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RESEARCH ARTICLE

ESSENTIAL OILS: ITS MEDICINAL AND PHARMACOLOGICAL USES

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ABSTRACT

Essential oils have been important substances since early times. The review of its extraction methods and applications were treated in this study. These include hydrodistillation, hydrodiffusion, effleurage, steam distillation, cold pressing, solvent extraction, microwave assisted process and carbondioxide extraction. Its applications both the medicinal and therapeutics, such as aromatherapy, phytotherapy, antibacterial and antifungal uses, hypolipidemic, antitumor etc. were also reviewed.

INTRODUCTION

Plants produce primary and secondary metabolites which encompasses a whole array of function (Croteau, 2000). Primary metabolites include amino acids, simple sugars, nucleic acids and lipids, are compounds that are necessary for cellular processes. Secondary metabolites include compounds produced in response to stress such as the case when acting as a deterrent against herbivores. Plants can manufacture many different types of secondary metabolites which have been subsequently exploited by humans for beneficial role in a diverse array of application. Waterman, (1992) suggested that the role of secondary metabolites may centre on the defence of the plants against predation, most especially the herbivores, pathogens or competitor or an acid to pollination or seed disposal or for the protection against or adaptation to extrinsic abiotic factors or a combination of these functions. The metabolites may be able to perform roles mentioned above because of their special attributes. These include odour, physiological actions and taste. Secondary metabolites may be referred to as plants natural products. There are three broad categories of plant secondary metabolites as natural products. Terpenes and Terpenoids (25,000 types), Alkaloids (12,000 types) and the Phenolic compounds (8,000 types) (Croteau, 2000). Most of these metabolites have been isolated and characterized. For instance, Madagascan frog of mantellid genus. Mantellid has been a rich source of alkaloid derived directly from arthropods. Eight new phenolic glycosides, cucurbitosides F.M were isolated from the seed of cucurbita pepo. Four sterols and ten triterpenes were isolated from the fruiting bodies of Ganoderma pfeifferi including the three new triterpenes 3, 7, 11-trioxo-5a-lanoster-8-ene-24-diene-26-al (Luciadehyde D, 1), 5a lanoster-8-ene-

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24, 25 epoxy-26-hydroxy-3, 7-dione (ganoderone C, 30). Some monoterpeniods, sesquitrpenoids and aromatic compounds have been isolated and characterized in essential oils extracted from different odoriferous plants.

Essential oils

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenou layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant. Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelica archangelica), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated factors, climatic, seasonal and geographical conditions, harvest period and extraction techniques (Pannizi *et al.,* 1993). The yield of oils from the plants can also be affected by the stages of the plant growth.

Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators. The oxygenated molecules of essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers (Buchbauer *et al.,*1993b, Federspil *et al.,*1997, Rajesh and Howard, 2003). These properties were established after the oils have been extracted from the plant materials.

Extraction of essential oils

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process (Bruneton, 1995). The oil droplets being stored in the oil glands or sacs can be removed by either accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are;

- Hydrodistillation
- Hydrodiffusion
- Effleurage.
- Cold pressing
- Steam distillation
- Solvent extraction
- Microwave Assisted Process (MAP)
- Carbondioxide extraction.

Hydrodistillation

The technique involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapour and oil are removed through a water cool condenser.

Hydrodiffusion

Hydrodiffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma of the plants (Buchbauer, 2000).

Enfleurage

This process is applicable to flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odourless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called Enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind only the essential oil, hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

Cold pressing

Another method of extracting essential oil that has not found high application in scientific research is cold pressing. It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

Steam distillation

This is the most common method of extracting oils and is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel. Next steam is added and passed through the plant that contains the plants aromatic molecules or oils. Once upon, the plant releases these aromatic molecules and in the state, the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapours. As they cool, they condense and transform into a liquid state.

Solvent extraction

This method involves the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used for extraction. For instance, leaves, roots, fruits are extracted with benzene with or without mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and colour. After the extraction, the solvent is removed by distillation under reduced pressure leaving behind the semisolid concentrate, this concentrate are extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil.

