KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

BIOPHYSICO-CHEMICAL STUDIES ON THE ESSENTIAL OILS AND HYDROSOLS FROM SOME PLANTS USED TRADITIONALLY AS MOSQUITO REPELLENT

BY

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Bsc. Hons (Chemistry)

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DECLARATION

I, Osei-Owusu Jonathan, do hereby declare that this thesis is a product of a research work I carried out towards the M.Phil degree except references to other people's work which have been duly acknowledged, and that neither the whole nor part of this thesis has been presented for another degree or programme elsewhere.

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This thesis is dedicated to my family and all my love ones for their support and motivation especially Rev. John Osei, Mrs. Martha Opoku and my daughter Gifty Osei-Owusu Ansah.

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ABSTRACT

Plant based repellents have been used for generations in traditional practice as protection measures against mosquitoes. Knowledge on traditional repellent plants is a valuable resource for the development of new natural products. For this reason some biophysicochemical data were obtained for the essential oils from some eight selected local plants known to be used traditionally to repel mosquitoes. The oils were hydrodistilled and gave average yields for *Citrus aurantifolia* (fresh peels 1.38%, dried peels 1.98%), *Ocimum gratissimum* (fresh leaves 1.16%, dried leaves 4.05%), *Cymbopogon citratus* (fresh leaves 0.53%, dried leaves 1.91%), *Cymbopogon nardus* (fresh leaves 1.09%, dried leaves 2.70%), *Citrus sinensis* (fresh peels 0.80%, dried peels 3.64%) and *C. odorata* (dried leaves 0.27%, fresh leaves 0.26%). Out of all the oils screened for their repellence ability against the tested mosquitoes, the most effective ones were *Cymbopogon nardus, Citrus sinensis* and *Azadirachta indica* with average repellence times of 120 min, 50 min and 50 min respectively. Antimicrobial potency of the plants hydrosols were also screened against five microorganisms. The hydrosols showed varied levels of inhibition. The hydrosol from fresh *Citrus aurantifolia* was the only hydrosol able to inhibit all the tested organisms with an inhibition zone of *Escherichia coli* (4mm), *Staphylococcus aureus* (5 mm), *Candida albicans* (2 mm), *Bacillus subtilis* (4mm) and *Escherichia faeculis* (1mm). Hydrosols from dried *C. nardus*, fresh C. *nardus*, dried *C. sinensis*, dried C. *odorata*, fresh *C. sinensis* and fresh *C. citrates* were not able to inhibit any of the tested organisms. Thin Layer Chromatography finger prints of the oils have also been established using three solvent systems.

Keywords: Essential oil, Mosquito repellence, Antimicrobial, Hydrosols, Thin Layer Chromatography

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

W H O	World Health Organisation
K.N.U.S.T	Kwame Nkrumah University of Science and Technology
RI	Refractive Index
R_F	Retention Factor
DEET	N-N-diethly-m-toluamide
GC -MS	Gas Chromatography Mass Spectroscopy
DMADP	Dimethylallyl diphosphate
IDP	Isopentenyl diphosphate
FDP	farnesyl diphosphate
GGDP	Geranyl diphosphate diphosphate
TLC	Thin Layer Chromatography
ppm	Part per million

CHAPTER ONE

INTRODUCTION

1.1 THE PROBLEM

Traditional Ghanaian Herbal Medicine contains multiple botanicals, each of which contains many compounds that may be relevant to the medicines putative activity. Therefore, analytical techniques that look at a suite of compounds, including their respective ratio, provide a more rational approach to the authentication and quality assessment of the herbal medicine. Despite the enormous advances made in health care, infectious diseases account for 25% of the mortality worldwide and 45% in low income countries. Moreover, the causative agents are also developing increasing resistance against many of the commonly used antibiotics and currently the costs of many drugs are not affordable for most people (Binder *et al*., 1999).

Malaria remains the most serious vector-borne disease affecting some 300 -500 million people and 1.4 to 2.6 million deaths annually throughout the world (Massebo et al., 2007). More than 40% of the world's populations live in malaria prone areas (Ghai et al., 200). Malaria is caused by single celled protozoan parasites called *Plasmodium* and transmitted to man through the anopheles mosquito. It is one of the major fatal diseases in the world, especially in the tropics and is endemic in some 102 countries with more than half of the world population at risk (Asase et al., 2005). Mosquitoes pose the greatest threat to public health because of their ability to act as vectors of pathogens causing malaria, yellow fever, encephalitis and filariasis (Service 2000). All of the *Plasmodium* species that cause human malaria are transmitted by mosquitoes of the genus *Anopheles*. The African malaria mosquito, *Anopheles gambiae sensu stricto* (*s.s.*), is an especially important vector because it is highly

anthropophilic and a very efficient carrier of the most potent malaria parasite, *Plasmodium falciparum.* Only female adult mosquitoes transmit the malaria parasite into the human body (Li et al. 2005). The discovery, development and use of synthetic insecticides have reduced the interest in plant origin products. However, due to the high environmental and health related problems with the synthetic insecticides there has been an increasing concern in developing products that are environmentally safe and biodegradable. Plant-based repellents have been used for generations in traditional practice as a personal protection measure against host-seeking mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies is a valuable resource for the development of new natural products. Recently, commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers, as these are commonly perceived as "safe" in comparison to long-established synthetic repellents although this is sometimes a misconception (Maia et al., 2011).

Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Essential oils are found in glandular hairs or secretary cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants (Koul et al., 2008).

Essential oils are usually obtained via steam distillation of aromatic plants, specifically those used as fragrances and flavourings in the perfume and food industries, respectively, and more recently for aromatherapy and as herbal medicines. Essential oil constituents are primarily lipophilic compounds that act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect and pests.

Insecticidal properties of several monoterpenoids to the housefly, red flour beetle and southern corn root-worm have been reported (Koul et al., 2008). Insecticidal activities of different plant essential oils have been reported against mosquito species (Massebo at el., 2007).

