.3	2.03±0.1	-
E. coli	4.48±0.2	2.34±0.2	-
P. multocida	5.62±0.3	2.03±0.1	-
A. alternata	5.0±0.3	-	2.34±0.2
A. flavus	**1.17±0.1**	-	**2.81±0.2**
A. niger	1.25±0.1	-	0.94±0.1
G. lucidum	5.62±0.4	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.5.4 FFA of edible oil stabilized by *Citrus reticulata* essential oil

IPD Induction Period in Days	% FFA			
	Citrus reticulata ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.15±0.02	0.11±0.012	0.08±0.01	0.16±0.014
30	0.18±0.013	0.13±0.014	0.09±0.015	0.22±0.016
45	0.24±0.016	0.17±0.018	0.11±0.012	0.34±0.03
60	0.29±0.02	0.2±0.02	0.14±0.014	0.48±0.02
75	0.34±0.03	0.25±0.03	0.18±0.018	0.57±0.03
90	0.39±0.025	0.29±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

Table 4.5.5 PV of edible oil stabilized by *Citrus reticulata* essential oil

IPD Induction Period in Days	PV			
	Citrus reticulata ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.9±0.02	0.6±0.02	0.4±0.01	1±0.03
30	1.4±0.02	0.9±0.02	0.6±0.16	1.81±0.12
45	2.0±0.3	1.5±0.21	0.9±0.21	2.5±0.24
60	2.8±0.2	2.3±0.21	1.4±0.29	3.4±0.36
75	3.6±0.3	2.9±0.24	1.9±0.24	4.7±0.21
90	4.8±0.27	3.4±0.21	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

Table 4.5.6

***para*-Anisidine Value of edible oil stabilized by *Citrus reticulata* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	Citrus reticulata ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.15	2.7±0.12	2.7±0.12	2.7±0.12
15	5.6±0.22	4.6±0.31	3.4±0.27	10.2±0.52
30	8.2±0.34	6.8±0.55	5.8±0.36	17.5±0.74
45	10.7±0.65	9.5±0.27	8.5±0.27	24.1±0.81
60	14.9±0.81	13.6±0.31	10.9±0.55	29.6±0.63
75	18.8±1.0	17.5±0.47	13.6±0.47	37.4±1.2
90	26.6±1.2	21.4±0.55	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

Table 4.5.7

Cytotoxicity (% hemolysis) of *Citrus reticulata* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	3.12%	2.33%
5mg/mL	5.90%	5.22%
10mg/mL	9.96%	8.84%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Citrus reticulata*, analyzed individually in triplicate.

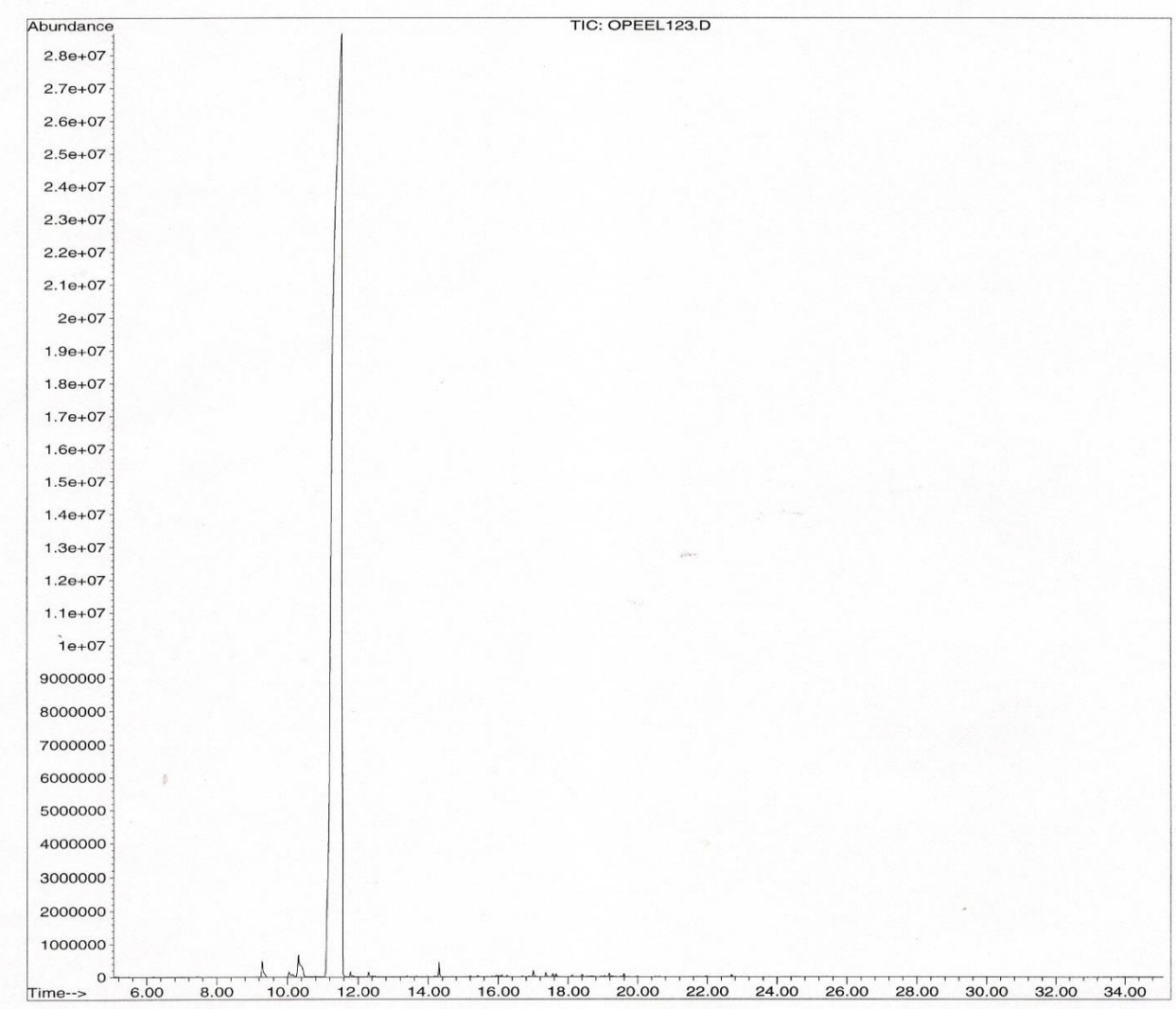


Figure 4.5.2. Typical GC-MS chromatogram of essential oil from *Citrus reticulata*

Table 4.5.8. Chemical composition of *Citrus reticulata* essential oil

Components[1]	**RI**[2]	**% age**	**Mode of Identification**[3]
α-Pinene	934	0.19	RT, RI, MS
Sabinene	972	0.13	RT, RI, MS
β-Pinene	978	0.15	RT, RI, MS
β -Myrcene	994	0.69	RT, RI, MS
3-Carene	1011	0.24	RT, RI, MS
Limonene	**1033**	**92.83**	**RT, RI, MS**
β-Ocimene	1044	0.22	RI, MS
γ-Terpinen	1072	0.21	RT, RI, MS
trans-Linalool oxide	1088	0.26	RT, MS
Linalool	1096	0.31	RT, RI, MS
α- Terpinolen	1187	0.14	RT, RI, MS
Citronellol	1228	0.18	RT, RI, MS
3-p-Menthene	1234	0.20	RI, MS
Linalyl formate	1237	0.15	RT, MS
Neryl acetate	1344	0.19	RT, RI, MS
Eugenol	1356	0.21	RT, RI, MS
Copaene	1366	0.19	RT, RI, MS
Patchoulane	1378	0.24	RI, MS
α-Terpineol acetate	1407	0.17	RT, MS
Isocaryophyllene	1413	0.17	RT, MS
β-Gurjunene	1432	0.20	RT, MS
Aromadendrene	1440	0.25	RI, MS
Germacrene D	1451	0.22	RT, RI, MS
α-caryophyllene	1454	0.19	RT, RI, MS
α-Farnesene	1509	0.18	RT, RI, MS
δ-Cadinene	1522	0.21	RT, MS
γ-Eudesmol	1623	0.24	RT, RI, MS
Total (27)		98.57	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.5.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Citrus reticulata* was found to be 0.91 % as listed in Table 4.5.1. The oil was colourless with strong aromatic smell and refractive index 1.355. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.81. Hamdan *et al.* (2009) found that hydrodistillation of fruit rind of *C. jambhiri and C. pyriformis* resulted in colourless and pale yellow oils having strong aromatic odour with yields 4 and 1.2% (v/w), respectively. In another study Chutia *et al.* (2009) recorded the yield of *C. reticulata* (0.6 ml/100g peel). In another study Baik *et al.* (2008) examined essential oils extracted from peel of 14 citrus species collected from Jeju island (Korea). The yields varied between 0.6 % to 3.5 %. Vasudeva and Sharma (2012) found the essential oil of *Citrus limettioides* colourless to light greenish yellow having citrusy and pleasant odour. Its specific gravity, refractive index, optical rotation at 25 °C were 0.840 g/mL, 1.470 and + 95.3°, respectively.

The DPPH radical scavenging by *C. reticulata* essential oil was represented in terms of 50% scavenging (IC_{50}) as shown in Table 4.5.2, which was found to be 24.77 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.5.2 shows the % inhibition in linoleic acid system by *C. reticulata* essential oil taken at three different concentrations. It is clear that maximum inhibition was 70.03 % at concentration 50µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution

using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *C. reticulata* is shown in Figure 4.5.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *C. reticulata* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Hamdan *et al.* (2009) evaluated the antioxidant activity of two citrus species using DPPH assay. He calculated IC_{50} values for essential oils of *C. pyriformis and C. jambhiri* to be 28.91 and 37.69 mg/mL, respectively. Baik *et al.* (2008) found the specie collected from Dongjunggyul (Korea) to be the most potential antioxidant among the 14 citrus species.

The antimicrobial activity of the essential oil of *C. reticulata* against various microbes including bacteria and fungi is shown in Table 4.5.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *C. reticulata* showed potential as an antimicrobial agent with larger inhibition zones (5.3-19.0 and 5.5-20.1 mm) and smaller MIC values (1.41-5.62 and 1.17-5.62 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *B. subtilis* (Gram positive) with largest inhibition zone 19.0 mm followed by minimum MIC value 1.41 mg/mL. These results are much better than that of antibiotic, Rifampicin, which showed the inhibition zone 10.8 mm and MIC 1.72 mg/mL. In case of fungi, *A. flavus* showed to be most sensitive with largest inhibition zone 20.1 mm followed by minimum MIC value 1.17 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 11.4 mm and MIC 2.81 mg/mL. Vasudeva and Sharma (2012) tested essential oil of *Citrus limettioides* against various microbes and maximum activity in case of bacteria was found against *Propionibacterium acnes* with MIC value 3.12 µL/mL, while in case of fungal strains against *Aspergillus niger* with MIC 6.25 µL/mL.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.5.4 shows the effect of *C. reticulata* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the

length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *C. reticulata* essential oil at 1000ppm which was 0.29 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.5.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.4 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.5.6 for *C. reticulata*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *C. reticulata* essential oil sample of 1000ppm with value 21.4 while in case of BHT it was found to be 17.9.

The effect of *C. reticulata* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.5.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *C. reticulata* exhibited the maximum hemolysis i.e. 8.84 % at 10 mg/mL concentration and the minimum of 2.33 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.96 % at 10 mg/mL concentration and the minimum of 3.12 % at 0.5 mg/mL.

The data of chemical composition of the essential oil from *C. reticulata* was listed in Table 4.5.8. Total 27 compounds were identified representing 98.57 % of the oil. The starring component was found to be limonene (92.83 %). Chutia *et al.* (2009) collected fresh and matured fruit of *C. reticulata* Blanco from Jorhat, Assam (India). The peels were subjected to essential oil extraction with major chemical constituents; limonene (46.7 %), geranial (19.0 %), Neral (14.5 %) and Geranyl acetate (3.9 %). In another study Baik *et al.* (2008) examined essential oils extracted from peel of 14 citrus species collected from Jeju island (Korea). Among them limonene (82.43 %), α-terpinene (6.83 %), β-myrcene (3.42 %) and α-pinene (1.32 %) were found to be the major components among others. Hamdan *et al.* (2009) evaluated fruit rind of two of citrus species; *C. jambhiri and C. pyriformis* collected from Egypt. Their essential oil was subjected to chemical characterization and biological activities. Limonene was found to be the starring compound 92.48 and 75.56 % from essential oils of *C. jambhiri and C. pyriformis,* respectively.

4.6. *Syzygium aromaticum*

Table 4.6.1

Physical properties of *Syzygium aromaticum* essential oil

Parameter	***Syzygium aromaticum***
Colour	Colourless
Yield (%)	6.70±0.3
Refractive Index (30 °C)	1.364±0.004
Specific Gravity	0.71±0.03

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

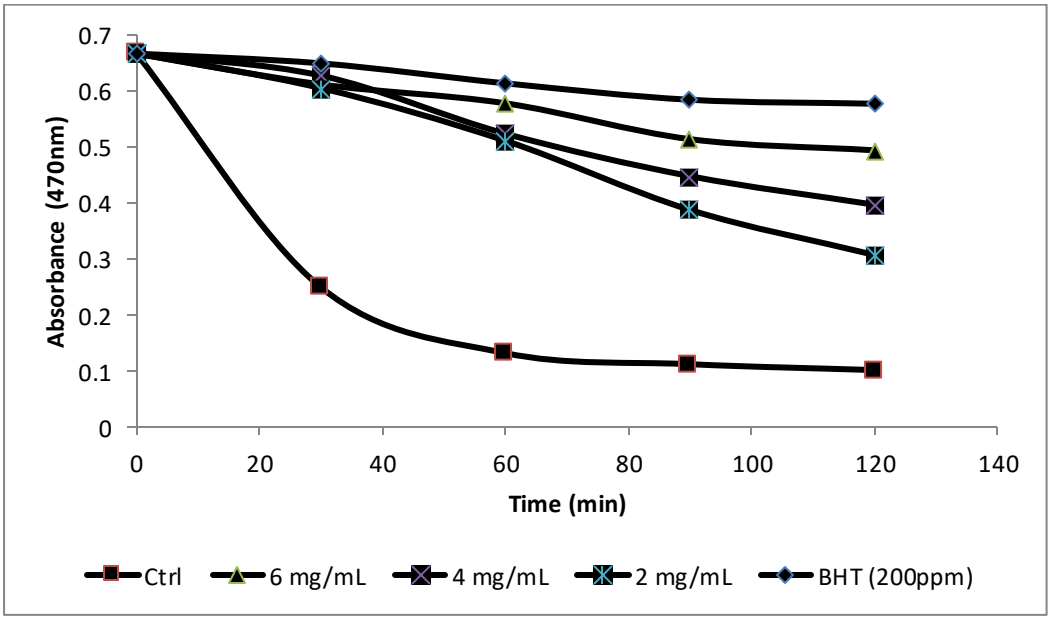
Table 4.6.2

Antioxidant activity of *Syzygium aromaticum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*S. aromaticum*			BHT
IC_{50} (µg/mL)	14.58±1.43			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	81.15±3.2	69.59±2.6	52.25±2.8	85.10±3.2

Values are mean ± standard deviation of *Syzygium aromaticum*, analyzed individually in triplicate.

Fig. 4.6.1



Antioxidant activity of *Syzygium aromaticum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.6.3

Antimicrobial activity of *Syzygium aromaticum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Syzygium aromaticum		
Inhibition zone (mm)			
B. subtilis	**27.0±1.0**	**10.8±2.1**	-
L. rhamnosus	8.3±0.7	15.5±0.8	-
S. aureus	9.7±0.4	15.1±1.0	-
S. mutans	**17.3±0.5**	**13.4±0.7**	-
E. coli	7.2±0.6	9.6±1.3	-
P. multocida	**16.2±0.8**	**11.6±1.5**	-
A. alternata	**19.3±1.0**	-	**14.2±0.8**
A. flavus	7.4±0.3	-	11.4±1.4

A. niger	10.0±1.0	-	21.7±1.2
G. lucidum	**20.1±1.2**	-	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	**1.25±0.12**	**1.72±0.2**	-
L. rhamnosus	3.12±0.2	2.81±0.3	-
S. aureus	4.37±0.3	2.19±0.2	-
S. mutans	**1.56±0.1**	**2.03±0.1**	-
E. coli	3.75±0.2	2.34±0.2	-
P. multocida	**1.88±0.1**	**2.03±0.1**	-
A. alternata	**1.41±0.1**	-	**2.34±0.2**
A. flavus	3.44±0.2	-	2.81±0.2
A. niger	2.5±0.1	-	0.94±0.1
G. lucidum	**1.25±0.1**	-	**1.88±0.1**

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.6.4 FFA of edible oil stabilized by *Syzygium aromaticum* essential oil

IPD Induction Period in Days	% FFA			
	Syzygium aromaticum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.11±0.012	0.09±0.015	0.08±0.01	0.16±0.014
30	0.15±0.02	0.1±0.02	0.09±0.015	0.22±0.016
45	0.2±0.022	0.13±0.02	0.11±0.012	0.34±0.03
60	0.24±0.025	0.16±0.014	0.14±0.014	0.48±0.02
75	0.31±0.015	0.21±0.018	0.18±0.018	0.57±0.03
90	0.39±0.03	0.25±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

Table 4.6.5 PV of edible oil stabilized by *Syzygium aromaticum* essential oil

IPD Induction Period in Days	PV			
	Syzygium aromaticum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.9±0.1	0.5±0.03	0.4±0.01	1±0.03
30	1.5±0.14	0.7±0.02	0.6±0.16	1.81±0.12
45	2.1±0.26	1.31±0.12	0.9±0.21	2.5±0.24
60	2.7±0.21	1.9±0.16	1.4±0.29	3.4±0.36
75	3.3±0.27	2.4±0.24	1.9±0.24	4.7±0.21
90	4.0±0.3	3.1±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

Table 4.6.6

***para*-Anisidine value of edible oil stabilized by *Syzygium aromaticum* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	Syzygium aromaticum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	4.8±0.24	3.9±0.36	3.4±0.27	10.2±0.52
30	7.5±0.38	6.3±0.47	5.8±0.36	17.5±0.74
45	11.2±0.64	9.8±0.31	8.5±0.27	24.1±0.81
60	14.6±0.81	13.5±0.55	10.9±0.55	29.6±0.63
75	19.6±0.72	18.2±0.31	13.6±0.47	37.4±1.2
90	25.5±1.1	22.4±0.47	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

Table 4.6.7

Cytotoxicity (% hemolysis) of *Syzygium aromaticum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.38%	0.14%
5mg/mL	3.05%	1.79%
10mg/mL	9.89%	6.24%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Syzygium aromaticum*, analyzed individually in triplicate.

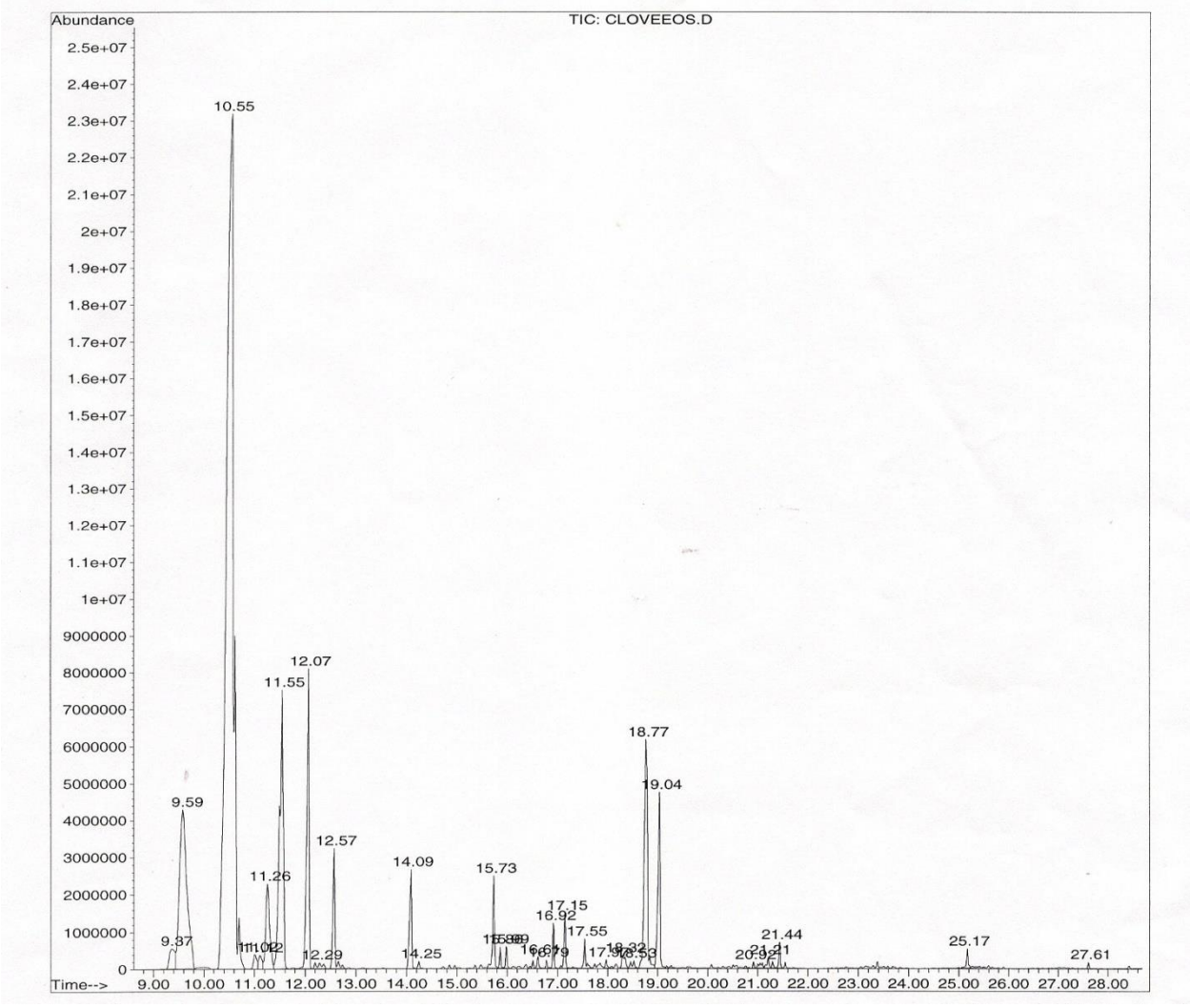


Figure 4.6.2. Typical GC-MS chromatogram of essential oil from *Syzygium aromaticum*

Table 4.6.8. Chemical composition of *Syzygium aromaticum* essential oil

Components[1]	**RI**[2]	**% age**	**Mode of Identification**[3]
Cumene	926	0.32	RT, RI, MS
α-pinene	934	0.29	RT, RI, MS
α-Fenchene	952	0.34	RI, MS
β-Thujene	970	0.21	RT, RI, MS
β -Myrcene	994	0.25	RT, RI, MS
4-carene	1002	0.36	RT, RI, MS
Limonene	1033	0.47	RT, RI, MS
Eucalyptol	1037	0.41	RT, RI, MS
γ-Terpinen	1072	0.22	RT, RI, MS
m-Cymene	1081	0.42	RI, MS
β-Citronellal	1162	0.34	RT, RI, MS
L-terpinen-4-ol	1178	0.19	RI, MS
Linalyl formate	1237	0.37	RT, MS
Eugenol	**1356**	**51.02**	**RT, RI, MS**
cis-Jasmone	1394	0.37	RI, MS
α-Bergamotene	1436	0.23	RI, MS
Caryophyllene	**1454**	**13.97**	**RT, RI, MS**
α -Farnesene	1509	0.31	RT, RI, MS
Eugenyl acetate	**1524**	**13.09**	**RT, RI, MS**
Caryophyllene Oxide	1578	0.17	RT, RI, MS
Isoeugenol acetate	**1610**	**11.16**	**RI, MS**
Benzyl Benzoate	1764	0.24	RI, MS
Total (23)		95.41	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.6.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Syzygium aromaticum* was found to be 6.70 % as listed in Table 4.6.1. This was the maximum yield obtained among all the plants taken in the following present study. The very high percentage showed that this plant was very much enriched with essential oil. The oil was colourless with strong aromatic smell and refractive index 1.364. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.71.

The essential oil of *S. aromaticum* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *S. aromaticum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.6.2, which was found to be 14.58 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.6.2 shows the % inhibition in linoleic acid system by *S. aromaticum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 81.15 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470nm. Bleaching of β-carotene as a

function of antioxidant activity of the essential oil of *S. aromaticum* is shown in Figure 4.6.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *S. aromaticum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Nassar *et al.* (2007) tested various clove buds extracts for their antioxidant activity where ethanol extract showed the maximum scavenging ability towards DPPH free radical which was found comparable with BHT. Alitonou *et al.* (2012) evaluated essential oil of clove for scavenging ability of DPPH radical and IC_{50} value was calculated for both essential oil and its main component eugenol which was found to be 10.3 and 1.6 mg/L, respectively.

The antimicrobial activity of the essential oil of *S. aromaticum* against various microbes including bacteria and fungi is shown in Table 4.6.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *S. aromaticum* showed potential as an antimicrobial agent with larger inhibition zones (7.2-27.0 and 7.4-20.1 mm) and smaller MIC values (1.25-4.37 and 1.41-3.44 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *B. subtilis* (gram positive) with largest inhibition zone 27.0 mm followed by minimum MIC value 1.25 mg/mL. These results are much better than that of antibiotic, Rifampicin, which showed the inhibition zone 10.8mm and MIC 1.72 mg/mL. In case of fungi, *G. lucidum* showed to be most sensitive with largest inhibition zone 20.1 mm followed by minimum MIC value 1.25 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 9.1mm and MIC 1.88 mg/mL. Alitonou *et al.* (2012) evaluated essential oil of clove against a panel of bacteria and fungi and maximum inhibition was found against *Staphylococcus aureus* MIC and MBC values, 0.025 and 1.56 mg/mL, respectively.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.6.4 shows the effect of *S. aromaticum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum

stabilization was exhibited by *S. aromaticum* essential oil at 1000ppm which was 0.25 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.6.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.1 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.6.6 for *S. aromaticum*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *S. aromaticum* essential oil sample of 1000 ppm with value 22.4 while in case of BHT it was found to be 17.9.

The effect of *S. aromaticum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.5.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *S. aromaticum* exhibited the maximum hemolysis i.e. 6.24 % at 10 mg/mL concentration and the minimum of 0.14 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.89 % at 10 mg/mL concentration and the minimum of 0.38 % at 0.5 mg/mL. Marya *et al.* (2012) tested clove essential oil and its main components eugenol and eugenyl acetate for their cytotoxicity and hemolysis was found to be 48, 41 and 57 %, respectively.

The data of chemical composition of the essential oil from *S. aromaticum* was listed in Table 4.6.8. Total 23 compounds were identified representing 95.41 % of the oil. The major components were found to be eugenol (51.02 %), caryophyllene (13.97 %), eugenyl acetate (13.09 %) and isoeugenol acetate (11.16 %). Nassar *et al.* (2007) collected the buds of *S. aromaticum* from local market of Cairo (Egypt) which were subjected to essential oil extraction and further for their chemical characterization. 16 components were identified with eugenol (71.56 %), eugenyl acetate (8.99 %), caryophyllene oxide (1.67 %) and nootkatin (1.05 %) as major components. In another study Alitonou *et al.* (2008) collected plant material from Abomey-Calavi (Benin) and examined essential oil using GC-MS. Eugenol (60.4%), *trans*-*β*-caryophyllene (24.0 %) and eugenol acetate (10.0 %) were found to be the major components among others.

4.7. *Myristica fragrans* (Nutmeg)

Table 4.7.1

Physical properties of *Myristica fragrans (N)* essential oil

Parameter	***Myristica fragrans (N)***
Colour	Pale Yellow
Yield (%)	1.70±0.2
Refractive Index (30 °C)	1.342±0.001
Specific Gravity	0.70±0.02

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N)*, analyzed individually in triplicate.

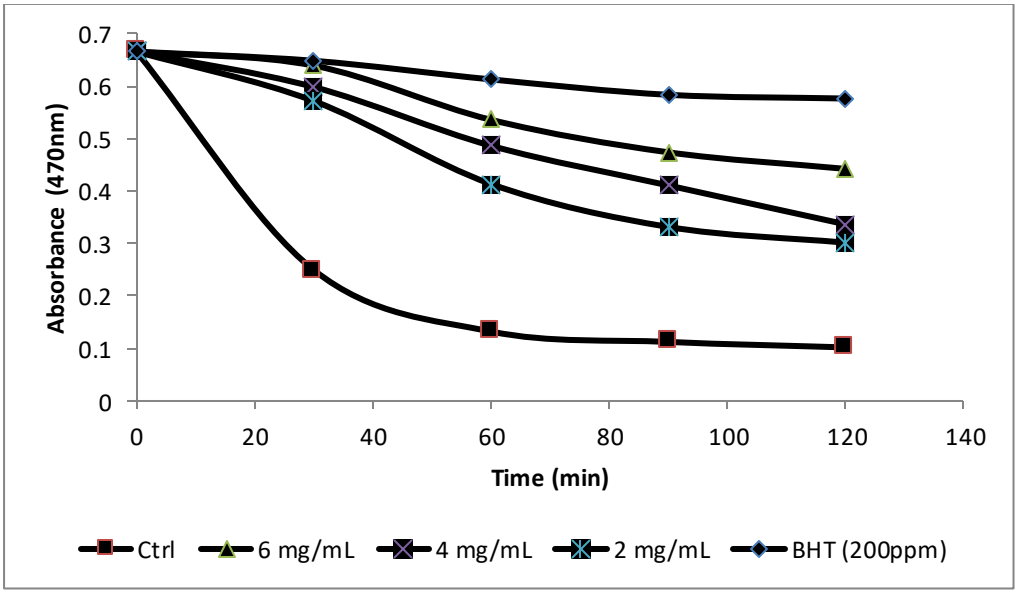
Table 4.7.2

Antioxidant activity of *Myristica fragrans (N)* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*M. fragrans* (N)			BHT
IC_{50} (µg/mL)	38.29±0.67			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	71.19±3.1	67.13±2.1	48.26±3.1	85.10±3.2

Values are mean ± standard deviation of *Myristica fragrans (N)*, analyzed individually in triplicate.

Fig. 4.7.1



Antioxidant activity of *Myristica fragrans (N)* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.7.3

Antimicrobial activity of *Myristica fragrans (N)* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Myristica fragrans (N)		
Inhibition zone (mm)			
B. subtilis	4.3±0.2	10.8±2.1	-
L. rhamnosus	6.4±0.3	15.5±0.8	-
S. aureus	4.2±0.5	15.1±1.0	-
S. mutans	9.6±0.1	13.4±0.7	-
E. coli	4.9±0.3	9.6±1.3	-
P. multocida	8.5±0.5	11.6±1.5	-
A. alternata	13.4±0.3	-	14.2±0.8
A. flavus	10.7±0.3	-	11.4±1.4
A. niger	20.3±2.0	-	21.7±1.2

G. lucidum	8.6±0.2	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	4.48±0.3	1.72±0.2	-
L. rhamnosus	4.06±0.3	2.81±0.3	-
S. aureus	5.0±0.4	2.19±0.2	-
S. mutans	2.81±0.2	2.03±0.1	-
E. coli	4.37±0.3	2.34±0.2	-
P. multocida	3.12±0.2	2.03±0.1	-
A. alternata	2.03±0.1	-	2.34±0.2
A. flavus	2.5±0.1	-	2.81±0.2
A. niger	1.25±0.1	-	0.94±0.1
G. lucidum	3.12±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N),* analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.7.4 FFA of edible oil stabilized by *Myristica fragrans (N)* essential oil

IPD Induction Period in Days	% FFA			
	Myristica fragrans (N) ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.15±0.012	0.11±0.02	0.08±0.01	0.16±0.014
30	0.17±0.016	0.13±0.014	0.09±0.015	0.22±0.016
45	0.23±0.02	0.16±0.016	0.11±0.012	0.34±0.03
60	0.27±0.023	0.2±0.016	0.14±0.014	0.48±0.02
75	0.31±0.027	0.25±0.018	0.18±0.018	0.57±0.03
90	0.37±0.03	0.29±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Myristica fragrans*, analyzed individually in triplicate.

Table 4.7.5 PV of edible oil stabilized by *Myristica fragrans (N)* essential oil

IPD Induction Period in Days	PV			
	Myristica fragrans (N) ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	1.1±0.1	0.8±0.02	0.4±0.01	1±0.03
30	1.7±0.3	1.31±0.12	0.6±0.16	1.81±0.12
45	2.5±0.26	1.9±0.21	0.9±0.21	2.5±0.24
60	3.1±0.31	2.4±0.29	1.4±0.29	3.4±0.36
75	3.7±0.38	2.8±0.24	1.9±0.24	4.7±0.21
90	4.3±0.4	3.4±0.29	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N)*, analyzed individually in triplicate.