Microwave assisted process (MAP)

The MAP process uses microwave to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants (Belanger *et al.,* 1995). This technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction (Pare *et al.,* 1989, Collin *et al.,* 1991, Bouzid *et al.,* 1997, Chiasson *et al.,* 2001, Ghoulami *et al.,* 2001). This technique has also been applied for the extraction of saponins from some medicinal plants (Safir *et al.,* 1998).

Carbondioxide extraction

In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition of the oil.

CHEMICAL COMPOSITION OF ESSENTIAL OILS

Most constituents of oil belong to the large group of terpenes. Terpenes usually refer to hydrocarbon

molecules consisting of isoprene (2-methylbuya-1, 3-diene). The isoprene unit, which can build upon it in various ways, is a five-carbon molecule. Two of the molecules of isoprene give monoterpenes, sisquiterpenes contain three molecule of isoprene, four isoprene gives diterpene, five isoprene gives sesquiterpenes. Isoprene units (Fig. 1) are obtained biosynthetically via mevalonate3 pathway (Swansom and Hohl 2006).

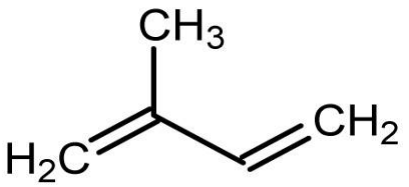

Fig. 1. Isoprene

MEVALONATE PATHWAY

This mechanism involves the consideration of two molecules of Acetyl-CoA subunit via Acetyl-CoA transferase to form Acetoacetyyl-CoA . Acetyl-CoA condenses with acetoacetyl-CoA to form 3-hydroxy-3-methyl glut aryl-CoA (HMG-CoA) (Fig. 2). HMG-CoA is reduced to mavelonate by NADPH. This reaction occurs in the crytosol. Followed by mevalonate kinase catalyses of the first ATP-dependent phosphorilation of 5-phosphomevalonate is produced by the further action of phosphomevalonate kinase. This reaction leads to the formation of isopentyl diphosphate (IPP) . The IPP is isomerized to dimethyl allyl diphosphate. In contrast to the classical mevalonate pathway of isoprenoids biosynthesis, plants and

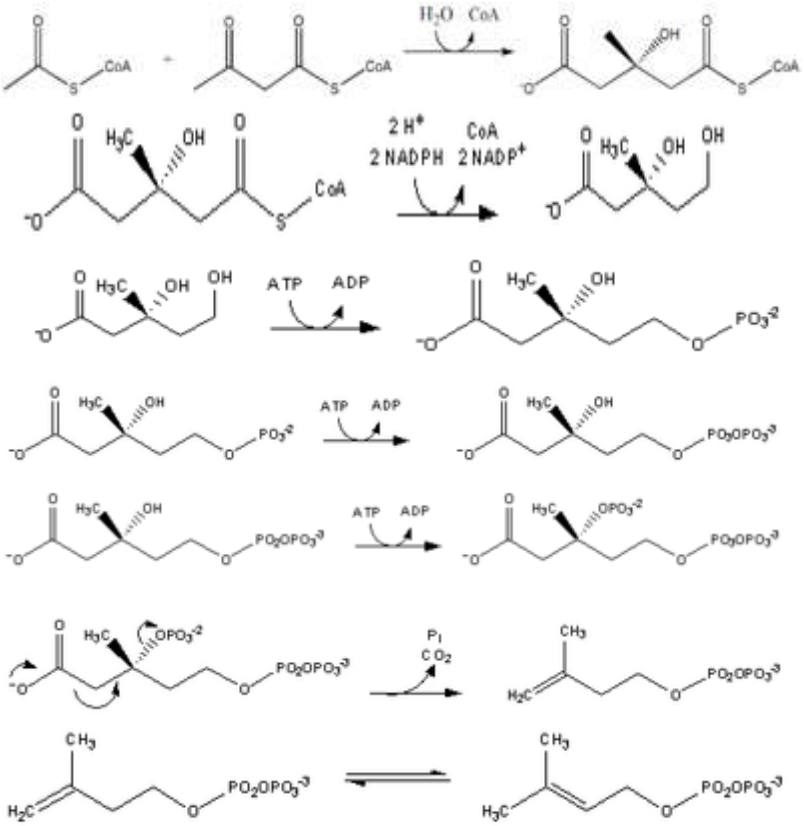

Fig. 2. Mevalonate pathway.

