CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF SELECTED *Alpinia* (ZINGIBERACEAE) SPECIES

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ABSTRAK

KOMPOSISI KIMIA DAN AKTIVITI ANTIMIKROORGANISMA DARIPADA MINYAK PATI SPESIS *Alpinia* YANG TERPILIH

Minyak pati rizom bagi enam spesis *Alpinia* iaitu *A. galanga, A. purpurata, A. latilabris, A. nieuwenhuzii, A. ligulata dan A. aquatica* telah diekstrak dengan menggunakan kaedah penyulingan hidro. Kemudian kandungan minyak pati ini telah dikaji dengan menggunakan Kromatografi Gas – Pengesan Nyalaan Ion (GC-FID). Kuantiti minyak pati yang dihasilkan adalah 1.3%, 0.1%, 0.4% 3.8%, 0.9% dan 0.9% bagi minyak pati *A. galanga, A. purpurata, A. nieuwenhuzii, A.latilabris, A.ligulata* dan *A. aquatica*. *A. nieuwenhuzii* mempunyai 120 komponen di dalam minyak pati dan 5 – oksimetilfurfurol (18%) adalah komponen utama. Ini diikuti oleh *A.ligulata* dengan 84 jenis komponen di dalam minyak pati dan tetradesil asetat (15%) sebagai komponen utama. Manakala minyak pati *A. galanga* mempunyai 50 komponen di dalamnya dan 2 – asetiltetrahidropiradin (57%) sebagai komponen utama. *A. aquatica* pula mempunyai 1 – heptane –3-one (22%) sebagai komponen yang paling banyak dan terdapat 41 komponen di dalam minyak pati *A. aquatica.* Minyak pati *A. latilabris* pula mengandungi 25 jenis komponen dan metil sinamat (84%) adalah komponen utama. Minyak pati *A. purpurata* pula mempunyai kandungan komponen yang paling sedikit iaitu 20 komponen dan komponen utama di dalamnya adalah metilisoheksil keton (25.3%). Kajian bioasei bagi menentukan aktiviti mikroorganisma melalui penentuan diameter zon perencatan telah dijalankan. Keputusan menunjukkan bahawa bagi aktiviti antibakteria dan antifungal, minyak pati bagi *A. galanga* dan *A. latilabris* merencat 100% pertumbuhan kesemua spesis bakteria dan fungal yang terpilih dengan zon perencatan yang paling tinggi adalah 7.0 ± 2.1mm dan 9.0 ± 0.6mm bagi bakteria dan 20.0 ± 0.0mm dan 18.0 ± 1.7mm bagi fungal. *A. purpurata* pula menunjukkan zon perencatan yang paling tinggi pada 3.0 ± 0.6mm bagi bakteria. *A. nieuwenhuzii, A. aquatica* dan *A. ligulata* pula masing-masing mempunyai zon perencatan maksimum 8.0 ± 0.6 mm, 7.0 ± 1.7 mm dan 1.0 ± 2.6 mm bagi bakteria. *A. purpurata, A. nieuwenhuzii, A. aquatica* dan *A. ligulata* tidak aktif kepada perencatan pertumbuhan fungal. Bagi penentuan kepekatan perencatan minimum pula menunjukkan bahawa *A. galanga* dan *A. latilabris* mempunyai aktiviti antibakteria dan antifungal yang tinggi dengan kepekatan minimum pada 1.8 mg/mL dan 2.2mg/mL. *A. purpurata, A. aquatica* dan *A. ligulata* menunjukkan aktiviti yang sederhana dengan kepekatan minimum pada 27.0 mg/mL, 9.00 mg/mL dan 32.00mg/mL. Manakala *A.nieuwenhuzii* pula menunjukkan aktiviti yang paling lemah iaitu pada 30.00 mg/mL.