Table 4.7.6

***para*-Anisidine Value of edible oil stabilized by *Myristica fragrans (N)* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	Myristica fragrans (N) ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	6.7±0.41	5.4±0.27	3.4±0.27	10.2±0.52
30	10.4±0.73	8.7±0.62	5.8±0.36	17.5±0.74
45	13.7±0.54	12.3±0.55	8.5±0.27	24.1±0.81
60	18.5±0.61	16.6±0.36	10.9±0.55	29.6±0.63
75	21.8±0.92	20.1±0.55	13.6±0.47	37.4±1.2
90	27.8±1.3	22.2±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N)*, analyzed individually in triplicate.

Table 4.7.7

Cytotoxicity (% hemolysis) of *Myristica fragrans (N)* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	4.40%	2.74%
5mg/mL	5.79%	3.96%
10mg/mL	8.61%	6.62%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N)*, analyzed individually in triplicate.

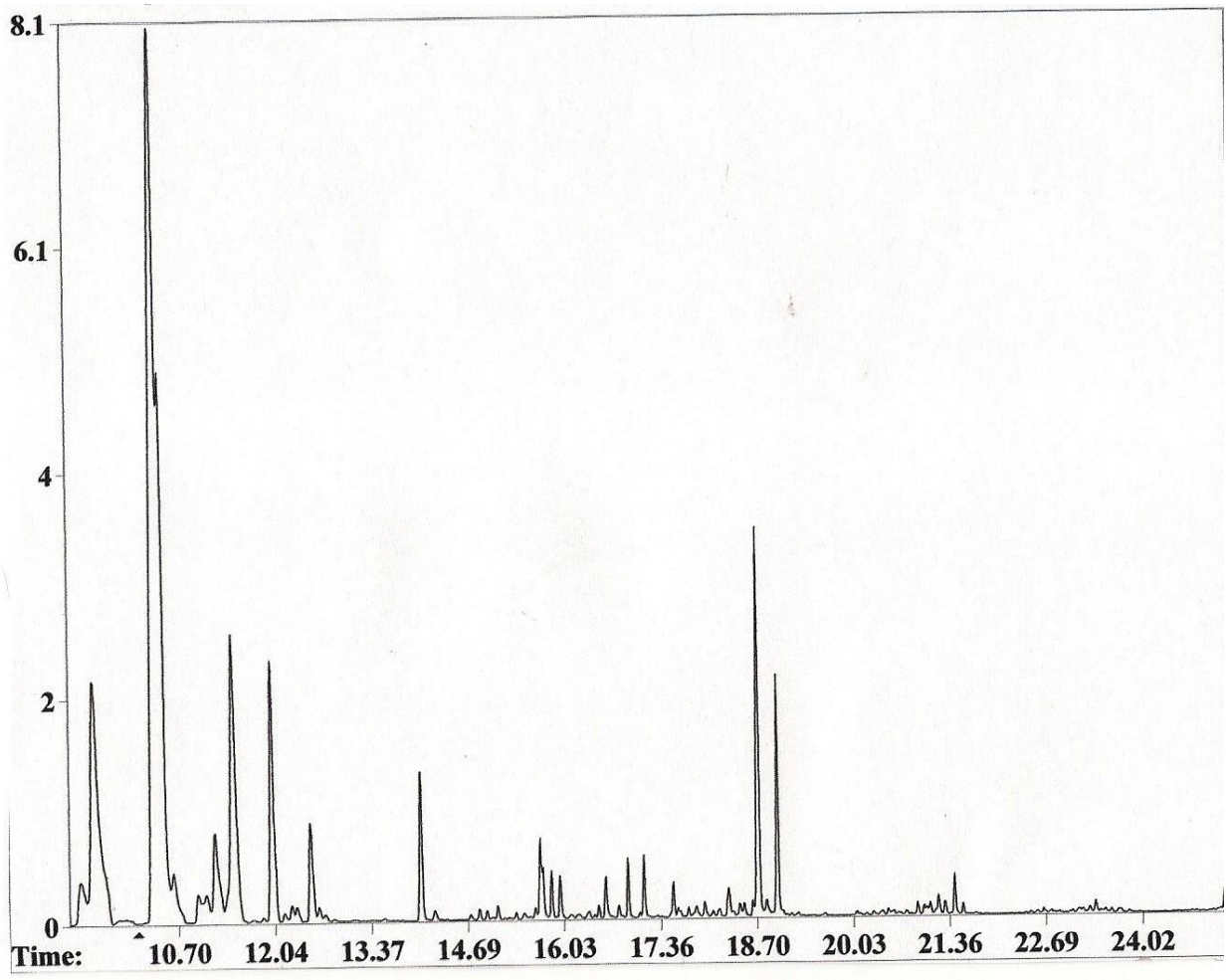


Figure 4.7.2. Typical GC-MS chromatogram of essential oil from *Myristica fragrans (N)*

Table 4.7.8. Chemical composition of *Myristica fragrans* (N) essential oil

Components[1]	RI[2]	% age	Mode of Identification[3]
Cumene	**926**	**5.73**	**RT, RI, MS**
α-Pinene	**934**	**18.06**	**RT, RI, MS**
α-Fenchene	952	0.34	RI, MS
Camphene	**955**	**5.18**	**RT, RI, MS**
Sabinene	972	2.26	RT, RI, MS
β -Myrcene	994	1.23	RT, RI, MS
α -Phellandrene	1005	0.25	RT, RI, MS
3-Carene	1011	0.45	RT, RI, MS
α-Terpinene	1020	1.42	RT, RI, MS
β-cis-Ocimene	1043	0.26	RI, MS
γ-Terpinen	1072	1.96	RT, RI, MS
α- Terpinolene	1086	0.57	RI, MS
L-Pinocarveol	**1148**	**7.58**	**RI, MS**
α-Terpinol	1187	0.49	RT, RI, MS
3-p-Menthene	**1234**	**25.56**	**RI, MS**
Linalyl alcohol	1237	1.08	RT, MS
cis-Geraniol	1258	0.34	RT, RI, MS
(E)-p-Menth-2-en-1-ol	1262	0.41	RI, MS
Bornyl acetate	1285	0.52	RT, RI, MS
Isopregol	-	6.69	RT, MS
α-Terpinyl acetate	1350	0.29	RI, MS
Eugenol	1356	0.22	RT, RI, MS
Myristicin	**1521**	**14.68**	**RI, MS**
Nerolidol	1533	0.38	RT, RI, MS
Elemicin	1555	0.37	RI, MS
Isoeugenol acetate	1610	0.64	RT, RI, MS
Isoeugenol	2250	0.40	RT, MS
Total (27)		98.60	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.7.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Myristica fragrans* (Nutmeg) was found to be 1.70 % as listed in Table 4.7.1 which showed that this plant was enriched with essential oil. The oil was pale yellow in colour with strong aromatic smell and refractive index 1.342. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.70. Muchtaridi *et al.* (2010) found the yield of essential oil 6.85 % w/w isolated from nutmeg seeds. Piras *et al.* (2012) examined that yield of volatile oil extracted from nutmeg was 1.4% obtained by supercritical CO_2 extraction. Kapoor *et al.* (2013) got 3.4 % of colourless volatile oil of nutmeg with characteristic odour and sharp taste.

The essential oil of *M. fragrans (N)* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *M. fragrans (N)* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.7.2, which was found to be 38.29 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.7.2 shows the % inhibition in linoleic acid system by *M. fragrans (N)* essential oil taken at three different concentrations. It is clear that maximum inhibition was 71.19 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *M. fragrans (N)* is shown in Figure 4.7.1. The least antioxidant activity was exhibited by control with the maximum colour depletion.

Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *M. fragrans (N)* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Piaru *et al.* (2012) found that essential oil of nutmeg showed significant antioxidant activity with IC_{50} value 136 µg/mL. In another study Piaru *et al.* (2012) evaluated that nutmeg essential oil effectively inhibited the oxidation of linoleic acid by 88.68 %.

The antimicrobial activity of the essential oil of *M. fragrans (N)* against various microbes including bacteria and fungi is shown in Table 4.7.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *M. fragrans (N)* showed potential as an antimicrobial agent with larger inhibition zones (4.2-9.6 and 8.6-20.3 mm) and smaller MIC values (2.81-5.0 and 1.25-3.12 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *S. mutans* (Gram positive) with largest inhibition zone 9.6 mm followed by minimum MIC value 2.81 mg/mL. These results are quite comparable to that of antibiotic, Rifampicin, which showed the inhibition zone 13.4 mm and MIC 2.03 mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 20.3mm followed by minimum MIC value 1.25mg/mL which was closer to that of antibiotic, Terbinafine, with inhibition zone 21.7mm and MIC 0.94 mg/mL. Pal *et al.* (2011) tested essential oil of nutmeg against various microbes and it was found that it showed activity against all microbes tested except *Pseudomonas aeruginosa* and *Candida albicans.*

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.7.4 shows the effect of *M. fragrans (N)* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *M. fragrans (N)* essential oil at 1000ppm which was 0.29 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.7.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 3.4 meq/kg after induction period of 90 days, which was 2.3 and 6.1

meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.7.6 for *S. aromaticum*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *M. fragrans (N)* essential oil sample of 1000 ppm with value 22.2 while in case of BHT it was found to be 17.9.

The effect of *M. fragrans (N)* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.7.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *M. fragrans (N)* exhibited the maximum hemolysis i.e. 6.62 % at 10 mg/mL concentration and the minimum of 2.74 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.61 % at 10 mg/mL concentration and the minimum of 4.40 % at 0.5 mg/mL. In another study nutmeg essential oil was tested for its cytotoxicity against Vero cells using MTT assay and it was found that it showed minute cytotoxicity with IC_{50} at 24.83 μL/mL (Piaru, *et al.*, 2012). Malti *et al.* (2008) tested the cytotoxicity of *M. fragrans* extract on mice and found that its extract was toxic at doses ≥3 mg/g.

The data of chemical composition of the essential oil from *M. fragrans (N)* was listed in Table 4.7.8. Total 27 compounds were identified representing 98.60 % of the oil. The major components were found to be 3-p-menthene (25.56 %), α-pinene (18.06 %), myristicin (14.68 %), L-pinocarveol (7.58 %), cumene (5.73 %) and camphene (5.18 %). Piaru *et al.* (2011) collected fresh fruits of *M. fragrans* from Balik Pulau, Penang (Malaysia) which was subjected to essential oil extraction and further for their chemical characterization. 37 components were identified with terpineol 4 (21.3 %), γ -Terpinene (9.9 %), α-Terpinene (9.8 %) and limonene (8.8 %). In another study Muchtaridi *et al.* (2010) obtained nutmeg seeds from Bogor (West Java) and examined essential oil using GC-MS. Sabinene (21.38 %), 4-terpineol (13.92 %), myristicin (13.57 %) and α-pinene (10.23 %) were found to be the major components among others. Kostic *et al.* (2013) collected nutmeg from Ovcarsko Kablarska Gorge (Serbia). Among 24 identified components the major were found to be α-pinene (25.07 %), β-pinene (18.79 %), sabinene (18.73 %) and myristicin (5.12 %).

4.8. *Myristica fragrans* (Mace)

Table 4.8.1

Physical properties of *Myristica fragrans (M)* essential oil

Parameter	***Myristica fragrans (M)***
Colour	Light brown
Yield (%)	1.75±0.04
Refractive Index (30 °C)	1.374±0.002
Specific Gravity	0.95±0.02

Values are mean ± standard deviation of three samples of each *Myristica fragrans (M)*, analyzed individually in triplicate.

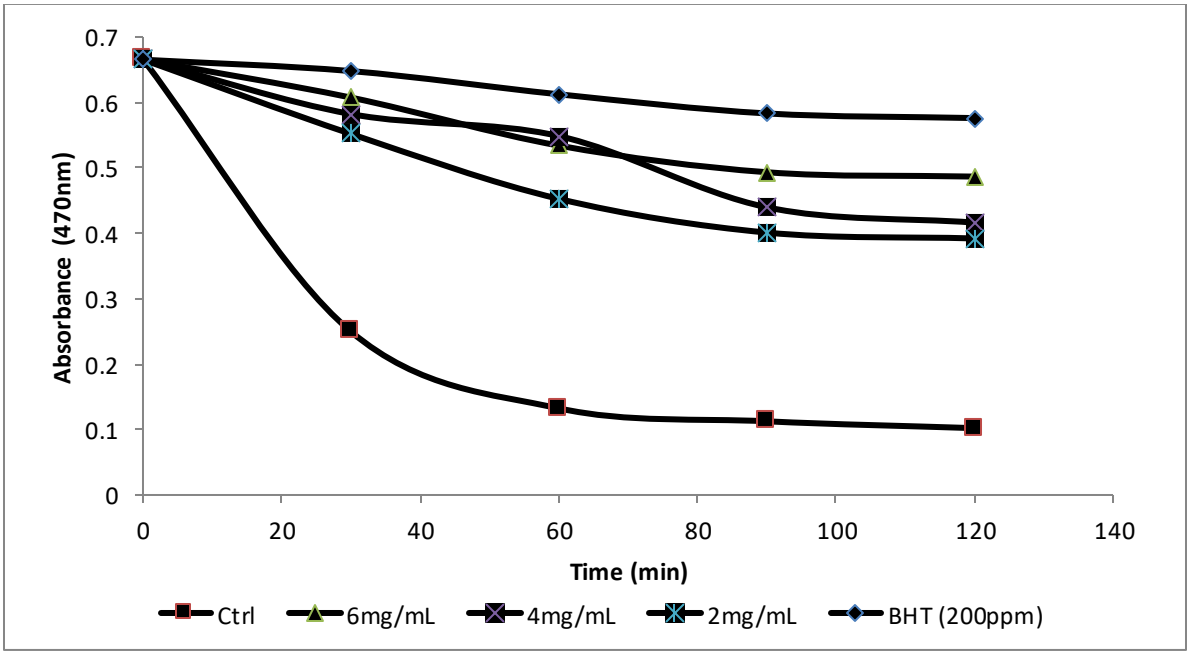
Table 4.8.2

Antioxidant activity of *Myristica fragrans (M)* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*M. fragrans (M)*			BHT
IC_{50} (µg/mL)	21.95±2.14			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	62.13±1.6	44.21±1.4	19.01±1.1	85.10±3.2

Values are mean ± standard deviation of *Myristica fragrans (M)*, analyzed individually in triplicate.

Fig. 4.8.1



Antioxidant activity of *Myristica fragrans (M)* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.8.3

Antimicrobial activity of *Myristica fragrans (M)* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Myristica fragrans (M)		
Inhibition zone (mm)			
B. subtilis	6.7±1.5	10.8±2.1	-
L. rhamnosus	12.6±0.4	15.5±0.8	-
S. aureus	10.7±1.5	15.1±1.0	-
S. mutans	**14.3±0.7**	**13.4±0.7**	-
E. coli	**10.3±1.1**	**9.6±1.3**	-
P. multocida	8.3±1.1	11.6±1.5	-
A. alternata	11.7±0.6	-	14.2±0.8
A. flavus	6.3±0.5	-	11.4±1.4

A. niger	21.6±1.5	-	21.7±1.2
G. lucidum	8.7±0.7	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	3.44±0.3	1.72±0.2	-
L. rhamnosus	2.19±0.2	2.81±0.3	-
S. aureus	2.81±0.1	2.19±0.2	-
S. mutans	**1.72± 0.2**	**2.03±0.1**	-
E. coli	**1.88±0.1**	**2.34±0.2**	-
P. multocida	2.5±0.1	2.03±0.1	-
A. alternata	2.81±0.2	-	2.34±0.2
A. flavus	3.75±0.1	-	2.81±0.2
A. niger	1.01±0.2	-	0.94±0.1
G. lucidum	2.03±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Myristica fragrans (M)*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.8.4 FFA of edible oil stabilized by *Myristica fragrans (M)* essential oil

IPD Induction Period in Days	% FFA			
	Myristica fragrans (M) ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.14±0.011	0.11±0.012	0.08±0.01	0.16±0.014
30	0.18±0.015	0.12±0.014	0.09±0.015	0.22±0.016
45	0.21±0.014	0.15±0.02	0.11±0.012	0.34±0.03
60	0.25±0.02	0.19±0.014	0.14±0.014	0.48±0.02
75	0.29±0.017	0.22±0.018	0.18±0.018	0.57±0.03
90	0.34±0.02	0.27±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Myristica fragrans*, analyzed individually in triplicate.

Table 4.8.5 PV of edible oil stabilized by *Myristica fragrans (M)* essential oil

IPD Induction Period in Days	PV			
	Myristica fragrans (M) ppm		BHT 200ppm	Control
	500	**1000**		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	1.0±0.02	0.7±0.03	0.4±0.01	1±0.03
30	1.6±0.1	1.2±0.21	0.6±0.16	1.81±0.12
45	2.3±0.17	1.7±0.24	0.9±0.21	2.5±0.24
60	3.0±0.2	2.4±0.16	1.4±0.29	3.4±0.36
75	3.7±0.22	2.9±0.21	1.9±0.24	4.7±0.21
90	4.4±0.25	3.5±0.24	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Myristica fragrans (N)*, analyzed individually in triplicate.

Table 4.8.6

***para*-Anisidine Value of edible oil stabilized by *Myristica fragrans (M)* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	Myristica fragrans (M) ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	6.1±0.24	5.1±0.31	3.4±0.27	10.2±0.52
30	8.9±0.33	7.8±0.36	5.8±0.36	17.5±0.74
45	12.7±0.37	10.9±0.27	8.5±0.27	24.1±0.81
60	16.4±0.28	14.6±0.55	10.9±0.55	29.6±0.63
75	21.4±0.83	18.8±0.47	13.6±0.47	37.4±1.2
90	26.8±0.75	22.5±0.55	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Myristica fragrans (M)*, analyzed individually in triplicate.

Table 4.8.7

Cytotoxicity (% hemolysis) of *Myristica fragrans (M)* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.52%	0.31%
5mg/mL	8.95%	4.67%
10mg/mL	9.93%	7.24%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Myristica fragrans (M)*, analyzed individually in triplicate.

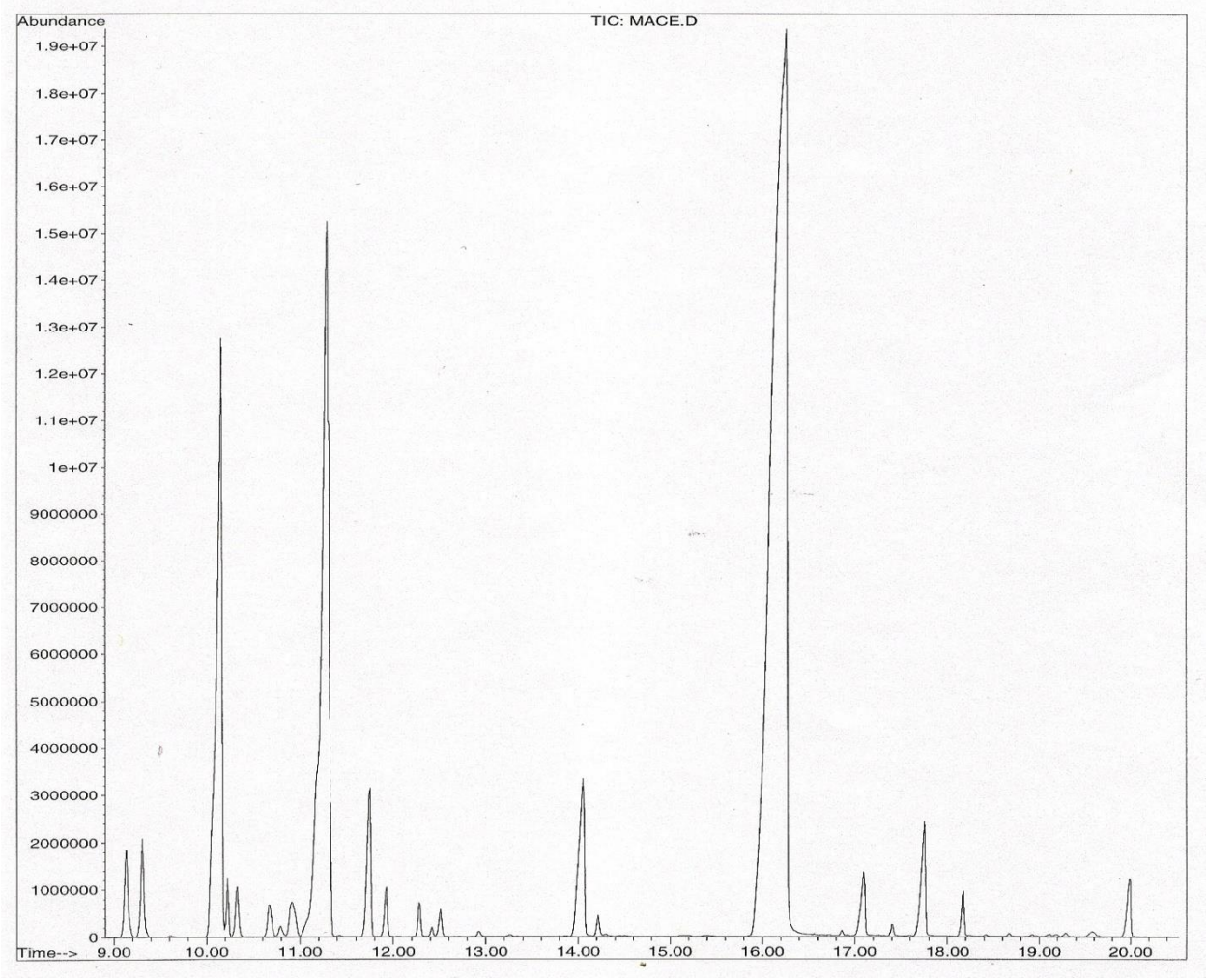


Figure 4.8.2. Typical GC-MS chromatogram of essential oil from *Myristica fragrans (M)*

Table 4.8.8. Chemical composition of *Myristica fragrans (M)* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
Cyclofenchene	896	0.28	RI, MS
α-Thujene	926	1.21	RT, RI, MS
α-pinene	934	1.19	RT, RI, MS
Camphene	955	0.25	RT, RI, MS
Sabinene	972	0.48	RI, MS
β-Myrcene	994	0.62	RT, RI, MS
α-Phellandrene	1005	0.49	RT, RI, MS
3-Carene	1011	0.72	RT, RI, MS
α-Terpinene	1020	0.75	RT, RI, MS
1-Methyl-2-propylcyclopentane	1031	0.24	RI, MS
β-Phellandrene	**1032**	**18.27**	**RT, RI, MS**
Limonene	1034	0.83	RT, RI, MS
α- Terpinolen	1089	0.36	RT, RI, MS
Linalool	1096	0.31	RT, RI, MS
L-Pinocarveol	1140	0.49	RI, MS
dihydro α-terpineol	1162	0.46	RI, MS
L-Terpinen-4-ol	1178	3.35	RI, MS
3-p-Menthene	**1234**	**10.76**	**RT, RI, MS**
trans-p-Menth-2-en-1,8-diol	1266	0.56	RI, MS
β-Methylallylbenzene	1270	0.36	RI, MS
Safrole	**1287**	**49.09**	**RT, RI, MS**

Geranyl formate	1300	0.29	RT, RI, MS
Eugenol	1356	0.98	RT, RI, MS
Patchoulane	1378	0.38	RI, MS
α-Copaene	1396	0.56	RT, RI, MS
Methyl Eugenol	1401	1.83	RT, RI, MS
α-Caryophyllene	1454	0.49	RT, RI, MS
α-Farnesene	1509	0.31	RT, RI, MS
Myristicin	1521	0.52	RI, MS
Spathulenol	1577	0.26	RT, RI, MS
β-Eudesmol	1652	0.64	RI, MS
Myristic acid	1720	0.83	RT, RI, MS
Stearic acid	2123	0.35	RT, RI, MS
Total (33)		98.51	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.8.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Myristica fragrans* (Mace) was found to be 1.75 % as listed in Table 4.8.1 which shows that plant was enriched with essential oil. The oil was light brown in colour with strong aromatic smell and refractive index 1.374. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.95. Kapoor *et al.* (2013) got 3.4 % of colourless volatile oil of *M. fragrans* with characteristic odour and sharp taste.

The essential oil of *M. fragrans (M)* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *M. fragrans (M)* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.8.2, which was found to be 21.95 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.8.2 showed the % inhibition in linoleic acid system by *M. fragrans (M)* essential oil taken at three different concentrations. It is clear that maximum inhibition was 62.13 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can

easily be examined by depleting the colour of solution using spectrophotometer at 470nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *M. fragrans (M)* is shown in Figure 4.8.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *M. fragrans (M)* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Chatterjee *et al*. (2007) evaluated that Mace acetone extract exhibited a higher antioxidant activity. Higher activity observed was attributed due to the presence of other components such as lycopene that contributes to the antioxidant activity of the total extract.

The antimicrobial activity of the essential oil of *M. fragrans (M)* against various microbes including bacteria and fungi is shown in Table 4.8.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *M. fragrans (M)* showed potential as an antimicrobial agent with larger inhibition zones (6.7-14.3 and 6.3-21.6 mm) and smaller MIC values (1.72-3.44 and 1.01-3.75 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *S. mutans* (Gram positive) with largest inhibition zone 14.3mm followed by minimum MIC value 1.72 mg/mL. These results are quite better than that of antibiotic, Rifampicin, which showed the inhibition zone 13.4 mm and MIC 2.03 mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 21.6 mm followed by minimum MIC value 1.01 mg/mL which was closer to that of antibiotic, Terbinafine, with inhibition zone 21.7mm and MIC 0.94 mg/mL. Shafiei , et al. (2012) tested extracts flesh, mace and seed of *Myristica fragrans* against different pathogens. It is clear that extract of flesh showed better activity than that of extract of seed and mace. In another study the extract of mace was found effective against majority of tested bacteria; MIC ranged between 9.4 to 37.5 mg/mL (Malti *et al*., 2008).

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.8.4 shows the effect of *M. fragrans (M)* essential oil on

formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *M. fragrans (M)* essential oil at 1000 ppm which was 0.27 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.8.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.5 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.8.6 for *S. aromaticum*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *M. fragrans (M)* essential oil sample of 1000 ppm with value 22.5 while in case of BHT it was found to be 17.9.

The effect of *M. fragrans (M)* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.8.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *M. fragrans (M)* exhibited the maximum hemolysis i.e. 7.24 % at 10 mg/mL concentration and the minimum of 0.31 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.93% at 10 mg/mL concentration and the minimum of 0.52 % at 0.5 mg/mL. Malti *et al*. (2008) tested the cytotoxicity of mace extract on mice and found that its extract was toxic at doses ≥ 3 mg/g.

The data of chemical composition of the essential oil from *M. fragrans (M)* was listed in Table 4.8.8. Total 33 compounds were identified representing 98.51 % of the oil. The major components were found to be safrole (49.09 %), β-Phellandrene (18.27%), 3-p-Menthene (10.76 %) and L-terpinen-4-ol (3.35 %). Chatterjee *et al.* (2007) obtained mace from fresh fruits of *M. fragrans* collected from Kottakal, Kerala (India). The extract was prepared in 10% methanol and subjected for chemical characterization by GC-MS. Major compounds were found to be isoeugenol acetate (29.18 %), elemicin (13.63 %) and 6-methoxy-eugenol acetate (10.30 %).

4.9. *Murraya koenigii*

Table 4.9.1

Physical properties of *Murraya koenigii* essential oil

Parameter	***Murraya koenigii***
Colour	Greenish yellow
Yield (%)	1.12±0.1
Refractive Index (30 °C)	1.357±0.002
Specific Gravity	0.95±0.01

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

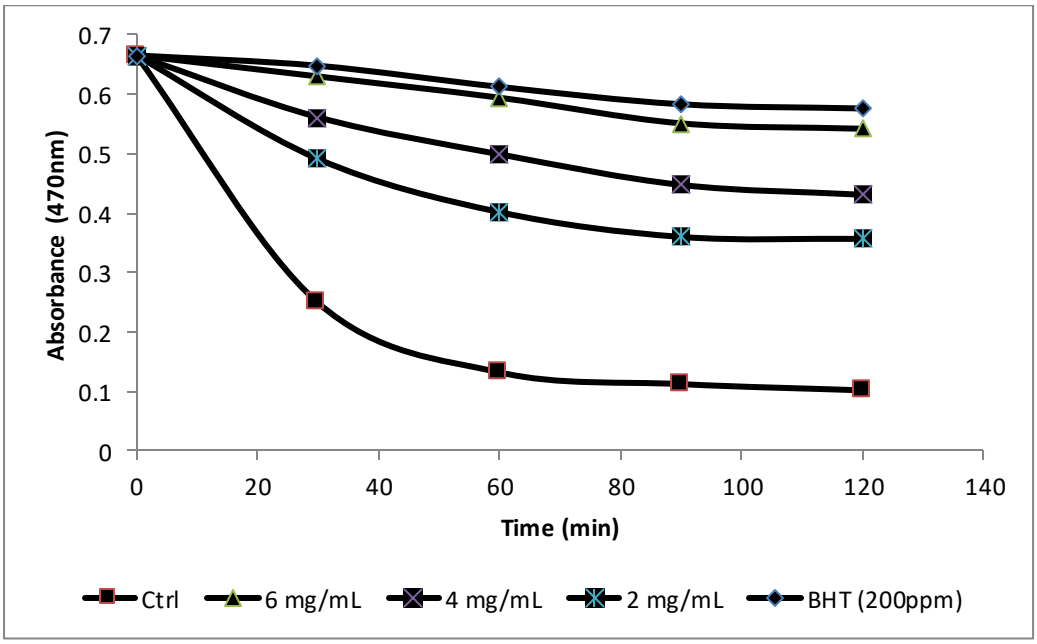
Table 4.9.2

Antioxidant activity of *Murraya koenigii* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*M. koenigii*			BHT
IC_{50} (µg/mL)	26.68±2.72			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	82.78±2.7	71.44±3.1	49.26±1.2	85.10±3.2

Values are mean ± standard deviation of *Murraya koenigii*, analyzed individually in triplicate.

Fig. 4.9.1



Antioxidant activity of *Murraya koenigii* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.9.3

Antimicrobial activity of *Murraya koenigii* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Murraya koenigii		
Inhibition zone (mm)			
B. subtilis	5.2±0.6	10.8±2.1	-
L. rhamnosus	12.4±0.6	15.5±0.8	-
S. aureus	14.3±0.7	15.1±1.0	-
S. mutans	**13.4±0.2**	**13.4±0.7**	-
E. coli	9.3±0.5	9.6±1.3	-
P. multocida	10.4±0.5	11.6±1.5	-
A. alternata	**18.3±1.2**	-	**14.2±0.8**
A. flavus	**11.6±0.5**	-	**11.4±1.4**

A. niger	11.7±1.2	-	21.7±1.2
G. lucidum	**10.7±0.3**	-	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	4.06±0.3	1.72±0.2	-
L. rhamnosus	2.34±0.1	2.81±0.3	-
S. aureus	2.03±0.1	2.19±0.2	-
S. mutans	2.34±0.2	2.03±0.1	-
E. coli	2.81±0.2	2.34±0.2	-
P. multocida	2.5±0.1	2.03±0.1	-
A. alternata	**1.56±0.1**	-	**2.34±0.2**
A. flavus	**2.34±0.2**	-	**2.81±0.2**
A. niger	2.34±0.1	-	0.94±0.1
G. lucidum	2.5±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.9.4 FFA of edible oil stabilized by *Murraya koenigii* essential oil

IPD Induction Period in Days	% FFA			
	Murraya koenigii ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.1±0.011	0.08±0.01	0.08±0.01	0.16±0.014
30	0.13±0.013	0.1±0.012	0.09±0.015	0.22±0.016
45	0.18±0.016	0.13±0.016	0.11±0.012	0.34±0.03
60	0.23±0.018	0.15±0.02	0.14±0.014	0.48±0.02
75	0.27±0.02	0.19±0.018	0.18±0.018	0.57±0.03
90	0.34±0.02	0.23±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

Table 4.9.5 PV of edible oil stabilized by *Murraya koenigii* essential oil

IPD Induction Period in Days	PV			
	Murraya koenigii ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.7±0.013	0.4±0.01	0.4±0.01	1±0.03
30	1.1±0.15	0.71±0.12	0.6±0.16	1.81±0.12
45	1.6±0.19	1.1±0.16	0.9±0.21	2.5±0.24
60	2.1±0.18	1.6±0.21	1.4±0.29	3.4±0.36
75	2.9±0.2	2.2±0.24	1.9±0.24	4.7±0.21
90	3.6±0.2	2.6±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

Table 4.9.6 *para*-Anisidine Value of edible oil stabilized by *Murraya koenigii* essential oil

IPD Induction Period in Days	*para*-Anisidine Value			
	Murraya koenigii ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	4.9±0.22	4.1±0.36	3.4±0.27	10.2±0.52
30	7.6±0.35	6.5±0.31	5.8±0.36	17.5±0.74
45	10.6±0.51	9.3±0.47	8.5±0.27	24.1±0.81
60	15.4±0.83	13.7±0.36	10.9±0.55	29.6±0.63
75	19.6±1.1	17.6±0.55	13.6±0.47	37.4±1.2
90	25.3±1.4	22.1±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

Table 4.9.7

Cytotoxicity (% hemolysis) of *Murraya koenigii* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.08%	0.34%
5mg/mL	1.80%	3.14%
10mg/mL	8.26%	9.53%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Murraya koenigii*, analyzed individually in triplicate.