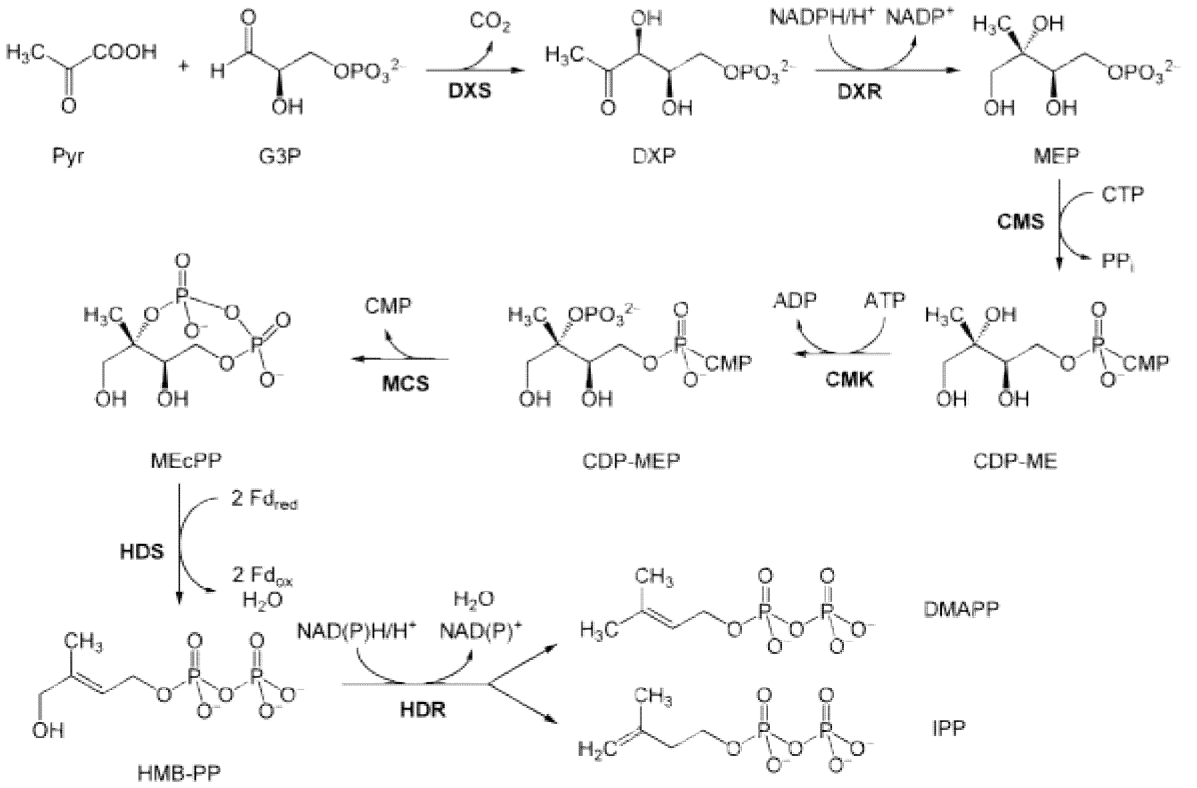

Fig.3. Non-mevalonate pathway

apicomplexan protozoa such as malaria parasite have the ability to produce their own isoprenoids (terpeniods) using an alternative pathway called the non-mevalonate pathway (Rohmer *et al.,* 1999, Lichtenthaler 1999).

NON-MEVALONATE PATHWAY

The first reaction of this pathway is a transketolase-like condensation between pyruvate and D-glyceraldehyde 3-phosphate to form 1-Deoxy-D-xylulose-5-phosphate (DOXP) which is transformed to 2-C-methylerythritol-4-phosphate (MEP) (Fig. 3). The anticipated intermediate (2-C-methylerythrose-4-phosphate) is not released from the enzyme but simultaneously reduced by NADPH. Subsequently, reactions can lead to the formation of DMAPP and (IPP) isopentenyl diphosphate (Eisenreich *et al.,* 2004).