1.2 THE NEED FOR THE RESEARCH

The tropical rainforests provide a lot of medicinal plants with potential medicinal properties but their phytochemicals have not fully been exploited. The potential medicinal importance is due not only to the species richness of the tropical flora but also the diversity of pathogens, parasites and herbivores against which the plants provide defensive mechanism. Many of the defense chemicals secreted can be used to treat human and prevent or eradicate other organisms (Falodun et al., 2009). Plants and plant-derived substances have been used to repel or kill mosquitoes and other domestic pests and insects for a long time before the advent of synthetic chemicals (Curtis et al., 1989). In rural African communities, thermal expulsion and direct burning of aromatic plants before sleeping continues to play a very important role in house protection against mosquito vector of dangerous diseases such as malaria and lymphatic filariasis (Norbert et al., 2003). Medicinal and aromatic plants are accessible, affordable and culturally appropriate source of primary health care for more than 80% of world's population. Plant secondary metabolites have been a fertile area of chemical investigation (Abbaszadeh et al., 2009). Essential oil of a large number of plants has been found to have repellent properties and some have formed the basis of commercial repellent formulation (Curtis et al., 1989). The identification and eventual use of local plants in control of mosquitoes may be very valuable for a developing country like Ghana since these plants are readily available and can be

obtained in commercial quantities. Thus, the research seeks to evaluate the chemical composition and mosquito's repellency of the essential oils from some selected local plants. Since the hydrosols are the by-product of the distillation and they normally contain traces of essential oils and several water soluble component of essential oils, their antimicrobial potencies would be investigated to serve as adjuvant for herbal medicine industrial preparations.

1.3 AIMS AND OBJECTIVES

The work has two main aims:

Firstly, it seeks to obtain analytical data on the essential oils of the plants under study and secondly, to investigate the essential oil potential to repel mosquitoes and the probable antimicrobial properties of the hydrosols thereof.

Specific objectives of the study are:

- Determination of the essential oil content of each plant under study.
- Obtain Thin Layer Chromatographic (TLC) Finger print or profile for each essential oil as a way of establishing the authenticity of the oil.
- Screening the essential oil for their mosquito's repellency.
- Test antimicrobial potencies of the hydrosols or aromatic water from the hydrodistillation.

CHAPTER TWO

LITERATURE REVIEW

2.1 ESSENTIAL OILS

Essential oils are volatile, natural, complex compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites. They are obtained from plants by steam or hydrodistillation (Ravandeh et al., 2011). They are considered as a concentrated hydrophobic liquid containing volatile aroma compounds from some plants (Abbaszedeh et al., 2009).The total essential oil content of plants is generally very low (<1%). However many therapeutic oils are so potent they are still active in herbal preparations. Upon isolation, these oils are highly concentrated, and are widely used in this form by aromatherapists, - mainly for external application but sometimes for internal consumption in diluted forms (Pengelly, 2003). The aroma of many naturally occurring plants is due to the volatile oil present in them. These essential oils make up the characteristic odor of many plants, among them eucalyptus, citronella, garlic, orange, roses, peppermint and many more (Hauser, 2008). The essential oils and extracts of many plants species have become popular in recent years and attempt to characterize their bioactive principle(s) have recently gained momentum in many pharmaceutical processing applications (Alim et al., 2009). Essential oils have been reported to be useful in aromatherapy, food preservation and fragrance industries (Afolayan et al., 2009). The oils of certain plants have been shown to posses antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Mmbengwa et al., 2008). The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents.

The volatile oils obtained from *Cyclotrichium niveum (Boiss*) showed a remarkable

antibacterial activity against *Klebsiella pneumonia*, *Staphylococcus aureus* and also as a strong antifungal activity against *Candida albicans* (Alim et al., 2009). In traditional African communities, repellent volatiles from certain plants generated by direct burning or by thermal expulsion have played an important role in protecting households against vectors of malaria and other diseases. The essential oil from the leaves of *Suregada zanzibariensis* was found to be repellent against the mosquito *Anopheles gambiae* (Innocent et al., 2010). The oils which have been reported as potential sources of insect repellents includes citronella, cedar, verbena, pine, pennyroyal, geranium, lavender, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint (Trongtokit et al., 2005). Sharma et al. (1993) reported the effectiveness of neem oil as method of protection from mosquitoes which is safer compared to synthetic chemicals, Xie et al. (1995) also reported that neem extracts were toxic to *Sitophilus oryzae*, *Cryptolestes ferruginous* and *Tribolium castaneum.*

The volatile oils occur in the most varied parts of the plant anatomy, in some cases being found throughout the various organs and others being restricted to one special portion of the plant. In conifers, much volatile oil is found in most of the tree, whereas in rose, the oil is confined to the flower (Parry, 1922). In plants, essential oil has a function which regulates the rate of transpiration. Moisture which is saturated with oil has different heat conductivity from that of moisture alone, so that a plant which gives off much perfume may be protected during the day from too great transpiration and during the night from too great reduction of temperature (Parry, 1922). Climate, genetic material, geographical and environmental conditions change the essential oil percentage, composition and content in different plant species (Mazroa et al., 2010). Essential oils are produced by different types of plants to serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. These oils

can be extracted from different parts of the plant and are made up of different chemical compounds (Gundidza et al., 2008). Essential oils do not as a group needs to have any specific chemical properties in common (Abbaszadeh et al., 2009). Typically, these oils are liquid at room temperature and get easily transformed from a liquid to a gaseous state at room or slightly higher temperature without undergoing decomposition. The amount of essential oil found in most plants is 1 to 2%, but can contain amounts ranging from 0.01 to 10% (Koul et al., 2008). Essential oils differ from fixed oils. Essential oils that can be distilled from their natural sources, do not consist of glyceryl esters of fatty acids, do not leave a permanent oily spot on paper and are unsaponifiable with alkalies. Unlike fixed oils, essential oils do not become rancid. Instead, they get oxidized and resinified on exposure to light and air (Joy et al., 2001). Essential oils are colourless or lightly coloured and free flowing when they are fresh. The quality of the oil spoils during prolonged storage. This deterioration in quality of the oil is attributed to a number of chemical reactions such as; oxidation, resinification, polymerization and interaction of functional groups. These processes are activated by heat, presence of oxygen or air and moisture. These reactions are considered to be catalyzed by light in some cases and possibly by metals (Joy et al., 2001). To prevent this, they are stored in a cool and dry place in tightly stopper amber glass bottles. Exclusion of air by completely filling the container with oil prolongs its storage life.