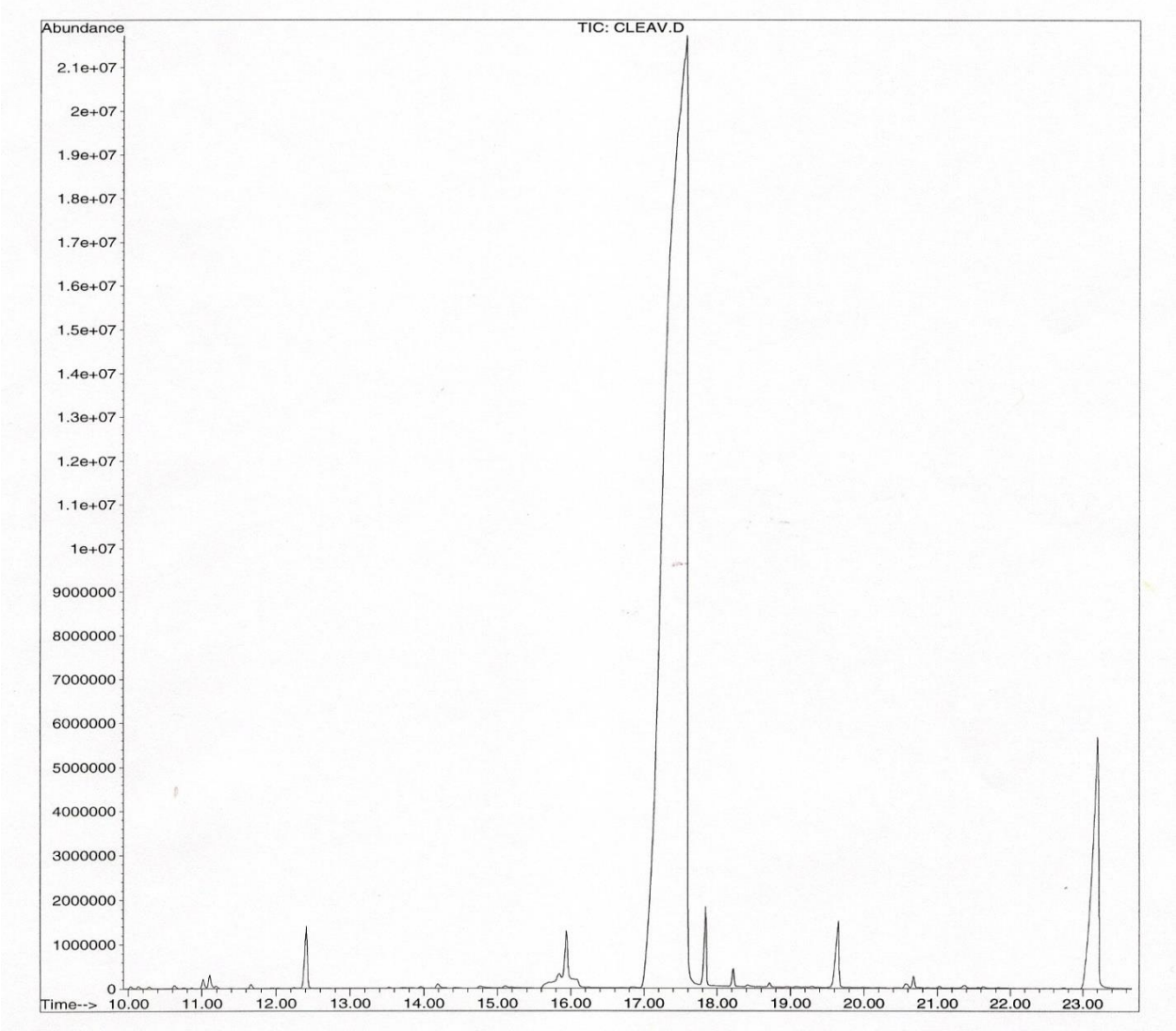


Figure 4.9.2. Typical GC-MS chromatogram of essential oil from *Murraya koenigii*

Table 4.9.8. Chemical composition of *Murraya koenigii* essential oil

Components[1]	**RI**[2]	**% age**	**Mode of Identification**[3]
Cyclofenchene	896	0.41	RI, MS
Cumene	926	0.22	RT, RI, MS
Camphene	955	0.46	RT, RI, MS
β-Thujene	970	0.38	RT, RI, MS
Sabinene	972	0.34	RI, MS
α -Phallendrene	1005	0.28	RT, RI, MS
α-Terpinene	1020	0.29	RT, RI, MS
Limonene	1034	0.24	RT, MS
γ-Terpinen	1072	0.43	RT, RI, MS
2-phenylethyl alcohol	1116	0.26	RI, MS
3-p-Menthene	1234	0.3	RT, RI, MS
Linalyl formate	1237	0.65	RI, MS
Cumyl alcohol	1296	0.19	RT, MS
Linalyl propanoate	1321	0.37	RT, MS
α-Terpinyl acetate	1350	0.44	RT, RI, MS
Eugenol	**1356**	**81.61**	**RT, RI, MS**
Cinnamic acid	1387	0.52	RT, RI, MS
Iso-caryophyllene	1442	0.18	RT, MS
α –himachalene	1451	0.44	RI, MS
Myristicin	**1521**	**1.00**	**RI, MS**
Eugenyl acetate	1524	0.84	RT, RI, MS
Geranyl butyrate	1562	0.33	RT, MS
1-tert-Butyl-*1,5*-*Cyclooctadiene*	1752	0.11	RT, MS
Benzyl Benzoate	**1764**	**7.13**	**RI, MS**
Isoeugenol	2250	0.9	RT, MS
Total (25)		98.43	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.9.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Murraya koenigii* was found to be 1.12 % as listed in Table 4.9.1. The oil was greenish yellow in colour with strong aromatic smell and refractive index 1.357. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.95. Nagappan *et al.* (2011) found the yield of colourless oil (0.12 %) based on fresh weight of *M. koenigii* with characteristic odour and sharp taste.

The essential oil of *M. koenigii* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *M. koenigii* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.9.2, which was found to be 26.68 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.9.2 shows the % inhibition in linoleic acid system by *M. koenigii* essential oil taken at three different concentrations. It is clear that maximum inhibition was 82.78 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10% inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting

the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *M. Koenigii* is shown in Figure 4.9.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *M. koenigii* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. The antioxidant properties of the leaf extracts of *M. Koenigii* using different solvents were evaluated based on the oil stability index OSI together with their radical scavenging ability against DPPH (Kureel *et al.,* 1969)**.** Rao *et al.* (2006) extracted two carbazole alkaloids, Mahanimbine and koenigine, from the leaves of *M. koenigii*, showed antioxidant activity. Koenigine also showed a high degree of radical-scavenging properties

The antimicrobial activity of the essential oil of *M. koenigii* against various microbes including bacteria and fungi is shown in Table 4.9.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *M. koenigii* showed potential as an antimicrobial agent with larger inhibition zones (5.2-14.3 and 10.7-18.3 mm) and smaller MIC values (2.03-4.06 and 1.56-2.5 mg/mL) against various bacterial and fungal strians, respectively. In case of bacteria maximum activity was found against *S. aureus* (Gram positive) with largest inhibition zone 14.3 mm followed by minimum MIC value 2.03 mg/mL. These results are quite comparable to that of antibiotic, Rifampicin, which showed the inhibition zone 15.1 mm and MIC 2.03 mg/mL. In case of fungi, *A. alternata* showed to be most sensitive with largest inhibition zone 18.3 mm followed by minimum MIC value 2.34 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 14.2 mm and MIC 2.81 mg/mL. Nagappan *et al.* (2011) tested the essential oil of *M. koenigii* against various bacterial strains and found that the range of diameter of inhibition was between 10.00 mm to 18.50 mm, showing greater activity towards *Streptococcus pneumoniae*. The minimal value of inhibition (MIC) was 25.00 µg/mL against *Psedomonas aeruginosa* and *Klebsiella pneumoniae* while 200.00 µg/mL was the minimal bactericidal concentration (MBC) against *S. pneumoniae*.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well

checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.9.4 shows the effect of *M. koenigii* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *M. koenigii* essential oil at 1000ppm which was 0.23 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.9.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 2.6 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value is an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.9.6 for *M. koenigii*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *M. koenigii* essential oil sample of 1000ppm with value 22.1 while in case of BHT it was found to be 17.9.

The effect of *M. koenigii* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked using three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.8.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *M. koenigii* exhibited the maximum hemolysis i.e. 9.53% at 10 mg/mL concentration and the minimum of 0.34 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.26 % at 10 mg/mL concentration and the minimum of 0.08 % at 0.5 mg/mL. Manfred *et al*. (2008) evaluated that carbazole alkaloid extracted from *M. koenigii* showed significant cytotoxic effect.

The data of chemical composition of the essential oil from *M. koenigii* is listed in Table 4.9.8. Total 25 compounds were identified representing 98.43 % of the oil. The major components were found to be eugenol (81.61 %), benzyl benzoate (7.13 %) and myristicin (1.0 %). Nagappan *et al.* (2011) obtained collected *M. koenigii* leaves from Kota Belud, Sabah (Malaysia), which were subjected to extraction of essential oil followed by chemical characterization. Major compounds were found to be β-caryophyllene (19.50 %), α-humulene (15.24 %), *p*-cymen-8-ol (10.31 %), phytol (10.07 %) and α-selinene (6.10 %).

4.10. *Piper nigrum*

Table 4.10.1

Physical properties of *Piper nigrum* essential oil

Parameter	***Piper nigrum***
Colour	Dark brown
Yield (%)	3.75±0.3
Refractive Index (30 °C)	1.333±0.003
Specific Gravity	0.72±0.03

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

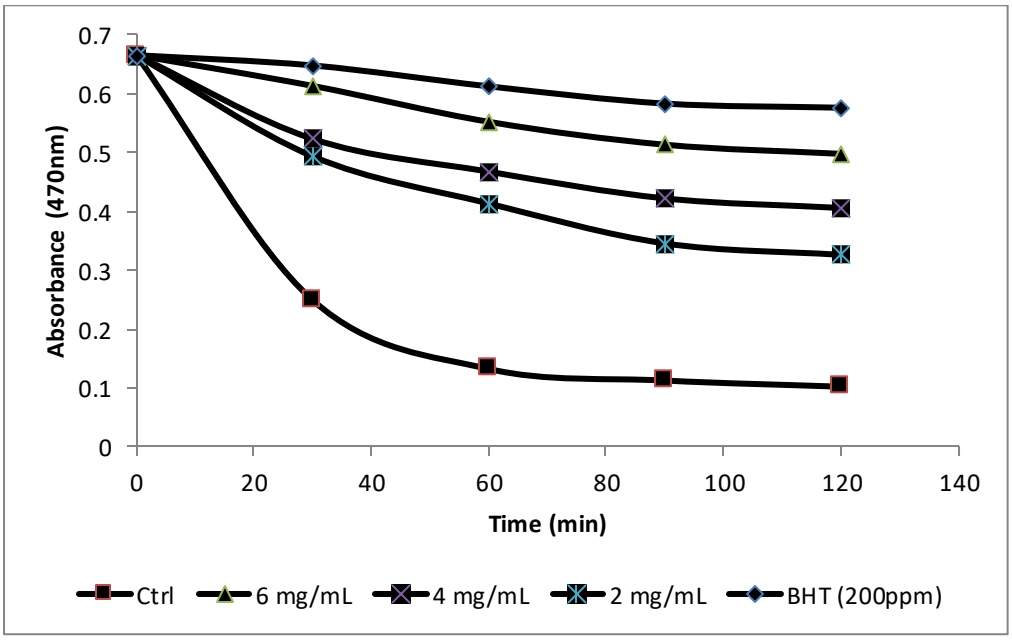
Table 4.10.2

Antioxidant activity of *Piper nigrum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*P. nigrum*			BHT
IC_{50} (µg/mL)	47.43±1.86			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	61.03±1.5	49.28±1.4	42.96±0.9	85.10±3.2

Values are mean± standard deviation of *Piper nigrum*, analyzed individually in triplicate.

Fig. 4.10.1



Antioxidant activity of *Piper nigrum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.10.3

Antimicrobial activity of *Piper nigrum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Piper nigrum		
Inhibition zone (mm)			
B. subtilis	8.5±0.3	10.8±2.1	-
L. rhamnosus	5.5±0.1	15.5±0.8	-
S. aureus	5.1±0.4	15.1±1.0	-
S. mutans	8.9±0.4	13.4±0.7	-
E. coli	**10.1±0.6**	**9.6±1.3**	-
P. multocida	**13.2±0.6**	**11.6±1.5**	-
A. alternata	11.7±0.4	-	14.2±0.8
A. flavus	**19.3±1.2**	-	**11.4±1.4**

A. niger	13.7±0.8	-	21.7±1.2
G. lucidum	**11.4±0.7**	-	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	3.12±0.2	1.72±0.2	-
L. rhamnosus	5.0±0.3	2.81±0.3	-
S. aureus	4.06±0.3	2.19±0.2	-
S. mutans	2.81±0.2	2.03±0.1	-
E. coli	2.5±0.1	2.34±0.2	-
P. multocida	2.34±0.2	2.03±0.1	-
A. alternata	2.34±0.1	-	2.34±0.2
A. flavus	**1.25±0.1**	**-**	**2.81±0.2**
A. niger	2.03±0.1	-	0.94±0.1
G. lucidum	2.34±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.10.4 FFA of edible oil stabilized by *Piper nigrum* essential oil

IPD Induction Period in Days	% FFA			
	Piper nigrum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.14±0.012	0.1±0.012	0.08±0.01	0.16±0.014
30	0.19±0.015	0.12±0.012	0.09±0.015	0.22±0.016
45	0.24±0.02	0.16±0.016	0.11±0.012	0.34±0.03
60	0.28±0.018	0.19±0.018	0.14±0.014	0.48±0.02
75	0.33±0.014	0.24±0.018	0.18±0.018	0.57±0.03
90	0.39±0.02	0.28±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

Table 4.10.5 PV of edible oil stabilized by *Piper nigrum* essential oil

IPD Induction Period in Days	PV			
	Piper nigrum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	1.0±0.1	0.7±0.03	0.4±0.01	1±0.03
30	1.7±0.13	1.21±0.12	0.6±0.16	1.81±0.12
45	2.3±0.17	1.7±0.16	0.9±0.21	2.5±0.24
60	2.7±0.18	2.31±0.12	1.4±0.29	3.4±0.36
75	3.7±0.2	3±0.21	1.9±0.24	4.7±0.21
90	4.4±0.22	3.8±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

Table 4.10.6

***para*-Anisidine Value of edible oil stabilized by *Piper nigrum* essential oil**

IPD Induction Period in Days	*para*-Anisidine Value			
	Piper nigrum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	6.5±0.21	5.6±0.31	3.4±0.27	10.2±0.52
30	9.6±0.43	8.4±0.36	5.8±0.36	17.5±0.74
45	14.7±0.35	12.7±0.31	8.5±0.27	24.1±0.81
60	17.5±0.62	16.2±0.36	10.9±0.55	29.6±0.63
75	22.2±0.58	20.7±0.55	13.6±0.47	37.4±1.2
90	27.4±0.96	24.4±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

Table 4.10.7

Cytotoxicity (% hemolysis) of *Piper nigrum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.27%	0.52%
5mg/mL	1.44%	1.67%
10mg/mL	9.65%	3.34%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Piper nigrum*, analyzed individually in triplicate.

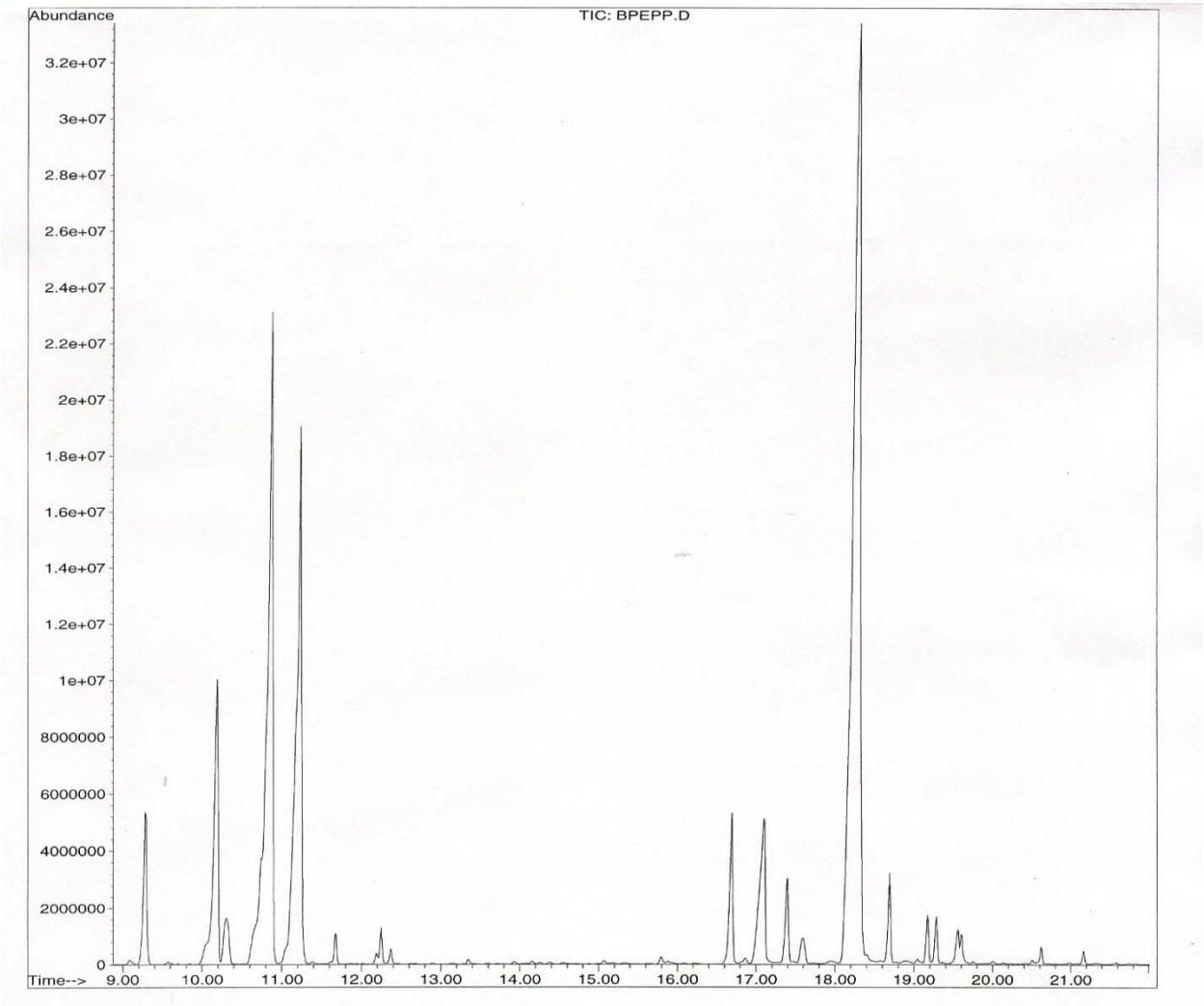


Figure 4.10.2. Typical GC-MS chromatogram of essential oil of *Piper nigrum*

Table 4.10.8. Chemical composition of *Piper nigrum* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
Cyclofenchene	896	0.26	RI, MS
α -Pinene	934	2.66	RT, RI, MS
α-Fenchene	952	0.27	RI, MS
β-Thujene	970	1.27	RT, RI, MS
Sabinene	972	0.31	RI, MS
β-Pinene	978	0.28	RT, RI, MS
3-Carene	**1011**	**18.22**	**RT, RI, MS**
Limonene	**1033**	**14.51**	**RT, RI, MS**
γ-Terpinen	1072	0.41	RT, RI, MS
L-Pinocarveol	**1148**	**6.74**	**RI, MS**
β-Citronellal	1162	0.31	RT, RI, MS
α- Terpinolen	1187	0.61	RT, RI, MS
Cumaldehyde	1240	0.19	RT, RI, MS
Piperitone	1282	0.67	RI, MS
trans-Anethole	1283	0.18	RI, MS
Linalyl propanoate	1321	0.20	RT, MS
α -Cubebene	1347	0.25	RT, RI, MS
Eugenol	**1356**	**4.45**	**RT, RI, MS**
Copaene	1366	1.53	RI, MS
β-Caryophyllene	**1418**	**39.14**	**RT, RI, MS**
δ-EIemene	1434	2.41	RT, RI, MS
α-Humulene	1448	0.22	RI, MS
α -caryophyllene	1454	1.27	RT, RI, MS
Cuparene	1503	0.66	RI, MS
7-epi-α-Cadinene	1522	0.27	RT, RI, MS
Eugenyl acetate	1524	0.99	RT, RI, MS
Spathulenol	1574	0.62	RT, RI, MS
Caryophyllene oxide	1578	0.24	RT, RI, MS
Cedrol	1598	0.16	RI, MS
α-cadinol	1653	0.28	RT, RI, MS
Hexadecanoic acid	1984	0.19	RT, RI, MS
Total (31)		98.88	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.10.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Piper nigrum* was found to be 3.75 % as listed in Table 4.10.1 which shows that plant was enriched with essential oil. The oil was dark brown in colour with strong aromatic smell and refractive index 1.333. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.72. Fan *et al.* (2011) extracted colourless essential oil rich in pungent fragrance with many volatile components from the fresh fruits of *P. nigrum* 0.8 % (v/w). Sasidharan and Menon (2010) obtained 2.2 % essential oil from fresh pepper berries, 2 % from dried pepper berries and 1.2 % from pepper leaves.

Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *P. nigrum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.10.2, which was found to be 47.43 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.10.2 shows the % inhibition in linoleic acid system by *P. nigrum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 61.03 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to

β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *P. nigrum* is shown in Figure 4.10.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *P. nigrum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. In another study *P. nigrum* inhibited lipid peroxidation by scavenging different radicals like hydroxyl and superoxide radicals (Muhtaseb *et al.* 2008; Neha and Mishra, 2011).

The antimicrobial activity of the essential oil of *P. nigrum* against various microbes including bacteria and fungi is shown in Table 4.10.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *P. nigrum* showed potential as an antimicrobial agent with larger inhibition zones (5.1-13.2 and 11.4-19.3 mm) and smaller MIC values (2.34-5.0 and 1.25-2.34 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *P. multocida* (Gram negative) with largest inhibition zone 13.2mm followed by minimum MIC value 2.34 mg/mL. These results are quite better than that of antibiotic, Rifampicin, which showed the inhibition zone 11.6mm and MIC 2.03 mg/mL. In case of fungi, *A. flavus* showed to be most sensitive with largest inhibition zone 19.3 mm followed by minimum MIC value 1.25 mg/mL which was much better to that of antibiotic, Terbinafine, with inhibition zone 11.4mm and MIC 2.81 mg/mL. Naz *et al.* (2009) tested different extracts of the roots of *Piper chaba* against various bacteria and fungi and found that activity of extracts was lower than that of the antibiotics i.e. Kanamycin in case of bacteria and Nystatin for fungi. Sasidharan and Menon (2010) evaluated that essential oil from fresh pepper berries, dry pepper berries and pepper leaves were more effective against *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.10.4 shows the effect of *P. nigrum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the

length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *P. nigrum* essential oil at 1000ppm which was 0.28% even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.10.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 3.8 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.10.6 for *M. koenigii*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *P. nigrum* essential oil sample of 1000ppm with value 24.4 while in case of BHT it was found to be 17.9.

The effect of *P. nigrum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.8.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *P. nigrum* exhibited the maximum hemolysis i.e. 3.34 % at 10 mg/mL concentration and the minimum of 0.52 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.65% at 10 mg/mL concentration and the minimum of 0.27 % at 0.5 mg/mL. Naz *et al.* (2009) tested cytotoxicity of various root extracts of *Piper chaba* against *Artemia salina* nauplii and found that petroleum ether, chloroform and ethyl acetate extracts were very potent cytotoxic in comparison to gallic acid.

The data of chemical composition of the essential oil from *P. nigrum* is listed in Table 4.10.8. Total 31 compounds were identified representing 98.88% of the oil. The major components were found to be β-caryophyllene (39.14%), 3-carene (18.22%), limonene (14.51%), L-Pinocarveol (6.74 %) and eugenol (4.45 %). Fan *et al.* (2011) collected fresh and dried fruits of black pepper from Sibu, Sarawak (Malaysia), which was subjected to extraction of essential oil followed by chemical characterization. Major compounds were found to be limonene (35.06 %), β-pinene (12.95 %), linalool (9.55 %), α-pinene (4.31 %) and caryophyllene (3.98 %). In another study Sasidharan and Menon (2010) collected plant material from Trivandrum, Kerala (India) and examined essential oil using GC-MS. limonene (18.0 %), β-pinene (14.2 %), β-caryophyllene (13.2 %), α-pinene (12.1 %) and 3-carene (3.2 %) were found to be the major components in fresh berries essential oil.

4.11. *Elettaria cardamomum*

Table 4.11.1

Physical properties of *Elettaria cardamomum* essential oil

Parameter	***Elettaria cardamomum***
Colour	Pale green
Yield (%)	5.40±0.3
Refractive Index (30 °C)	1.392±0.012
Specific Gravity	0.84±0.02

Values are mean ± standard deviation of three samples of each *Elettaria cardamomum*, analyzed individually in triplicate.

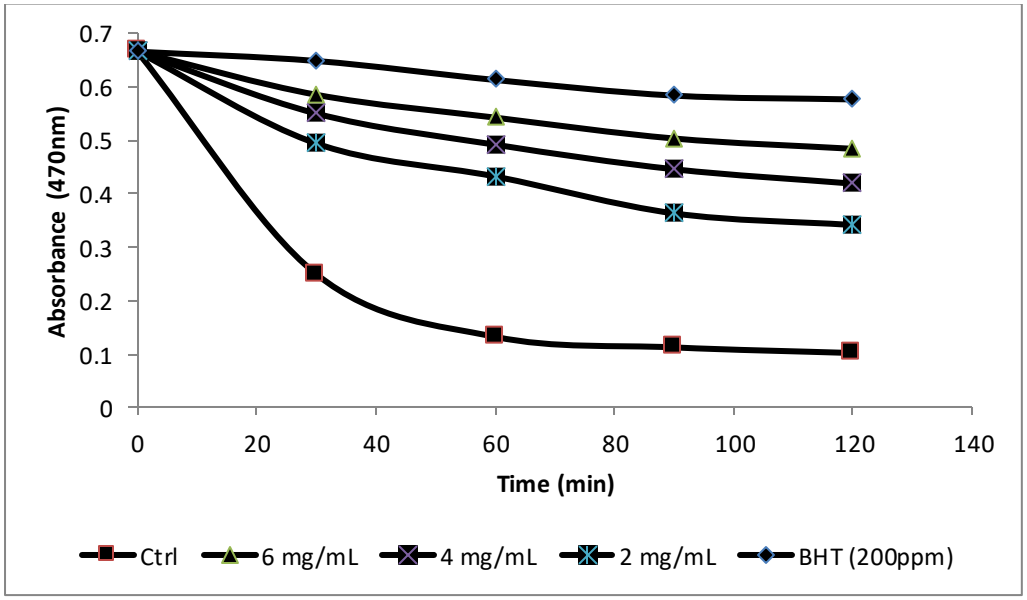
Table 4.11.2

Antioxidant activity of *Elettaria cardamomum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*E. cardamomum*			BHT
IC_{50} (µg/mL)	33.6±0.22			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	74.44±2.6	62.94±1.8	38.84±0.7	85.10±3.2

Values are mean ± standard deviation of *Elettaria cardamomum*, analyzed individually in triplicate.

Fig. 4.11.1



Antioxidant activity of *Elettaria cardamomum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.11.3

Antimicrobial activity of *Elettaria cardamomum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Elettaria cardamomum		
Inhibition zone (mm)			
B. subtilis	7.8±0.5	10.8±2.1	-
L. rhamnosus	11.6±0.3	15.5±0.8	-
S. aureus	4.3±0.5	15.1±1.0	-
S. mutans	12.7±0.7	13.4±0.7	-
E. coli	8.3±0.2	9.6±1.3	-
P. multocida	8.7±0.5	11.6±1.5	-
A. alternata	8.2±0.1	-	14.2±0.8
A. flavus	6.3±0.7	-	11.4±1.4
A. niger	**21.7±2.0**	**-**	**21.7±1.2**

G. lucidum	7.2±0.5	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	3.44±0.3	1.72±0.2	-
L. rhamnosus	2.34±0.2	2.81±0.3	-
S. aureus	5.0±0.3	2.19±0.2	-
S. mutans	2.34±0.2	2.03±0.1	-
E. coli	3.12±0.2	2.34±0.2	-
P. multocida	3.44±0.3	2.03±0.1	-
A. alternata	3.12±0.2	-	2.34±0.2
A. flavus	4.37±0.3	-	2.81±0.2
A. niger	1.17±0.1	-	0.94±0.1
G. lucidum	3.44±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Elettaria cardamomum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.11.4 FFA of edible oil stabilized by *Elettaria cardamomum* essential oil

IPD Induction Period in Days	% FFA			
	Elettaria cardamomum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.13±0.011	0.09±0.015	0.08±0.01	0.16±0.014
30	0.17±0.014	0.11±0.014	0.09±0.015	0.22±0.016
45	0.22±0.013	0.15±0.014	0.11±0.012	0.34±0.03
60	0.26±0.017	0.19±0.016	0.14±0.014	0.48±0.02
75	0.32±0.02	0.24±0.02	0.18±0.018	0.57±0.03
90	0.4±0.022	0.27±0.018	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Elettaria cardamomum*, analyzed individually in triplicate.

Table 4.11.5 PV of edible oil stabilized by *Elettaria cardamomum* essential oil

IPD Induction Period in Days	PV			
	Elettaria cardamomum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.8±0.015	0.6±0.02	0.4±0.01	1±0.03
30	1.4±0.018	1±0.16	0.6±0.16	1.81±0.12
45	2.0±0.02	1.6±0.21	0.9±0.21	2.5±0.24
60	2.8±0.022	2.1±0.16	1.4±0.29	3.4±0.36
75	3.7±0.026	2.8±0.24	1.9±0.24	4.7±0.21
90	4.5±0.03	3.4±0.21	0.4±0.01	6.1±1.24

Values are mean ± standard deviation of three samples of each *Elettaria cardamomum*, analyzed individually in triplicate.

Table 4.11.6

***para*-Anisidine Value of edible oil stabilized by *Elettaria cardamomum* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Elettaria cardamomum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	0.4±0.01	2.7±0.12
15	6.3±0.19	5.4±0.27	0.4±0.01	10.2±0.52
30	9.4±0.33	8.3±0.47	0.6±0.16	17.5±0.74
45	13.3±0.41	11.7±0.27	0.9±0.21	24.1±0.81
60	16.8±0.39	15.4±0.55	1.4±0.29	29.6±0.63
75	21.6±0.51	21.8±0.47	1.9±0.24	37.4±1.2
90	33.4±1.4	26.1±0.55	2.3±0.16	45.8±1.8

Values are mean ± standard deviation of three samples of each ***Elettaria cardamomum***, analyzed individually in triplicate.

Table 4.11.7

Cytotoxicity (% hemolysis) of *Elettaria cardamomum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	1.51%	1.74%
5mg/mL	5.06%	6.13%
10mg/mL	9.72%	9.26%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Elettaria cardamomum*, analyzed individually in triplicate.