ANALYSIS OF ESSENTIAL OILS

The two main purposes of analysing essential oils are:

(i) To identify and quantify as many constituents as possible.

(ii) To evaluate the quality of the oils and detect any possible adulteration that may affect their usage. Analysis of essential oils is generally performed using Gas chromatography (qualitative analysis) and Gas chromatography-mass spectroscopy (qualitative analysis)(Keravis, 1997). Gas chromatography analysis is a common confirmation test.

Gas Chromatography Analysis (GC)

Gas chromatography analysis is a chemical instrument used for separating chemicals in a

complex sample and provides a representative spectral output. The gas chromatography instrument vapourizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak. The time elapsed between injection and evaluation is called "Retention time".

The sample is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separates before eluting from the column end. The detector measure different compounds as they emerge from the column.

Gas Chromatography-Mass Spectroscopy Analysis (GC/MS)

Gas chromatography-Mass spectroscopy analysis is a method which combines the features of gas, liquid chromatography and mass spectroscopy to identify different substances within a test sample. The gas chromatography-mass spectroscopy instrument is made of two parts: The gas chromatography (GC) portion separates the chemical mixture into pulses of pure chemicals and mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed through the GC, the chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions. This is important because the particles must be charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. This information is sent to the computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that travelled through the filter. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent, so that it can be put into various uses.

MEDICINAL AND PHAMACOLOGICAL USES OF ESSENTIAL OILS

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides (Buchbauer 2000). Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure or mitigate or prevent diseases, infection and indisposition by means of inhalation (Buchbauer *et al.,* 1993a). Inhalation of essential oils or their individual volatile terpenes has a significant role in controlling the central nervous system. For instance, aroma inhibit of storax pill essential oil and pre inhalation of *Aconus gramineus* rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy (Koo *et al.,* 2003, Koo *et al.,* 2004). The fragrance compounds, cis-jasmonate, which characterized the aroma of *Jasminum grandiflorum* have a tranquilizing effect on the brain upon inhalation (Hossain *et al.,* 2004). They significantly increased the sleeping time of mice induced by pentobarbital. Cendrol, which is a major component of cardwood essential oil, shows a sedative effect and prolonged pentobarbital-induced sleeping time on rats upon inhalation (Kagawa *et al.,* 2003). The vapour of lavender essential oil or one of its main component linalool may also be applicable to the treatment of menopausal disorder through inhalation (Yamada *et al.,* 2005). Lavender essential oil demonstrated an analgesic activity, mainly relevant after inhalation at the doses devoid of sedative side effects (Barocelli *et al.,* 2004). Medical professionals are more interested in the medicinal properties of essential oils. Many oils show antibacterial, fungicidal, relaxant, stimulating, anti-depressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both by pathogenic and non-pathogenic diseases. Pathogenic diseases

caused by bacterial, virus, and the fungi can be treated with essential oils.

