PART-A

Utilization of Terpenoids and Allied Molecules for Value Added Products
1. INTRODUCTION TO OLFATORY PERCEPTS

From time immemorial man has been interested in the diverse and fragrant odors associated with certain plants. He discovered the active principles responsible for the odors of plants which he separated from the plants by gentle heating and later by steam distillation. The oil thus isolated was named as “essential oil”. The importance of these steam volatile oils gained significance in the sixteenth century when the oils of anise, spike, cinnamon, clove etc., were used for medicinal purposes in various pharmaceuticals. In the beginning of the nineteenth century, the chemical investigation by Alender Dumas found more volatile fractions of oil containing a number of hydrocarbons of the formula C_{10}H_{16}.

Nature has blessed the word with its endless treasures leading to the basic comforts of human life. It has provided multiple parameters of beautification and attraction. The most common and popular is the nature’s sense of smell which one realizes in many ways. The blooming gardens, growing crops and running creatures, even the mosquitoes and flies spread different odors in atmosphere. The odor which brings feelings of pleasure, environment of freshness and even imbibes the soul in the extreme of joy, is probably perfume.

A perfume is normally a complex mixture of naturally or synthetically produced substances which are blended along with the odorless solvent such as ethanol to produce the typical odor. The core ingredients of these perfumes are essential oils.

The knowledge of using flowers, fruits, leaves, barks and roots of many plants containing essential oil (odorous substances) is in practice since ancient times. Essential oils which
were once considered inactive waste products of plants metabolism and have no significant biological function, are now being realized for their importance as a means of chemical communication which the plants keep itself against competitors, predator and pathogens (Mahindru, 1992).

Gilgamesh wrote in one of his poems; Fragrance obtained by burning cedarwood and myrrh should cajole the Gods and put them in pleasant feeling. Plutarch mentioned in Morali myrrh- its pleasant refreshing fumes made human body readier for the delight of sleep, sorrow which oppresses him all over the day will get banished. The use of fragrance obtained from the Poppy flowers is well known for its sedative effect. Even Cleopatra used pillow filled with rose petals (Wladslaw et al., 1989). It showed its stimulating effect on the central nervous system (Kirk-Smith and Booth, 1987)

The transmission of complex and selective biological message to distant another organism from the source plant points to the volatile nature of the essential oil which arises from the complexity of composition and structure of the constituents. Organoleptic nature of many of the terpenes constituent for the flowering plants serves to attract specific insect for the purpose of cross fertilization when the plant is in boom

Apart from the olfactions, the odour of the chemical compound is also related to the molecular structure of the compound. It has been observed that different types of compounds can possess almost identical odor while the closely related compounds even the enantiomers possess different odor. According to Bechman, “A substance may be odorous if it is in sufficiently soluble in both water and in the lipid fats of the nose cell” (Mahindru, 1992).
Literature scan reveals that monoterpenes often exist in nature as cyclic ethers, substituted cyclic ethers or esters derived are artefacts in nature and possess better perfumery value than alicyclic or straight chain alcohols. Cyclic ethers with small side chain up to C$_3$ atoms have better odor value than long chain ones. If the side chain is – C$_3$H$_7$ or C$_4$H$_9$, the odor is typical spicy. It is due to the fact that long chain cyclic ethers having higher molecular weight are less volatile than the small chain ones and hence possess poor odor value (Mahindru et al., 1984)

Several cyclic ethers, which probably are derived biosynthetically by acid catalysed cyclization products of oxidation of geraniol, nerol, citronellol and linalool have been isolated from natural sources namely cis and trans-linalool oxides, rose furan, rose oxide and perillene (Sathikge, 2008; Cori et al., 1986).