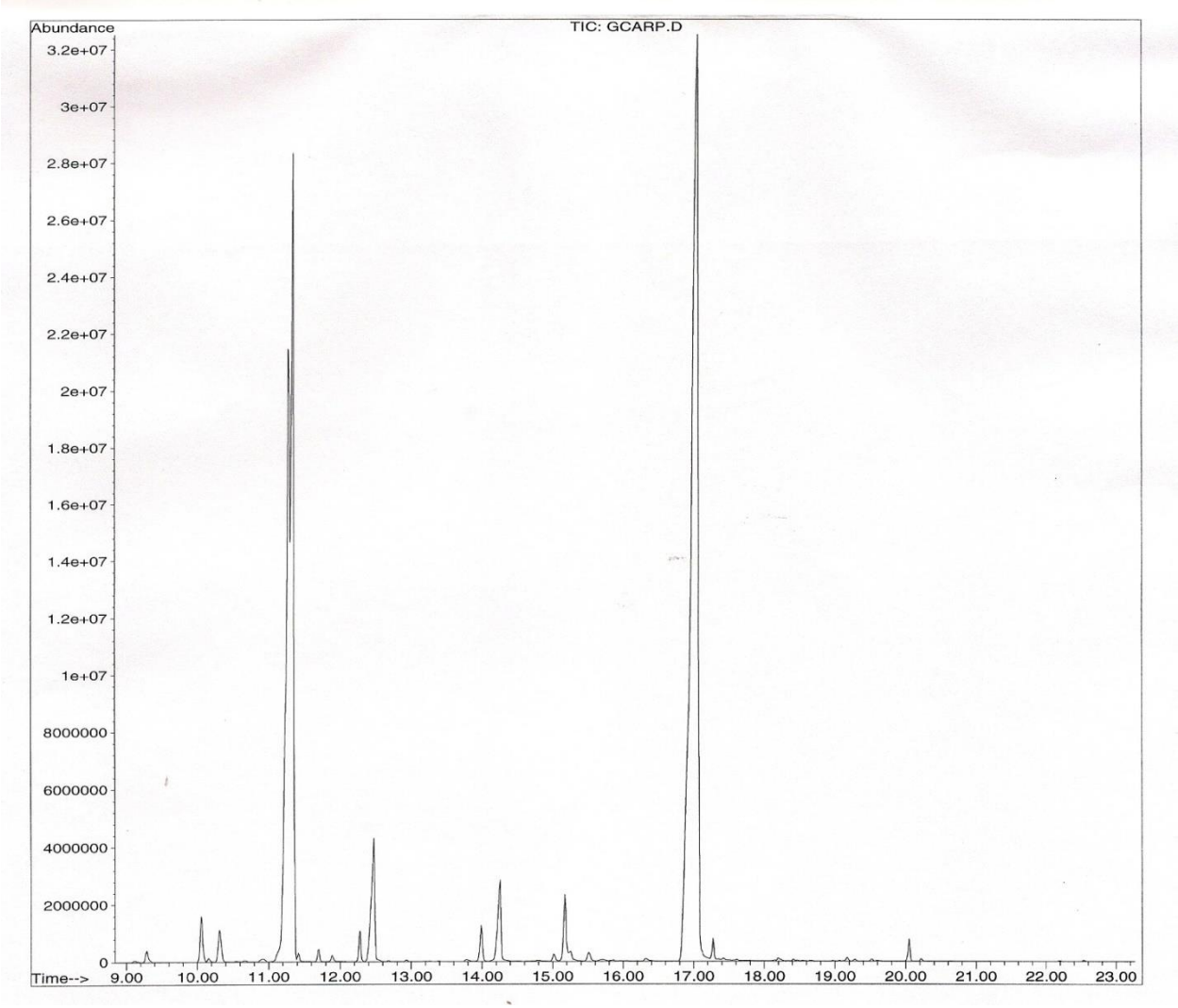


Figure 4.11.2. Typical GC-MS chromatogram of essential oil of *Elettaria cardamomum*

Table 4.11.8. Chemical composition of *Elettaria cardamomum* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
Cyclofenchene	896	0.30	RI, MS
α-Pinene	934	1.74	RT, RI, MS
Camphene	955	0.28	RT, RI, MS
Sabinene	972	0.86	RI, MS
β-Pinene	978	0.21	RT, RI, MS
β -Myrcene	994	0.73	RT, RI, MS
4-Carene	1002	0.18	RT, RI, MS
3-carene	1011	0.24	RT, RI, MS
α-Terpinene	1018	0.26	RT, RI, MS
D-Limonene	**1033**	**19.37**	**RT, RI, MS**
Eucalyptol	**1037**	**14.88**	**RT, RI, MS**
γ-Terpinen	1072	0.23	RT, RI, MS
Linaool	**1096**	**3.19**	**RT, MS**
L-terpinen-4-ol	1178	0.65	RI, MS
α- Terpinolen	1187	0.50	RT, RI, MS
3-p-Menthene	1234	0.35	RI, MS
Citral	1237	1.34	RT, RI, MS
trans-p-Menth-2-en-1-ol	1268	0.19	RI, MS
Geranial	1270	0.29	RT, RI, MS
α-Terpineol acetate	1335	1.99	RI, MS
Neryl acetate	1344	0.34	RT, RI, MS
α-Terpinyl acetate	**1350**	**48.32**	**RT, RI, MS**
Patchoulane	1378	0.32	RI, MS
α-Caryophyllene	1454	0.17	RT, RI, MS
β-Farnesene	1458	0.31	RT, RI, MS
γ-Gurjunene	1473	0.25	RI, MS
α -Farnesene	1509	0.23	RT, RI, MS
β-Bisabolene	1513	0.35	RI, MS
trans-nerolidol	1564	0.20	RT, RI, MS
Geranyl velerate	1625	0.24	RI, MS
Total (30)		98.40	

[1] Compounds are listed in order of elution from a ZB-5MS column.
[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.
[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.11.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Elettaria cardamomum* was found to be 5.40 % as listed in Table 4.11.1 which was the second most yield obtained among the plants taken in present study. The oil was pale green in colour with strong aromatic smell and refractive index 1.392. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.84. Marongiu *et al.* (2004) found the yield of *E. cardamomum* by hydrodistillation to be 5 %. Sereshti *et al.* (2011) found extraction yield of *E. cardamomum* was 3.1 % (w/w).

The DPPH radical scavenging by *E. cardamomum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.11.2, which was found to be 33.60 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.11.2 shows the % inhibition in linoleic acid system by *E. cardamomum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 74.44 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *E. cardamomum* is shown in Figure 4.11.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their

antioxidant potential, there was less depletion of colour. *E. cardamomum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Prakash *et al.* (2010) tested ethanol and water extracts of greater cardamom for their antioxidant potential and found the scavenging ability in terms of IC_{50} values to be 8.25 and 21.6 µg/mL, respectively. Both samples showed inhibition of oxidation of linoleic acid 41.2 and 35.96 %, respectively.

The antimicrobial activity of the essential oil of *E. cardamomum* against various microbes including bacteria and fungi is shown in Table 4.11.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *E. cardamomum* showed potential as an antimicrobial agent with larger inhibition zones (4.3-12.7 and 6.3-21.7 mm) and smaller MIC values (2.34-5.0 and 1.17-4.37 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *S. mutans* (Gram positive) with largest inhibition zone 12.7 mm followed by minimum MIC value 2.34 mg/mL. The activity was quite lesser than that of antibiotic, Rifampicin, which showed the inhibition zone 13.4 mm and MIC 2.03 mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 21.7 mm followed by minimum MIC value 1.17mg/mL which was equal to that of antibiotic, Terbinafine, with inhibition zone 21.7mm and MIC 0.94 mg/mL. Satyal *et al.* (2012) found that the seed and rind oils of black cardamom showed marginal activity against Gram-positive bacteria: *Bacillus cereus* (MIC = 625 and 313 µg/mL) and *Staphylococcus aureus* (MIC = 313 and 625 µg/mL), and antifungal activity against *Aspergillus niger* (MIC = 313 and 19.5 µg/mL, respectively). Agnihotri and Wakode (2010) found *Escherichia coli and Aspergillus niger* to be most sensitive microbes against essential oil of black cardamom.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.11.4 shows the effect of *E. cardamomum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum

stabilization was exhibited by *E. cardamomum* essential oil at 1000ppm which was 0.27% even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.11.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 3.4 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.11.6 for *M. koenigii*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *E. cardamomum* essential oil sample of 1000 ppm with value 26.1 while in case of BHT it was found to be 17.9.

The effect of *E. cardamomum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.11.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *E. cardamomum* exhibited the maximum hemolysis i.e. 9.26 % at 10 mg/mL concentration and the minimum of 1.74 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.72 % at 10 mg/mL concentration and the minimum of 1.51 % at 0.5 mg/mL. Satyal *et al.* (2012) evaluated that the seed and rind oil of black cardamomum also exhibited reasonable brine shrimp lethality (LC_{50}=28.1 and 15.0 µg/mL, respectively).

The data of chemical composition of the essential oil from *E. cardamomum* is listed in Table 4.11.8. Total 30 compounds were identified representing 98.40% of the oil. The major components were found to be α-terpinyl acetate (48.32 %), D-limonene (19.37 %), eucalyptol (14.88 %), linalyl alcohol (3.19 %) and α-terpineol acetate (1.99 %). Marongiu *et al.* (2004) obtained green cardamom seeds from Minardi (Italy), which were subjected to extraction of essential oil using different methods followed by their chemical characterization. Major compounds in hydrodistilled oil were found to be α-terpinyl acetate (37.7 %), eucalyptol (27.4 %), linalool (6.6 %), α-terpineol (5.0 %) and limonene (3.5 %). In another study Sereshti *et al.* (2012) collected plant material from local market of Tehran (Iran) and examined essential oil using GC-MS. α-terpinyl acetate (46.01 %), eucalyptol (27.73 %), linalool (5.27 %), α-terpineol (4.0 %) and linalyl acetate (3.52 %) were found to be the major components.

4.12. *Amomum subulatum*

Table 4.12.1

Physical properties of *Amomum subulatum* essential oil

Parameter	***Amomum subulatum***
Colour	Reddish brown
Yield (%)	1.47±0.1
Refractive Index (30 °C)	1.458±0.010
Specific Gravity	0.87±0.04

Values are mean ± standard deviation of three samples of each *Amomum subulatum*, analyzed individually in triplicate.

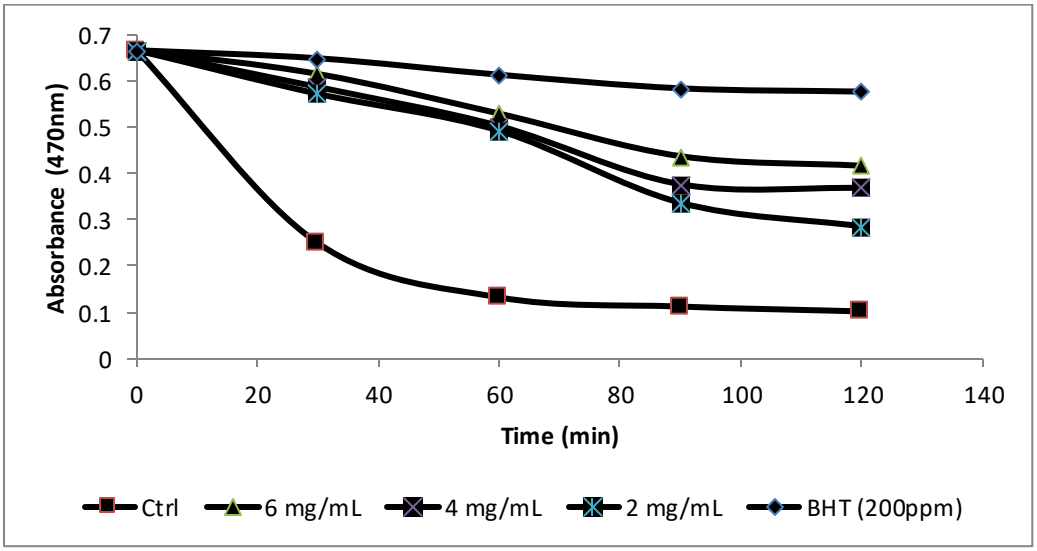
Table 4.12.2

Antioxidant activity of *Amomum subulatum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*A. subulatum*			BHT
IC_{50} (µg/mL)	46.57±2.81			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	69.40±2.3	46.59±2.1	32.05±1.1	85.10±3.2

Values are mean ± standard deviation of *Amomum subulatum*, analyzed individually in triplicate.

Fig. 4.12.1



Antioxidant activity of *Amomum subulatum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.12.3

Antimicrobial activity of *Amomum subulatum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Amomum subulatum		
Inhibition zone (mm)			
B. subtilis	5.4±0.3	10.8±2.1	-
L. rhamnosus	6.7±0.3	15.5±0.8	-
S. aureus	4.5±0.2	15.1±1.0	-
S. mutans	8.7±0.5	13.4±0.7	-
E. coli	6.2±0.5	9.6±1.3	-
P. multocida	8.5±0.6	11.6±1.5	-
A. alternata	6.5±0.2	-	14.2±0.8
A. flavus	9.3±0.5	-	11.4±1.4

A. niger	14.2±1.2	-	21.7±1.2
G. lucidum	8.3±0.8	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	3.75±0.2	1.72±0.2	-
L. rhamnosus	4.48±0.3	2.81±0.3	-
S. aureus	4.48±0.4	2.19±0.2	-
S. mutans	3.12±0.2	2.03±0.1	-
E. coli	3.75±0.2	2.34±0.2	-
P. multocida	3.12±0.2	2.03±0.1	-
A. alternata	4.37±0.3	-	2.34±0.2
A. flavus	2.81±0.1	-	2.81±0.2
A. niger	2.03±0.1	-	0.94±0.1
G. lucidum	3.44±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Amomum subulatum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70% : 30%) oil

Table 4.12.3 FFA of edible oil stabilized by *Amomum subulatum* essential oil

IPD Induction Period in Days	% FFA			
	Amomum subulatum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.14±0.011	0.1±0.02	0.08±0.01	0.16±0.014
30	0.18±0.013	0.13±0.012	0.09±0.015	0.22±0.016
45	0.25±0.017	0.15±0.016	0.11±0.012	0.34±0.03
60	0.29±0.02	0.19±0.018	0.14±0.014	0.48±0.02
75	0.35±0.022	0.24±0.018	0.18±0.018	0.57±0.03
90	0.42±0.025	0.29±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Amomum subulatum*, analyzed individually in triplicate.

Table 4.12.4 PV of edible oil stabilized by *Amomum subulatum* essential oil

IPD Induction Period in Days	PV			
	Amomum subulatum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	1.0±0.1	0.8±0.03	0.4±0.01	1.0±0.03
30	1.6±0.13	1.41±0.12	0.6±0.16	1.81±0.12
45	2.4±0.31	1.8±0.24	0.9±0.21	2.5±0.24
60	3.3±0.26	2.5±0.29	1.4±0.29	3.4±0.36
75	4.0±0.33	3.1±0.16	1.9±0.24	4.7±0.21
90	4.7±0.28	3.7±0.24	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Amomum subulatum*, analyzed individually in triplicate.

Table 4.12.5

***para*-Anisidine Value of edible oil stabilized by *Amomum subulatum* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Amomum subulatum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	6.5±0.27	5.7±0.31	3.4±0.27	10.2±0.52
30	10.1±0.35	9.1±0.36	5.8±0.36	17.5±0.74
45	15.9±0.31	13.6±0.31	8.5±0.27	24.1±0.81
60	19.4±0.73	18.2±0.62	10.9±0.55	29.6±0.63
75	22.9±0.91	24.3±0.55	13.6±0.47	37.4±1.2
90	34.7±1.4	29.1±0.47	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Amomum subulatum* analyzed individually in triplicate.

Table 4.12.7

Cytotoxicity (% hemolysis) of *Amomum subulatum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	1.71%	1.37%
5mg/mL	6.71%	4.86%
10mg/mL	9.40%	9.23%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Amomum subulatum*, analyzed individually in triplicate.

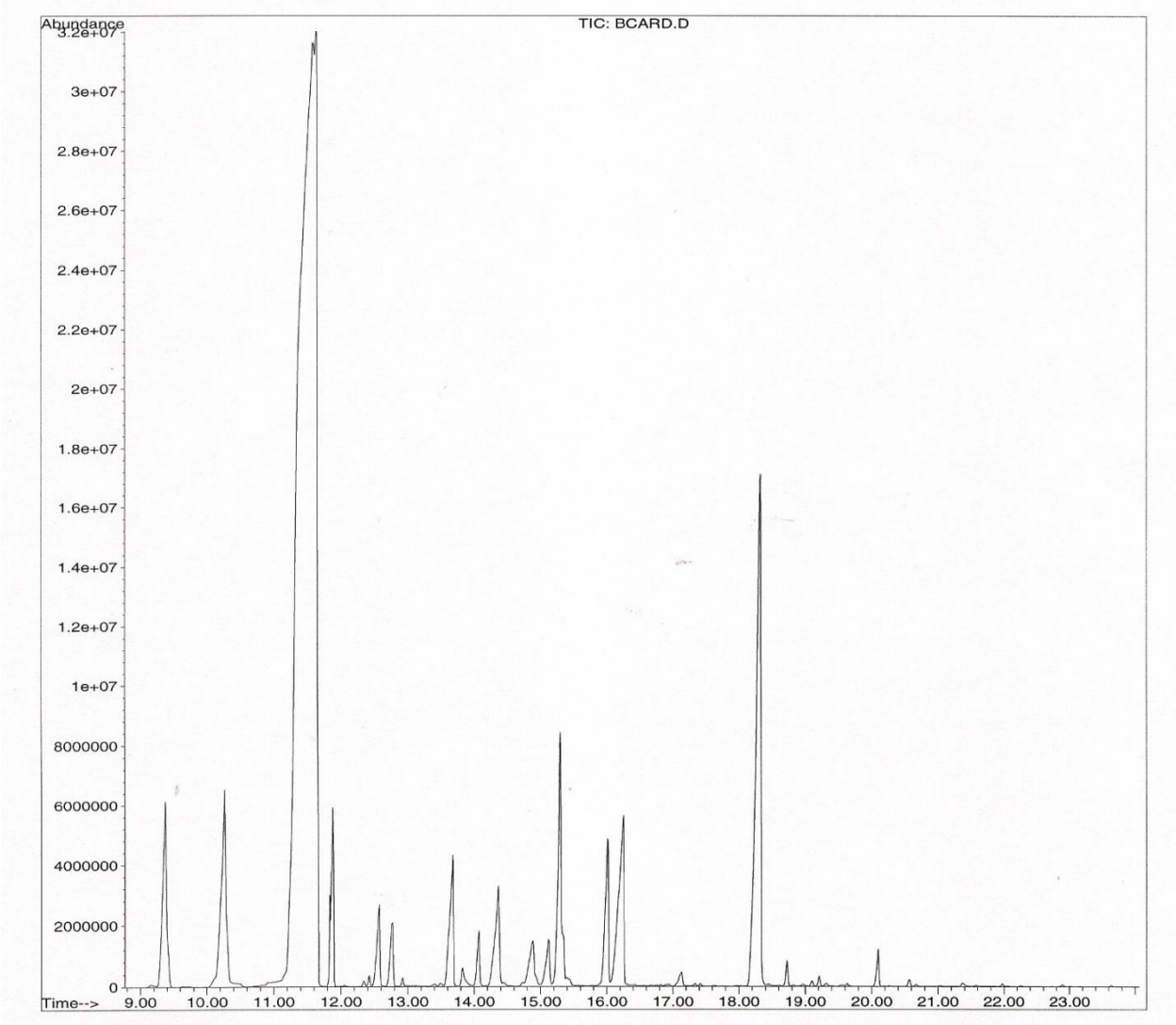


Figure 4.12.2. Typical GC-MS chromatogram of essential oil of *Amomum subulatum*

Table 4.12.8. Chemical composition of *Amomum subulatum* essential oil

Components[1]	**RI**[2]	**% age**	**Mode of Identification**[3]
α-Pinene	934	2.77	RT, RI, MS
Camphene	**955**	**3.27**	**RI, MS**
β -Myrcene	994	0.24	RT, RI, MS
α-Phallendrene	1005	0.18	RI, MS
α-Terpinene	1008	0.25	RT, RI, MS
3-Carene	1011	0.17	RT, RI, MS
Limonene	1033	0.62	RT, RI, MS
Eucalyptol	**1037**	**61.86**	**RT, RI, MS**
Linalool	1092	1.79	RI, MS
Fenchone	1096	0.20	RT, RI, MS
L-Pinocarveol	1148	0.19	RT, MS
Borneol	1169	0.26	RT, RI, MS
L-terpinen-4-ol	1178	0.51	RT, RI, MS
δ-Terpineol	1191	0.67	RT,RI, MS
Myrtenol	1196	0.63	RI, MS
trans-Piperitol	1208	0.23	RI, MS
3-p-Menthene	**1234**	**3.65**	**RT, RI, MS**
Citral	1237	1.41	RT, RI, MS
Isogeraniol	1275	0.21	RT, RI, MS
Bornyl acetate	1285	0.19	RI, MS
Carvacrol	**1299**	**3.33**	**RT, RI, MS**
Linalyl propanoate	1321	0.88	RT, MS
α-Terpenyl acetate	1344	0.26	RT, RI, MS
Eugenol	1356	0.19	RT, RI, MS
Copaene	1366	1.8	RI, MS
Patchoulane	1378	0.32	RI, MS
β-Elemene	1392	0.22	RI, MS
α-Humulene	1448	0.18	RT, RI, MS
Germacrene D	1451	0.23	RT, RI, MS
Caryophyllene	**1454**	**7.94**	**RT, RI, MS**
Nerolidol	1533	0.28	RI, MS
Spathulenol	1574	0.35	RT, RI, MS
L-Menthol	1623	1.87	RT, MS
α-Cadinol	1654	0.17	RI, MS
Myristic acid	1768	1.63	RT, RI, MS
Total (35)		98.91	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.12.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Amomum subulatum* was found to be 1.47% as listed in Table 4.12.1. The oil was reddish brown in colour with strong aromatic smell and refractive index 1.458. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.87. Joshi *et al.* (2012) obtained pale-coloured oil extracted from cardamom seeds collected from various regions in Himachal Pradesh with yield 9.8 to 19.5 g/kg (w/w). Satyal *et al.* (2012) obtained 4.5 % (seed oil) and 1.0 % (rind oil) of *A. subulatum*.

The essential oil of *A. subulatum* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. The DPPH radical scavenging by *A. subulatum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.12.2, which was found to be 46.57 µg/mL. The scavenging effect in case of synthetic antioxidant i.e. BHT was found to be 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.12.2 shows the % inhibition in linoleic acid system by *A. subulatum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 69.40 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *A. subulatum* is shown in Figure 4.12.1. The least antioxidant activity was

exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *A. subulatum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Prakash *et al.* (2010) tested ethanol and water extracts of greater cardamom for their antioxidant potential and found the scavenging ability in terms of IC_{50} values to be 8.25 and 21.6 µg/mL, respectively. Both samples showed inhibition of oxidation of linoleic acid 41.2 and 35.96 %, respectively.

The antimicrobial activity of the essential oil of *A. subulatum* against various microbes including bacteria and fungi is shown in Table 4.12.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *A. subulatum* showed potential as an antimicrobial agent with larger inhibition zones (4.5-8.7 and 6.5-14.2 mm) and smaller MIC values (3.12-4.48 and 2.03-4.37 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *S. mutans* (Gram positive) with largest inhibition zone 8.7mm followed by minimum MIC value 3.12 mg/mL. The activity was quite lesser than that of antibiotic, Rifampicin, which showed the inhibition zone 13.4 mm and MIC 2.03 mg/mL. In case of fungi, *A. niger* showed to be most sensitive with largest inhibition zone 14.2mm followed by minimum MIC value 2.03mg/mL which was less effective than that of antibiotic, Terbinafine, with inhibition zone 21.7 mm and MIC 0.94 mg/mL. Satyal *et al.* (2012) found that the seed and rind oils of *A. subulatum* showed marginal activity against Gram-positive bacteria : *Bacillus cereus* (MIC = 625 and 313 µg/mL) and *Staphylococcus aureus* (MIC = 313 and 625 µg/mL), and antifungal activity against *Aspergillus niger* (MIC = 313 and 19.5 µg/mL, respectively). Agnihotri and Wakode (2010) found *Escherichia coli and Aspergillus niger* to be most sensitive microbes against essential oil of *A. subulatum*.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.12.4 shows the effect of *A. subulatum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum

stabilization was exhibited by *A. subulatum* essential oil at 1000 ppm which was 0.29 % even after induction period of 90 days, while with BHT it was 0.22%. Peroxide value (PV) is presented in table 4.12.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 3.7 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.12.6 for *M. koenigii*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *A. subulatum* essential oil sample of 1000 ppm with value 29.1 while in case of BHT it was found to be 17.9.

The effect of *A. subulatum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.12.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *A. subulatum* exhibited the maximum hemolysis i.e. 9.23 % at 10 mg/mL concentration and the minimum of 1.37 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.40 % at 10 mg/mL concentration and the minimum of 1.71 % at 0.5 mg/mL. Satyal *et al.* (2012) evaluated that the seed and rind oil of black cardamomum also exhibited reasonable brine shrimp lethality (LC_{50}=28.1 and 15.0 µg/mL, respectively).

The data of chemical composition of the essential oil from *A. subulatum* is listed in Table 4.12.8. Total 35 compounds were identified representing 98.91 % of the oil. The major components were found to be eucalyptol (61.86 %), caryophyllene (7.94 %), 3-p-menthene (3.65 %), carvacrol (3.33 %), camphene (3.27 %) and α-Pinene (2.77 %). Joshi *et al.* (2012) obtained black cardamom capsules from different places of Himachal Pradesh (India), which were subjected to extraction of essential oil followed by chemical characterization. Major compounds found in sample obtained from Kullu were eucalyptol (57.31 %), α-terpineol (15.84 %), D-limonene (11.76 %), 4-terpineol (4.89 %) and δ-terpineol (2.86 %). In another study Satyal *et al.* (2012) collected plant material from Terahthum district (Nepal) and examined essential oil using GC-MS. eucalyptol (60.8 %), α-terpineol (9.8 %), β-pinene (8.3 %) and α-pinene (6.4 %) were found to be the major components in seed essential oil.

4.13. *Trachyspermum ammi*

Table 4.13.1

Physical properties of *Trachyspermum ammi* essential oil

Parameter	***Trachyspermum ammi***
Colour	Brown
Yield (%)	2.94±0.2
Refractive Index (30 °C)	1.327±0.006
Specific Gravity	0.87±0.03

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

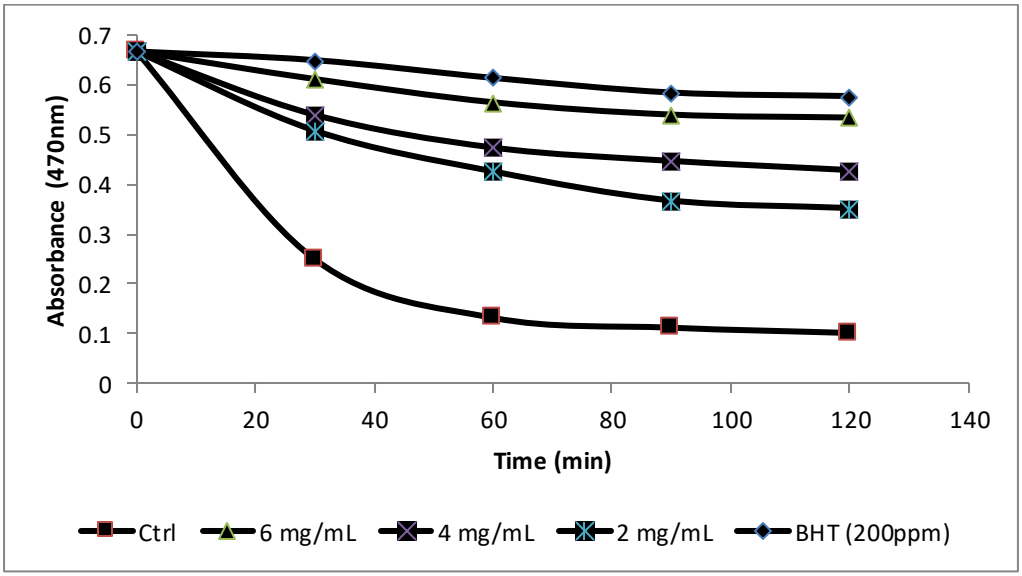
Table 4.13.2

Antioxidant activity of *Trachyspermum ammi* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*T. ammi*			BHT
IC_{50} (µg/mL)	2.61±0.15			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	80.73±2.7	65.38±1.6	48.28±2.5	85.10±3.2

Values are mean ± standard deviation of *Trachyspermum ammi*, analyzed individually in triplicate.

Fig. 4.13.1



Antioxidant activity of *Trachyspermum ammi* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.13.3

Antimicrobial activity of *Trachyspermum ammi* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Trachyspermum ammi		
Inhibition zone (mm)			
B. subtilis	**14.6±0.6**	**10.8±2.1**	**-**
L. rhamnosus	**18.3±1.1**	**15.5±0.8**	**-**
S. aureus	13.7±0.5	15.1±1.0	-
S. mutans	**19.7±1.2**	**13.4±0.7**	**-**
E. coli	**13.7±0.4**	**9.6±1.3**	**-**
P. multocida	**17.5±0.6**	**11.6±1.5**	**-**
A. alternata	10.3±0.5	-	14.2±0.8
A. flavus	**12.3±0.8**	-	**11.4±1.4**

A. niger	15.7±1.5	-	21.7±1.2
G. lucidum	**17.0±0.5**	-	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	**1.72±0.1**	**1.72±0.2**	-
L. rhamnosus	**1.56±0.1**	**2.81±0.3**	-
S. aureus	2.19±0.1	2.19±0.2	-
S. mutans	**1.41±0.1**	**2.03±0.1**	-
E. coli	**2.19±0.1**	**2.34±0.2**	-
P. multocida	**1.88±0.1**	**2.03±0.1**	-
A. alternata	2.5±0.2	-	2.34±0.2
A. flavus	**2.19±0.1**	-	**2.81±0.2**
A. niger	1.88±0.1	-	0.94±0.1
G. lucidum	**1.72±0.1**	-	**1.88±0.1**

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.13.4 FFA of edible oil stabilized by *Trachyspermum ammi* essential oil

IPD Induction Period in Days	% FFA			
	Trachyspermum ammi ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.1±0.012	0.08±0.01	0.08±0.01	0.16±0.014
30	0.13±0.014	0.09±0.015	0.09±0.015	0.22±0.016
45	0.17±0.015	0.12±0.012	0.11±0.012	0.34±0.03
60	0.2±0.019	0.15±0.014	0.14±0.014	0.48±0.02
75	0.24±0.02	0.19±0.02	0.18±0.018	0.57±0.03
90	0.3±0.022	0.22±0.03	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

Table 4.13.5 PV of edible oil stabilized by *Trachyspermum ammi* essential oil

IPD Induction Period in Days	PV			
	Trachyspermum ammi ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.6±0.02	0.4±0.01	0.4±0.01	1±0.03
30	1.1±0.10	0.7±0.02	0.6±0.16	1.81±0.12
45	1.6±0.15	1.1±0.21	0.9±0.21	2.5±0.24
60	2.1±0.18	1.61±0.12	1.4±0.29	3.4±0.36
75	2.8±0.28	2.2±0.29	1.9±0.24	4.7±0.21
90	3.6±0.33	2.6±0.21	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

Table 4.13.6

***para*-Anisidine Value of edible oil stabilized by *Trachyspermum ammi* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Trachyspermum ammi ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	4.6±0.22	3.8±0.27	3.4±0.27	10.2±0.52
30	7.3±0.28	6.4±0.31	5.8±0.36	17.5±0.74
45	9.8±0.31	8.7±0.36	8.5±0.27	24.1±0.81
60	12.7±0.38	11.4±0.55	10.9±0.55	29.6±0.63
75	16.8±0.81	14.7±0.47	13.6±0.47	37.4±1.2
90	21.3±1.1	18.6±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

Table 4.13.7

Cytotoxicity (% hemolysis) of *Trachyspermum ammi* essential oil

Concentration	**Human erythrocytes**	**Bovine erythrocytes**
0.5mg/mL	0.79%	0.23%
5mg/mL	7.11%	4.25%
10mg/mL	9.82%	7.64%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Trachyspermum ammi*, analyzed individually in triplicate.

Table 4.13.8. Chemical composition of *Trachyspermum ammi* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
Cyclofenchene	896	0.42	RI, MS
α-Thujene	926	0.18	RT, RI, MS
Cumene	**928**	**18.57**	**RT, RI, MS**
α-Pinene	934	1.36	RT, RI, MS
Camphene	955	0.33	RT, RI, MS
Sabinene	972	0.31	RI, MS
β -pinene	978	2.14	RT, RI, MS
β -Myrcene	994	0.20	RT, RI, MS
3-carene	**1011**	**22.33**	**RT, RI, MS**
α-Terpinen	**1020**	**4.45**	**RT, RI, MS**
p-Cymene	1026	0.22	RT, RI, MS
1,8-cineole	1029	0.67	RI, MS
Limonene	**1040**	**5.16**	**RT, RI, MS**
β-Ocimene	1044	0.18	RI, MS
γ-Terpinene	1062	0.25	RT, RI, MS
L-Pinocarveol	1148	0.86	RI, MS
terpinen-4-ol	1178	0.83	RI, MS
α- Terpinolen	1187	0.29	RT, RI, MS
3-p-Menthene	1234	0.37	RI, MS
Cumaldehyde	1240	0.56	RT, RI, MS
Thymol	**1290**	**37.75**	**RT, RI, MS**
Carvacrol	1298	0.18	RI, MS
Grenyl formate	1300	0.21	RI, MS
Neryl acetate	1344	0.17	RT, RI, MS
Vanillin	1391	0.24	RI, MS
Piperitone	1342	0.23	RI, MS
trans-nerolidol	1564	0.19	RT, RI, MS
L-Menthol	1624	0.26	RT, MS
Total (28)		98.73	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.13.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Trachyspermum ammi* was found to be 2.94 % as listed in Table 4.13.1. The oil was brown in colour with strong aromatic smell and refractive index 1.327. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.87. Joshi *et al.* (2012) obtained the yield of pale-coloured oil extracted from cardamom seeds collected from different regions of Himachal Pradesh ranged from 9.8 to 19.5 g/kg (w/w). Ishikawah *et al.* (2001) found that *T. ammi* fruit yield 2-4 % brownish coloured essential oil.