Strong in vitro evidence indicates that essential oil can act as antibacterial agent against a wide spectrum of pathogenic bacteria strains including; *Listeria monocytogenes, Linnocua, Salmonella typhimurium, Shigella dysentria, Bacilluscerus*, and *Staphylococcus aureus* (Jirovetz *et al.,* 2005, Burt 2004, Dadalioglu and Evrendile 2004, Nguefack *et al.,* 2004, Hulin *et al.,* 1998). Thyme and oregano essential oils can inhibit some pathogenic bacteria strains such as E.coli, *Salmonella typhimurium, Salmonella entritidis* and *Salmonella choleraesuis* (Penalver *et al.,* 2005), with the inhibition directly correlated to the phenolic components carvacrol and thymol. Eugenol and carvocrol showed an inhibitory effect against the growth of four strains of Escherichia coli 0157:H7 and Listeria monocytogens (Gaysinsky *et al.,* 2005). Also, the presence of phenolic hydroxyl group in carvacrol particularly is credited with its activity against pathogens such as Bacillus cereus (Ultee *et al.,* 1999, Ultee *et al.,* 2006). Essential oil with high concentration of thymol and carvacrol e.g. oregano, savory and thyme, usually inhibit gram positive more than gram-negative pathogenic bacteria (Nevas *et al.,* 2004). However the antibacterial activity against gram- negative *Haemophilus influenza* and *Pseudomonas aeruginosa* respiratory pathogens, while gram-positive streptococcus pyrogens was the most resistant to the oil (Skocibusic *et al.,* 2004). Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural mouth control (Yengopal 2004a), general improvement of oral health (Yengopal 2004b), interdental hygiene (Yengopal 2004c) and to control oral malodour (Yengopal 2004d). Mouth rinses containing essential oils with chlorhexine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decreases chances of postoperative infections, decreases oral bacterial load and decrease aerolization of bacteria (Hennessy and Joyce 2004). Mouth washes containing essential oils could also be used as part of plaque-control routine since they can penetrate the plaque biofilm, kill pathogenic-wall and inhibiting their enzymatic activity (Ouhayoun 2003). In addition, essential oil in mouth washes prevent bacterial aggregation slows the multiplication and extract bacterial endotoxins (Seymour 2003). *Croton cajucara* benth essential oil was found to be toxic to some pathogenic bacteria and fungi associated with oral cavity diseases (Alviano *et al.,* 2005).

Besides their antibacterial and antifungal activities, essential oils have also been reported to posses interesting antiviral activities alternative to synthetic antiviral drugs. They have demonstrated virucidal properties with the advantages of low toxicity (Baqui *et al.,* 2001); Herpes simplex virus (type III) causes some of the most common viral infections in human and can be fatal. Synthetic antiviral drugs have been used to treat Herpes infection (Wagstaff *et al.,* 1994), but not all are efficacious in treatment of genital herpes infections. Incorporation of *Artemisia arboreseens* essential oils in multi lamella liposomes greatly improved its activity against intra cellular herpes simplex virus type 1 (HSV-1) (Sinico *et al.,* 2005). Due to the presence of Citra and citronellal in *Melissa officinalis* L. essential oil, it also inhibits the replication of HSV-2 (Allahverdiyev *et al.,* 2004) and the ability to replicate of HSV-1 can be suppressed incubation with different essential oils *in vitro*. Bammi *et al.,* (1997) also demonstrated the effect of five essential oils on Epstein-Barr virus (EBV) (viridae) which caused the infectious mononucleosis associated with Burkitt lymphoma and naso-pharynx carcinoma. The study aimed the effect of these oils on the expression of EBV viral capside antigen (VCA) in the marmouset B95-8 lymphoblastoid cellular line using the indirect immune-flourescence technique. The result showed a cytotoxic effect of tested oils at a dilution factor lower than 1: 500. Moreover, the vapour of cellular viability was not affected. Treatment of B95-8 cells with 1: 1000 dilution of thymus oil increased the fluorescence intensity VCA-positive cells in two separate experiments. In three other tests, only fluorescence intensity was increased by oil from thymus *sp* while the percentage of the fluorescent cells did not increase significantly.

Essential oils can also be used for the treatment of non-pathogenic diseases. For instance, Garlic