2.2 BIOSYTHESIS AND THE MAJOR CONSTITUENT OF ESSENTIAL OIL

Essential oils are made up of many chemical ingredients of which some play a major role and others a minor role. The chemical compositions of essential oil are organic compounds due to their molecular structures which are based on carbon atoms held together by hydrogen atoms. Oxygen atoms and sometimes nitrogen and sulphur atoms may also be present. Most essential oils comprises of monoterpenes – compounds that contain 10 carbon atoms often arranged in a ring or in acyclic form, as well as sesquiterpenes which are hydrocarbons comprising of 15 carbon atoms. Higher terpenes may also be present as minor constituents. The most predominant groups are cyclic compounds with saturated or unsaturated hexacyclic or an aromatic system. Bicyclic (1, 8-cineole) and acyclic (linalool, citronellal) examples also make the components of essential oils (Koul et al., 2008). Terpenoids have been reported to be the major chemical composition of most essential oils in some plants (Dongmo et at., 2009). Terpenoids are enzymatically synthesized from acetyl CoA and pyruvate provided by the carbohydrate pools in plastids and the cytoplasm. Terpenoids constitute one of the most diverse families of natural products, with over 40 000 different structures of terpenoids discovered so far. Many of the terpenoids produced are non-volatile and are involved in important plant processes such as membrane structure (sterols), photosynthesis (chlorophyll side chains, carotenoids), redox chemistry (8uinines) and growth regulation (gibberellins, abscisic acid, brassinosteroids). The volatile terpenoids – hemiterpenoids (C_5), monoterpenoids (C_{10}), sesquiterpenoids (C_{15}) and some diterpenoids (C_{20}) are involved in interactions between plants and insect herbivores or pollinators and are also implicated in general defense or stress responses (Schwab et al., 2008). Terpenoids are derived from a common building unit's isopentenyl diphosphate (IDP) and its isomer dimethylallyl

diphosphate (DMADP). In plants, both IDP and DMADP are synthesized via two parallel pathways, the mevalonate (MVA) pathway, which is active in the cytosol, and the methylerythritol 4- phosphate (MEP) pathway, which is active in the plastids (Schwab et al., 2008, Rohdich et al., 2000)). The direct precursors of terpenoids, linear geranyl diphosphate (GDP, diphosphate (GGDP, C_{20}), are produced by the activities of C_{10}), farnesyl diphosphate (FDP, C_{15}) and geranylgeranylthree prenyl transferases. Terpene synthases are the primary enzymes responsible for catalyzing the formation of hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}) or diterpenes (C_{20}) from the substrates DMADP, GDP, FDP or GGDP, respectively (Schwab et al., 2008). Biosynthesis involves conversion of the various prenyl diphosphates DMADP, GDP, FDP and GGDP to hemiterpenes, monoterpenes, sesquiterpenes and diterpenes, respectively, by the large family of the terpene synthases.