ABSTRACT

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF SELECTED Alpinia (ZINGIBERACEAE) SPECIES

Essential oil from rhizomes of six Alpinia species; A. galanga, A. purpurata, A. nieuwenhuzii, A. latilabris, A. ligulata and A. aquatica were extracted by hydrodistillation, analysed via Gas Chromatography-Flame Ionization Detector (GC-FID) and tested for antimicrobial activities against range of human pathogenic microbes. Quantity of essential oil obtained are as follows; 1.3%, 0.1%, 0.4%, 3.8%, 0.9%, and 0.9% for A. galanga, A. purpurata, A. nieuwenhuzii, A. latilabris, A. ligulata and A. aquatica respectively. Essential oil of A. nieuwenhuzii had the highest variety of the compound, 120 components in total with 5-oxymethylfurfurole (18%) as the major component. This is followed by essential oil of A. ligulata with 84 components and tetradecyl acetate as the major compound (15%). While essential oil of A. galanga has 50 compounds total of all with 2- acetyltetrahydropyradine (57%) as the major compound. Essential oil of A. aquatica has 1- heptane-3-one (22%) as a major compound with total 41 compounds in the essential oil. Essential oils of A. latilabris contained 25 components in total with methyl cinnamate (83.8%) as the major compound. Essential oil of A. purpurata shows the less variety with total 20 compounds and the major compound is methylisohexyl ketone (25.3%). Obtained crude essential oil was tested against six species of pathogenic bacteria and five species of pathogenic fungi. Essential oil of A. galanga and A. latilabris shows 100% inhibition against tested microorganism with the maximum inhibition zone of 7.0 $\pm$ 2.1mm and 9.0 $\pm$ 0.6 mm for bacteria, and 20.0 $\pm$ 0.0mm and 18.0 $\pm$ 1.7mm for fungi. A. purpurata showed a maximum inhibition zone of 3.0 $\pm$ 0.6mm. While A. nieuwenhuzii, A. aquatica and A. ligulata maximum inhibition zone is 8.0 $\pm$ 0.6 mm, 7.0 $\pm$1.7 mm and 1.0 $\pm$ 2.6 mm, respectively, for bacteria. A. purpurata, A. nieuwenhuzii, A, aquatica and A. ligulata did not inhibit the growth of the fungi. The Minimal Inhibiton Concentration (MIC) was determined from the result. The essential oil from A. galanga and A. latilabris showed to be the most effective against all selected microorganism. A. galanga and A. latilabris showed MIC at 1.8mg/mL and 22.00mg/mL respectively. A. purpurata, A. aquatica and A. ligulata showed moderate activity for the antimicrobial properties with MIC at 27.00 mg/mL, 9.00mg/mL and 32.00mg/mL. A. nieuwenhuzii showed the weakest activity among all the essential oils with MIC at 30.00mg/mL.

LIST OF ABBREVIATION

%	Percentage
α	Alpha
β	Beta
δ	Delta
ρ	Rho
°C	Degree Celsius
μm	Micrometer
>	More than
Abs	Absorbance
BCE	Before Common Era
cm	Centimeter
g	Gram
GC-FID	Gas chromatography flame ionization detector.
km	Kilometer
m	Meter
MIC	Minimal inhibition Concentration
min	Minutes
mL	Milliliter
mm	Millimeter
NA	Nutrient agar
nm	Nanometer
PDA	Potato dextrose agar
ppm	Parts per million
SDA	Sabouraud Dextrose Agar
v/v	Volume per volume

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CHAPTER 1

INTRODUCTION

Plant extracts have aroused great interest from scientists all over the world. Plants contain important values in the form of medicine, foods, flavours, cosmetic and various other products. Malaysia's tropical forest contains many plants with important chemical compounds. From the estimated 12 000 species in Malaysia, more than 1 000 species are said to have therapeutic property and are used in the local traditional medicine system (Ikram, 1995). Plants have gained prominence due to long term use of plants by societies in traditional healthcare. Many plants have been recognized for their antifungal, antimicrobial, insecticidal, cytostatic and other therapeutic properties (Gherman *et al.*, 2000).

Modern trends in medicine, research and consumer preference have fuelled the demand for natural products from plants (Mohanlall, 2002). In order to promote the proper use of natural products from plants and to determine their potential as sources for new drugs, it is essential to begin by systematically analyzing, among others, plants recorded in folklore (Mothana and Lindequist, 2005).

Essential oils and extracts obtained from many plants have been used for a variety of purposes such as food, drugs and perfumery (Tepe *et al.,* 2003). An estimated 3 000 essential oils are known and more than 300 have found commercial importance as flavours and fragrances. Essential oils are aromatic liquids obtained from various plant parts such as flowers, rhizome, leaves, buds, seeds, barks and fruits. They can be obtained by expression, fermentation or extraction. Steam distillation is commonly used for the commercial production of essential oils.