The essential oil of *T. ammi* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *T. ammi* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.13.2, which was found to be 2.61 µg/mL, which was much better than scavenging effect of synthetic antioxidant i.e. BHT i.e. 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.13.2 shows the % inhibition in linoleic acid system by *T. ammi* essential oil taken at three different concentrations. It is clear that maximum inhibition was 80.73 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with

peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *T. ammi* is shown in Figure 4.13.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *T. ammi* was used at three different concentrations where minimum depletion was observed at 6mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. In another study it was evaluated that essential oil of *T. ammi* also exhibited antioxidant activity assessed by DPPH assay, with IC_{50} of 34 μg/mL (Gandomi *et al.,* 2013). Similarly, in β-carotene/linoleic acid assay, the EO was effectively able to inhibit the linoleic acid oxidation, exhibiting 82.16 % inhibition (Gandomi *et al.,* 2013).

The antimicrobial activity of the essential oil of *T. ammi* against various microbes including bacteria and fungi is shown in Table 4.13.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *T. ammi* showed potential as an antimicrobial agent with larger inhibition zones (13.7-18.3 and 10.3-17.0 mm) and smaller MIC values (1.41-2.19 and 1.72-2.5 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *L. rhamnosus* (Gram positive) with largest inhibition zone 18.3mm followed by minimum MIC value 1.56 mg/mL. The activity was much better than that of antibiotic, Rifampicin, which showed the inhibition zone 15.5 mm and MIC 2.81 mg/mL. In case of fungi, *G. lucidum* showed to be most sensitive with largest inhibition zone 17.0 mm followed by minimum MIC value 1.72 mg/mL which was less effective than that of antibiotic, Terbinafine, with inhibition zone 9.1 mm and MIC 1.88 mg/mL. In another study it was revealed that the essential oil of *T. ammi* exhibited strong activity against both bacteria and fungi, with greater inhibition of bacterial growth compared with fungi (Gandomi *et al.,* 2013).

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.13.4 shows the effect of *T. ammi* essential oil on formation

of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *T. ammi* essential oil at 1000ppm which was 0.22 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.13.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 2.6 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.13.6 for *M. koenigii*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *T. ammi* essential oil sample of 1000ppm with value 18.6 while in case of BHT it was found to be 17.9.

The effect of *T. ammi* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.13.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *T. ammi* exhibited the maximum hemolysis i.e. 7.64 % at 10 mg/mL concentration and the minimum of 0.23 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 9.82 % at 10 mg/mL concentration and the minimum of 0.79 % at 0.5 mg/mL.

The data of chemical composition of the essential oil from *T. ammi* is listed in Table 4.13.8. Total 28 compounds were identified representing 98.73 % of the oil. The major components were found to be thymol (37.75 %), 3-carene (22.33 %), cumene (18.57 %), limonene (5.16 %), α-terpinen (4.45 %) and α-pinene (1.36 %). Gandomi *et al.* (2013) obtained *T. ammi* seeds from Isfahan (Iran), which were subjected to extraction of essential oil followed by chemical characterization. Major components were found to be thymol (63.42 %), *p*-cymene (19.01 %) and γ-terpinene (16.89 %). In another study it has been reported that thymol was the most abundant compound of this oil and make 39 % of the whole oil contents (Saei-Dehkordi *et al.*, 2010).

4.14. *Foeniculum vulgare*

Table 4.14.1

Physical properties of *Foeniculum vulgare* essential oil

Parameter	***Foeniculum vulgare***
Colour	Light brown
Yield (%)	1.08±0.04
Refractive Index (30 °C)	1.345±0.002
Specific Gravity	0.81±0.02

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

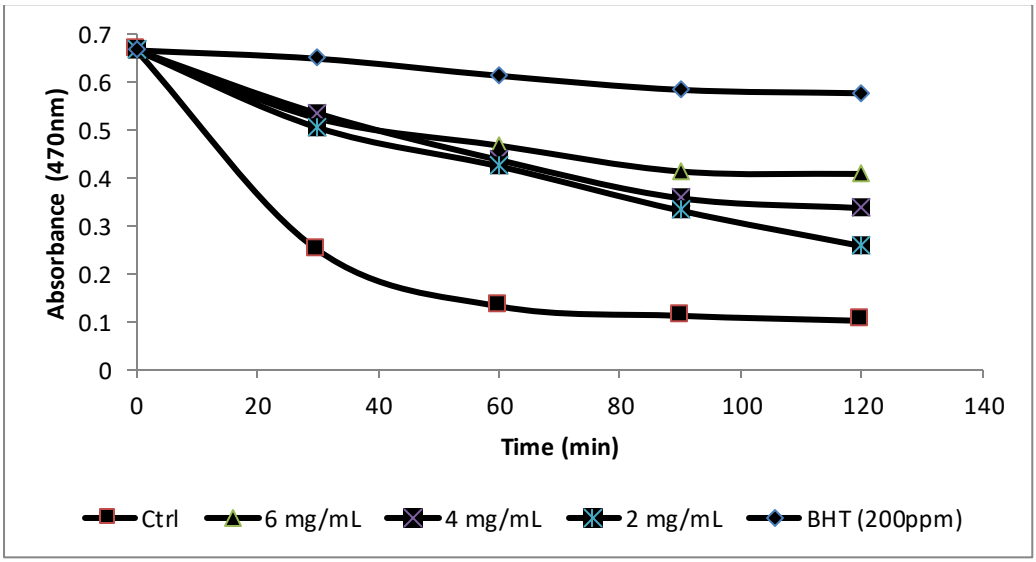
Table 4.14.2

Antioxidant activity of *Foeniculum vulgare* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*F. vulgare*			BHT
IC_{50} (µg/mL)	64.75±2.56			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	57.67±1.3	34.53±1.4	28.71±1.1	85.10±3.2

Values are mean ± standard deviation of *Foeniculum vulgare*, analyzed individually in triplicate.

Fig. 4.14.1



Antioxidant activity of *Foeniculum vulgare* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.14.3

Antimicrobial activity of *Foeniculum vulgare* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Foeniculum vulgare		
Inhibition zone (mm)			
B. subtilis	8.0±0.3	10.8±2.1	-
L. rhamnosus	10.6±0.2	15.5±0.8	-
S. aureus	4.7±0.1	15.1±1.0	-
S. mutans	**16.7±0.1**	**13.4±0.7**	-
E. coli	3.8±0.1	9.6±1.3	-
P. multocida	**20.3±1.1**	**11.6±1.5**	-
A. alternata	7.9±0.4	-	14.2±0.8
A. flavus	**19.3±2.3**	-	**11.4±1.4**

A. niger	19.0±1.5	-	21.7±1.2
G. lucidum	7.6±0.4	-	9.1±0.4
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	3.12±0.2	1.72±0.2	-
L. rhamnosus	2.34±0.2	2.81±0.3	-
S. aureus	4.37±0.3	2.19±0.2	-
S. mutans	**1.88±0.1**	**2.03±0.1**	**-**
E. coli	5.62±0.4	2.34±0.2	-
P. multocida	**1.25±0.1**	**2.03±0.1**	-
A. alternata	3.44±0.2	-	2.34±0.2
A. flavus	**1.25±0.1**	**-**	**2.81±0.2**
A. niger	1.41±0.1	-	0.94±0.1
G. lucidum	3.75±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.14.4 FFA of edible oil stabilized by *Foeniculum vulgare* essential oil

IPD Induction Period in Days	% FFA			
	Foeniculum vulgare ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.15±0.013	0.11±0.012	0.08±0.01	0.16±0.014
30	0.19±0.012	0.13±0.012	0.09±0.015	0.22±0.016
45	0.23±0.018	0.17±0.016	0.11±0.012	0.34±0.03
60	0.28±0.017	0.2±0.016	0.14±0.014	0.48±0.02
75	0.34±0.02	0.25±0.018	0.18±0.018	0.57±0.03
90	0.41±0.022	0.29±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

Table 4.14.5 PV of edible oil stabilized by *Foeniculum vulgare* essential oil

IPD Induction Period in Days	PV			
	Foeniculum vulgare ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.9±0.11	0.6±0.03	0.4±0.01	1.0±0.03
30	1.6±0.15	1.2±0.16	0.6±0.16	1.81±0.12
45	2.3±0.17	1.8±0.24	0.9±0.21	2.5±0.24
60	2.8±0.12	2.3±0.21	1.4±0.29	3.4±0.36
75	3.7±0.2	2.9±0.29	1.9±0.24	4.7±0.21
90	4.4±0.24	3.4±0.16	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

Table 4.14.6

***para*-Anisidine Value of edible oil stabilized by *Foeniculum vulgare* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Foeniculum vulgare ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	5.8±0.32	4.8±0.31	3.4±0.27	10.2±0.52
30	8.7±0.38	7.3±0.47	5.8±0.36	17.5±0.74
45	11.6±0.61	9.8±0.27	8.5±0.27	24.1±0.81
60	15.3±0.82	13.4±0.62	10.9±0.55	29.6±0.63
75	19.6±1.0	19.2±0.55	13.6±0.47	37.4±1.2
90	32.1±1.4	25.8±0.47	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

Table 4.14.7

Cytotoxicity (% hemolysis) of *Foeniculum vulgare* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.52%	0.23%
5mg/mL	7.53%	4.13%
10mg/mL	8.85%	6.72%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Foeniculum vulgare*, analyzed individually in triplicate.

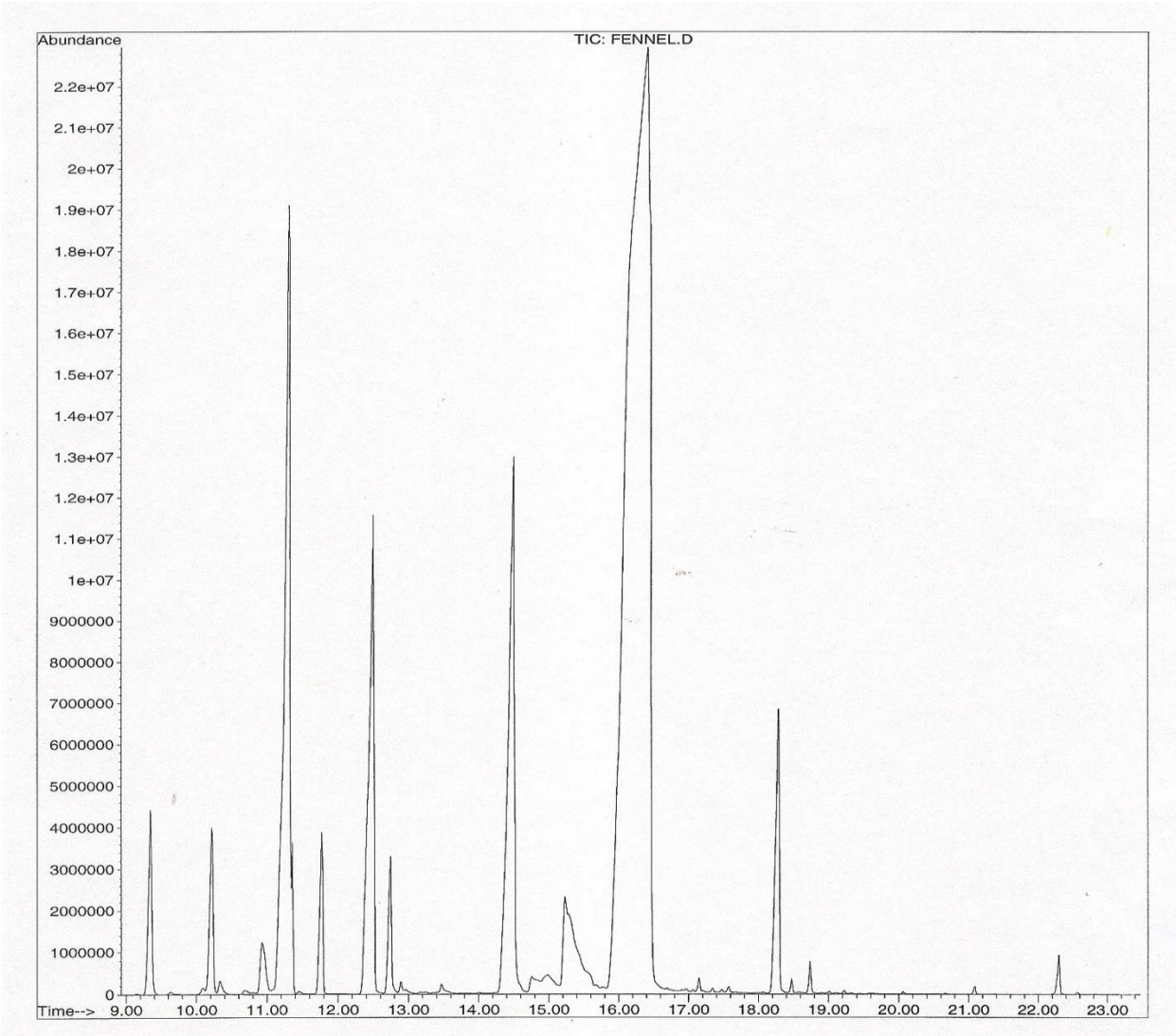


Figure 4.14.2. Typical GC-MS chromatogram of essential oil of *Foeniculum vulgare*

Table 4.14.8. Chemical composition of *Foeniculum vulgare* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
α-pinene	934	1.53	RT, RI, MS
Sabinene	972	0.32	RI, MS
β -Pinene	978	0.17	RT, RI, MS
β -Myrcene	994	0.18	RT, RI, MS
α-Phellandrene	1005	0.18	RT, RI, MS
3-carene	1011	1.24	RT, RI, MS
α-Terpinene	1020	0.67	RT, RI, MS
Limonene	**1033**	**11.01**	**RT, RI, MS**
Eucalyptol	1037	0.41	RT, RI, MS
γ-terpinene	1062	0.17	RT, RI, MS
m-cymene	1082	0.22	RT, RI, MS
Fenchone	**1096**	**6.17**	**RI, MS**
3-Thujanone	1116	1.06	RI, MS
trans-verbenol	1141	0.24	RI, MS
L-camphor	1143	0.19	RT, RI, MS
L-Pinocarveol	1148	3.29	RI, MS
Estragole	1195	0.20	RI, MS
Fenchyl acetate	1224	0.25	RI, MS
3-p-Menthene	1234	1.44	RT, RI, MS
Anisaldehyde	1252	0.17	RI, MS
Chavicol	**1255**	**8.22**	**RT, RI, MS**
Anethole	**1283**	**55.78**	**RT, RI, MS**
Thymol	1290	0.54	RT, RI, MS
Geranyl formate	1300	0.16	RI, MS
Isocaryophyllene	1413	0.32	RT, RI, MS
β-trans-Ocimene	1451	0.27	RI, MS
Caryophyllene	1454	2.84	RT, RI, MS
β-bisabolene	1513	0.17	RI, MS
Myristicin	1521	0.32	RI, MS
Germacrene B	1559	0.21	RT, RI, MS
Apiol	1680	0.53	RT, RI, MS
Total (31)		98.57	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.14.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Foeniculum vulgare* was found to be 1.08 % as listed in Table 4.14.1. The oil was light brown in colour with strong aromatic smell and refractive index 1.345. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.81. Roby *et al.* (2013) obtained 1.95 % yield of hydrodistilled fennel seed essential oil.

The essential oil of *F. vulgare* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. During the process, the examined essential oil transferred electron or hydrogen atoms towards DPPH• (purple colour) which was reduced to form DPPH-H (yellow colour) and this ability of essential oil was investigated. The DPPH radical scavenging by *F. vulgare* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.14.2, which was found to be 64.75 µg/mL. Whereas the scavenging effect of synthetic antioxidant i.e. BHT was 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.14.2 shows the % inhibition in linoleic acid system by *F. vulgare* essential oil taken at three different concentrations. It is clear that maximum inhibition was 57.67 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *F. vulgare* is shown in Figure 4.14.1. The

least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *F. vulgare* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. In another study it was evaluated that fennel extracts showed excellent radical scavenging activity, with IC_{50} values ranging from 0.0031 to 0.0047 µg/mL (Roby *et al.*, 2013). *Foeniculum vulgare* var. *azoricum* showed the highest activity in scavenging of DPPH radical, even higher than either ascorbic acid or BHT (Shahat *et al.*, 2011).

The antimicrobial activity of the essential oil of *F. vulgare* against various microbes including bacteria and fungi is shown in Table 4.14.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *F. vulgare* showed potential as an antimicrobial agent with larger inhibition zones (3.8-20.3 and 7.6-19.3 mm) and smaller MIC values (1.25-5.62 and 1.25-3.75 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *P. multocida* (Gram negative) with largest inhibition zone 20.3 mm followed by minimum MIC value 1.25 mg/mL. The activity was much better than that of antibiotic, Rifampicin, which showed the inhibition zone 11.6 mm and MIC 2.3 mg/mL. In case of fungi, *A. flavus* showed to be most sensitive with largest inhibition zone 19.3 mm followed by minimum MIC value 1.25 mg/mL which showed much effectiveness even than that of antibiotic, Terbinafine, with inhibition zone 11.4 mm and MIC 2.81 mg/mL. Shahat, *et al.* (2011) tested essential oils of *F. vulgare* from three different cultivars for their antimicrobial potential. *F. vulgare azoricum* proved to be most effective oil against Gram negative bacteria while *F. vulgare vulgare* was effective against Gram positive bacteria even than the antibiotic, ampicillin. Roby *et al.* (2013) tested potential of essential oil of *F. vulgare* against panel of microbes like *Aspergillus flavus, Candida albicans, Bacillus cereus,* and *Staphylococcus aureus.*

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.14.4 showed the effect of *F. vulgare* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the

length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *F. vulgare* essential oil at 1000ppm which was 0.29 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.14.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.4 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.14.6 for *F. vulgare*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *F. vulgare* essential oil sample of 1000 ppm with value 25.8 while in case of BHT it was found to be 17.9.

The effect of *F. vulgare* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.14.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *F. vulgare* exhibited the maximum hemolysis i.e. 6.72 % at 10 mg/mL concentration and the minimum of 0.23 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.85 % at 10 mg/mL concentration and the minimum of 0.52 % at 0.5 mg/mL.

The data of chemical composition of the essential oil from *F. vulgare* was listed in Table 4.14.8. Total 31 compounds were identified representing 98.57 % of the oil. The major components were found to be anethole (55.78 %), limonene (11.01 %), chavicol (8.22 %), fenchone (6.17 %), L-Pinocarveol (3.29 %) and caryophyllene (2.84 %). Yu *et al.* (2012) obtained *F. vulgare* from Changchun, Jilin Province (China), which was subjected to extraction of essential oil followed by chemical characterization. Major components were found to be *trans*-anethole (75.67 %), limonene (5.82 %), fenchone (4.58 %) and estragole (2.87 %). Qiu *et al.* (2012) collected *F. vulgare* from Guangxi Province (China). The major components were found to be *trans*-anethole (88.91 %), anisole (2.89 %), anisaldehyde (2.54 %) and D-limonene (1.64%). Chowdhary *et al.* (2009) collected fennel seeds from Chittagong (Bangladesh) and examined essential oil using GC-MS. anethole (58.54 %), limonene (19.63 %), fenchone (7.72 %) and β-pinene (1.80 %) were found to be the major components in seed essential oil.

4.15. *Cuminum cyminum*

Table 4.15.1

Physical properties of *Cuminum cyminum* essential oil

Parameter	***Cuminum cyminum***
Colour	Colourless
Yield (%)	2.25±0.1
Refractive Index (30 °C)	1.351±0.004
Specific Gravity	0.78±0.02

Values are mean ± standard deviation of three samples of each *Cuminum cyminum*, analyzed individually in triplicate.

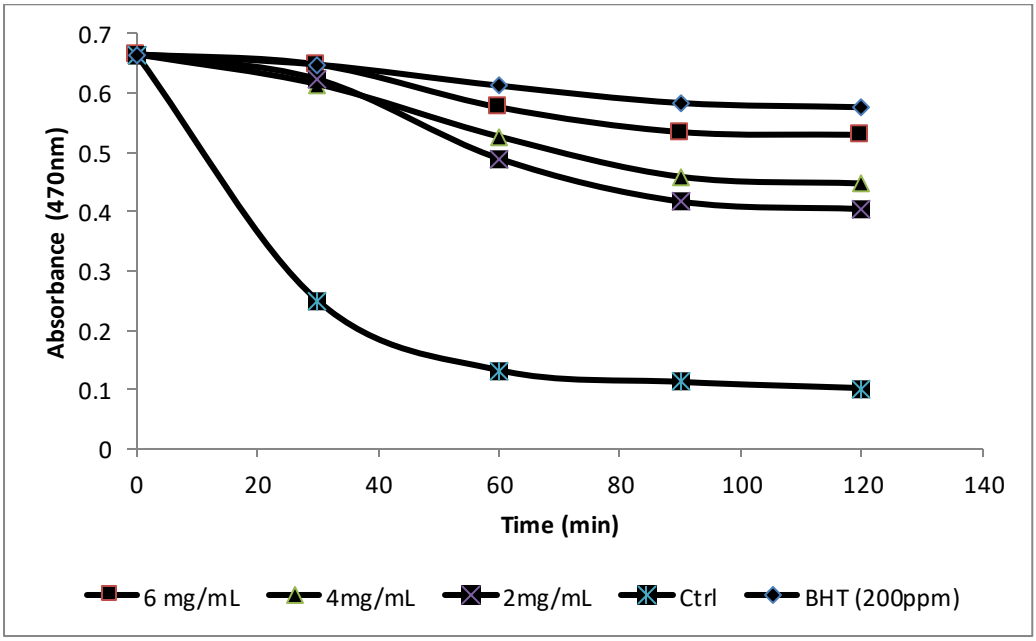
Table 4.15.2

Antioxidant activity of *Cuminum cyminum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*C. cyminum*			BHT
IC_{50} (µg/mL)	16.86±0.85			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	79.36±2.5	67.52±2.4	53.00±2.6	85.10±3.2

Values are mean ± standard deviation of *Cuminum cyminum*, analyzed individually in triplicate.

Fig. 4.15.1



Antioxidant activity of *Cuminum cyminum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.15.3

Antimicrobial activity of *Cuminum cyminum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Cuminum cyminum		
Inhibition zone (mm)			
B. subtilis	**27.3±2.3**	**10.8±2.1**	**-**
L. rhamnosus	8.0±0.5	15.5±0.8	-
S. aureus	**18.0±1.0**	**15.1±1.0**	**-**
S. mutans	9.7±0.5	13.4±0.7	-
E. coli	**15.3±0.5**	**9.6±1.3**	**-**
P. multocida	**21.7±0.5**	**11.6±1.5**	**-**
A. alternata	**16.7±0.5**	-	**14.2±0.8**
A. flavus	8.3±0.57	-	11.4±1.4
A. niger	19.3±2.09	-	21.7±1.2

G. lucidum	**27.0±1.6**	**-**	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	**1.1±0.2**	**1.72±0.2**	**-**
L. rhamnosus	4.2±0.1	2.81±0.3	-
S. aureus	**2.1±0.1**	**2.19±0.2**	**-**
S. mutans	2.1± 0.3	2.03±0.1	-
E. coli	**3.2±0.1**	**2.34±0.2**	**-**
P. multocida	**1.4±0.2**	**2.03±0.1**	**-**
A. alternata	**2.3±0.2**	**-**	**2.34±0.2**
A. flavus	3.9±0.3	-	2.81±0.2
A. niger	2.3±0.2	-	0.94±0.1
G. lucidum	**1.4±0.2**	**-**	**1.88±0.1**

Values are mean ± standard deviation of three samples of each *Cuminum Cyminum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.15.4 FFA of edible oil stabilized by *Cuminum cyminum* essential oil

IPD Induction Period in Days	% FFA			
	Cuminum cyminum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.09±0.012	0.08±0.01	0.08±0.01	0.16±0.014
30	0.13±0.011	0.09±0.015	0.09±0.015	0.22±0.016
45	0.18±0.016	0.11±0.012	0.11±0.012	0.34±0.03
60	0.22±0.017	0.14±0.014	0.14±0.014	0.48±0.02
75	0.27±0.02	0.18±0.018	0.18±0.018	0.57±0.03
90	0.31±0.014	0.21±0.018	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Cuminum cyminum*, analyzed individually in triplicate.

Table 4.15.5 PV of edible oil stabilized by *Cuminum cyminum* essential oil

IPD Induction Period in Days	PV			
	Cuminum cyminum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.5±0.015	0.4±0.01	0.4±0.01	1±0.03
30	0.9±0.019	0.6±0.02	0.6±0.16	1.81±0.12
45	1.6±0.1	1.2±0.21	0.9±0.21	2.5±0.24
60	2.0±0.13	1.6±0.16	1.4±0.29	3.4±0.36
75	2.8±0.17	2.1±0.29	1.9±0.24	4.7±0.21
90	3.5±0.2	2.6±0.21	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Cuminum cyminum*, analyzed individually in triplicate.

Table 4.15.6

***para*-Anisidine Value of edible oil stabilized by *Cuminum cyminum* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Cuminum cyminum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	4.1±0.21	3.6±0.27	3.4±0.27	10.2±0.52
30	7.3±0.65	6.1±0.36	5.8±0.36	17.5±0.74
45	10.5±0.81	8.9±0.47	8.5±0.27	24.1±0.81
60	12.8±1.0	11.5±0.55	10.9±0.55	29.6±0.63
75	16.7±0.9	13.9±0.47	13.6±0.47	37.4±1.2
90	22.4±1.4	17.8±0.62	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Cuminum cyminum*, analyzed individually in triplicate.

Table 4.15.7

Cytotoxicity (% hemolysis) of *Cuminum cyminum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.45%	0.21%
5mg/mL	8.33%	3.32%
10mg/mL	8.54%	6.89%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Cuminum Cyminum*, analyzed individually in triplicate.

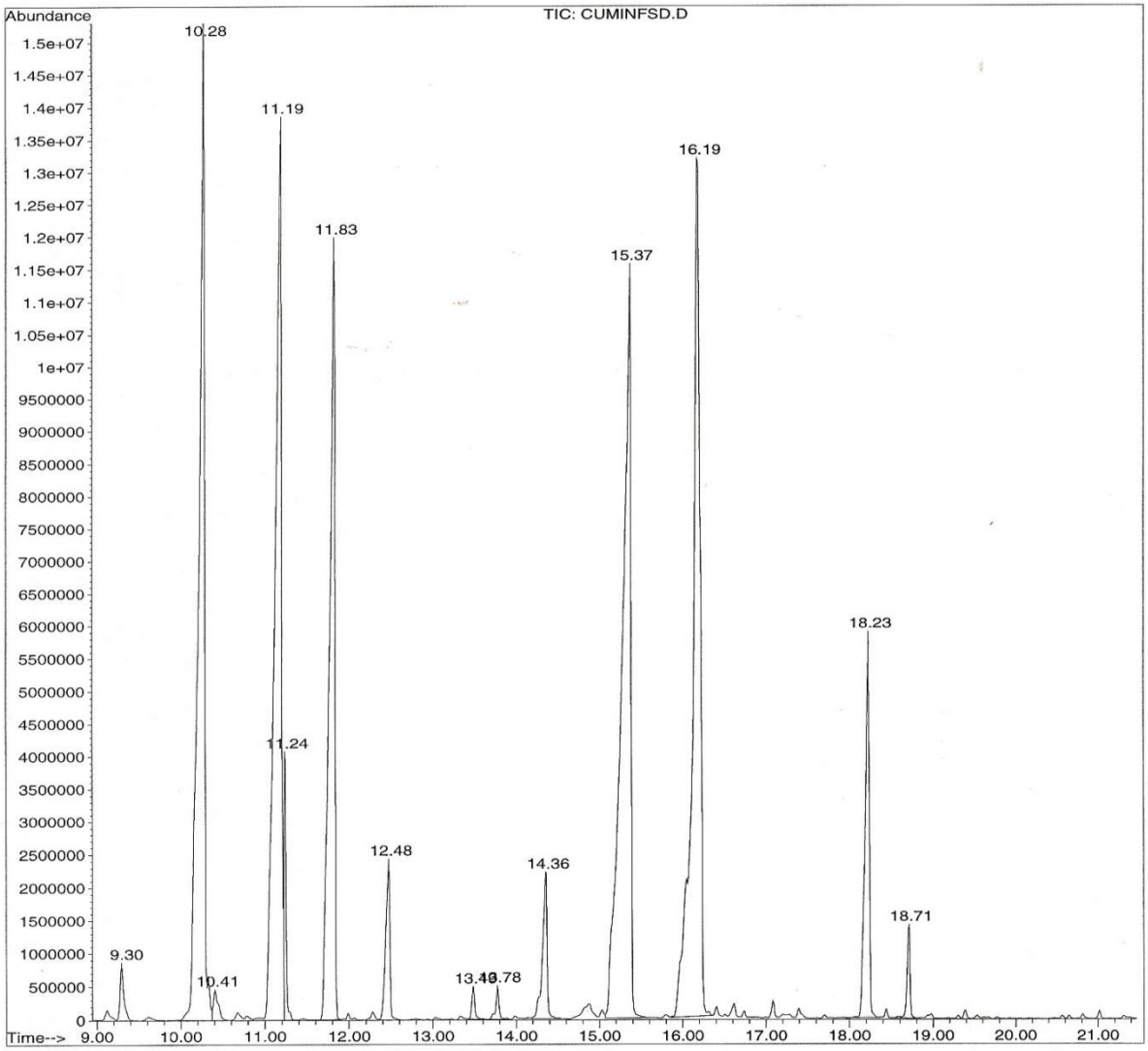


Figure 4.15.2. Typical GC-MS chromatogram of essential oil of *Cuminum cyminum*

Table 4.15.8. Chemical composition of *Cuminum cyminum* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
Isobutyl isobutyrate	892	0.23	RI, MS
Bornylene	898	0.19	RT, RI, MS
α-Tricyclene	925	0.57	RT, RI, MS
Cumene	927	0.4	RT, RI, MS
Camphene	955	0.27	RT, RI, MS
p-Menthene	977	0.33	RT, RI, MS
β-Pinene	**982**	**12.57**	**RT, RI, MS**
α-phellandrene	1005	0.25	RT, RI, MS
3-carene	1011	0.20	RT, RI, MS
p-Cymene	1026	1.39	RT, RI, MS
1,8-Cineole	1029	2.95	RT, RI, MS
Limonene	**1033**	**12.02**	**RT, RI, MS**
β-trans-Ocimene	1051	0.37	RT, RI, MS
γ-Terpinene	**1060**	**7.24**	**RT, RI, MS**
α-Terpinolene	1089	0.42	RT, RI, MS
L-fenchone	1094	0.38	RI, MS
Linalool	1096	0.26	RT, RI, MS
trans-Pinocarveol	1140	0.24	RI, MS
Terpinene-4-ol	1178	0.34	RI, MS
3,4-Xylenol	1187	0.28	RI, MS
Myrtenol	1196	0.46	RI, MS
Safranol	1201	0.32	RT, RI, MS
Trans carveole	1219	0.22	RT, RI, MS
3-p-Menthene	1233	0.19	RI, MS
Trans Anethole	1238	1.83	RT, RI, MS
Cumaldehyde	**1240**	**24.10**	**RT, RI, MS**
Linalyl Acetate	1248	0.89	RT, RI, MS
Geraniol	1254	0.27	RT, RI, MS
2-Caren-10-al	**1291**	**20.03**	**RI, MS**
Eugenol	1356	0.21	RT, RI, MS
cis-Carvyl acetate	1364	0.30	RI, MS
Isocaryophyllene	1410	0.18	RT, RI, MS
thujopsene	1429	0.27	RI, MS
β-Caryophyllene	1431	0.23	RT, RI, MS
γ-elemene	1434	0.32	RT, RI, MS
α-Caryophyllene	1454	0.18	RT, RI, MS
α-Humulene	1467	0.21	RT, RI, MS
Valencene	1490	0.32	RI, MS
α- Farnesene	1509	4.22	RT, RI, MS
Trans-Nerolidol	1563	0.25	RI, MS

Spathulenol	1577	0.32	RT, RI, MS
Isoaromadendrene	1578	0.75	RI, MS
Caryophyllene Oxide	1583	0.19	RT, RI, MS
Carotol	1595	0.18	RI, MS
Phellandral	1601	0.22	RT, RI, MS
α-Humulene epoxide	1606	0.24	RT, RI, MS
α-Eudesmol	1651	0.17	RT, RI, MS
Total (47)		97.97	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS = identification based on comparison of mass spectra

4.15.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Cuminum cyminum* was found to be 2.25 % as listed in Table 4.15.1. The oil was colourless with strong aromatic smell and refractive index 1.351. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 0.78. The extraction yield of Iranian variety of *C. cyminum* was 1.45 % (Mehdi *et al*., 2007) whereas the essential oil yield of the *C. cyminum* seeds from the local market of India was 2.33 % (Sowbhagya *et al*., 2008).