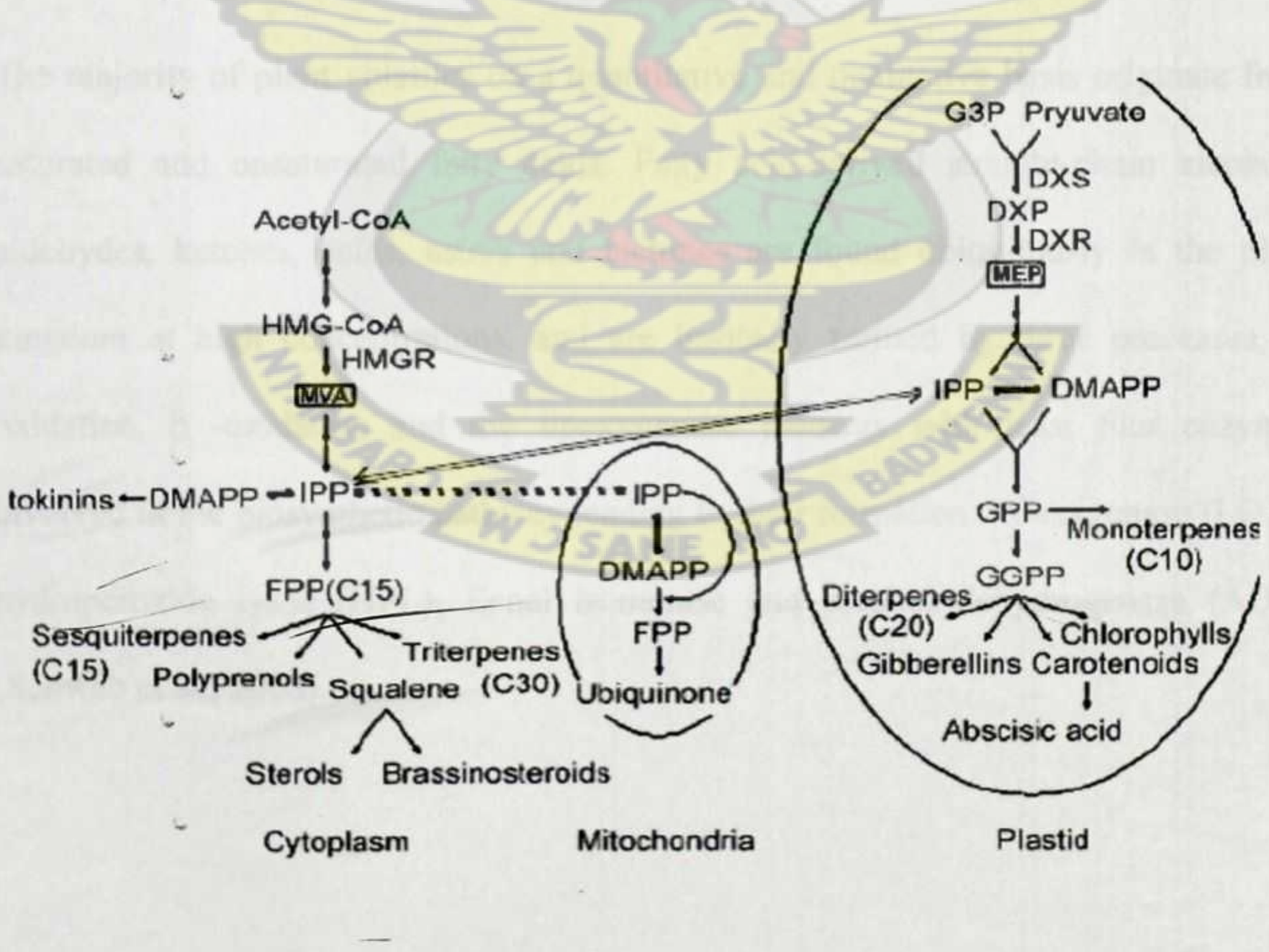


Fig 2.1(a) Biosynthesis of terpeniods from acetyl CoA

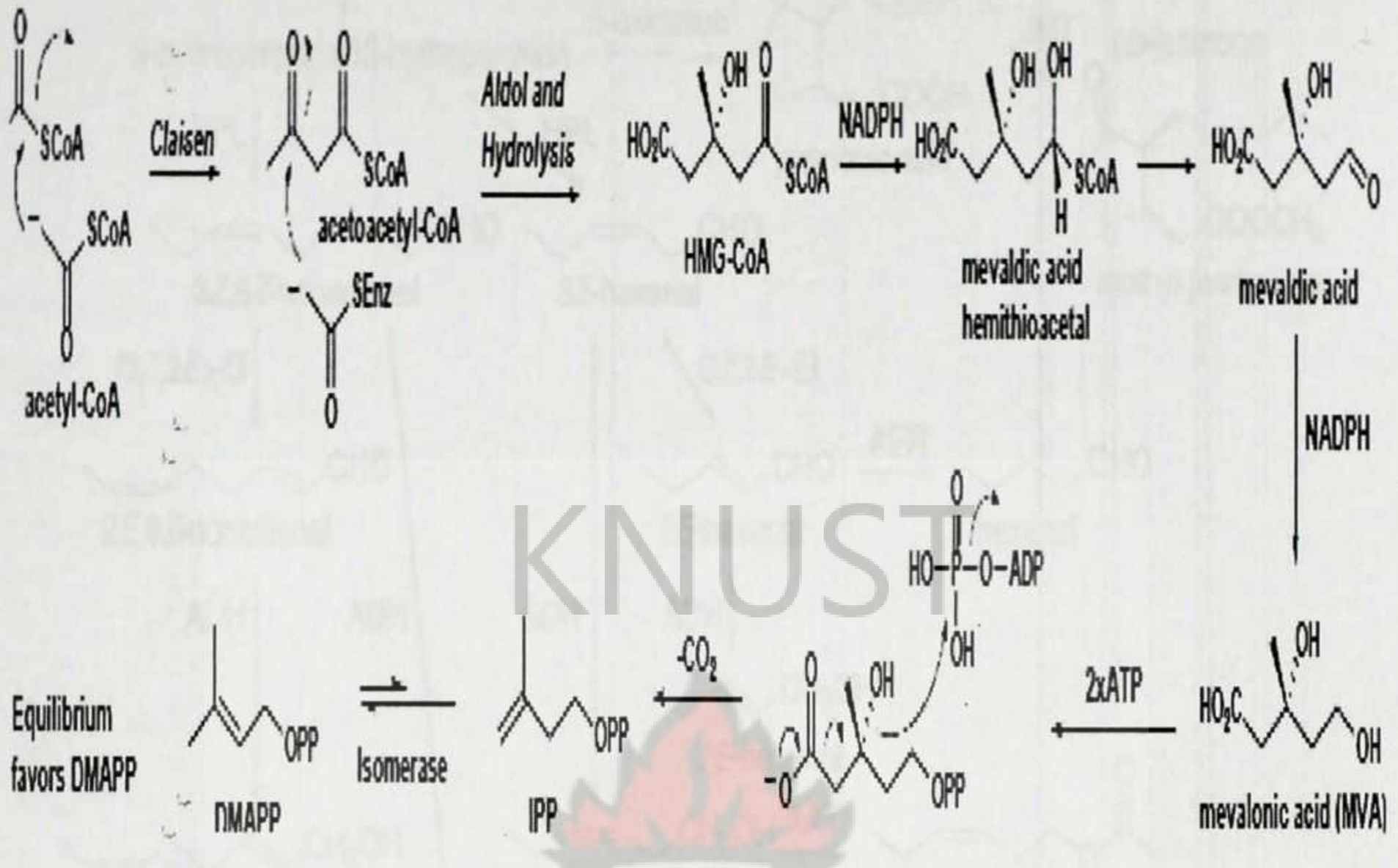


Fig 2.1 (b) mevalonate (MVA) pathways for terpeniods biosynthesis

The majority of plant volatiles on a quantitative and qualitative basis originate from saturated and unsaturated fatty acids. Fatty acid-derived straight-chain alcohols, aldehydes, ketones, acids, esters and lactones are found ubiquitously in the plant kingdom at high concentrations, and are basically formed by three processes, a-oxidation, β -oxidation and the lipoxygenase pathway with least four enzymes involved in the biosynthetic pathway leading to their formation: lipoxygenase (LOX), hydroperoxide lyase (HPL), Eenal isomerase and alcohol dehydrogenase (ADH) (Schwab et al., 2008).