Alpinia, a genus of the family Zingiberaceae, comprises of 250 species in tropical Asia. They can be found as medium sized to large forest plants, some species reaching a height over three meters (Larsen *et al.,* 1999). The most widely documented is *Alpinia galanga* or Lengkuas in Malay. The extensive use of several aromatic plants in the cosmetic and food industries demands an extensive screening of the biologically active compounds and genotoxic activities (Gherman *et al.,* 2000).

Microorganisms have unfavorable effects on quality, safety and shelf life of foods (Baydar *et al.,* 2004). While synthetic additives have been used to overcome this problem, recent interest in using natural extracts from plants has increased. Extracts from herbs and spices are the most common plant materials evaluated for this purpose (Baydar *et al.,* 2004).

The antimicrobial properties of some plant-derived essential oils have been recognized for hundred of years and have been documented in scientific studies. The knowledge and studies of gingers in Malaysia in terms of their chemical composition or biological activities are still lacking. As far as literature could ascertain, antimicrobial activities of gingers is still limited to *A. galanga*.

.1 Objectives

The objectives of this study are to obtain essential oils of six selected *Alpinia* species that can be found in Malaysia. Thus, this research is to identify and compare the chemical composition of the essential oils of selected species of genus *Alpinia*. The compounds of the essential oils will be analyzed and identified using gas chromatography. The essential oils compositions will then be compared. The identification of the essential oil constituents is important in analyzing the chemical composition in the essential oil.

The essential oils of selected *Alpinia* species will be used to qualitatively and quantitatively assess the antimicrobial activities of essential oils against selected pathogenic microbes. It will be tested against the growth of the selected pathogenic bacteria using the disc diffusion assay method and broth micro dilution in finding the minimal inhibition concentration (MIC).

CHAPTER 2

LITERATURE RIVIEW

2.1 Zingiberaceae

Zingiberaceae are part of order Zingiberales. Zingiberaceae comprises about 1 200 species of which about 1 000 occur in tropical Asia (Larsen *et al.,* 1999; Kress *et al.,* 2005). Zingiberaceae species are mostly found in tropical Asia, *viz*., the Malesian region, which include Malaysia, Indonesia, Brunei, Singapore, Philippine, Papua New Guinea (Jones, 2002; Larsen *et al.,* 1999) and from Sri Lanka, India, China, Japan, the Pacific region, Caroline Islands, Australia, and Northern New South Wales (Kress *et al.,* 2005). Ginger flora in Borneo and Sumatra are largely unrecorded.

Zingiberaceae vary in height and size. Some species such as *Etlingera elatior* or known as 'kantan' in Malay, can reach about 8 m height. Some of them are very small such as *Kaemferia galanga* (cekur) with the height can be as small as 10 cm. It has showy inflorescence and often brightly coloured bracts and floral parts. The flowers of most Zingiberaceae species are very short lived and lasting a few hours. Gingers are generally abundant in lowland to hill forest, notably between 200 m to 500 m but many species do not thrive well outside the moist environment of a tropical rain forest, therefore a few species has been cultivated. Members of Zingiberaceae are usually aromatic in all or most parts or at least one of the plant parts. This feature is an advantage as the plants are easily recognizable by the smell of crushed leaves, roots or other parts of the plants.

Most of the Zingiberaceae species have been cultivated and used as spices, condiments, flavouring agents, medicine and ornamental (Kress *et al.,* 2005; Larsen *et al.,* 1999; Jones, 2002; Kamaruddin and Latiff, 2002; Habsah *et al.*, 2000). Gingers were also one of the earliest used as spice. The three species with commercial importance are *Zingiber officinale* (ginger), *Curcuma domestica* (turmeric) and *Elettaria cardamomum* (cardamom).

Larsen *et al.* (1999) reported that gingers are rich in essential oil and phytochemicals studies show that selected species contained flavonoids, terpenoids and alkaloids. The presence of compounds like limonene, eugenol, pinene and geraniol in essential oils add great value to the gingers as spice (Larsen *et al.,* 1999). Volatile constituents are useful as taxonomic characters especially at generic or family level. Phytochemical screening of selected species has revealed that flavonoids and terpenoids are ubiquitous but alkaloids detectable in *Alpinia* (Larsen *et al.,* 1999).