essential oil significantly lowered serum cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases (Bordia 1981). The hypolipidemic action of garlic oil is primarily due to a decrease in hepatic cholestrogenesis (Mathew *et al.,* 1996). Some essential oils also exert hypotensive activity when applied in vivo and they are used for treating hypertension. Oral administration of combination of oregano, cinnamon, cumin, and other essential oils decreases systolic blood pressure in rats (Talpul *et al.,* 2005) and intravenous administration of the essential oil from the aerial parts of *Menthax villosa* induced in a significant and dose-dependent hypotension associated with decrease in heart rate (Guedes *et al.,* 2005). This activity was attributed to volatile component, piperitenone oxide which represents 55.4% of the oil. The hypotensive effect induced by the oil is probably due to its direct cardiodeplesant action and peripheral vasolidation, which can be attributed to both endothelium-dependent and endothelium-independent mechanism. Intravenous administration of essential oil of basil (*Ocimum gratissium*) induced an immediate and significant hypotension and bradycardia (Lahlou *et al.,* 2004). The hypotensive activity of the essential oil resulted from its vasodilator effect, acting directly upon vascular smooth muscles. This effect was attributed to the suction of eugenol known about 80%; (Deyama and Horiguchi 1971) but from a safety point of view, care must be taken in dealing with eugenol due to its suspected carcinogenicity and hepatotoxicity (National Toxicology Program 1983). Intravenous injection of the monoterpene alcohol terpinen-4-ol decrease main aortic blood pressure in a dose related manner, in a conscious DOCA-salt hypertensive rats. The mechanism of action was related to the induction of vascular smooth muscle relaxation rather than enhanced sympathetic nervous system activity. Terpinene-4-ol is a major constituent of several essential oil, particularly tea tree (Brophy *et al.,* 1989) and sweet marjoram essential oils. Some essential oils may aggravate diabetes, for instance rosemary essential oil showed hyperglycaemic and insulin release inhibitory effect in diabetic rabbits (Al-Hader *et al.,*1994, Broadhurst *et al.,* 2000) has emphasised that the lipophilic fraction of aromatic plants are not generally responsible for any anti-diabetic activity showed by these plants, but it was also indicated that an oral administration of a combination of essential oils including cinnamon, cumin, oregano, fennel, myrtle besides others was able to enhance insulin sensitivity in type II diabetes, in addition to lowering circulating glucose in the tolerance testing a rat. The essential oil of satureja khuzestanica results in significant decreases in fasting blood glucose level in diabetic rats (Abdollahi *et al.,* 2003).

Essential oils and their individual aroma components showed cancer suppressive inactivity when tested on a number of human cancer cells lines including glioma, tumours, breast cancer, leukaemia and others. Glioma is one of the most malignant human tumours (De Angelis 2001). A significant effect on the treatment of glioma using the sesquiterpene hydrocarbon element which is found in small amounts in many essential oils, it prolonged quality survival time of patients with glioma (Tan *et al.,* 2000). Antiangiogenic therapy is one of the most promising approaches to control cancer. Perillyl alcohol (POH) which is the hydroxlated analogue of d-limonene has the ability to interfere with angiogenesis (Loutrari *et al.,* 2004). POH either alone or with PA (perillic acid, the major metabolite of POH in the body), has the potential use as an anticancer drug that stimulates different types of tumour to apoptosis inhibit their proliferation of overcomes their resistance to chemo/radiotherapy (Rajesh and Howard 2003). Treatment of human leukaemia cells with eucalyptus oil showed morphological changes (fragmentation of DNA) indicating an induction of apoptosis (Motakii *et al.,* 2002). The essential oil of lemon balm (mellisa oficinalis L) was found to be effective against a series of human (A549, MCF-7, Caco-2, HL-60, K562) and a mouse cell line (B16F10) (De Sousa *et al.,* 2004) and that of *Artemisia annua* L. Induced apoptosis of cultured SMMC-7721 hepatocarcinoma cells (Li *et al.,* 2004). The essential oils of Australian tea tree (*Melaleuca alternifolia*) and its major monoterpene alcohol, terpinen-4-ol, were able to induce caspase-dependent apoptosis in human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamicin-resistant (Calcabrin *et al.,* 2004). There was evidence to suggest that the effect of the total

oil of terpinen-4-ol was meditated by their interaction within the plasma membrane and subsequent reorganisation of membrane lipids. Hepatic arterial infusion with Curcuma oil had a similar positive effect in treating primary liver cancer as that of the chemical drugs (Cheng *et al.,* 2001). The essential oil of *Tetraclinis articulate*, (a conifer tree) showed the hallmarks of apoptosis when tested on a number of human cancer cell lines including melanoma, breast and ovarian cancer in addition to peripheral blood lymphocytes (Buhagiar *et al.,* 1999).