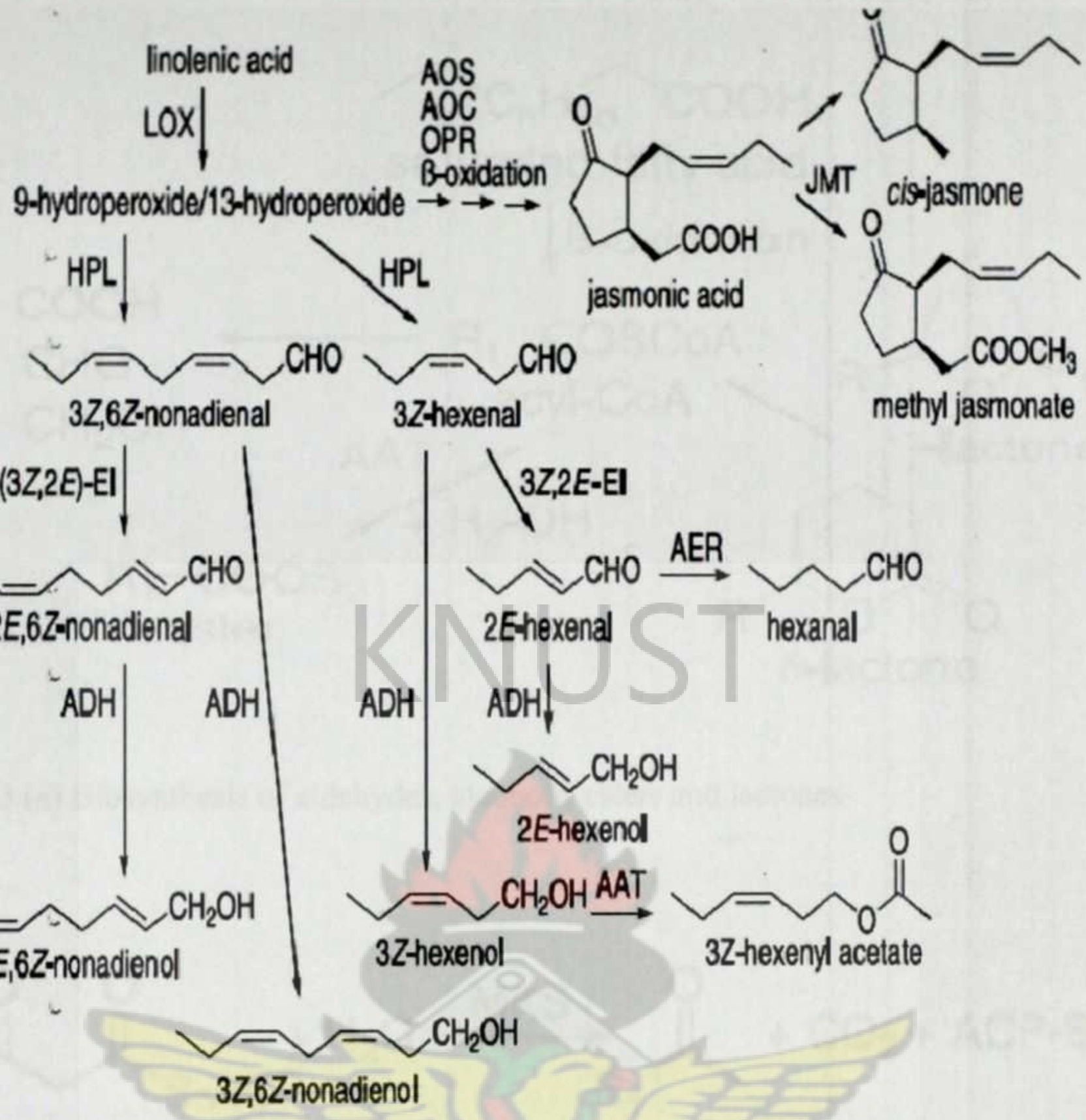


Fig 2.2 Biosynthesis from fatty acids

The reaction below shows the biosynthesis of (a) short-chain acids, aldehydes, alcohols, esters and lactones, and (b) methylketones using the enzyme AAT, alcohol acyl CoA transferase; MKS, methylketone synthase; ACP, acyl carrier protein (Schwab et al., 2008). Aldehydes and alcohols derived from the degradation of branched-chain and aromatic amino acids or methionines constitute a class of highly abundant plant volatiles (Schwab et al., 2008)