The essential oil of *C. cyminum* was assessed for its antioxidant potential using various assays being in practice these days. Free radical scavenging ability was tested using DPPH assay and the potential was noted to be increased with the increase in concentration of essential oil. The DPPH radical scavenging by *C. cyminum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.15.2, which was found to be 16.86 µg/mL. Whereas the scavenging effect of synthetic antioxidant i.e. BHT was 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Due to unsaturation in its structure, it may form peroxides upon oxidation which have ability to oxidize Fe^{+2} to Fe^{+3} that may form complex with SCN^{-} present in reaction medium. The concentration of this complex was measured using spectrophotometer at 500 nm. Higher the concentration of peroxides higher will be the value of absorbance and ultimately lower will be the antioxidant activity. Table 4.15.2 shows the % inhibition in linoleic acid system by *C. cyminum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 79.36 % at concentration 50µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *C. cyminum* is shown in Figure 4.15.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *C. cyminum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Our results have compatibility with that of found by Damasius *et al*. (2007) and Thippeswamy and Naidu (2005) who showed that *C. cyminum* was a potent

antioxidant capable of scavenging DPPH free radicals and thus suppresses peroxidation of lipids interceded by free radicals. Hajlaoui *et al.* (2010) found that scavenging potential of essential oil of *C. cyminum* and standard (BHT) on the DPPH radical as IC_{50} values was 31 µg/mL and 11.5 µg/mL respectively. Gachkar *et al.* (2007) compared the lipid peroxidation inhibitory activities of the *C. cyminum* and rosemary essential oils using the β-carotene in linoleic acid system.

The antimicrobial activity of the essential oil of *C. cyminum* against various microbes including bacteria and fungi is shown in Table 4.15.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *C. cyminum* showed potential as an antimicrobial agent with larger inhibition zones (8.0-27.3 and 8.3-27.0 mm) and smaller MIC values (1.1-4.2 and 1.4-3.9 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *B. subtilis* (Gram positive) with largest inhibition zone 27.3 mm followed by minimum MIC value 1.10 mg/mL. The activity was much better than that of antibiotic, Rifampicin, which showed the inhibition zone 10.8 mm and MIC 1.72 mg/mL. In case of fungi, *G. lucidum* showed to be most sensitive with largest inhibition zone 27.0 mm followed by minimum MIC value 1.40 mg/mL which showed much effectiveness even than that of antibiotic, Terbinafine, with inhibition zone 9.1mm and MIC 1.88 mg/mL. In another study *in vitro* antibacterial activities of different essential oils were performed against various microorganisms and it was found that *C. cyminum* essential oil was the most effective essential oil after the oregano one, which showed inhibition zones between 31.23 mm on *Lactobacillus sakei* and 38.17 mm (Viuda-Martos *et al.*, 2008). Hajlaoui *et al.* (2010) found that *C. cyminum* oil exhibited higher potential as an antibacterial and antifungal agent with a maximum effectiveness against *Vibrio spp*. strains with a diameter of inhibition zones growth ranging from 11 to 23 mm and MIC and MBC values (0.078–0.31 mg/ml) to (0.31–1.25 mg/ml), respectively.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.15.4 shows the effect of *C. cyminum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the

length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *C. cyminum* essential oil at 1000ppm which was 0.21 % even after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.15.5 and it showed that maximum stabilization was obtained at 1000 ppm with minimum PV i.e. 2.6 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.15.6 for *C. cyminum*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *C. cyminum* essential oil sample of 1000 ppm with value 17.8 while in case of BHT it was found to be 17.9.

The effect of *C. cyminum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.15.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *C. cyminum* exhibited the maximum hemolysis i.e. 6.89% at 10 mg/mL concentration and the minimum of 0.21 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.54% at 10 mg/mL concentration and the minimum of 0.45 % at 0.5 mg/mL. Allahghadri *et al.* (2010) conducted a 30 days oral toxicity study in Wistar rats to conclude the potential of cumin essential oil in producing toxic effects.

The data of chemical composition of the essential oil from *C. cyminum* is listed in Table 4.15.8. Total 47 compounds were identified representing 97.97 % of the oil. The major components were found to be cumaldehyde (24.10 %), 2-caren-10-al (20.03 %), β-pinene (12.57 %), limonene (12.02 %), γ-terpinene (7.24 %) and α- farnesene (4.22 %). Gachkar *et al.* (2007) obtained *C. cyminum* from national botanical garden (Iran), which was subjected to extraction of essential oil followed by chemical characterization. Major components were found to be α-pinene (29.1 %), limonene (21.5 %), eucalyptol (17.9 %) and linalool (10.4 %). Hajlaoui *et al.* (2010) collected cumin seeds from Swassi (Tunisia). The major components were found to be cinnamaldehyde (39.48 %), γ-terpinene (15.21 %), O-cymene (11.82 %) and β-pinene (11.13 %).

4.16. *Cinnamomum zeylanicum*

Table 4.16.1

Physical properties of *Cinnamomum zeylanicum* essential oil

Parameter	***Cinnamomum zeylanicum***
Colour	Light brown
Yield (%)	0.78±0.02
Refractive Index (30 °C)	1.342±0.005
Specific Gravity	1.00±0.01

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

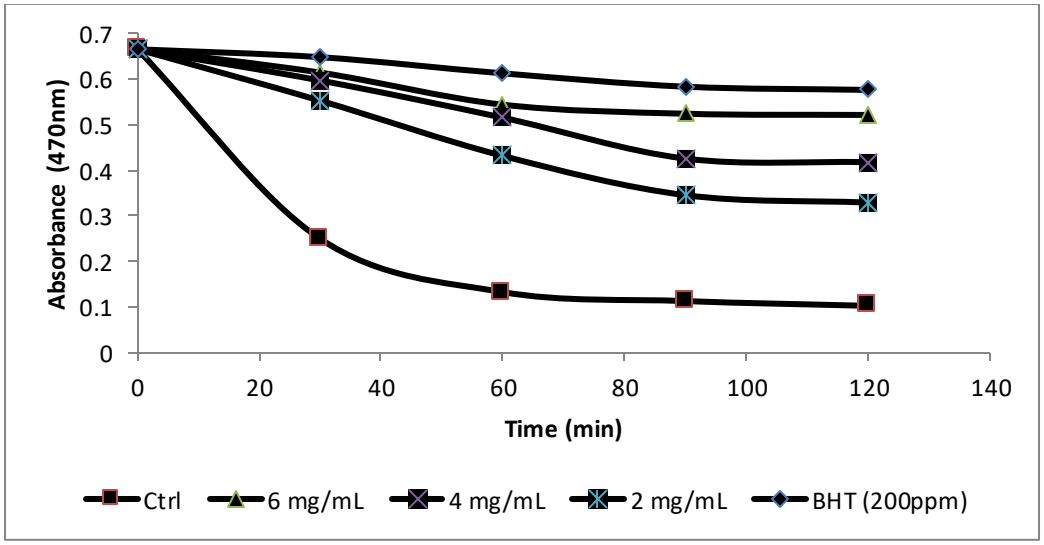
Table 4.16.2

Antioxidant activity of *Cinnamomum zeylanicum* essential oil measured by DPPH assay and % inhibition in linoleic acid system

DPPH				
Parameter	*C. zeylanicum*			BHT
IC_{50} (µg/mL)	54.77±2.87			3.46±0.3
% Inhibition in linoleic acid system				
Concentrations	50µL/mL	30µL/mL	10µL/mL	BHT (200ppm)
Inhibition of linoleic acid peroxidation (%)	73.19±1.7	52.90±2.3	42.09±2.3	85.10±3.2

Values are mean ± standard deviation of *Cinnamomum zeylanicum*, analyzed individually in triplicate.

Fig. 4.16.1



Antioxidant activity of *Cinnamomum zeylanicum* essential oil measured by bleaching of β-carotene-linoleic acid emulsion

Table 4.16.3

Antimicrobial activity of *Cinnamomum zeylanicum* essential oil

Tested organism	Essential oils	Rifampicin	Terbinafine
	Cinnamomum zeylanicum		
Inhibition zone (mm)			
B. subtilis	8.2±0.4	10.8±2.1	-
L. rhamnosus	**22.5±0.9**	**15.5±0.8**	-
S. aureus	10.7±1.5	15.1±1.0	-
S. mutans	**18.1±0.8**	**13.4±0.7**	-
E. coli	**11.3±0.6**	**9.6±1.3**	-
P. multocida	**14.3±1.1**	**11.6±1.5**	-
A. alternata	6.7±0.4	-	14.2±0.8
A. flavus	**19.3±2.5**	-	**11.4±1.4**

A. niger	13.7±0.5	-	21.7±1.2
G. lucidum	**10.7±0.2**	-	**9.1±0.4**
Minimum inhibitory concentration (MIC) mg/mL			
B. subtilis	2.81±0.2	1.72±0.2	-
L. rhamnosus	**1.09±0.1**	**2.81±0.3**	-
S. aureus	2.5±0.2	2.19±0.2	-
S. mutans	**1.56±0.1**	**2.03±0.1**	-
E. coli	**2.34±0.2**	**2.34±0.2**	-
P. multocida	2.81±0.2	2.03±0.1	-
A. alternata	4.37±0.3	-	2.34±0.2
A. flavus	**1.41±0.1**	-	**2.81±0.2**
A. niger	2.03±0.1	-	0.94±0.1
G. lucidum	2.5±0.2	-	1.88±0.1

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

Stabilization of Canola + Rapeseed (70%:30%) oil

Table 4.16.4 FFA of edible oil stabilized by *Cinnamomum zeylanicum* essential oil

IPD Induction Period in Days	% FFA			
	Cinnamomum zeylanicum ppm		BHT 200ppm	Control
	500	1000		
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
15	0.13±0.012	0.09±0.015	0.08±0.01	0.16±0.014
30	0.14±0.011	0.11±0.012	0.09±0.015	0.22±0.016
45	0.2±0.013	0.14±0.014	0.11±0.012	0.34±0.03
60	0.25±0.02	0.18±0.02	0.14±0.014	0.48±0.02
75	0.31±0.023	0.23±0.018	0.18±0.018	0.57±0.03
90	0.37±0.027	0.27±0.02	0.22±0.018	0.64±0.02

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

Table 4.16.5 PV of edible oil stabilized by *Cinnamomum zeylanicum* essential oil

IPD Induction Period in Days	PV			
	Cinnamomum zeylanicum ppm		BHT 200ppm	Control
	500	1000		
00	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
15	0.9±0.1	0.7±0.03	0.4±0.01	1±0.03
30	1.7±0.15	1.21±0.12	0.6±0.16	1.81±0.12
45	2.3±0.18	1.7±0.24	0.9±0.21	2.5±0.24
60	3.0±0.2	2.2±0.29	1.4±0.29	3.4±0.36
75	3.7±0.23	2.8±0.21	1.9±0.24	4.7±0.21
90	4.5±0.28	3.4±0.24	2.3±0.16	6.1±1.24

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

Table 4.16.6

***para*-Anisidine Value of edible oil stabilized by *Cinnamomum zeylanicum* essential oil**

IPD Induction Period in Days	para-Anisidine Value			
	Cinnamomum zeylanicum ppm		BHT 200ppm	Control
	500	1000		
00	2.7±0.12	2.7±0.12	2.7±0.12	2.7±0.12
15	6.2±0.32	4.9±0.36	3.4±0.27	10.2±0.52
30	8.8±0.51	7.5±0.47	5.8±0.36	17.5±0.74
45	12.5±0.83	10.6±0.31	8.5±0.27	24.1±0.81
60	15.8±0.64	14.3±0.62	10.9±0.55	29.6±0.63
75	19.7±1.1	17.4±0.55	13.6±0.47	37.4±1.2
90	24.6±1.3	21.7±0.47	17.9±0.62	45.8±1.8

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

Table 4.16.7

Cytotoxicity (% hemolysis) of *Cinnamomum zeylanicum* essential oil

Concentration	Human erythrocytes	Bovine erythrocytes
0.5mg/mL	0.95%	0.31%
5mg/mL	1.77%	1.26%
10mg/mL	8.78%	7.21%
PBS	0.00%	0.00%
Triton X-100	100%	100%

Values are mean ± standard deviation of three samples of each *Cinnamomum zeylanicum*, analyzed individually in triplicate.

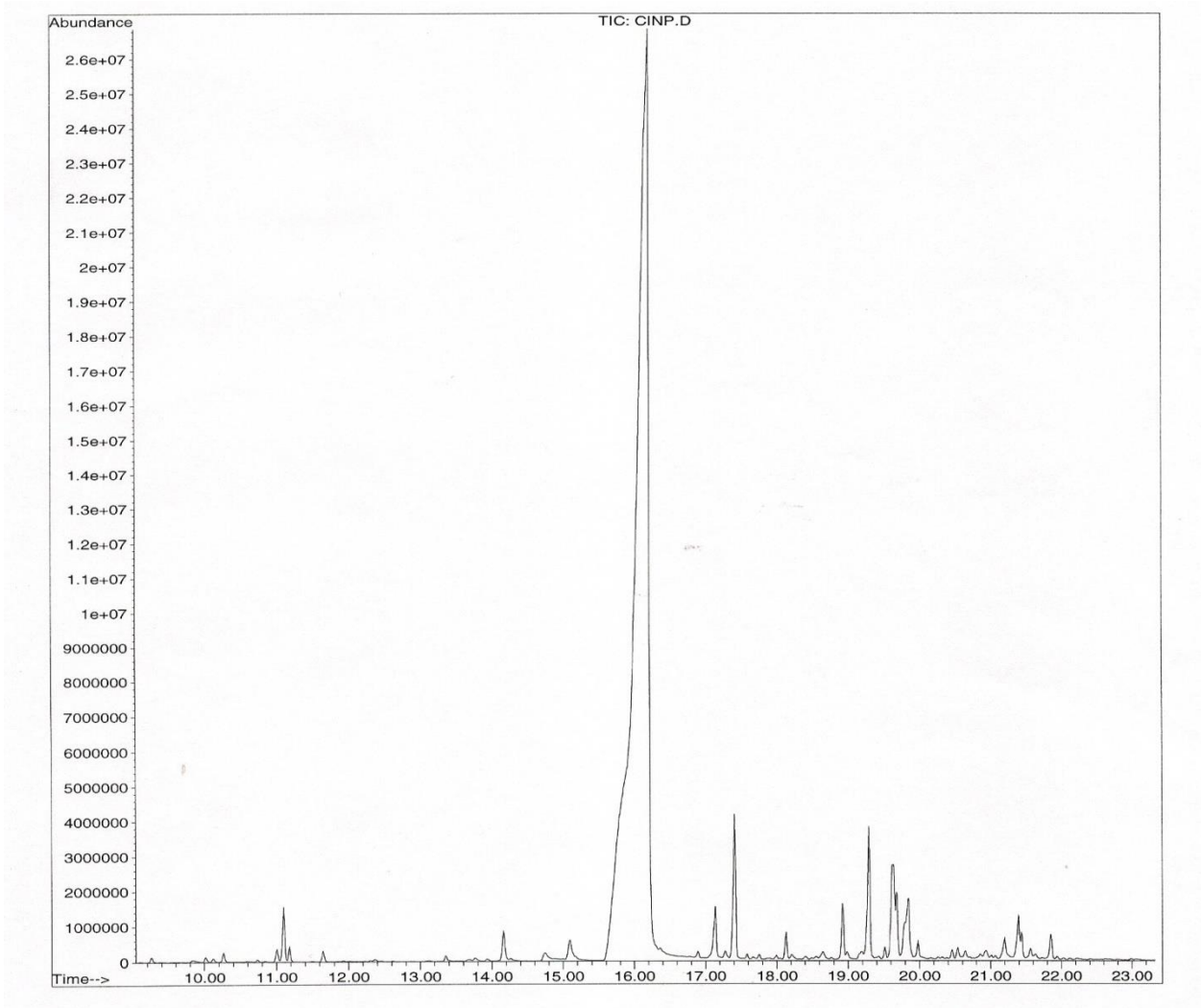


Figure 4.16.2. Typical GC-MS chromatogram of essential oil of *Cinnamomum zeylanicum*

Table 4.16.8. Chemical composition of *Cinnamomum zeylanicum* essential oil

Components[1]	**RI[2]**	**% age**	**Mode of Identification[3]**
α -Pinene	934	0.46	RT, RI, MS
Sabinene	972	0.34	RI, MS
β -Pinene	978	0.35	RT, RI, MS
3-Carene	1011	0.27	RT, RI, MS
p-Cymene	1026	0.23	RT, RI, MS
1,8-Cineole	1029	0.30	RI, MS
Limonene	1033	0.71	RT, RI, MS
Eucalyptol	1037	0.19	RT, RI, MS
γ-Terpinen	1072	0.18	RT, RI, MS
β-Citronellal	1161	0.45	RT, RI, MS
Borneol	1169	0.21	RI, MS
L-terpinen-4-ol	1178	0.23	RI, MS
α- Terpinolen	1187	0.51	RT, RI, MS
Cinnamaldehyde	**1233**	**77.86**	**RT, RI, MS**
3-p-Menthene	1234	0.17	RI, MS
p-Cumic aldehyde	1246	0.50	RI, MS
Chavicol	1255	0.19	RT, RI, MS
trans.-Anethole	1283	1.74	RT, RI, MS
Azulene	1300	0.45	RI, MS
α -Cubebene	1347	0.91	RT, RI, MS
Eugenol	1356	0.89	RT, RI, MS
Copaene	**1366**	**2.15**	**RI, MS**
Isolongifolene	1387	0.14	RI, MS
Isocaryophyllene	1413	0.20	RT, RI, MS
β-Caryophyllene	1418	0.32	RT, RI, MS
Aromadendrene	1440	0.19	RI, MS
Germacrene-D	1451	0.18	RI, MS
α -Caryophyllene	1454	0.28	RT, RI, MS
α-Ylangene	1471	0.33	RI, MS
α-Amorphene	1485	0.44	RI, MS
α -Farnesene	1509	0.22	RT, RI, MS
Calamenene	1520	0.25	RI, MS
7-epi-α-Cadinene	**1522**	**2.26**	**RT, RI, MS**
δ-Cadinene	**1524**	**3.02**	**RT, RI, MS**
Nerolidol	1533	0.26	RT, RI, MS
Spathulenol	1574	0.32	RT, RI, MS
Viridiflorol	1590	0.16	RI, MS
Hexadecanoic acid	1984	0.20	RT, RI, MS

Total (38)		98.17	

[1] Compounds are listed in order of elution from a ZB-5MS column.

[2] Retention indices relative to C_9-C_{28} *n*-alkanes on the ZB-5MS column.

[3] RT = identification based on retention time, RI = Identification based on retention index, MS= identification based on comparison of mass spectra

4.16.a. Discussion

The yield (g/100g of dry plant materials) of essential oil of *Cinnamomum zeylanicum* was found to be 0.78 % as listed in Table 4.16.1. The oil was light brown in colour with strong aromatic smell and refractive index 1.342. Essential oil was found to be less dense floating on the surface of water during the process of hydrodistillation with specific gravity 1.0. Mallavarapu *et al*. (1995) obtained the yield of volatile oil content of the Hyderabad sample (4.7 %) was higher than the Bangalore sample (1.8 %) of *C. zeylanicum*. Singh *et al.* (2007) subjected cinnamon leaves and bark to hydrodistillation to get yield of essential oils (3.1 % and 2.5 %, respectively).

The DPPH radical scavenging by *C. zeylanicum* essential oil was represented in terms of 50 % scavenging (IC_{50}) as shown in Table 4.16.2, which was found to be 54.77µg/mL. Whereas the scavenging effect of synthetic antioxidant i.e. BHT was 3.46 µg/mL. Linoleic acid was also used to test the antioxidant capacity of essential oils. Table 4.16.2 shows the % inhibition in linoleic acid system by *C. zeylanicum* essential oil taken at three different concentrations. It is clear that maximum inhibition was 73.19 % at concentration 50 µL/mL which decreased with the decrease in concentration and therefore could be attributed to decrease in concentration of bioactive compound accordingly. The activity shown by essential oil was found to be quite lesser than that of synthetic antioxidant BHT which showed 85.10 % inhibition. β-carotene has ability to form a stable β-carotene radical with peroxyl radical (LOO•), formed by the lipids such as linoleic acid in result of their oxidation which causes in reduction of amount of β-carotene. However this reduction would have been inhibited due to presence of some antioxidant in the reaction medium which could react with peroxyl radical competitively to β-carotene. Thus antioxidant effect can easily be examined by depleting the colour of solution using spectrophotometer at 470 nm. Bleaching of β-carotene as a function of antioxidant activity of the essential oil of *C. zeylanicum* is shown in Figure 4.16.1. The least antioxidant activity was exhibited by control with the maximum colour depletion. Whereas in samples, due to their antioxidant potential, there was less depletion of colour. *C. zeylanicum* was used at three different concentrations where minimum depletion was observed at 6 mg/mL. In case of BHT the depletion was the least showing the maximum antioxidant activity. Singh *et al.* (2007) evaluated the radical scavenging activity of leaf and bark volatile oils and oleoresins of

Cinnamomum zeylanicum. Bark oleoresin showed the best result through all concentrations for DPPH assay. In the same study lipid inhibitory activities of leaf and bark volatile oils and oleoresins of *C. zeylanicum* were also tested using ferric thiocyanate method of measuring the amounts of peroxides formed in emulsion. Bark oleoresin was found to be most effective among others. Schmidt *et al.* (2006) found that antioxidant activity showed by essential oil from *C. zeylanicum* was much better than BHT and BHA.

The antimicrobial activity of the essential oil of *C. zeylanicum* against various microbes including bacteria and fungi is shown in Table 4.16.3. It is clear that essential oil showed average activity against most of the fungi and bacteria in comparison with the antibiotic. The results obtained from the disc diffusion method and MIC made it clear that *C. zeylanicum* showed potential as an antimicrobial agent with larger inhibition zones (10.7-22.5 and 6.7-19.3 mm) and smaller MIC values (1.56-2.81 and 1.41-4.37 mg/mL) against various bacterial and fungal strains, respectively. In case of bacteria maximum activity was found against *L. rhamnosus* (Gram positive) with largest inhibition zone 22.5 mm followed by minimum MIC value 1.09 mg/mL. The activity was much better than that of antibiotic, Rifampicin, which showed the inhibition zone 15.5 mm and MIC 2.81 mg/mL. In case of fungi, *A. flavus* showed to be most sensitive with largest inhibition zone 19.3mm followed by minimum MIC value 1.41mg/mL which showed much effectiveness even than that of antibiotic, Terbinafine, with inhibition zone 11.4 mm and MIC 2.81 mg/mL. Singh *et al.* (2007) tested leaf and bark volatile oils of *C. zeylanicum* against various microbes like *Aspergillus ochraceus, Penicillium citrinum, Aspergillus flavus, A. ochraceus, Aspergillus niger, Aspergillus terreus, P. citrinum and Penicillium viridicatum*. Gupta *et al.* (2008) found oil of *C. zeylanicum* to be very effective against *Bacillus* sp., *Listeria monocytogenes, E. coli* and *Klebsiella* sp.

Oils and fats become rancid upon hydrolysis of triglycerides to give rise free fatty acids (FFAs) in presence of oxygen, moisture or enzymes. Rancidity of fatty foods can be well checked by measuring the quantity of free fatty acids formed which has become an important parameter for manufacturers. Table 4.16.4 shows the effect of *C. zeylanicum* essential oil on formation of FFAs from where it is clear that their concentration continuously increased with the length of storage period. Highest FFA was exhibited by control (0.64 %), while maximum stabilization was exhibited by *C. zeylanicum* essential oil at 1000ppm which was 0.27 % even

after induction period of 90 days, while with BHT it was 0.22 %. Peroxide value (PV) is presented in table 4.16.5 and it showed that maximum stabilization was obtained at 1000ppm with minimum PV i.e. 3.4 meq/kg after induction period of 90 days, which was 2.3 and 6.1 meq/kg for BHT and control, respectively. *p*-anisidine value was an important parameter used to measure the secondary products in lipid oxidation process which is presented in Table 4.15.6 for *C. zeylanicum*. Control showed *p*-anisidine value 45.8 after 90 days which was much stabilized by *C. zeylanicum* essential oil sample of 1000ppm with value 21.7 while in case of BHT it was found to be 17.9. In another study oxidative deterioration of mustered oil was evaluated by measuring the antioxidant parameters like PV, TBA, p-An and TC values while *C. zeylanicum* essential oil and oleoresins were used for its stabilization (Singh *et al.*, 2007).

The effect of *C. zeylanicum* essential oil on the viability of human and bovine erythrocytes was evaluated to investigate the cytotoxicity of the essential oil. The effect was checked on three different concentrations of essential oils and % hemolysis were calculated which is presented in table 4.16.7. It is clear from the results that sensitivity of both erythrocytes increased with the increase in concentration of essential oil. For bovine erythrocytes, *C. zeylanicum* exhibited the maximum hemolysis i.e. 7.21% at 10 mg/mL concentration and the minimum of 0.31 % at 0.5 mg/mL concentration, while with human erythrocytes maximum hemolysis i.e. 8.78 % at 10 mg/mL concentration and the minimum of 0.95 % at 0.5 mg/mL. Budavari *et al.* (1989) have reported *Cinnamon* to show minute toxicity in the animals.

The data of chemical composition of the essential oil from *C. zeylanicum* is listed in Table 4.16.8. Total 38 compounds were identified representing 98.17 % of the oil. The major components were found to be Cinnamaldehyde (77.86 %), δ-Cadinene (3.02 %), 7-epi-α-Cadinene (2.26 %), Copaene (2.15 %) and trans.-Anethole (1.74 %). Singh *et al.* (2007) obtained cinnamon leaves and bark from local market of Gorakhpur, Utter pradesh (India), which was subjected to extraction of essential oil followed by chemical characterization. Major components were found to be eugenol (87.3 %), β-caryophyllene (1.9 %), α-phellandrene (1.9 %) and amorphene (1.1 %). Ooi *et al.* (2006) analyzed essential oil of *C. cassia*. The major components were found to be *trans*-cinnamaldehyde (85.06 %), o-methoxy-cinnamaldehyde (8.79 %) and cis-cinnamaldehyde (1.33 %).

Chapter 5

SUMMARY

The research work presented in this dissertation was conducted in the Central Hi-tech Lab, University of Agriculture, Faisalabad; Protein and Molecular Biology Lab, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan and Lab of Medicinal and Aromatic Plants, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA. Total 16 plants including a weed, some spices and eucalyptus species were collected from Pothohar region, which is the northern eastern part of Pakistan with its specific weather. Experiments were conducted to study different parameters like % yield, chemical composition and biological activities including antioxidant, antimicrobial activities and cytotoxicity of essential oils of collected plants including *Parthenium hysterophorus*. To the best of our knowledge the essential oil of *Parthenium hysterophorus* has never been reported with regard to its composition as well as biological activities before present study.

Among all the plants tested maximum essential oil yield was obtained in case of *Syzygium aromaticum* (6.70 g/100g) while minimum in case of *Parthenium hysterophorus* (0.02 g/100g). The essential oils were evaluated for antioxidant potential using DPPH assay where maximum scavenging capability was shown by *Trachyspermum ammi* (IC_{50}=2.61µg/mL) which was even better than that of BHT, the synthetic antioxidant (IC_{50}=3.46 µg/mL). % inhibition in linoleic acid system was another assay to test the antioxidant activity of essential oils. The inhibition was tested on three different concentrations and all samples showed maximum inhibition at maximum concentration i.e. 50 µL/mL. *Trachyspermum ammi, Murraya koenigii, Syzygium aromaticum and Parthenium hysterophorus* inhibited linoleic acid oxidation more than 80% which was found comparable to BHT with 85% inhibition. Antioxidant activity of all the samples were also tested using bleachability of β-carotene in linoleic acid system and again the

above mentioned plants inhibited the bleachability of β-carotene, showing the greater antioxidant potential.

Stabilization studies of edible oil by essential oils were performed using a blend of oils i.e. canola and rapeseed (70:30). Three different parameters like % FFA, PV and *p*-Anisidine value were used to evaluate the stabilization capability of essential oils. Minimum FFA were found in case of *Cuminum cyminum* treated oils, 0.21% which was even much better than that of BHT 0.22%. Minimum PV was again found in case of *Cuminum cyminum,* 2.6 meq/kg while that of BHT it was 2.3 meq/kg. *Cuminum cyminum* showed minimum *p*-Anisidine value of 17.8. In this way *Cuminum cyminum* stabilized the edible oil more effectively as compared to others.

Antimicrobial activity of essential oils tested against various bacteria and fungi revealed that *Cuminum cyminum* and *Trachyspermum ammi* both showed good antibacterial activity which is evident from their largest inhibition zones (13.3-27.0mm) for *Cuminum cyminum* and (13.7-19.7mm) for *Trachyspermum ammi* with lowest MIC values (1.1-4.2 mg/mL) for *Cuminum cyminum* and (1.41-2.19 mg/mL) for *Trachyspermum ammi*. The activity could be attributed to the presence of cumaldehyde and thymol. Against fungal strains best activity was shown by *Eucalyptus camaldulensis* with largest zones of inhibition (5.3-22.7mm) and lowest MIC values (1.17-2.5 mg/mL).

Cytotoxicity study was performed using hemolytic assay which was performed at three different concentrations of essential oils. It was noted that all the samples showed maximum cytotoxicity at highest concentration i.e. 10 mg/mL and minimum at lowest concentration i.e. 0.5 mg/mL. Maximum cytotoxicity at minimum concentration was shown by *Myristica fragrans (N),* 44.09% while minimum cytotoxicity at minimum concentration was shown by *Murraya koenigii,* 0.79%. Maximum cytotoxicity at maximum concentration was exhibited by *Citrus reticulata,* 99.65 while minimum cytotoxicity at maximum concentration was exhibited by *Parthenium hysterophorus,* 32.63% which shows that it can also be used at higher concentrations.

To see that which components were responsible for the activity of essential oils, GC-MS analysis was performed. The samples which showed best potential for various biological activities include *Trachyspermum ammi* (Thymol, 37.75%), *Cuminum cyminum* (cumaldehyde, 24.10%), *Murraya koenigii* (eugenol, 81.61%), *Syzygium aromaticum* (eugenol, 51.02%), and *Eucalyptus camaldulensis* (*p*-cymene, 46.12%).

REFERENCES

Abbasi, B.H., N. Ahmad, H. Fazal and T. Mahmood. 2010. Conventional and modern propagation techniques in *Piper nigrum*. J. Med. Plant Res., 4:007-012.

Adams, R.P. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Carol stream, Allured, IL, USA.

Agar, N.S., Ogawa, E., O'Callaghan, S.S. and Hume, I.D. 1998. The Effect of Eucalyptus Oils on the Erythrocytes of Koalas. Comp. Haematol. Int., 8:225-229

Agnihotri, S.A., S.R. Wakode and M. Ali. 2012. Chemical composition, antimicrobial and topical anti-inflammatory activity of essential oil of *Amomum subulatum* fruits. Acta Polonoae Pharm. Drug Res., 69(6):1177-1181

Ahmadi, F., S. Sadeghi, M. Modarresi, R. Abiri and A. Mikaeli. 2010. Chemical composition, *in vitro* antimicrobial, antifungal and antioxidant activities of the essential oil and methanolic extract of *Hymenocrater longiflorus* Benth., of Iran. Food Chem. Toxicol., 48:1137-1144.

Ahmad, N., H. Fazal, B.H. Abbasi, M. Rashid, T. Mahmood and N. Fatima. 2010. Efficient regeneration and antioxidant potential in regenerated-tissues of *Piper nigrum* L. Plant Cell, Tissue and Organ Cult., 102:129-134.

Akerel, O. and B.A. Ayinde. 1998. Antibacterial Activity Of The Volatile Oil And Aqueous Extract Of *Murraya koenigii* Leaves, Niger. J. Nat. Prod. Med., 2:44-45.

Aki, H. and M. Yamamoto. 1991. Drug binding to human erythrocytes in the process of ionic drug-induced hemolysis. Biochem. Pharmacol., 41(1):133-138.

Alam, K., D. Pathak and S.H. Ansari. 2011. Evaluation of anti-inflammatory activity of *Amomium subulatum* fruit extract. Int J Pharm Sci Drug Res**., 3**:35-37.

Alitonou, G.A., F.P. Tchobo, F. Avlessi, B. Yehouenou, P. Yedomonhan, A.Y. Koudoro, C. enut and D.K. Sohounhloue. 2012. Chemical and biological investigations of *Syzygium aromaticum* L. essential oil from Benin. Int. J. Biol. Chem. Sci. 6(3):1360-1367

Allahghadri, T., I. Rasooli, P. Owlia, M.J. Nadooshan, T. Ghazanfari, M. Taghizadeh And S.D.A. Astaneh. 2010. Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. J. of Food Sci., 75(2): 54-61.