2.2 Genus *Alpinia*

Alpinia is the largest genus in the Alpinieae. It consists of 250 species in tropical Asia from medium size to large forest plants. Some species of *Alpinia* reach heights of over three metres. The *Alpinia* is easy to identify because it is the only genus in Alpinieae having the terminal inflorescence on the leafy shoot (Larsen *et al.,* 1999). The flowers of most *Alpinia* are yellowish green to cream or red. The staminodes are reduced to large teeth, several millimeters long at the base of the lip. The lip is more or less saccade and not divided and if pale coloured it is with yellow blotches or red lines. The capsules are smooth spherical or ellipsoid. The name *Alpinia* commemorates Prosper Alpinus, the 17th century Italian botanist (Larsen *et al.,* 1999; Jones, 2002).

Essential oils of *Alpinia latilabris, Alpinia nieuwenhuizii* and *Alpinia ligulata* are undocumented to date. Many wild species, especially those from Sabah and Sarawak remained undescribed and to large extent undocumented. The chemical composition and bioactivity of the essential oil of *A. purpurata* are also unavailable.

2.2.1 *Alpinia galanga* Willd.

Alpinia galanga Willd. or greater galangal is one of the economically important species. It is also called 'lengkuas' in Malay. *A. galanga* is not native to Malaysia but is widely cultivated (Larsen *et al.,* 1999) throughout South East Asia for its aromatic rhizome (Jones, 2000). The Malays use its rhizomes as spice especially in dishes called 'rendang' and in the North Peninsular Malaysia to decrease the smell of fish stock in a dish called 'Laksa'. It is also consumed as condiments or 'ulam'

A. galanga can grow up to two meters tall and from its thickened rhizome arise stout leafy stems. The inflorescences are borne at the ends of these stems and comprise numerous short branches with 3 – 5 flowers each. Each flower consists of a tubular calyx, pale green petal and notched white lip streaked with pink and red. A single massive anther prostrutes from the throat of the corolla tube. The fruit is a round red capsule containing a few seeds (Ridley, 1924; Jones, 2002).

Figure 2.1: *A. galanga* plant

Figure 2.2: *A. galanga* rhizome

A. galanga is the most well documented species compared to other *Alpinia* species. *A. galanga* has been proven to have medicinal value. Its essential oil was reported to have a more woody, minty and floral aroma quality (Nurdijati *et al.,* 1999). The Kenyah's tribe in Apo Kayan Region of Kalimantan in Indonesia use *A. galanga* rhizome in treating ringworm (Ficker *et al.*, 2003). Besides that, previous research reported that it can inhibit tumor promoters and has exhibited potential as insecticide.

A. galanga is frequently cultivated in villages from where they often spread to disturbed habitats (Larsen *et al.,* 1999). It grows in abundance along bunds of rice field, near ditches and in rubber estates. The essential oil composition of the dried stalk and rhizome vary (Nurdijati *et al.,* 1999). *A. galanga* constituents such as 1'-acetoxy chavicol acetate; 1,8-cineole; linalool; geranyl acetate and eugenol are odorants and are important in perfumery and aromatherapy (Nurdijati *et al.,* 1999). De Pooter *et al.* (1985) reported about 49 compounds in the essential oil of *A. galanga* from Kuala Lumpur, while Larson *et al.* (1999) reported 31 compounds in the essential oil of *A. galanga.*

Previous workers (De Pooter *et al.,* 1985) reported *A. galanga* to smell as strongly campherous, floral and spicy. The essential oil of *A. galanga* from Malaysia was also reported to contain α-pinene; 1,8-cineole; bornyl acetate; geranyl acetate; α-gergamontene; trans-β-farnesene and β-bisabolene. Galanga oil from Java was reported to contain 1,8-cineole; (+)-pinene (probable); camphor and methyl cinnamate. An analyses of galangal oil from India found large amounts of 1,8-cineole; methyl cinnamate and a large amount of unidentified sesquiterpenes. Camphor or pinenes were undetected in the galangal oil of India (De Pooter *et al.,* 1985).

Ficker *et al.* (2003) reported significant antifungal activity of the ethanolic extract of the rhizome of *A. galanga*. Besides that, the rhizome and seed of *A. galanga* have been reported to suppress tumor promoters by inducing Epstein-Barr virus activation and inhibit the skin tumor promotion in mice (Nurdijati *et al.,* 1999). The ethanolic extract of *A. galanga* also exhibit free radical scavenging properties and acted as lipoxygenase inhibitor (Juntachote and Berghofer, 2005).

A. galanga has been used in traditional medicine for treating ringworm (Ficker *et al.,* 2003) flatulence, colic, dysentery, diarrhea, dyspepsia, food poisoning, rheumatism, sinus and skin disease (Nurdijati *et al.,* 1999).