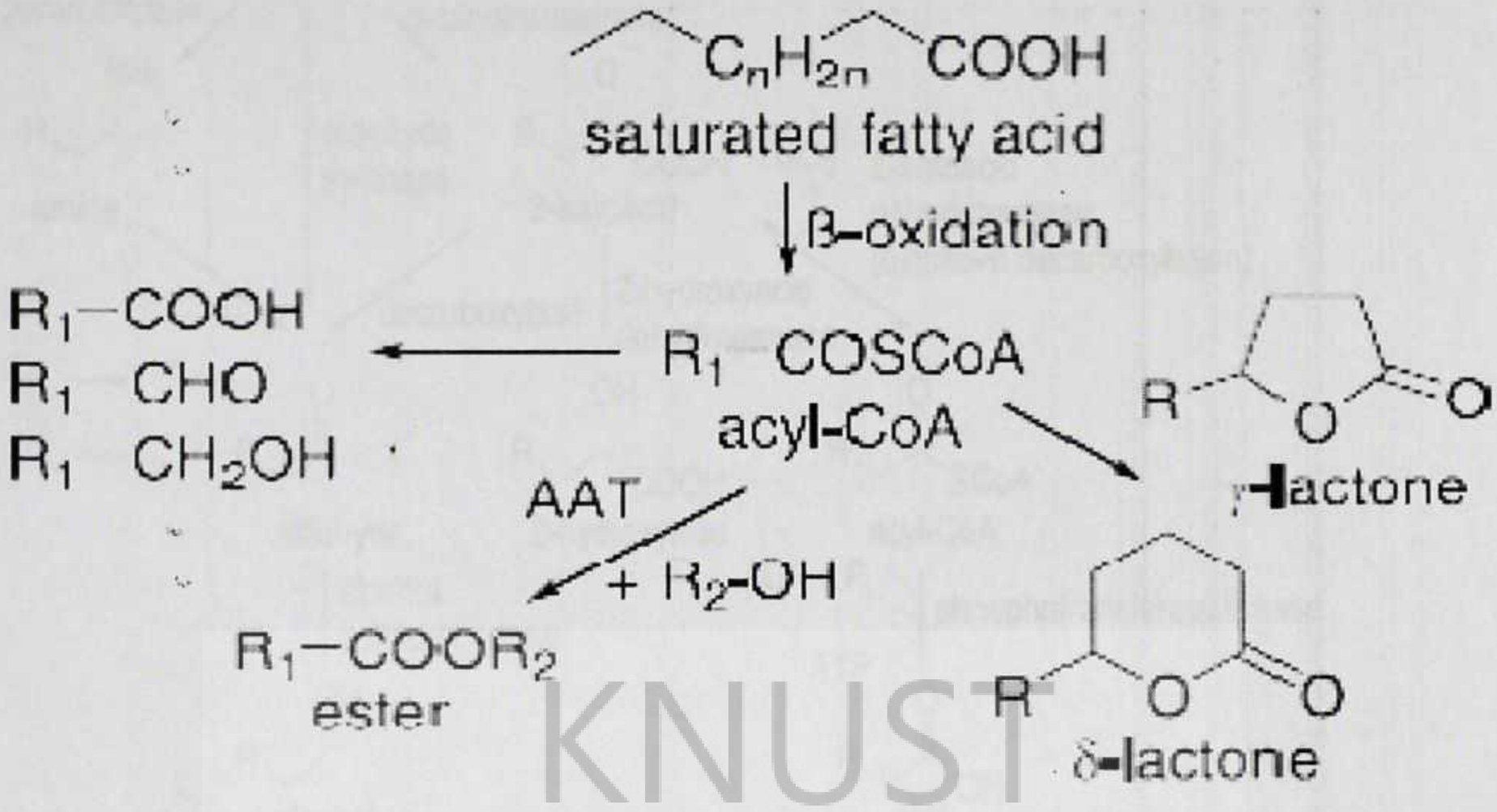


Fig 2.3 (a) Biosynthesis of aldehydes, alcohols, esters and lactones

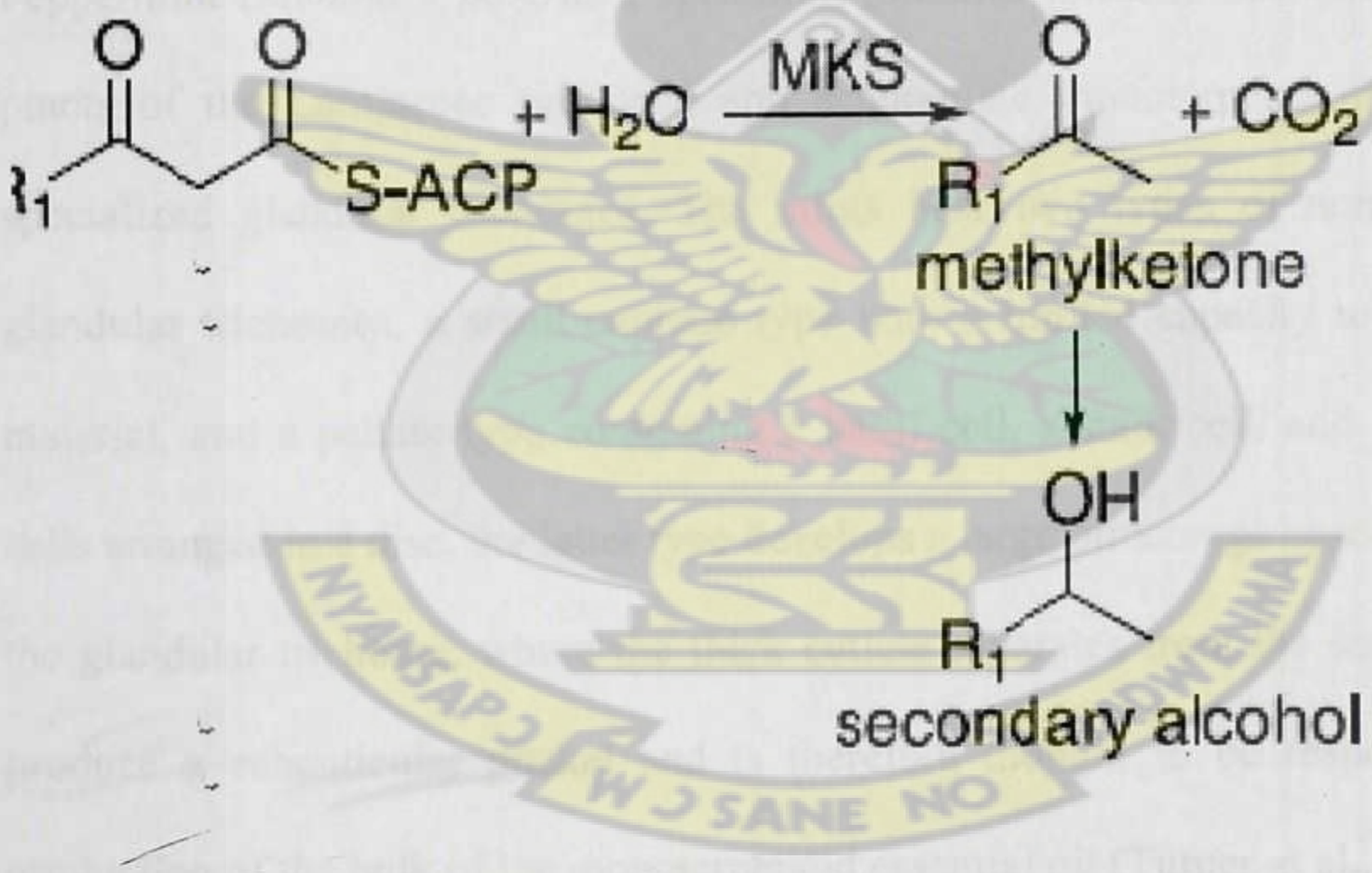


Fig 2.3 (b) Biosynthesis of methylketones

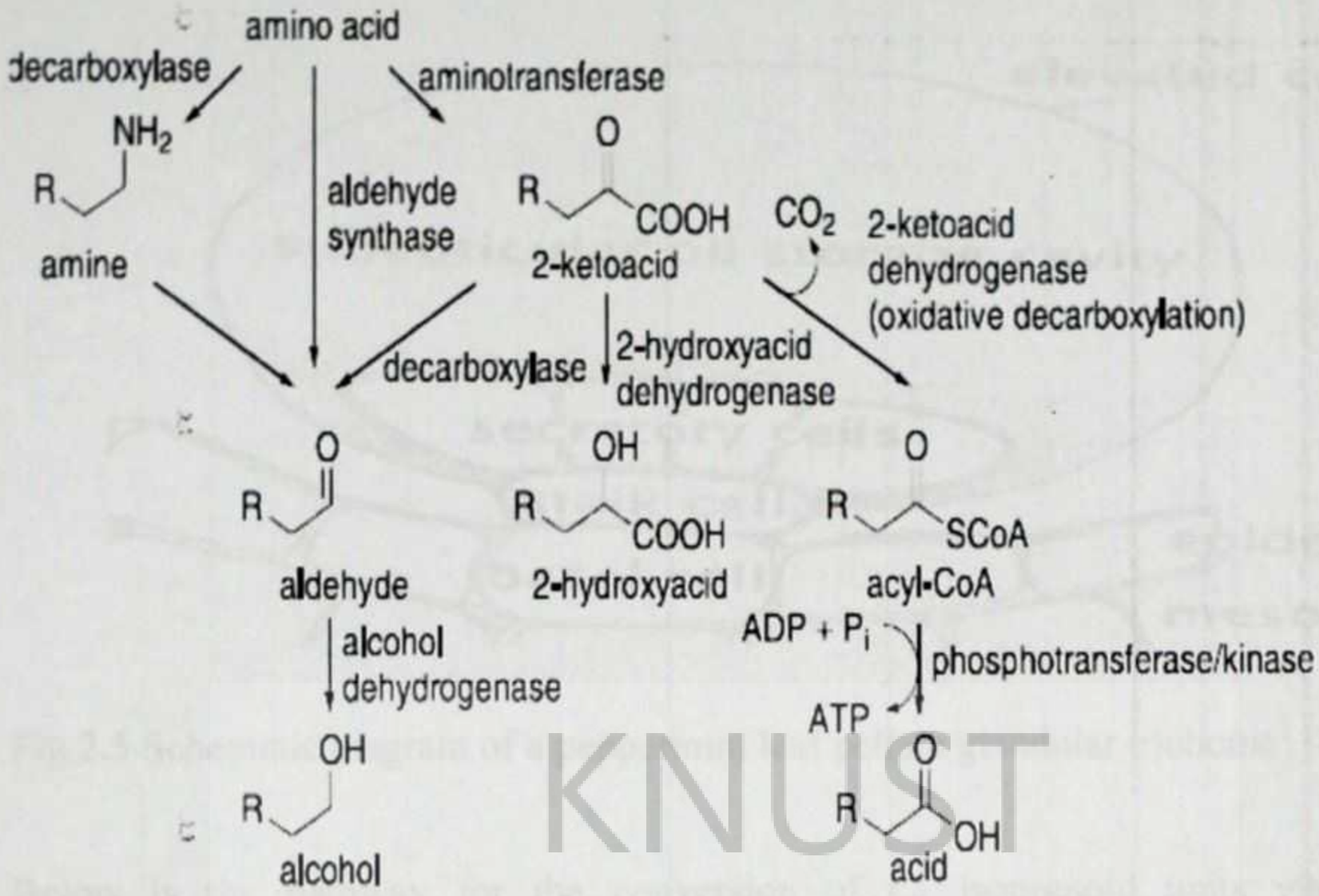


Fig 2.4 Biosynthesis of alcohol and Aldehydes from amino acids

Peppermint (*Mentha* 3 *piperita*), spearmint (*Mentha spicata*), and other essential oil plants of the Lamiaceae produced and accumulate monoterpenes in anatomically specialized glandular trichomes. The mints bear two types of nonphotosynthetic glandular trichomes, a small capitate type with a limited capacity to store secreted material, and a peltate type containing a basal cell, a stalk cell, and eight secretory cells arranged in a disc, the latter type develops a large oil-storage space at the apex of the glandular trichome, where the thick cuticle separates from the secretory cells to produce a subcuticular pocket and is therefore thought to be responsible for the production of the bulk of the monoterpenoid essential oil (Turner et al., 1999).