The tetrahydropyran nucleus is a common structural feature of many naturally occurring compounds. The six membered oxygenated ring is an important olfactory ingredient of rose otto and geranium. Cyclization of alcohols to substituted tetrahydropyrans opens a new chapter in the field of the synthetic aroma such as rose oxide (Wagner et al., 1989)
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compounds</th>
<th>Odor</th>
<th>Ref.</th>
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<tr>
<td>1</td>
<td>Rose furan</td>
<td>Rose like</td>
<td>Buechi et al., 1968</td>
</tr>
<tr>
<td>2</td>
<td>Thujone</td>
<td>Menthol odor</td>
<td>Perry et al., 1999</td>
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<td>3</td>
<td>Citral</td>
<td>Lemony</td>
<td>The merck</td>
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<tr>
<td>4</td>
<td>Rose oxide</td>
<td>Rose like</td>
<td>Vidari et al., 1989</td>
</tr>
<tr>
<td>5</td>
<td>Furfuryl alcohol</td>
<td>Rosy aroma</td>
<td>Buechi et al., 1968</td>
</tr>
<tr>
<td>6</td>
<td>Menthofuran</td>
<td></td>
<td>Perfumery Dubey et al., 2003</td>
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Table 1 A brief list of some of the potent fragrance compounds
**Bioflavors** have been defined as “natural and naturally produced flavors” (Demyttenaere, 2001) this includes the biotechnological production of flavors by means of microorganism and enzymes, as the microbial biotransformation of terpenes as one of the most promising techniques. Industrially important bioflavors, such as α-terpeniol, perillyl alcohol, perillic acid, carviond and carveone, for example might be produced by this method (Bicas et al., 2009).

![Chemical structures](image)

perillyl alcohol  
Perillic Acid  
Carvone

**Figure1.** List of some bioflavors

1.1 ESSENTIAL OILS

Essential oils (EOs) (also called volatile or ethereal oils; (Guenther, 1948) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs (Van de Braak and Leijten, 1999). The term ‘essential oil’ is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he named the effective component of a drug Quinta essentia (Guenther, 1948). An estimated 3000 EOs are known, of which about 300 are commercially important—destined chiefly for the flavors and fragrances market. (Van de Braak and Leijten, 1999).
1.1.1 Medicinal attributes of essential oils

It has long been recognised that some EOs have antimicrobial properties (Guenther, 1948; Boyle, 1955) and these have been reviewed in the past (Shelef, 1983; Nychas, 1995) as have the antimicrobial properties of spices (Shelef, 1983) but the relatively recent enhancement of interest in ‘green’ consumerism has lead to a renewal of scientific interest in these substances (Nychas, 1995; Tuley de Silva, 1996).

Besides antibacterial properties (Deans and Ritchie, 1987; Carson et al., 1995; Mourey and Canillac, 2002), EOs or their components have been shown to exhibit antiviral (Bishop, 1995), antimycotic (Azzouz and Bullerman, 1982; Akgul and Kivanc, 1988; Jayashree and Subramanyam, 1999; Mari et al., 2003), antitoxigenic (Akgul et al., 1991; Ultee and Smid, 2001; Juglal et al., 2002), antiparasitic (Pandey et al., 2000; Pessoa et al., 2002), and insecticidal (Konstantopoulou et al., 1992; Karpouhitis et al., 1998) properties.

These characteristics are possibly related to the function of these compounds in plants (Guenther, 1948; Mahmoud and Croteau, 2002). The phenolic components are chiefly responsible for the antibacterial properties of essential oils (Cosentino et al., 1999). Essential oils also have hepatoprotective activity for e.g. Nutmeg, (*Myristica fragrans*), showed a potent hepatoprotective activity against liver damage caused by certain chemicals (Morita et al., 2003). The protective activity was correlated with a major constituent myristicin (Ahmed et al., 1997)
1.1.2 Historical uses of essential oils

Although spices have been used for their perfume, flavor and preservative properties since antiquity (Bauer et al., 2001), of the known EOs, only oil of turpentine was mentioned by Greek and Roman historians (Guenther, 1948). Distillation as a method of producing EOs was first used in the East (Egypt, India and Persia) (Guenther, 1948) more than 2000 years ago and improved in the 9th century by the Arabs (Bauer et al., 2001). The first authentic written account of distillation of essential oil is ascribed to Villanova (ca. 1235–1311), a Catalan physician (Guenther, 1948). By the 13th century EOs were being made by pharmacies and their pharmacological effects were described in pharmacopoeias (Bauer et al., 2001) but their use does not appear to have been widespread in Europe until the 16th century, from which time they were traded in the city of London (Crosthwaite, 1998).