2.2.2 *Alpinia purpurata* K.Schum.

Alpinia purpurata K. Schum. is known as a beautiful landscape plant. It has red inflorescence bracts, which makes it as the choice pick for landscape (Jones, 2002) and is easy to grow (Chantrachit and Paull, 1998). It has leafy shoots between 1 m to 5 m tall. The leaves oblong, 30 cm to 70 cm long, glabrous, ligules unequally lobed, 7 mm to

20mm long, sheaths pubescent at apex margins. Inflorescences terminal on leafy shoots, unbranched stamen 6-7mm long; ovary 3-4 mm long (Jones, 2002). Antimicrobial activity of its essential oil is undocumented.

Figure 2.3: **Flower of *A. purpurata***

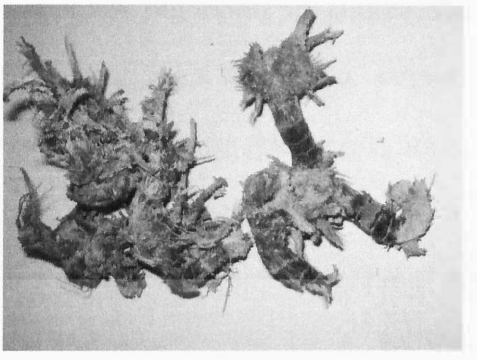

Figure 2.4: **Rhizome of *A. purpurata***

2.2.3 *Alpinia aquatica* Rosc.

Alpinia aquatica Rosc. can be found in South and East Peninsular Malaysia and in Sabah and Sarawak. It is used as decoctions for post-natal treatment. It is a small tree and the stems are not sturdy. It has small leaves and a pink or purplish flower 2cm long, terminal inflorescence on leafy shoot. The staminodes are reduced to large teeth. It is also known as 'meroyan siamang' in Malay (Kamarudin and Latiff, 2002). Analysis of the essential oil shows that β-sesquiphellandrene is the major constituent (Norazah *et al.*, 2002). Antibacterial screening of *A. aquatica* crude extract, essential oil and pure compound isolated to exhibit moderate inhibition to bacterial growth (Norazah *et al.*, 2002).

2.2.4 *Alpinia latilabris* Ridl.

Alpinia latilabris Ridl. is about 3m tall, stout, except midrib, petiole and ligule, 70cm long, 7cm wide, narrow lanceolate; petioles 3cm long. Panicles pubescent, large, outer bracts oblong lanceolate, 5cm long 3cm across, rosy white. Calyx dilates upward white, rose tipped. Corolla tube long. Lip 5 cm long in wide cordate not lobed, tip shortly bifid, lobes acute, orange, densely red spotted, central bar and veins deep red, edged yellow. Staminodes at base horn-shaped 0.5mm long crimson. Fruits globose orange, nearly glabrous (Ridley, 1924). The previous workers reported that essential oil of the *A. latilabris* rhizomes consist of 33 identified compounds; the major constituent is methyl (*E*)-cinnamate (Wong *et al.,* 2004).

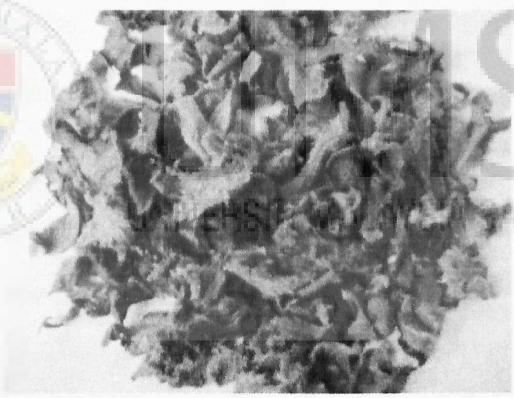

Figure 2.5: Dried rhizome of *A. latilabris*

2.2.5 *Alpinia nieuwenhuzii* Val.

Alpinia nieuwenhuzii is also one of the *Alpinia* species that can be found in Borneo in swampy areas. This species is about 1m to 5m in height. The flower is pinkish in colour. Lip with prominent transverse darker red lines on each half. The fruits are yellowish brown. It has paniculate inflorescence branches and short ligules between 5mm to 6mm. Report on chemical composition and bioassay for this species is unavailable.