Anand, P., A. Kunnumakara, C. Sundaram, K. Harikumar, S. Tharakan, O. Lai, B. Sung and B. Aggarwal. 2008. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceut. Res., 25:2097-2116.

Angioni, A., A. Barra, V. Coroneo, S. Dessi and P. Cabras. 2006. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. J. Agric. and Food Chem., 54:4364-4370.

Anwar, F., M. Ali, A. I. Hussain and M. Shahid. 2009. Antioxidant and antimicrobial activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. Flav. and Frag. J., 24:170-176.

Anwar, F., R. Naseer, M.I. Bhanger, M. Ashraf, F.N. Talpur and F.A. Aladedunye. 2008. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. J. Am. Oil Chem. Soc. 85:321-330.

Anwar, F., M. Ali, A.I. Hussain and M. Shahid. 2009. Antioxidant and antimicrobial activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. Flav. and Frag. J., 24:170-176.

Arima, Y., Y. Nakai, R. Hayakawa and T. Nishino. 2003. Antibacterial effect of beta-thujaplicin on staphylococci isolated from atopic dermatitis: relationship between changes in the number of viable bacterial cells and clinical improvement in an eczematous lesion of atopic dermatitis. J Antimicrob. Chemother., 51(1): 113-122.

Asolkar, L.V., K.K. Kakkar and O.J. Chakra. 1992. Second Supplement to glossary of Indian medicinal plants with active principles. Part I (A-K). Publication and Information Division, CSIR, New Delhi, India.

Atal, C.K. and B.M. Kapur. 1982. Cultivation and Utilization of Aromatic plants. CSIR, Jammu-Tawi, India. 136-741.

Baik, J.S., S.S. Kim, J.A. Lee, T.H. Oh, J.Y. Kim, N.H. Lee and C.G. Hyun. 2008. Chemical composition and biological activities of essential oils extracted from Korean endemic citrus species. J. Microbiol. Biotechnol. 18(1):74-79

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. 2008. Biological effects of essential oils – A review. Food and Chem Toxicol., 46:446–475

Ball, J.B. 1995. Development of Eucalyptus plantations – an overview. In: White K, Ball J, Kashio M (eds), Proceedings of the Regional Expert Consultation on Eucalyptus Vol. I. 15-27.

Barra, A., V. Coroneo, S. Dessi, P. Cabras and A. Angioni. 2010. Chemical variability, antifungal and antioxidant activity of *Eucalyptus camaldulensis* essential oil from Sardinia. Nat. Prod. Commun., 5:329-335.

Barton, D.H.R. and A.S. Lindsay. 1951. Sesquiterpenoids. Part I. Evidence for a nine-membered ring in caryophyllene. J. Chem. Soc. Lond., 2988-2991.

Barceloux, D.G. 2008. Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Toxic Plants, and Venomous Animals. Hoboken, N.J., John Wiley & Sons. 67–70.

Baser, K.H.C. 2005. In: Sacred Incense to Fragrant Elixir: Perfume. YKY, Istanbul, pp 123–125

Baser, K.H.C. 1995. In: de Silva KT (ed) A Manual on Essential Oil Industry. UNIDO, Vienna, p 155

Basak, S.S. and F. Candan. 2010. Chemical composition and *In vitro* antioxidant and antidiabetic activities of *Eucalyptus Camaldulensis* Dehnh. essential oil. J. Iran. Chem. Soc., 7(1): 216-226.

Bassole, I.H.N., A.S. Ouattara, R. Nebie, C.A.T. Ouattara, Z.I. Kabore and S.A. Traore. 2003. Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. Phytochem., 62:209–212.

Batish, D.R., H.P. Singh, N. Setia, S. Kaur and R.K. Kohli. 2006. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. J. Biosci., 61(8):465-471

Baytop, T. 1999. Therapy with Medicinal Plants in Turkey, Past and Present, Nobel Tıp Kitapevi, Istanbul, Turkey, 2nd edition. Pp 118-119.

Bergougnoux, V., J.C. Caissard, F. Jullien, J.L. Magnard, G. Scalliet, J.M. Cock, P. Hugueney, and S. Baudino, 2007. Both the adaxial and abaxial epidermal layers of the rose petal emit volatile scent compounds. Planta, 226: 853–866.

Betts, T.J. 2001. Chemical characterization of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas hromatographic stationary phases. J. Chromatogr., 936:33-46.

Bisht, V.K., J.S. Negi, A.K. Bhandari and R.C. Sundriyal. 2011. *Amomum subulatum* Roxb.: Traditional, phytochemical and biological activities – An overview. Africa J Agric Res**.,** 24: 5386–5390.

Bowles, E.J. 2003. Chemistry of Aromatherapeutic Oils. Allen & Unwin, ISBN 9781741140514. 3rd Ed.

Braak,V.D.S.A.A.J. and G.C.J.J. Leijten. 1999. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, p. 116.

Branen, A.L., 1983. Introduction to use of antimicrobials. In: Davidson, P.M., Branen, A.L. (Eds.), Antimicrobials in Foods. Marcel Dekker, Inc., New York, pp. 1-9.

Brand-Williams, W., M.E. Cuvelier and C. Berset. Use of a free radical method to evaluate antioxidant activity. Lebensm. -Wiss. Technol., 28:25–30.

Breitmaier, E. 2005. Terpene: Aromen, Dute, Pharmaka, Pheromone. Wiley-VCH, Weinheim

Bren, L.J. and N.L. Gibbs. 1986. Relationships between flood frequency, vegetation and topography in a river red gum forest. Aus Forest Res., 16:357-370.

Briozzo, J. 1989. Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J. App Bacteriol. 66:69-75.

Buchel, J.A. 1989. Flavoring with citrus oil. Perfumer and Flavorist, 14:22–26.

Budavari, S., M.J. O'Neil, A. Smith and P.E. Heckelman. 1989. The Merck Index, 11th Ed. Merck & Co., Inc., Rahway, NJ, p. 242.

Burt, S.A and R.D. Reinders. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Letters in App Microbiol., 36(3):162– 167.

Burt, S. 2004. Essential oils: their antimicrobial properties and potential application in foods-A review. Int J Food Microbiol., 94:223-253.

Butz, P. and B. Tauscher. 2002. Emerging technologies: chemical aspects. Food Res. Int., 35: 279-284.

Calliste, C.A., P. Trouillas, D.P. Allais, A. Simon, and J.L. Duroux. 2001. Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. J. Agric. Food Chem., 49: 3321–3327.

Cantore, P.L., N.S. Iacobellis, A.D. Marco, F. Capasso and F. Senatore. 2004. Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller Var. vulgare (Miller) essential oils. J. Agric. Food Chem., 52:7862–7866

Cao, L., J.Y. Si, Y. Liu, H. Sun, W. Jin and Z. Li. 2009. Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. Food Chem., 115:801–805.

Celiktas, O.Y., E.E.H. Kocabas, E. Bedir, F.V. Sukan, T. Ozek and K.H.C. Baser. 2006. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem., 100:553-559.

Chaillou, L. L. and M.A. Nazareno. 2006. New method to determine antioxidant activity of polyphenols. J. Agric. Food Chem., 54:8397–8402.

Champion, H.G., S.K. Sethi and G.M. Khattak. 1965. Forest types of Pakistan. Pakistan Forest Institute, Peshawar.

Chatha, S.A.S., A. I. Hussain, J.R. Bajwa, S.T.H. Sherazi and A. Shaukat. 2011. Wheat bran extracts: a potent source of natural antioxidants for the stabilization of canola oil. grasas y aceites, 62(2):190-197

Chatterjee, S., Z. Niaz, S. Gautam, S. Adhikari, P.S. Variyar, and A. Sharma. 2007. Antioxidant activity of some phenolic constituents from green pepper *(Piper nigrum L.)* and fresh nutmeg mace *(Myristica fragrans).* Food Chem., 101:515–523.

Cheewangkoon, R., J.Z. Groenewald, B.A. Summerell, K.D. Hyde, C. To-anun and P.W. Crous. 2009. Myrtaceae, a cache of fungal biodiversity. Persoonia. 23:55–85

Chericoni, S., J.A.. Prieto, P. Iacopini, P. Cioni and I. Morelli. 2005. In vitro activity of the essential oil of *Cinnamomum zeylanicum* and eugenol in peroxynitrite-induced oxidative processes. J. of Agric. and Food Chem., 53:4762-4765.

Chirathaworn, C., W. Kongcharoensuntorn, T. Dechdoungchan, A. Lowanitchapat, P. Sanguanmoo and Y. Poovorawan. 2007. *Myristica fragrans* Houtt. Methanolic Extract induces Apoptosis in a Human Leukemia Cell Line through SIRT1 mRNA Downregulation. J Med Assoc Thai., 90(11): 2422–2428.

Choo, L.C., S.M. Wong and K.Y. Liew. 1999. Essential oil of nutmeg pericarp. J Sci Food Agric., 79:1954–7.

Choi, E. and J. Hwang. 2004. Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia, 75: 557-565.

Chowdhury, J.U., M.D.H. Mobarok, N.I. Bhiyan and N.C. Nandi. 2009. Constituents of essential oils from leaves and seeds of *Foeniculum vulgare* Mill. Cultivated in Banmgladesh. Bang. J. Bot., 38(2):181-183

Chowdhry, J.U., M.H. Mobarok, M.N.I. Bhuiyan and N.C. Nandi. 2009. Constitunts of essential oils from leaves and seeds of *Foeniculum vulgare* MILL. Cultivated in Bangladesh. Bang. J. Bot., 38(2):181-183.

Chutia, M., P.D. Bhuyan, M.G. Pathak, T.C. Sarma and P. Boruah. 2009. Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. LWT - Food Sci. and Technol., 42: 777–780.

Cimanga, K., K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, J. Totte, L. Pieters and A.J. Vlietinck. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol., 79: 213– 220.

Clemente, M.A., O.C.M. Monteiro, M.G. Scoralik, F.T. Gomes, M.C. Prata and E. Daemon. 2010. Acaricidal activity of the essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). Parasitol Res., 107(4):987-92.

Coelho, D.S.L.N., C.j.H. Leal, A.M.F.J. De, S. Lahlou and P.J. Magalhaes. 2005. Relaxant effects of the essential oil of *Eucalyptus tereticornis* and its main constituent 1,8-cineole on guinea-pig tracheal smooth muscle. Planta Med., 71: 1173-1175.

Costa, R., M.R. De Fina, M.R. Valentino, P. Dugo and L. Mondello. 2007. Reliable identii cation of terpenoids and related compounds by using linear retention indices interactively with mass spectrometry search. Nat. Prod. Comm., 2: 413.

Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4):564-82.

Croteau, R., T.M. Kutchan and N.G. Lewis. 2000. Natural products (secondary metabolites). In Biochemistry & Molecular Biology of Plants, Chap. 24, 1st Ed., B. Buchanan, W. Gruissen, and R. Jones (eds). New Jersey: ASPB and Wiley.

Damasius, J., M. Skemaite, G. Kirkilaite, R. Vinauskiene and P.R. Venskutonis. 2007. Antioxidant and antimicrobial properties of caraway *(Carum carvi L.)* and cumin *(Cuminum cyminum L.)* extracts. Veterinarija IR Zootechnika, 40(62).

Damjanovic-Vratnica, B., T. Dakov, D. Sukovic and J. Damjanovic. 2011. Antimicrobial effect of essential oil isolated from *Eucalyptus globules Labill* from Montenegro. Czech J. Food Sci.**,** 29*:* 277–284.

Daniel, Z. and H. Maria. 2000. Domestication of Plants in the Old World, third ed. University Press, Oxford. p. 206.

Davidson, P.M. and M.A. Harrison. 2002. Resistance and Adaptation to Food Antimicrobials, Sanitizers, and Other Process Controls. Scientific Status Summary. 56:69-78

Das, B., V.S. Reddy, M. Krishnaiah, A.V.S. Sharma, K.K. Ravi, J.V. Rao and V. Sridhar. 2007. Acetylated pseudoguaianolides from *Parthenium hysterophorus* and their cytotoxic activity. Phytochem., 68:2029–2034

Das, K.C., D.P. Chakraborty and P.K. Bose. 1965. Antifungal Activity Of Some Constituents Of *Murraya koenigii*, Experimentia, 21(6):340

Dasti, A.A., S. Saima, M. Athar, A.R. Saeed and A. Malik. 2007. Botanical composition and multivariate analysis of vegetation on the pothowar plateau, Pakistan. J. Bot. Res., 1(1):557-568

Davies, F.S. and L.G. Albrigo. 1994. Citrus. Wallingford: CAB International. p. 1.

Deans, S.G. and G. Ritchie. 1987. Antibacterial properties of plant essential oils. Int. J. Food Microbiol., 5:165-180.

Dehkordi, S.S., H. Tajik., M. Moradi and K.F. Sigaroodi. 2010. Chemical composition of essential oils in *Zataria multiflora* Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem. Toxicol., 48:1562-1567.

Dewick, P.M. 2002. Medicinal Natural Products: A Biosynthetic Approach, 2nd ed. Wiley

Dhanya, K., J. Kizhakkayil, S. Syamkumar and B. Sasikumar. 2007. Isolation and amplification of genomic DNA from recalcitrant dried berries of black pepper (*Piper nigrum* L.). A medicinal spice. Mol. Biotechnol., 7:165-168.

Dharmawan, J., S. Kasapis, P. Curran and J.R. Johnson. 2007. Characterization of volatile compounds in selected citrus fruits from Asia. Part I: freshly-squeezed juice. Flav. and Frag. J., 22: 228–232.

Dongmo, P.M.J., L.N. Tatsadjieu, F. Tchournbougnang, M.L. Sameza, B.N. Dongmo, P.H.A. Zollo and C. Menut. 2007. Chemical composition, antiradical and antifungal activities of essential oil of the leaves of *Cinnamomum zeylanicum* blume from Cameroon. Nat. Prod. Comm., 2: 1287–1290.

Dorman, H.J.D., A. Peltoketo, R. Hiltunen and M.J. Tikkanen. 2003. Characterization of the antioxidant properties of deodorized aqueous extracts from selected Lamiaceae herbs. Food Chem., 83: 255-262.

Dorman, H.J.D and S.G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J App Microbiol., 88: 308–316.

Dorman, H.J.D., P. Surai and S.G. Deans. 2000. In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. J. Essent. Oil Res., 12:241–248.

Duke, J.A., M.J. Bogenschutz-Godwin, J. DuCelliar and P.K. Duke. 2002. Hand Book of Medicinal Herbs, second ed. CRC Press, Boca Raton, pp. 153–154.

Duke, J.A., 1992. Handbook of Phytochemical Constituents of GRAS Herbs and other Economical Plants. CRC Press, London, pp. 239–240.

Eikani, M.H., G. Iraj and M. Mehdi. 1999. Supercritical carbon dioxide extraction of cumin seeds (*Cuminum cyminum* L.). Flav. Frag. J., 14: 29-31.

El-Alfy, A.T., L. Wilson, M.A. El-Sohly and E.A. Abourashedd. 2009. Towards a better understanding of the psychopharmacology of nutmeg: Activities in the mouse tetrad assay. J Ethnopharmacol., 126(2): 280–286.

Elaissi, A., **H. Medini**, **M. Simmonds**, **F. Lynen**, **F. Farhat**, **R. Chemli**, **F. Harzallah-Skhiri** and M.L. **Khouja**. 2011. Variation in volatile leaf oils of seven eucalyptus species harvested from Zerniza arboreta (Tunisia). **Chem Biodivers.**, 8(2): 362-372.

Ergin, C., M. Ilkit, S. Hilmioglu, I. Kaleli, A.G. Gülbaba, M. Demirci, and S. Kaya. 2004. The first isolation of Cryptococcus neoformans from Eucalyptus trees in south aegean and Mediterranean regions of Anatolia in Turkey despite Taurus mauntains alkalinity. Mycopathol., 158: 43–47.

Fan, L.S., R. Muhammad, D. Omar and M. Rahmani. 2011. Insecticidal Properties of *Piper nigrum* Fruit Extracts and Essential Oils against *Spodoptera litura*. Int. J. Of Agric. and Biol., 1814–9596

Fan, J., X. Ding and W. Gu. 2007. Radical-scavenging proanthocyanidins from sea buckthorn seed. Food Chem., 102: 168-177.

Falahati, M., N.O. Tabrizib and F. Jahaniani. 2005. Anti dermatophyte Activities of *Eucalyptus camaldulensis* in Comparison with Griseofulvin. Iranian J. of Pharm. & Therap., 4: 80-83

Farag, R.S., Z.Y. Daw and A.S.H. Raya. 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci., 54: 74–76.

Fatimi, A.M., M. Wurster, G. Schroder and U. Lindequist. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol., 111: 657-666.

Firouzi, R., M. Azadbakht and A. Nabinedjad. 1998. Anti-listerial activity of essential oils of some plants. J App Animal Res., 14: 75– 80.

Fisher, K. and C. Phillips. 2008. Potential antimicrobial uses of essential oils in food: Is citrus the answer? Trends in Food Sci Technol., 19: 156–164.

Fonseca, J.M., J.W. Rushing, N.C. Rajapakse, R.L. Thomas and M.B. Riley. 2006. Potential implications of medicinal plant production in controlled environments: the case of feverfew (*Tanacetum parthenium*). Horti. Sci., 41: 531-535.

Francisco, J.C., E.P. Jarvenpaa, R. Huopalahti, and B. Sivik. 2001. Comparison of *Eucalyptus camaldulensis* Dehn. Oils from mozambique as obtained by hydrodistillation and supercritical carbon dioxide extraction. J. Agric. Food Chem., 49: 2339–2342.

Friedman, M., P.R. Henika and R.E. Mandrell. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot., 65: 1545-1560.

Gachkar, L., D. Yadegari, R.M. Bagher, M. Taghizadeh, A.S. Alipoor and I. Rasooli. 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem., 102: 898-904.

Gandomi, H., S. Abbaszadeh, A. Jebellijavan and A. Sharifzadeh. 2013. Chemical constituents, antimicrobial and antioxidative effects of *Trachyspermum ammi* essential oil. J. of Food Process. and Preserv., 1745-4549.

George, D.R., D. Masic, O.A.E. Sparagano and J. Guy. 2009. Variation in chemical composition and acaricidal activity against *Dermanyssus gallinae* of four eucalyptus essential oils. Exp Appl Acarol., 48: 43–50.

Gilani, A.H., Q. Jabeen, A.U. Khana and A.J. Shah. 2008. Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom. J. of Ethnopharmacol., 115: 463–472

Gopalakrishnan, M., C.S. Narayanan and M. Grenz. 1990. Non saponifiable lipid constituents of cardamom. J. of Agric. and Food Chem., 38: 2133–2136.

Grieve, M. 1997. A Modern Herbal. Dover publication Inc. 2: 823

Guenther, E. 1960. Determination of Physical and Chemical Properties. The essential oils (Vol III). Toronto, New York and London: D. Van Nostrand Comp., INC., pp. 236-262.

Guenther, E., 1948. The Essential Oils. D. Van Nostrand, New York.

Guenther, E. 1952. The essential Oils, D. Van Nostrand Company, Inc., New York, London, Vol. 1, pp, 19,50,65.

Gupta, V., S. Chauhan, A. Prakash and A. Mathur. 2013. Evaluation of *in vitro* and *in vivo* anti-inflammatory activities of *Parthenium camphora*. Recent Res. Sci. Technol., 5(1): 33-39

Gupta, C., A.P. Garg, R.C. Uniyal and A. Kumari. 2008. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on somefood-borne microbes. Afr. J. of Microbiol. Res., 2(9):247-251

Hajlaoui, H., H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri and A. Bakhrouf. 2010. Chemical composition and biological activities of Tunisian *Cuminum cyminum L.* essential oil: A high effectiveness against *Vibrio* spp. Strains. Food & Chem Toxicol., 48: 2186-2192

Hajhashemi, V., A. Ghannadi and B. Sharif. 2003. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. J. Ethnopharmacol., 89: 67-71.

Hamdan, D., M.Z. El-Redi, E. Nibret, F. Sporer, N. Farrag, A. El-Shazly and M. Wink. 2010. Chemical composition of essential oils of two citrus species and their biological activities. Pharmazie. 65: 141-147

Hammer, K.A., C.F. Carson T.V. Riley. 1999. Antimicrobial activity of essential oils and other plant extracts. J App Microbiol., 86: 985– 990.

Harrewijn, P., A.M.V. Oosten and P.G.M. Piron. 2001. Natural Terpenoids as Messengers. Dordrecht: Kluwer Academic Publishers.

Hasegawa, T., F. Takano, T. Takata, M. Niiyama and T. Ohta. 2008. Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of *Eucalyptus globules*. Phytochem., 69:714-753

Hassine, D.B., M. Abderrabba, Y. Yvon, A. Lebrihi, F. Mathieu, F. Couderc and J. Bouajila. 2012. Chemical Composition and in Vitro Evaluation of the Antioxidant and Antimicrobial Activities of *Eucalyptus gillii* Essential Oil and Extracts. Molecules, 17:9540-9558

Holley, J and K. Cherla. 1998. The Medicinal Plants Sector in India. International Development Research Centre. South Asia Regional Office, IDRC, Canada Medicinal and Aromatic Program in Asia (MAPPA), New Delhi, India. 1-114.

Hsieh, P.C. 2000. Antimicrobial effect of cinnamon extract. Tai. J. Agric. Chem. Food Sci., 38:184-193.

Huang, D., B. Ou and R.L. Prior. 2005. The Chemistry behind Antioxidant Capacity Assays. J. Agric. Food Chem., 53:1841-1856

Huang, Y., S.H. Ho, H.C. Lee and Y.L. Yap. 2002. Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Stored Prod. Res., 38:403-412.

Hussain, A., S. Naz, H. Nazir and Z.K. Shinwari. 2011. Tissue culture of Black pepp pepper (*Piper nigrum* L.) in Pakistan. Pak. J. Bot., 43:1069-1078.

Hussain, J., A.L. Khan, N. Rehman, Zainullah, F. Khan, S.T. Hussain and Z.K. Shinwari. 2009**.** Proximate and nutrient investigations on selected medicinal plant species of Pakistan. Pak. J. of Nutr., **8:**620-624.

Hussain, A.I., F. Anwar, S.T.H. Sherazi and R. Przybylski. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108:986-995.

Hussain, F. and H. Ilahi. 1991. Ecology and vegetation of lesser Himalayas, Pakistan. Department of Botany, Univ. of Pehawar, Pakistan.

Iacobellis, N.S., L.P. Cantore, F. Capasso F. Senatore. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J. of Agric. and Food Chem., 53(1): 57-61.

International Union of Pure and Applied Chemistry (IUPAC). 1987. Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th Rev. edited by C. Paquot and A. Hautfenne, Blackwell Scientific, London.

International Union of Pure and Applied Chemistry (IUPAC). 1987. Standard Method for the Analysis of Oils, Fats and Derivatives, 6th Ed. Pergamon Press, Oxford, U.K., New York, NY, Sydney, Australia. 20-118

Iqbal, S. and M.I. Bhanger. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem., 93:265-272.

Ishikawah, T., Y. Sega and J. Kitajima. 2001. Water soluble constituents of ajawan. Chem. Pharm. Bull., 49:840-844

Iyer, U.M. and U.V. Mani. 1990. Studies On The Effect Of Curry Leave Supplementation On Lipid Profile, Glycated Proteins And Amino Acids In NIDDM Patients, Plant Foods Hum. Nutr., 40(4): 275-282.

James, A.T. and A.J.P. Martin. 1952. Gas–liquid partition chromatography: The separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. Biochem. J., 50:679.

Jamal, A., K. Javed., M. Aslam and M.A. Jafri. 2006. Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats. J Ethnopharmacol. 103:149-153

Jafri, M.A.F., K. Javed and S. Singh. 2001. Evaluation of the gastric antiulcerogenic effect of large cardamom (fruits of *Amomum subulatum* Roxb.). J Ethnopharmacol**., 75**:89-94.

Jayaprakasha, G.K. and L.J.M. Rao. 2010. Chemistry, biogenesis, and biological activities of *Cinnamomum zeylanicum*. Crit. Rev. in food sci. and nutr., 51:547-562

Jirovetz, L., G. Buchbauer, M.B. Nagassoum and M. Geissler, 2002. Aroma compound analysis of *Piper nigrum* and *Piper guineese* essential oils from Cameroon using solid phase microextraction gas chromatography, solid phase micoextraction gas chromatography mass spectrometry and olfactometry. J. Chromatogr., 976:265–275

Joshi, R., P. Sharma, V. Sharma, R. Prasad, R.K. Sud and A. Gulati. 2013. Analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. J Sci Food Agric., 93:1303–1309

Joshi, S.C., A.R. Verma and C.S. Mathela. 2010. Antioxidant and antibacterial activities of the leaf essential oils of Himalayan Lauraceae species. J. Chem. Toxicol., 48:37-40.

Joy, P.P., J. Thomas., S. Mathew, G. Jose and J. Joseph. 2001. Aromatic plants in tropical horticulture, Calcutta (India); Naya Prokash Publisher. Pp 2.

Joshi, S.G. 2000. Medicinal Plants. 1st ed. Delhi (India): Oxford and IBH publisher.

Joy, P.P., J. Thomas, S. Mathew and B.P. Skaria. 1998. Medicinal Plants. Kerala Agricultural University, Aromatic and Medicinal Plants Research Station. Pp 3.

Juergens, U.R., M. Stober and H. Vetter. 1998. Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1,8-cineole) in human blood monocytes in vitro. Eur J Med Res. 3:508-510.

Kalaivani, T., C. Rajasekaran, K. Suthindhiran and L. Mathew. 2010. Free radical scavenging, cytotoxic and hemolytic activities from leaves of *Acacia nilotica* (L.) Wild. ex. Delile subsp. indica (Benth.) Brenan. Evidence-based compl alt med., http://dx.doi.org/10.1093/ecam/neq060.

Kannan, R.R.R., R. Arumugam, P. Iyapparaj, T. Thangaradjou and P. Anantharaman. 2013. *In vitro* antibacterial, cytotoxicity and haemolytic activities and phytochemical analysis of seagrasses from the Gulf of Mannar, South India. Food Chem., 136:1484–1489

Kapoor, I.P.S., B. Singh, G. Singh, C.S.D. Heluani, M.P.D. Lampasona and C.A.N. Catalan. 2013. Chemical composition and antioxidant activity of essential oil and oleoresins of nutmeg (*Myristica fragrans* Houtt.) fruits. Int. J. of Food Prop., 16:1059-1070.

Kaur, S., H.P. Singh, D.R. Batish and R.K. Kohli. 2011. Chemical characterization, antioxidant and antifungal activity of essential oil from *Eucalyptus tereticornis*. J. of Med. Plants Res., 5(19):4788-4793

Kennedy, T. A. and D.C. Liebler. 1991. Peroxy radical oxidation of *β*-carotene: formation of *β*-carotene epoxides. Chem. Res. Toxicol., 4:290–295.

Khaldun, A.O. 2006. Antibacterial action of ether oils of some plants. *Zh Mikrobiol Epidemiol Immunobiol., 3:*92-93.

Khalil, A.B. and B.F. Dababneth,, 2007. Inhibition of phytopathogenic fungi by extracts from medicinal plants in Jordan. J. Biol. Sci., 7: 579-581.

Khan, S., K.J. Mirza, F. Anwar and M. Zainul Abdin. 2010. Development of RAPD markers for authentication of *Piper nigrum* (L.). Environ. We Int. J. Sci. Tech., 5:47-56.

Khan, P.A., A. Abraham and Lellamma. 1997. Antioxidant effects of curry leaf (*Murraya koenigii*) and mustard seeds (*Brassica juncea*) in rats fed with high fat diet .Indian J. Exptl .Biol., 35(2):148-50.

Khan, N.A. and S.Z. Rahman. 1992. The screening of Majoon-e-Azaraqi for cardiovascular and peripheral activity. Hamdard Medicus. 35:102–109.

Khanum, H., K. Ramalakshmi, P. Srinivas and B.B. Borse. 2011. Synergistic antioxidant action of Oregano, Ajowan and Borage extracts. J. Food Nutr. Sci. *2:*387-392.

Khuntia, T.K. and D.S. Panda. 2011. Evaluation of antibacterial, antifungal and anthelmintic activity of *Murraya koenigii* Spreng. Pharma Sci Monit., 2(2):105-110.

Kostic, I., O. Petrovic, S. Milanovic, Z. Popovic, S. Stankovic, G. Todorovic, M. Kostic. 2013. Biological activity of essential oils of *Athamanta haynaldii* and *Myristica fragrans* to gypsy moth larvae. Indust. Crops and Prod., 41:17- 20

Kotsifopoulos, P.N. 1975. Electrophoretic study of normal erythrocyte membrane proteins after *in vitro* action of antimalarial agents. Pathol. Biol., 23(4):313-314.

Kureel, S.P., R.S. Kapil and S.P. Popli. 1969. Terpenoid alkaloids from *Murraya koenigii* Spreng.-II. The constitution of cyclomahanimbine, bicyclomahanimbine & mahanimbidine. Tetrahedrone Lett., 44:3857-3862.

Kuate, J., J. Foko, S.A. Ndindeng, P.M.J. Dongmo, E. Foure, and F. Damesse. 2006. Effect of essential oils from citrus varieties on in vitro growth and sporulation of *Phaeoramularia angolensis* causing citrus leaf and fruit spot disease. European J. of Plant Pathol., 114:151–161

Kulisic, T., A. Radonic, V. Katalinic and M. Milos. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem., 85:633-640.

Kumar, A., S. Joshi and T. Malik. 2013. Antimicrobial potential of *Parthenium hysterophorus* Linn. Plant extracts. J. Life Sci. Biotech. Pharm. Res., 2(3): 232-236

Kurita, N., M. Miyaji, R. Kurane and Y. Takahara. 1981. Antifungal activity of components of essential oils. Agric Biol Chem., 45:945-952.

Lado, B.H. and A.E. Yousef. 2002. Alternative food-preservation technologies: efficacy and mechanisms. Microbes Infect., 4:433-440.

Lee, K.G. and T. Shibamoto. 2001. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. Et Perry]. Food Chem., 74:443-448.

Lima, M.D.P., T.M.D. Silva and F.C. Sousa. 2005. Volatile constituents from leaves and branches of *Cinnamomum zeylanicum* Blume (Lauraceae). Acta Amazon., 35:363–365.

Lota, M.L., D.D. Serra, F. Tomi, C. Jacquemond and C.J. Casanova. 2002. Volatile components of peel and leaf oils of lemon and lime species. J. Agric Food Chem. 50:796-805

Low, D., B.D. Rawal and W.J. Griffin. 1974. Antibacterial action of the essential oils of some Australian myrtaceae with special references to the activity of chromatographic fractions of oil of *Eucalyptus citriodora*. Planta Med., 26(6):184-189

Lu, Y. and l.Y. Foo. 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chem., 68:81–85.

MacDonald-Wicks, L. K., L.G. Wood and M.L. Garg. 2006. Methodology for the determination of biological antioxidant capacity in vitro: a review. J. Sci. Food Agric., 86:2046–2056.

Maciel, M.A.M., A.C. Pinto, V.F. Veiga Jr., N.F. Grynberg, and A. Echevarria. 2002. Medicinal plants: the need for multidisciplinary scientific studies. Quim Nova., 25(3):429-38.

MacMillan, J. and M.H. Beale. 1999. In: Barton D, Nakanishi K (eds) Comprehensive Natural Product Chemistry, vol 2. Isoprenoids including Carotenoids and Steroids. Elsevier, London, 217–243

Maishi, A.I., P.K.S. Ali, S.A. Chaghtai and G. Khan. 1998. A proving of *Parthenium hysterophorus*, L. Brit Homoeopath J., 87:17–21

Mallavarapu, G.R., S. Ramesh, R.S. Chandrasekhara, B.R.R. Rao, P.N. Kaul and A.K. Bhattacharya. 1995. Investigation of the essential oil of cinnamon leaf grown at Bangalore and Hyderabad. Flav. Fragr. J., 10:239-242.

Malti, J.E., N. Bourhim and H. Amarouchi. 2008. Toxicity and antibacterial effect of mace of *Myristica fragrans* used in Morrocan gastronomy: biochemical and histological impact. J. of Food Saf., 28:422-441.

Manfred, F., M.P. John, D.S. Dajaja and A.K. Douglas. 1985. Koenoline, a further cytotoxic carbazole alkaloid from *Murraya koenigii*. Phytochem., 24:3041-3043.

Maroto, D.M.S., S. Perez-Coello, J. Esteban and J. Sanz. 2006. Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. J. Agric. Food Chem., 54:6814-6818.

Marongiu, B., A. Piras And S. Porcedda. 2004. Comparative Analysis of the Oil and Supercritical CO_2 Extract of *Elettaria cardamomum* (L.) Maton. J. Agric. Food Chem., 52: 6278-6282

Maroto, D.M.C., P.M.S. rez-Coello, J. Esteban and J. Sanz. 2006. Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. J. Agric. Food Chem., 54:6814–6818.