The use of tea tree oil for medical purposes has been documented since the colonisation of Australia at the end of the 18th century, although it is likely to have been used by the native Australians before that (Carson and Riley, 1993). However, in the course of the 19th and 20th centuries the use of Eos in medicine gradually became secondary to their use for flavor and aroma (Guenther, 1948).

![Parent carbon skeleton of drimane sesquiterpenes](image1)

![Polugodial, a drimane sesquiterpene](image2)

![Chrysomelidial, an iridoid monoterpene](image3)
1.1.3 Current use of essential oils

The greatest use of EOs in the European Union (EU) is in food (as flavorings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties) (Bauer and Garbe, 1985; Van Welie, 1997; Van de Braak and Leijten, 1999). The well-known use of EO in aromatherapy constitutes little more than 2% of the total market (Van de Braak and Leijten, 1999).

Individual components of EOs are also used as food flavourings, either extracted from plant material or synthetically manufactured (Oosterhaven et al., 1995).

Figure 2. Example of terpenes with established functions in nature.
The antibacterial properties of essential oils and their components are exploited in such diverse commercial products as dental root canal sealers (Manabe et al., 1987), antiseptics (Bauer and Garbe, 1985; Cox et al., 2000) and feed supplements for lactating sows and weaned piglets (Van Krimpen and Binnendijk, 2001; Ilsley et al., 2002).

A few preservatives containing EOs are already commercially available. ‘DMC Base Natural’ is a food preservative produced by DOMCA S. A., Alhendin, Granada, Spain and comprises 50% essential oils from rosemary, sage and citrus and 50% glycerol (Mendoza-Yepes et al., 1997). ‘Protecta One’ and ‘Protecta Two’ are blended herb extracts produced by Bavaria Corp. Apopka, FL, USA and are classed as generally recognized as safe (GRAS) food additives in the US.

1.1.4 Extraction of essential oils
a. Steam distillation is the most commonly used method for producing EOs on a commercial basis.

b. Extraction by means of liquid carbon dioxide produces a more natural organoleptic profile but is much more expensive (Moyler, 1998).

c. Enfleurage method is used for the isolation of essential oil from rose petals.

d. The microwave irradiation or microwave assisted process (–MAP–) has also been developed and reported as a technique for extraction of essential oils in order to obtain a good yield of the essence, and to reduce time of extraction (Collin et al., 1991, Chiasson et al., 2001)

This technique has also been applied for the extraction of saponins from some medicinal plants (Safir et al., 1998). The MAP process uses microwaves to excite water
molecules in the plant tissues causing plant cells to rupture and release the essential oils trapped in the extracellular tissues of the plant (Bélanger, et al., 1991).

e. Mechanical and thermochemical reaction (Bouzid et al., 1997).

1.1.5 Chemical analysis of essential oils:

Chemical analysis is generally performed using GC (quantitative analysis) and GC/MS (qualitative analysis). Identification of the main components is carried out by the comparison of both the GC retention times and MS data against those of the reference standards (with known source) (Lahlou and Berrada, 2001).

Analytical conditions and procedure description includes:

a) apparatus of oil analysis (make and model number of the equipment).

b) column type and dimensions.

c) carrier gas flow rate.

d) the temperature programming conditions including injector temperature, detector and column temperatures (Lahlou and Berrada, 2003).