Essential oils are reported to have insecticidal properties essentially as ovicidal, larvicidal, growth inhibitor, repellence and antifeedant (Isman *et al.,* 1990, Dale and Saradamma 1981, Saxena and Koul 1987). The influence of certain oils and their constituents on the reproduction of some insect species and on morphological changes in other has also been discussed (Smet *et al.,* 1986). According to Laurent (1997), 63 essential oils isolated from Bolivia plants were tested on *Triatomal infestants* for ovicidal and larvicidal properties. This insect is responsible for transmission of Chaga's disease to humans in the region extending from the arid Perivian highlands to the very dry north eastern Brazilian regions, and the plains of Argentina. Three types of test were used; topical application on insects; nymphs on impregnated paper and eggs on impregnated paper. In all tests, the essential oils were used as ethanol solution with concentration of 2% and 20% (v: v)

(i) For insects test, 1Mi of each solution was applied directly over the abdomen of 10 fourth instar nymphs of *T. infestans*. After observing daily for a week, the nymphs were treated again with 5µI of the same solution. Two sets of controls were utilized. One control was treated with ethanol only, while the other was not treated. The effect of the application was observed for another week and compared with the controls.

(ii) For nymphs, 200µl of each ethanol solution of essential oil were deposited over filter paper disk which were dried at room temperature for five minutes and placed in Petri dishes, and then 5 fourth instar nymphs of T. *infestans* were introduced in each dish. Insect control groups were treated in the same way but dosed only with ethanol. The effect knock-down was observed daily for four days and compared with controls.

(iii) For ovicidal test, 10 eggs of T. *infestans* were introduced in each dish were the filter paper disks impregnated with a known volume of oil was deposited. The hatching of the larvae and the effect on them (knock-down or mortality) were observed everyday until the control eggs completed hatching.

The result of this study indicated the effect of 20 oils on nymphs and eggs when the impregnated paper tests were used. These tests proved to be most sensitive and were therefore chosen for studying the action of twelve terpenes present in those active oils. Essential oils are used as flavouring agents. Flavours are added to food to enhance their taste and aroma. Flavouring in vanilla, is isolated from vanilla beans and methyl salicylate, which has a characteristic minty taste and odour. Essential oils and their terpene constituents may be accepted natural alternative to synthetic skin penetration enhancers. They are characterized by their relatively low price and promising penetration enhancing activities. The mechanism of skin penetration enhancing activities of terpenes was postulated (Barry 1991, Higaki *et al.,* 2003) due to the popularity of these essential oils. Their toxicities are well documented (Opdyke 1974-1976), and found to be relatively low compared with most synthetic penetration enhancers. Terpenes such as menthol and cineole were employed as enhancers to improve the skin penetration of propanol, a β-block, which has a short biological half-life and is subjected to extensive hepatic first-class metabolism (Amnuaikit *et al.,* 2005). Cineone and menthol are reported to improve the skin permeation of hydrophilic drugs better than other terpenes (Narishetty and Panchagnula 2004). Menthol and limonene produce maximum permeation of

melatonin and fatty acids (Kanikkanna *et al.,* 2004). On the other hand, menthol and menthone failed to enhance the penetration of high molecular weight, lipophilic drugs such as pacilitaxe (Panchagnula *et al.,* 2004). The combination of two penetration enhancers of two different classes such as terpenes (e.g. cineole) and fatty acids (e.g. oleic acid), synergistically enhanced transdermal flux of zidovudine in addition to reducing lag time. On the other hand, combinations of menthol with oleic and linolenic acid did not enhance transdermal delivery (Thomas and Panchagnula 2003). The proper choice of terpene enhancer is dictated by lipophilicity or hydrophicity of the drug (El-Kattan *et al.,* 2001). Some essential oils themselves have been investigated as potential skin penetration enhancers. Basil essential oil showed an enhancing activity for accelerating transdermal delivery of indometthacin (Fang *et al.,* 2004). Also Niaouli essential oil showed a high activity for the permeation of estradiol through hairless mouse skin *in vitro* (Monti *et al.,* 2002).

CONCLUSION

The application of essential oil has spread evenly throughout the whole world as well as its analysis, which had led to the tremendous increase in the yield and quality of essential oil production. Also in aromatherapy and medicaments, disinfectants and insect repellent, all of which are directly or indirectly applied to human life to suit peoples desires and demand. The general usefulness of essential oil cannot be over emphasised as it is more beneficial than synthetic drugs.

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