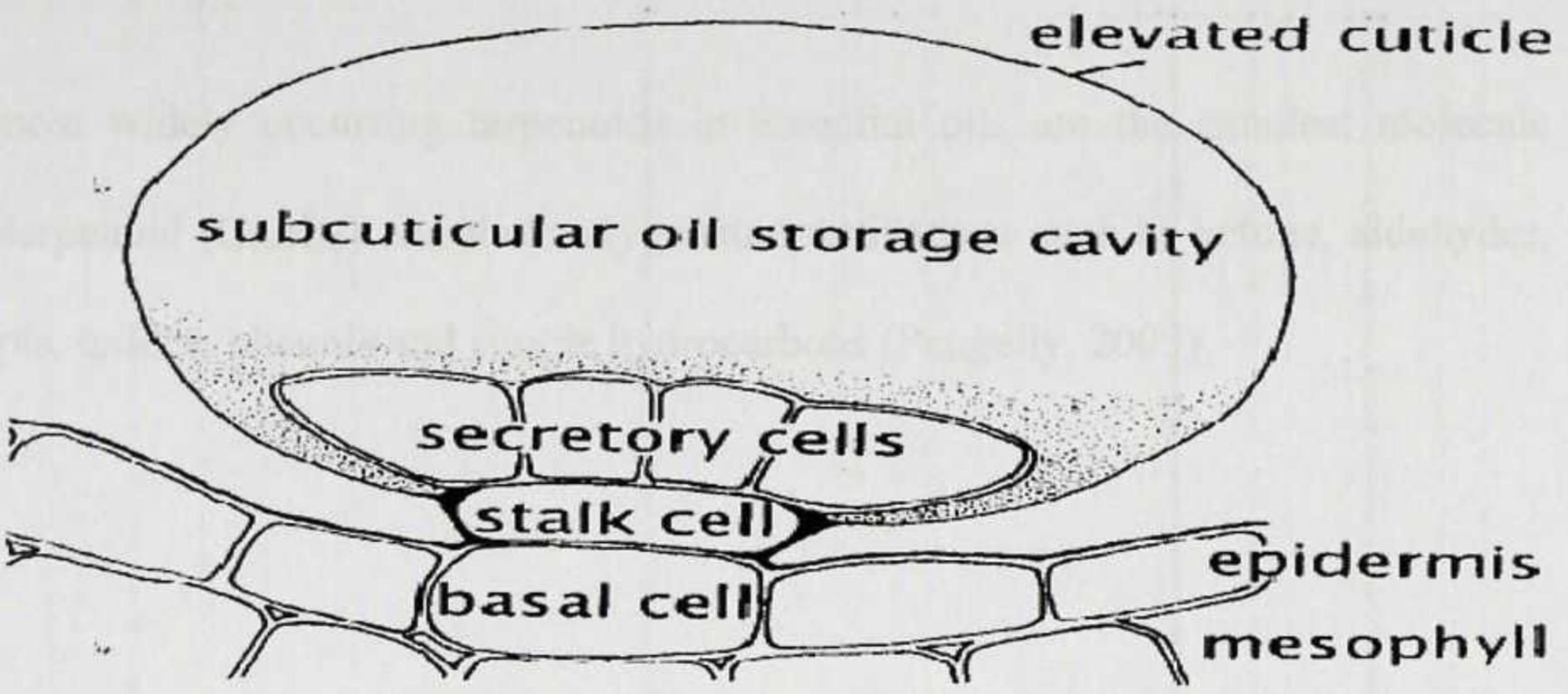


Fig 2.5 Schematic diagram of a peppermint leaf peltate glandular trichome

Below is the pathway for the conversion of C_5 isoprenoid units via geranyl diphosphate and limonene to the principal essential oil components (2)-menthol (peppermint) and (2)- carvone (spearmint). The responsible enzymes are: isopentenyl diphosphate isomerase (1); geranyl diphosphate synthase (2); 4S-limonene synthase (3); 4S-limonene-3-hydroxylase (4); and 4S-limonene-6-hydroxylase (5).