In addition to mass spectra (electronic impact), sometimes identification by GC/MS must be confirmed by retention indices (Kovats Indices) on two columns of different polarity, on the same column, but at a different temperature and for the identification of new constituents by co-injection or spiking with authentic compounds. Data should thus include essential oils optical rotation, density and refractive index. The compounds which are not easily separated by GC, and similar molecular structures like stereo-isomeric compounds of essential oils are analysed by $^{13}$C NMR. This technique is also applied to
the study of the chemical intraspecific variation and could also be used in the quality control of volatile oils (Tomi et al., 1997).

1.2 TERPENOIDS

Living organisms produce thousands and thousands of different structures of low-molecular-weight organic compounds. Many of these have no apparent function in the basic processes of growth and development, and have been historically referred to as natural products or secondary metabolites. The importance of natural products in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activities of these substances. Yet we still know comparatively little about their actual roles in nature. Such knowledge is especially lacking for terpenoids or isoprenoids, the largest group of natural products (Gershenzon and Dudareva, 2007).

Terpenoids (isoprenoids) encompass more than 40,000 structures and approximately 25,000 terpene structures reported; very few have been investigated from a functional perspective. Many terpenoids are essential for plant growth, development and general metabolism. These terpenoids are found in almost all plant species. Their physiological, metabolic and structural roles include, among others, those of light-harvesting pigments in photosynthesis or the regulatory activities of the many terpenoid plant hormones (Bohlmann and Christopher, 2008).

As the largest class of natural products, terpenes/terpenoids have a variety of roles in mediating antagonistic and beneficial interactions among organism (Chiasson et al., 2001) like reproduction, defence or symbiosis (Bohlmann and Christopher, 2008). They defend many species of plants, animals and microorganisms against predators, pathogens
and competitors, and are involved in conveying messages to conspecifics and mutualists regarding the presence of food, mates and enemies (Gershenzon and Dudareva, 2007). The terpenoid-mediated interactions of plants with other organisms involve species from all kingdoms and trophic levels (Bohlmann and Christopher, 2008).

Terpenoids are mixture of isomeric hydrocarbon along with their oxygenated derivatives, like alcohols, aldehydes, ketones etc. The isoprene unit i.e. C₅H₈ (2-methyl-1,3-butadiene) is the building block of all terpenoids, thus all terpenoids have multiple of (C₅) in their structure.

The thermal decomposition of almost all terpenoids give isoprene as one of the products, and this led to the suggestion that the skeleton structures of all naturally occurring terpenoids can be build up of isoprene units this is known as isoprene rule and was first pointed out by Wallach (1887). Thus the divisibility into isoprene units may be regarded as a necessary condition to be satisfied by the structure of any plant synthesized terpenoid.

1.2.1 Medicinal importance of Terpenoids

Despite the use of terpenoids as flavors and fragrances in foods and cosmetics (e.g. menthol, nootkatone and sclareol) or for the quality of agricultural products, such as the flavor of fruits and the fragrance of flowers (e.g. linalool) (Aharoni et al., 2004; Pichersky and Gershenzon, 1994) terpenoids can also have medicinal properties such as:

a) anti-carcinogenic (e.g. Taxol and perillyl alcohol)

b) antimalarial (e.g. Artemisinin)

c) anti-ulcer, antimicrobial or diuretic (e.g. glycyrrhizin) (Bertea, 2005)
1.2.2 The terpenoids have also been shown to be of ecological significance:

a) Bitter triterpenoid cucurbitacins and the pungent diterpenoid polygodial involved in insect resistance (Powell, 1997).

b) (E,E)-α-farnesene induced in cucumber by spider mite feeding is involved in interactions between plants, and microorganisms, and between plants and arthropod herbivores (Arimura, 2000).

Flavors and fragrances

Antimalarial

Sesquiterpenoids

Anti-carcinogenic (Mono/Diterpenoids)

Antiulcer/hepatoprotective (triterpenoid)
**Figure 3.** Examples of some medicinally important terpenoids