Figure 2.6: *A. nieuwenhuzii* leaves

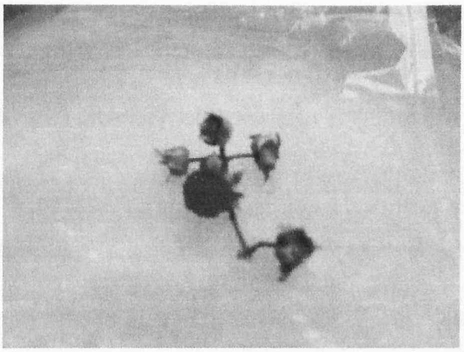

Figure 2.7: *A. nieuwenhuzii* fruit

2.2.6 *Alpinia ligulata* K.Schum.

Alpinia ligulata K. Schum. is one of the species, which is widely distributed in Borneo. It can be found in swampy habitat. The height of *A. ligulata* is about 1m to 3m. The ligule is 6cm long and strong reticulate leaf sheath. The flower is cream and red in colour, base of the lip with red lines on side lobes. The fruit diameter is 2.5cm, light brown in colour and has paniculate inflorescence. It has predominant long ligules. Report on chemical composition and bioassay for this species is unavailable.

2.3 Essential oils

Essential oil can be defined as a volatile and aromatic liquid or semisolid which generally constitute the odorous principles of plants (Lawless, 2002; Skočibušić *et al.*, 2005) and the fragrant essence of plants in their purest, most concentrated state, most of which are primarily composed of terpenes and their oxygenated derivatives. It can be obtained from the process of expression or distillation from a single botanical form or species. Different parts of plants can be used to obtain them, including the flowers, leaves, bark, roots, stems and wood (Lawless, 2002; Burt, 2004).

Essential oil was mentioned in 2000 BCE by the Vedic literature of India as well as Chinese literature. Through exploration and conquest, the knowledge was spread throughout the world (Burt, 2004). Essential oil is not merely simple perfumes but it is also important components of religious and ritualistic occasion and is believed to have the power of healing. The ancient Chinese have an ancient herbal tradition using essential oil that accompanies the practice of acupuncture (Lawless, 2002). Essential oil from plants has recently regained popularity and scientific interest (Tepe *et al.,* 2003). The term 'essential oil' is thought to be derived 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* (Burt, 2004).

The term essential oil is loosely applied to all aromatic products or extract that are derived from natural sources (Lawless, 2002). Essential oil is extracted from the plant using distillation processes. This method isolates only the volatile parts and water insoluble part (Lawless, 2002). Hexane is used as the solvent to collect the essential oil. In steam distillation, there are two products produced namely the oil and the hydrosol. The method of extraction for essential oil is dependent on the quality of the sample being used and the type of aromatic products that is required (Calkin and Jellinek, 1994). This means that the flower for example, should be extracted as fast as possible after harvesting compared to the root or stem (Burt, 2004). Nowadays the essential oils were found to have many functions especially in treating common infection. It is used as antiseptic, anti-inflammatory, deodorants, insect repellent, and has antioxidant and antimicrobial properties (Sahin *et al.* 2004; Calkin and Jellinek, 1994).

There are three modes of action of the essential oil in relation to the human body. Pharmacological effects are related to the chemical changes that take place when the essential oil enters the bloodstream and react with hormones and enzymes. The physiological mode is concerned with the way in which an essential oil affects the body. Psychological effect takes place when an essence is inhaled and an individual respond to its odour (Lawless, 2002).

Essential oil has been studied most from the viewpoint of their flavour and fragrance chemistry (Pauli, 2001b; Sacchetti *et al.,* 2005). Nowadays there is renewed interest in plant-derived essential oils, which are considered free from harmful synthetic additives (Sacchetti *et al.,* 2005).

2.4 Chemical properties of essential oil

Essential oils consist of volatile compounds from plants (Yayli *et al.,* 2005). The aromatic constituents of essential oil constitute hydrocarbons and heteroatom(s) containing hydrocarbons.

Terpenoid constituents of essential oils are constructed from one basic chemical building block namely the isoprene unit. Isoprene units (Figure 2.8) consist of five carbon atoms that form a basic chain or unit to form progressively larger chemical compounds that are volatile. Generally, there are two, three and four isoprene units known as monoterpene, sesquiterpene and diterpene. Triterpenes contain six isoprene units or 30 atom carbons. Compounds belonging to this series but containing oxygen is then described as an oxygenated terpene. The mono- and sesquiterpenes are compounds that stimulate a wide spectrum of aromas, mostly perceived as very pleasant (Belitz *et al.,* 2004).