Marya, C.M., G. Satija, J. Avinash, R. Nagpal, R. Kapoor and A. Ahmad. 2012. *In vitro* inhibitory effect of clove essential oil and its two active principles on tooth decalcification by apple juice. Int. J. Dentist., 1-6

Masotti, V., F. Juteau, J.M. Bessiere and J. Viano. 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. J. Agric. and Food Chem., 51:7115-7121.

Mathew, S. and T.E. Abraham. 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. Food Chem., **94**:520-528.

McNicholl, B.P., J.W. McGrath and J.P. Quinn. 2006. Development and application of a resazurin-based biomass activity test for activated sludge plant management. Water Res., 41: 127–133.

Mehdi, J.H., Z. Behrooz, and S. Hassan. 2007. Use of gas chromatography–mass spectrometry combined with resolution methods to characterize the essential oil components of Iranian cumin and caraway. J. Chrom., 11:215–226.

Mhaskar, K.S., E. Blatter, J.F. Caius, Kirtikar and Basu, 2000. Indian Medicinal Plants Vol. I. XI. 3rd Edn. Indian Medical Science Series # 86-96. Delhi, India.

Mighri, H., H. Hajlaoui, A. Akrout, H. Najjaa and M. Neffati. 2010. Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. C.R. Chim., 13:380-386.

Milan, K.S.M., H. Dholakia, P.K. Tiku and P. Vishveshwaraiah. 2008. Enhancement of digestive enzymatic activity by cumin (*Cuminum cyminum* L.) and role of spent cumin as a bionutrient. Food Chem., 110:678-83.

Mimica-Dukic, N., S. Kujundzic, M. Sokovic and M. Couladis. 2003. Essential oil composition and antifungal activity of *Foneiculum vulgare* Miller. obtained by different distillation conditions. Phytother. Res., 17:368-371.

Minakshi, M. and P. Monali, Antioxidant Activity And Organic Constituents Of Curry Leaf Tree, Asian J. Of Microbiol. Biotechnol. and Environ. Sci., 6(2):323-324.

Mohan, K., N.K. Ganguly, M.L. Dubey and R.C. Mahajan. 1992. Oxidative damage of erythrocytes infected with *P. falciparum*. An *in vitro* study. Annals of Hematol., 65(3)131-134.

Moore, G.A. 2001. Oranges and lemons: clues to the taxonomy of Citrus from molecular markers. *Trends Genet.,* 17(9):536-40

Morita, Y., E. Matsumura, T. Okabe, T. Fukui, T. Ohe, N. Ishida and Y. Inamori. 2004. Biological activity of beta-dolbrin, gamma-thujaplicin, and 4-acetyltropolone, hinokitiol-related compounds. Biol. Pharm. Bull., 27(10):1666-1669.

Morita, T., K. Jinno, H. Kawagishi, Y. Arimoto, H. Suganuma, T. Inakuma and K. Sugiyama. 2003. Heptoprotective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/dgalactosamine- induced liver injury. J Agric Food Chem., 51(6):1560–1565.

Mothana, R.A., A.J. Al-Rehaily and W. Schultze. 2010. Chemical analysis and biological activity of the essential oils of two endemic Soqotri *Commiphora* species. Molecules. 15:689-698.

Muchtaridi, A. Subarnas, A. Apriyantono and R. Mustarichie. 2010. Identification of compounds in the essential oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice. Int. J. Mol. Sci., 11:4771-4781

Muhtaseb, M.S., E.L.D. Talwar, A. Duncan, S.J.D. Oreilly, R.F. Mckee, J.H. Anderson and F.I.G. Foulisa. 2008. Free radical activity and lipid soluble antioxidant vitamin status in patients with long term ileal pouch-anal anastomosis. Colorectal dis., 11:67-72.

Musyimi, D.M. and J.A. Ogur. 2008. Comparative assessment of antifungal activity of extracts from *Eucalyptus globules* and *Eucalyptus citriodora*. Res. J. Phytochem., 2: 35-43.

Murthy, P.S., B. Borse., H. Khanum and P. Srinivas. 2009. Inhibitory effects of Ajowan *(Trachyspermum ammi)* ethanolic extract on *A. ochraceus* growth and ochratoxin production. Turk. J. Biol., 33:211–217.

Muthamma, M.K.S., D. Hemang, K.T. Purnima and V. Prakash. 2008. Enhancement of digestive enzymatic activity by cumin (*Cuminum cyminum* L.) and role of spent cumin as a bionutrient. Food Chem., 110:678-683.

Myoatasyoh, J.C., J.J. Kiplimo, N.M. Karubiu and T.P. Hailstroks. 2007. Chemical composition and antimicrobial activity of essential oil of *Tarchanthus camphorates*. Food Chem., 101:1183-1187.

Nadeem, M. and A. Riaz. 2012. Cumin (*Cuminum cyminum*) as a potential source of antioxidants. Pak. J. Food Sci., 22(2):101-107

Nadkarni, K.M. 1976. Indian Materia Medica, third ed. Popular Prakashan, Bombay, pp. 475–476.

Nagappan, T., P. Ramasamy, M.E.A. Wahid, T.C. Segaran and C.S. Vairappan. 2011. Biological activity of carbazole alkaloids and essential oil of *Murraya koenigii a*gainst antibiotic resistant microbes and cancer cell lines. Molecules, 16:9651-9664

Naik, J.P., M.T.M. Kumar, G. Sulochanamma, B.S. Ramesh and S.R. Sampathu. 2006. Studies on quality attributes of cultivars of large cardamom (*Amomum subulatum* Roxb.). J Food Sci Technol**.,** 43:308-310.

Nassar, M., A.H. Gaara, A.H. El-Ghorab, A.R.H. Farrag, H. Shen, E. Haq and T.J. Mabry. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. Rev. Latinoamer. Quím., 35(3)

Naz, T., A. Mosaddik and M.E. Haque. 2009. Antimicrobial and cytotoxic activities of root extracts of *Piper Chaba*. J. Sci. Res., 1(1):138-144

NCCLS, National committee for clinical laboratory standards. 2001. Performance standards for anti-microbial susceptibility testing: eleventh informational supplement. Document M1000-S11, National committee for clinical laboratory standards, Wayne PS, USA.

NCCLS, 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-Fifth edition. NCCLS document M7-A5. ISBN 1-56238-394-9, NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. USA.

Negi, P.S., G.K. Jayaprakasha, L.J.R. Mohan and K.K. Sakariah. 1999. Antibacterial activity of turmeric oil: a byproduct from curcumin. J Agri and Food Chem., 47:4297– 4300.

Neha, J. and R.N. Mishra. 2011. Antioxidant activity of Trikatu mega Ext inter. J Res. Pharma. Biomed. Sci., 2:624-628

Ningappaa, M.B., B.L. Dhananjayaa, R. Dineshaa, R. Harshaa and L. Srinivas. 2010. Potent antibacterial property of APC protein from curry leaves (*Murraya koenigii*). Food Chem., 118(3):747-750.

Ningappaa, M.B., R. Dineshaa and L. Srinivasa. 2008. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. Food Chem., 106(2):720-728.

Njoroge, S.M., H. Koaze, P.N. Karanja and M. Sawamura. 2005. Volatile constituents of redblush grapefruit (*Citrus paradisi*) and pummel (*Citrus grandis*) peel essential oils from Kenya. J. Agric. Food Chem., 53:9790-9794.

Novak, J., 2005. Lecture held on the 35th Int. Symp. on Essential Oils, Giardini Naxos, Sicily.

Nychas, G.J.E. 1995. Natural antimicrobials from plants. In: Gould, G.W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional, London, 58– 89.

Ogunwande, I.A., N.O. Olawore, K.A. Adeleke and W.A. Konig. 2003. Chemical composition of the essential oils from the leaves of three Eucalyptus species growing in Nigeria. J. Essent. Oil Res., 15:297-301.

Okugawa, H., M. Moriyasu, S. Matsushita, K. Saiki, Y. Hashimoto, K. Matsumoto, A. Fujioka and A. Kato. 1988. Evaluation of crude drugs by a combination of enfleurage and chromatography on flavor components in seeds of *Amomum cardamomum* and *Elettaria cardamomum*. Shoyakugaku Zasshi. 42:94–97.

Olajide, O.A., F.F. Ajayi, A.I. Ekhelar, S.O. Awe, J.M. Makinde and A.R. Alada. 1999. Biological effects of *Myristica fragrans* (nutmeg) extract. Phytother Res., 13:344–345.

Olivero-Verbel, L.S. Nerio and E.E. Stashenko. 2010. Bioactivity against Tribalism castaneum Herbst (Coleoptera: Tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. Pest Manag Sci., 66:664-668.

Olubunmi, A., Onayade, A.C. Adebajo. 2001. Composition of the Leaf Volatile Oil of *Murraya koenigii* growing in Nigeria. J. of Herbs, Spi. & Med, Plants, 7(4):59- 66.

Onawunmi, G.O., 1989. Evaluation of the antimicrobial activity of citral. Letters in Applied Microbiology 9:105– 108.

Ooi, L.S.M., Y. Li, S.L. Kam, H. Wang, E.Y.L. Wong and V.E.C. Ooi. 2006. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* blume. The Amer. J. of Chin. Med., 34(3): 511-522

Ozbek, H., S. Ugras, H. Dulger, I. Bayram, I. Tuncer and G. Ozturk. 2003. Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia, 74:317-319.

Ozcan, M.M., J.C. Chalchat, D. Arslan, A. Ates and A. Unver. 2006. Comparative essential oil composition and antifungal effect of bitter fennel (*Foeniculum vulgare* ssp. piperitum) fruit oils obtained during different vegetation. J. Med. Food., 9:552–561.

Ozel, M.Z., F. Gögüs and A.C. Lewis. 2008. Composition of *Eucalyptus camaldulensis* volatiles using direct thermal desorption coupled with comprehensive two-dimensional gas chromatography–time-of-flight-mass spectrometry. J. Chromatogr. Sci. 46:157-161

Packiyasothy, E.V. and S. Kyle. 2002. Antimicrobial properties of some herb essential oils. Food Aus. 54(9):384– 387.

Pal, M., M. Srivastava, D.K. Soni, A. Kumar and S.K. Tewari. 2011. Composition and anti-microbial activity of essential oil of *Myristica fragrans* from Andaman Nicobar Island. Int. J. Pharm. Life Sci., 2(10): 1115- 1117

Pandey, K., P.K. Sharma and R. Dudhe. 2012. Antioxidant and anti-inflammatory activity of ethanolic extract of *Parthenium hysterophorus* Linn. Asian J. Pharm. Clin. Res., 5(4): 28-31

Parashar, V., R. Parashar, B. Sharma and A. Pandey. 2009. Parthenium leaf extract mediated synthesis of silver nano particles: a novel approach towards weed utilization. Digest J Nanomater Biostruct., 4:45–50

Parganiha, R., S. Verma, S. Chandrakar, S. Pal, H.A. Sawarkar and P. Kashyap. 2011. In vitro anti- asthmatic activity of fruit extract of *Piper nigrum* (Piperaceae). Int. J. Herb. Drug Res., 1:15-18.

Parmar, M.Y., P. Shah, V. Thakkar and T.R. Gandhi. 2009. Hepatoprotective activity of *Amomum subulatum* Roxb. against ethanol-induced liver damage. Int J Green Pharm., 3:250-254.

Parmar, V.S., S.C. Jain, K.S. Bisht, R. Jain, P. Taneja, A. Jha and O.D. Tyagi. 1997. Phytochemistry of the genus Piper. Phytochem., 46:597-673.

Patel, V.S., V.P. Chitra, L. Prasanna and V. Krishnaraju. 2008. Hypoglycemic effect of aqueous extract of *Parthenium hysterophorus* L. in normal and alloxan induced diabetic rats. Ind J Pharmacol., 40:183–185

Patel, K., S. Ali, S. Sotheeswaran and J.P. Dufour. 2007. Composition of the leaf essential oil of *Cinnamomum verum* (Lauraceae) from Fiji Islands. J. of Essen. Oil-Bearing Plants., 10:374-377.

Perry, N.S., C. Bollen, E.K. Perry and C. Ballard. 2003. Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol. Biochem. Behav., 75:651-659.

Piaru, S.P., R. Mahmud, A.M.S.A. Majid, S. Ismailb and C.N. Man. 2012. Chemical composition, antioxidant and cytotoxicity activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*. J. Sci. Food Agric., 92:593-597

Piaru, S.P., R. Mahmud, A.M.S.A. Majid and Z.D.M. Nassar. 2012. Antioxidant and antiangiogenic activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*. Asian Paci. J. of Trop. Med., 294-298

Pichersky, E., J.P. Noel and N. Dudareva. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science, 311:808-811.

Piccaglia, R. and M. Marotti. 2001. Characterization of Some Italian Types of Wild Fennel (*Foeniculum vulgare* Mill.). J. Agric. Food Chem., 49:239-244.

Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R., Casanova, J. 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flav and Frag J. 17:15–19.

Pino, J.A., R. Marbot, R. Quert and H. Garcia. 2001. Study of essential oils of *Eucalyptus resinifera* Smith, *E. tereticornis* Smith and *Corymbia maculata* Hook. K. D. Hill and L.A.S. Johnson, grown in Cuba. Flavor Frag. J., 17:1-4.

Piras, A., A. Rosa, B. Marongiu, A. Atzeri, M.A. Dessi, D. Falconieri and S. Porcedda. 2012. Extraction and Separation of Volatile and Fixed Oils from Seeds of *Myristica fragrans* by Supercritical CO_2: Chemical Composition and Cytotoxic Activity on Caco-2 Cancer Cells. J of Food Sci., 77(4):448-453

Prakash, K.D., K. Brajesh, H. Arshad, V. Shikhar and M. Mala. 2012. Evaluation of antioxidant activity of large cardamom (leaves of *Amomum subulatum*). Int. J. Drug Develop. Res., 4(1): 175-179

Prudent, D., F. Perineau, J.M. Bessiere, G.M. Michel and J.C. Baccou. 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.)—evaluation of its bacterioatatic and fungistatic properties. J Ess. Oil Res., 7;165– 173.

Qiu, J., H. Li, H. Su, J. Dong, M. Luo, J. Wang, B. Leng, Y. Deng, J. Liu and X. Deng. Chemical composition of fennel essential oil and its impact on *Staphylococcus aureus* exotoxin production. World J. Microbiol. Biotechnol., 28:1399-1405

Rababah, T. M., N.S. Hettiarachy and R. Horax. 2004. Total phenolics and antioxidant activities of feurgreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and ter-butylhrdroquinone. J. Agric. and Food Chem., 52:5183–5186.

Raghavan, S. 2007. Handbook of spices, seasonings, and flavorings. 2nd Ed. CRC Press, Taylor & Francis Group, Boca Raton.

Rahman, A.U., M.I. Choudhary, A. Ahmed, M.Z. Iqbal, B. Demirci, F. Demirci and K.H.C. Baser. 2000. Antifungal activities and essential oil constituents of some spices from Pakistan. J. Chem. Soc. Pak., 22:60-65.

Raina, V.K., S.K. Srivastava, K.K. Aggrawal, S. Ramesh, Kumar and Shushil. 2001. Essential oil composition of *Cinnamon zeylinicum* Blume leaves from little Andaman, India. Flav. Frag. J., 16:374.

Ramos, A., R. Rivero, A. Visozo, J. Piloto and A. Garcia. 2002. Parthenin, a sesquiterpene lactone of *Parthenium hysterophorus* L. is a high toxicity clastogen. Mut Res 514:19–27

Rana, V.S., J.P. Juyal, Rashmi and M.A. Blazquez. 2004. Chemical constituents of the volatile oil of *Murraya koenigii* leaves. Int J Aromather., 14(1):23-25.

Rao, B.R.R., D.K. Rajput and G.R. Mallavarapu. 2011. Chemical diversity in curry leaf (*Murayya koenigii*) essential oil. Food Chem., 126(3):989-994.

Rao, L.J.M., K. Ramalakshmi, B.B. Borse and B. Raghavan. 2006. Chemical composition of volatiles from coconut sap neera and effect of processing. Food Chem., 100:742-747.

Rehman, Z. 2006. Citrus peel extract: A natural source of antioxidant. Food Chem., 99:450-454.

Renjie, L., L. Zhenhong and S. Shidi, S. 2010. GC-MS analysis of fennel essential oil and its effect on microbiology growth in rats' intestine. Afr. J. Microbiol. Res., 4:1319-1323.

Reynolds, J.E.F. 1982. Essential oils and aromatic carminatives, martindale the extra. Pharmacopeia, 670-676.

Rios, J.L., M.C. Recio and A. Villar. 1988. Screening methods for natural antimicrobial products with antimicrobial activity: a review of the literature. J Ethnopharmacol., 23:127– 149.

Roby, M.H.H., M.A. Sarhana, K.A.H. Selima and K.I. Khalela. 2013. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Indust. Crops and Prod., 44:437-445

Rodrigues, K.A.F., L.V. Amorim, J.M.G. Oliveira, C.N. Dias, D.F.C. Moraes, E.H.A. Andrade,J.G.S. Maia, S.P. Carneiro and F.A.A. Carvalho. 2013. *Eugenia uniflora* L. Essential Oil as a Potential Anti-*Leishmania* Agent: Effects on *Leishmania amazonensis* and Possible Mechanisms of Action. Evidence-Based Compl and Alt Med., Article ID 279726, http://dx.doi.org/10.1155/2013/279726

Rong, L. and J. Zi-Tao. 2004. Chemical composition of the essential oil of *Cuminum cyminum* L. from China. Flav. Frag. J., 19:311–313.

Ross, I.A and N.J. Totowa. 2001. Medicinal Plants of the World, Chemical Constituents, Traditional and Modern Medicinal Uses, Humana Press, Vol. 2.

Rota, M.C., A. Herrera, R.M. Martinez, J.A. Sotomayor and M.J. Jordan. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris, Thymus zygis* and *Thymus hyemalis* essential oils. Food Cont., 19:681-687.

Saei-Dehkordi, S., H. Tajik, M. Moradi and F. Khalighi-Sigaroodi. 2010. Chemical composition of essential oils in *Zataria multiflora* Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem. Toxicol., 48:1562–1567.

Saharkhiz, M.J., R. Omidbaigi and F. Sefidkon. 2007. The effect of phosphorus and irrigation treatments on the essential oil content and composition of feverfew (*Tanacetum parthenium* (L.) cv. Zardband). J. ess. oil-bearing plants. 10(5):391-398.

Salvat, A., L. Antonnacci, R.H. Fortunato, E.Y. Suarez and H.M. Godoy. 2001. Screening of some plants from Northern Argentina for their antimicrobial activity. Letters in Appl Microbiol., 32:293– 297.

Sanajy, S. and R.P. Singh. 2001. Anti Fungal Activity Of Essential Oil Of *Murraya koenigii* Spreng, Indian Perfumer, 45(1):49-51.

Sarker, S. D., L. Nahar and Y. Kumarasamy. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 42:321-324.

Sasidharan, I. and A.N. Menon. 2010. Comparative chemical composition and antimicrobial activity of berry and leaf essential oils of *Piper nigrum* L. Int. J. Biol. Med. Res., 1(4):215-218

Satyavati, G.V., A.K. Gupta and N. Tendon. 1987. Medicinal Plants of India. Indian council of medical research, New Delhi India, 2:289-299

Satyal, P., N.S. Dosoky, B.L. Kincer and W.N. Setzer. 2012. Chemical compositions and biological activities of *Amomum subulatum* essential oils from Nepal. Natur. Prod. comm., 7(9):1233-1236

Sawai, Y. and K. Sakata. 1998. NMR analytical approach to clarify the antioxidative molecular mechanism of catechins using 1,1- diphenyl-2-picrylhydrazyl. J. Agric. Food Chem., 46:111–114.

Schmidt, E., L. Jirovetz, G. Buchbauer, G.A. Eller, I. Stoilova, A. Krastanov and A. Stoyanova. 2006. Composition and antioxidant activities of the essential oil of cinnamon (*Cinnamomum zeylanicum* Blume) leaves from Sri Lanka. J of essen. oil bearing plants, 9(2): 170 – 182

Senatore, F., F. Napolitano and M. Ozcan. 2000. Composition and antibacterial activity of the essential oil from *Crithmum maritimum* L. (Apiaceae) growing wild in Turkey. Flav and Frag J., 15:186– 189.

Senevirathne, M., J. Youjin, H. Jinhwan and K. Soohyun. 2009. Effective drying of citrus by-product by high speed drying: a novel drying technique and their antioxidant activity. J. of Food Eng.,

Seo, S.M., H.M. Park and I.K. Park. 2012. Larvicidal activity of ajowan (*Trachyspermum ammi*) and Peru balsam (*Myroxylon pereira*) oils and blends of their constituents against mosquito, *Aedes aegypti*, Acute Toxicity on Water Flea, Daphnia magna, and Aqueous Residue. J. Agric. Food Chem., 60:5909-5914

Sereshti, H., A. Rohanifar, S. Bakhtiari and S. Samadi. 2012. Bifunctional ultrasound assisted extraction and determination of *Elettaria cardamomum* Maton essential oil. J. of chromat. A., 1238:46– 53

Sibanda, S., G. Chigwada, M. Poole, E.T. Gwebu, J.A. Noletto, J.M. Schmidt, A.I. Rea and W.N. Setzer. 2004. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. J. Ethnopharmacol., 92:107-111.

Shaban, M.A.E., K.M. Kandeel, G.A. Yacout and S.E. Mehaseb. 1987. The chemical composition of the volatile oil of *Elettaria cardamomum* seeds. Pharmazie. 42:207-208.

Shabbir, A. and R. Bajwa. 2006. Distribution of Parthenium weed (*Parthenium hysterophorus* L.), an alien invasive weed species threatening the biodiversity of Islamabad. Weed Biol and Manag., 6:89–95.

Shafiei, Z., N.N. Shuhairi, N.F.S. Yap, C.A.H. Sibungkil and J. Latip. 2012. Antibacterial Activity of *Myristica fragrans* against Oral Pathogens. Evi. Based Comp. and Alt. Med., doi:10.1155/2012/825362.

Shahat, A.A., A.Y. Ibrahim, S.F. Hendawy, E.A. Omer, F.M. Hammouda , F.H. Abdel-Rahman and M.A. Saleh. 2011. Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils from Organically Cultivated Fennel Cultivars. Molecules. 16:1366-1377

Sharma, N. and A. Tripathi. 2008. Effects of Citrus sinensis (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. Microbiol Res., 163(3):337-44.

Sharma, P. and J.D. Sharma. 2001. In vitro hemolysis of human erythrocytes-by plant extracts with antiplasmodial activity. J. of Ethnopharmacol., 74:239-243.

Sharma, E., R. Sharma, K.K. Singh and G. Sharma. 2000. A boon for mountain populations. Large cardamom farming in the Sikkim Himalaya. Mount. Res. and Develop., 20: 108-111.

Shaw, P.E. 1977. Essential oils. In S. Nagy, P. E. Shaw, & M. K. Veldhuis (Eds.), Citrus science and technology. Westport, CT: The AVI Publishing Co. Inc. pp. 427., 92(2):157-163

Shaw, P.E. and C.W. Wilson. 1976. Composition of extracted peel oil composition and juice flavor for rough lemon, Persian lime and a lemon-lime cross. J Agric Food Chem. 24:664-666.

Silva, S.L.D., J.D.S. Chaar, P.M.S. Figueiredo and T. Yano. 2008. Cytotoxic evaluation of essential oil from Casearia sylvestris Sw on human cancer cells and erythrocytes. Acta Amazon., 38(1):107-112.

Silva, J., W. Abebe, S.M. Sousa, V.G. Duarte, M.I. Machado, F.J. Matos. 2003. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol., 89(2-3):277-283.

Simic, A., M.D. Sokovic, M. Ristic, S.G. Jovanovic, J. Vukojevic and P.D. Marin. 2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytotherap. Res., 18:713-717.

Singh, H.P. S. Kaur, K. Negi, S. Kumari, V. Saini, D.R. Batish and R.K. Kohli. 2012. Assessment of *in vitro* antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalypt; Myrtaceae) and its major constituents. LWT-Food Sci. and Tech., 48:237-241

Singh, H.P., S. Mittal, S. Kaur, D.R. Batish and R.K. Kohli. 2009. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. J. Agric. Food Chem., 57:6962-6966.

Singh, G. and P. Marimuthu. 2006. Antioxidant and biocidal activities of *Carum nigrum* (seed) essential oil, oleoresin, and their selected components. J. of Agric. and Food Chem., 54: 174-181.

Singh, H.P., S. Mittal, S. Kaur, D.R. Batish and R.K. Kohli. 2009. Characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*. J. Agric. Food Chem., 57:6962-6966.

Singh, G., S. Maurya, M.P. deLampasona and C.A.N. Catalan. 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and chem. Toxicol., 45:1650–1661

Singh, G., P. Marimuthu, C. Catalan and M.P. deLampasona. 2004. Chemical composition, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. J. Sci. Food Agric., 84:1878–1884

Singh, G., S. Maurya., M.P. Lampasona and C. Catalan. 2004. Chemical constituents, antifungal and antioxidative effects of Ajowan essential oil and its acetone extract. J. agric. food chem., 52:3292-3296.

Singh, G., I.P. Kapoor, S.K. Pandey, U.K. Singh and R.K. Singh. 2002. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. Phytother. Res., 16(7), 680–682.

Sonavane, G.S., V.P. Sarveiya, V.S. Kasture and S.B. Kasture. 2002. Anxiogenic activity of *Myristica fragrans* seeds. Pharmacol. Biochem. Behav. 71:239–244.

Sorm, F., L. Dolejs and J. Pliva. 1950. Collect. Czechoslov. Chem. Commun., 3:187.

Sowbhagya, H.B., S.B.V. Rao and N. Krishnamurthy. 2008. Evaluation of size reduction and expansion on yield and quality of cumin *(Cuminum cyminum)* seed oil. J. of Food Eng., 84:595-600.

Srinivasan, K. 2007. Black pepper and its pungent principle-piperine. A review of diverse physiological effects. Crit. Rev. Food Sci. Nutr., 47:735-748.

Stashenko, E.E., R. Martinez, M.H. Pinzdn and J. Ramfrez. 1996. Changes in chemical composition of catalytically hydrogenated orange oil (*Citrus sinensis*). J Chrom., 752:217-222.

Stefano, D.V., R. Pitonzo and D. Schillaci. 2011 Antimicrobial and antiproliferative activity of *Athamanta sicula* L. (Apiaceae). Phcog Mag., 7(25):31–34.

Stevens, P. F. 2001. Angiosperm Phylogeny Website. Version 8, http://www.mobot.org/MOBOT/research/APweb/.

Sultan, M.T., M.S. Butt, F.M. Anjum, A. Jamil, S. Akhtar and M. Nasir. 2009. Nutritional profile of indigenous cultivar of black cumin seeds and antioxidants potential of its fixed and essential oil. 41(3):1321–1330.

Surib-Fakim A, M.D. Swerab, J. Gueho and E. Dullo. 1996. Medicinal plants of Rodrigues. Int J Pharmacogn., 34:2–14

Takada, H., K. Kokubo, K. Matsubayashi and T. Oshima. 2006. Antioxidant activity of supramolecular water-soluble fullerenes evaluated by β-carotene bleaching assay. Biosci., Biotechnol Biochem., 70:3088–3093.

Tembhurne, S.V. and D.M. Sakarkar. 2010. Protective effect of *Murraya koenigii* (L) leaves extract in streptozotocin induced diabetic rats involving possible antioxidant mechanism. J Med Plant Res., 4(22):2418-2423.

Thippeswamy, N.B. and K.A. Naidu. 2005. Antioxidant potency of cumin varieties-cumin, black cumin and bitter cumin-on antioxidant systems. Europ. Food Res. and Technol., 220: 472-476.

Thomas, V.P., M. Sabu and U. Gupta. 2009. Taxonomic studies on cultivars of *Amomum subulatum* (Zingiberaceae). *Rheedea*, **19**:25-36.

Tiwari, P. and D. Singh. 2008. Antitrichomonas activity of sapindus saponins, a candidate for development as microbicidal contraceptive. J. Antimicrob. Chemother., 62:526-534.

Todd, J.F.J. 1995. Recommendations for nomenclature and symbolism for mass spectroscopy. Int. J. Mass Spec., Ion Process, 142:209.

Toloza, A.C., A. Lucia, E. Zerba, H. Masuh and M.I. Picollo. 2008. Interspecific hybridization of Eucalyptus as a potential tool to improve the bioactivity of essential oils against permethrin-resistant head lice from Argentina. Bioresour Technol., 99(15):7341-7347.

Treibs, W. 1952. Uber bi und polycyclische Azulene. XIII. Das bicyclische Caryophyllen als Azulenbildner. Liebigs Ann. Chem., 576:125–131.

Tsuchihashi, H., M. Kigoshi, M. Iwatsuki and E. Niki. 1995. Action of β-carotene as an antioxidant against lipid peroxidation. Arch.Biochem. Biophys., 323:137–147.

Valko, M., H. Morris, M. Mazur, P. Rapta and F.R. Bilton. 2001. Oxygen free radical generating mechanisms in the colon: do the semiquinones of vitamin K play a role in the aetiology of colon cancer? Biochem. Biophys. Acta., 1527:161-166.

Vasudeva, N. and T. Sharma. 2012. Chemical composition and antimicrobial activity of essential oil of *Citrus limettioides* Tanaka. J. Pharma. Technol. & Drug Res., 1-7.

Velluti, A., V. Sanchis, A.J. Ramos and S. Mari. 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Int. J. Food Microbiol., 89:145-154.

Venkataiah B, C. Ramesh, N. Ravindranath and B. Das. 2003. Charminarone, a seco-pseudoguaianolide from *Parthenium hysterophorus*. Phytochem., 63:383–386

Viljoen, A.M., S. Petkar, S.F. Van-Vuuren. A.C. Figueiredo, L.G. Pedro and J.G. Barroso. 2006. Chemo-Geographical Variation in Essential Oil Composition and the antimicrobial

Properties of "Wild Mint" - *Mentha longifolia subsp. polyadena* (Lamiaceae) in Southern Afr. J. Essen. Oil Res., 18:60-65.

Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J.A. Pérez-Álvarez. 2008. Antibacterial activity from different essential oil obtained from spices widely used in Mediterranean diet. Int. Food Sci. and Technol., 43:526-531.

Vuuren,V.S.F., A.M. Viljoen, T. Ozek, B. Demirici and K.H.C. Baser. 2007. Seasonal and geographical variation of Heteropyxis natalensis essential oil and the effect thereof on the antimicrobial activity. S. Afr. J Bot., 73(3):441-448.

Wagner, G. 1899. J. Russ. Phys. Chem. Soc., 31: 690, cited in H. Meerwein, 1914. Liebigs Ann. Chem., 405:129–175.

Wallach, O. 1914. Terpene und Campher, 2nd ed., Leipzig: Veit & Co.

Wattanathorn, J., P. Chonpathompikunlert, S. Muchimapura, A. Priprem and O. Tankamnerdthai. 2008. Piperine, the potential functional food for mood and cognitive disorders. Food Chem. Tech., 46:3106-3110

Wise, M.L. and R. Croteau. 1999. In: Barton D, Nakanishi K (eds), Comprehensive Natural Product Chemistry, vol 2. Isoprenoids Including Carotenoids and Steroids. Elsevier, London, 97–153

Wilson, P.G., M.M. O'Brien, P.A. Gadek and C.J. Quinn. 2001. Myrtaceae revisited: a reassessment of infra familial groups. American J Bot., 88:2013–2025.

Woodward, R.B. 1941. Structure and the absorption spectra of α,β-unsaturated ketones. J. Am. Chem. Soc., 63:1123–1126.

Wong, K.C. and S.G. Chee. 1996. Volatile Constituents Of *Murraya koenigii* Spreng Flowers. J. Essent. Oil. Res., 8(5):545-547.

Yen, G.C., P.D. Duh and D.Y. Chuang. 2000. Antioxidant activity of anthraquinones and anthrone. Food Chem., 70:307–315.

Yousif, A.N., C.H. Scaman, T.D. Durance and B. Girard. 1999. Flavor volatiles and physical properties of vacuum-microwaves and air dried sweet basil (*Ocimum bacillicum* L.). J. Agric. Food Chem., 47:4777-4781.

Yuan, Z.Y., W.Z. Ying, Z.Z. Guo and Z. BinL. 2009. Analysis of essential oil from leaves and fruits of *Eucalyptus tereticornis* in Guangxi Province by GC-MS. J. Chin. Med. Mat., 1001-4454.

Yu, C., J. Yang, Y. Wang, H. Wei, H. Zhang and Y. Shi. 2013. Ultrasonic nebulisation extraction: extraction column coupled with liquid phase microextraction for analysis of the volatile organic compounds in *Foeniculum vulgare* Mill. as a Model. Phytochem. Anal., 24: 296-302

Zaheer, Z., S. Shazia, S. Sobiya and M. Tahir. (2012). Antifungal Potential of *Parthenium hysterophorus* L. Plant Extracts against *Fusarium solani*. *Sci. Res. Ess.*, 7(22): 2049-2054.

Zargari, A. 1989. Medicinal Plants. Tehran University Press, Tehran, 1:637-639.