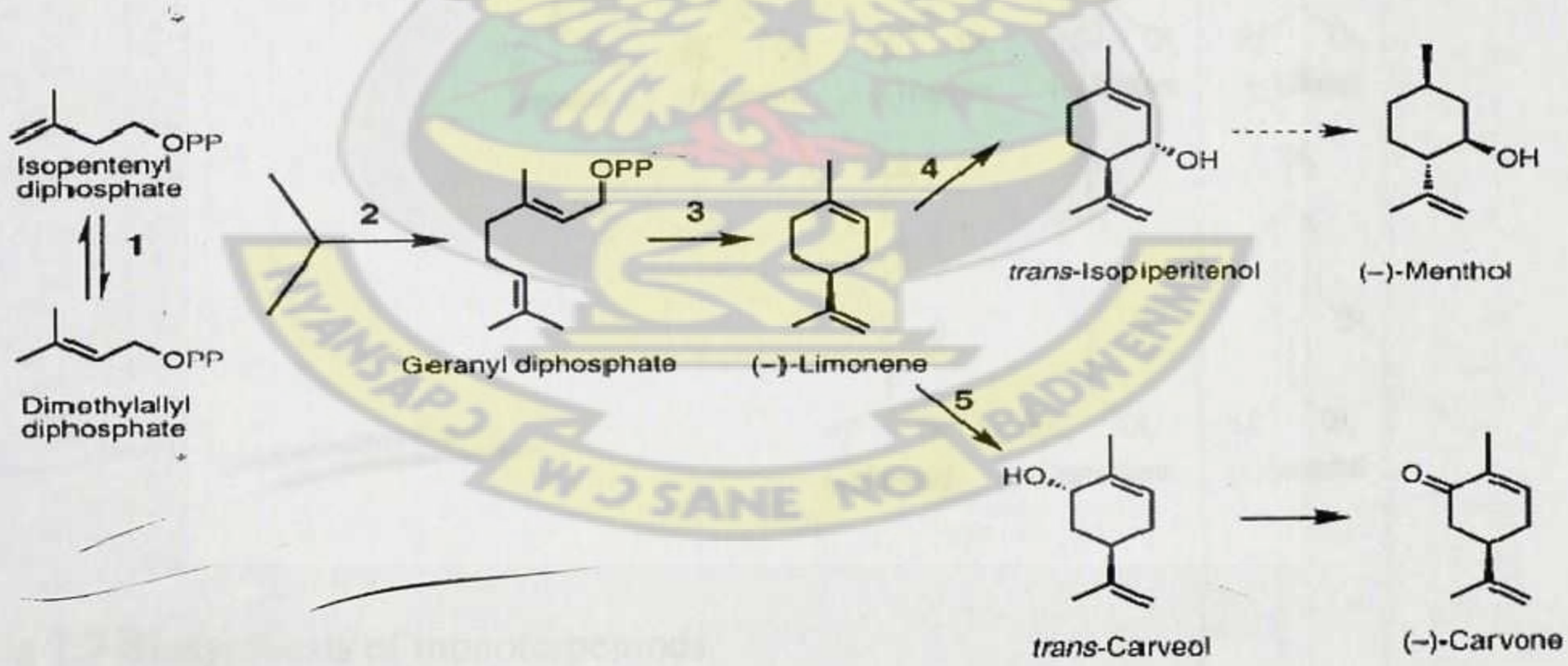


Fig 2.6 Pathway for the conversion of C_5 isoprenoid units via geranyl diphosphate

2.2.1 MONOTERPENOIDS

The most widely occurring terpenoids in essential oils are the smallest molecule monoterpenoid ($C_{10}H_{16}$) and their oxygenated derivatives such as ketone, aldehydes, alcohols, oxides, phenols and simple hydrocarbons (Pengelly, 2003).

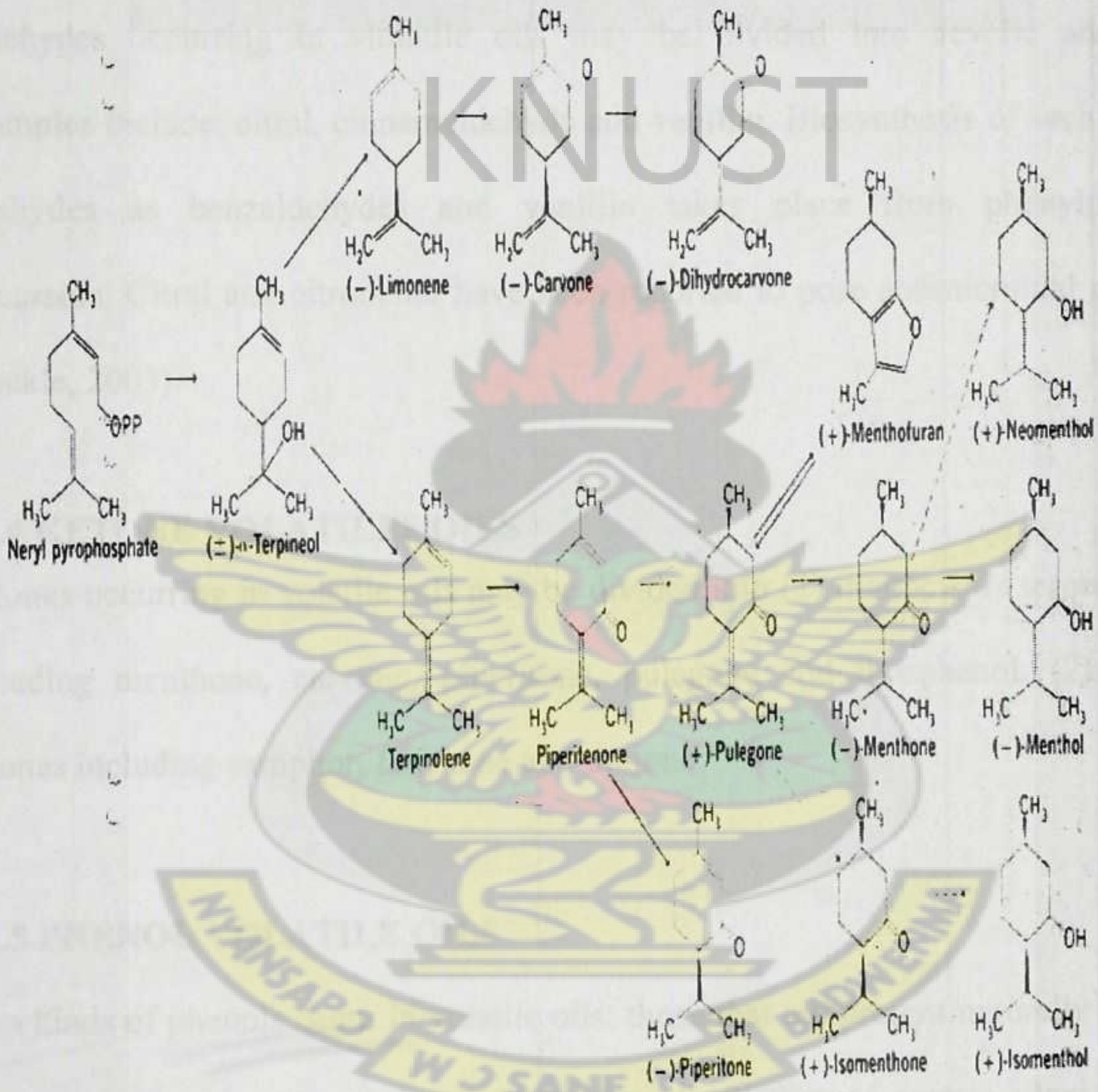


Fig 2.7 Biosynthesis of monoterpeniods

2.2.2 ALCOHOLS VOLATILE OIL

Alcohols found in volatile oils may be classified into acyclic, monocyclic and dicyclic alcohols. Methyl, ethyl, isobutyl, isoamyl, hexyl and higher aliphatic alcohols occur in volatile oils but because they are soluble in water, they are washed away in the process of steam distillation. An acyclic alcohol includes geraniol, linanool and citronellol. Monocyclic alcohols include menthol and terpineol.

2.2.3 ALDEHYDE VOLATILE OILS

Aldehydes occurring in vlolatile oils may be divided into acyclic and cyclic. Examples include; citral, cinnamaldehyde and vanillin. Biosynthesis of such aromatic aldehydes as benzaldehydes and vanillin takes place from phenylpropanoid precursors. Citral and citronellal have been reported to pose antimicrobial properties (Buckle, 2003).

2.2.4 KETONE VOLATILES OILS

Ketones occurring in volatile oils may be divided into (1) monocyclic terpene ketone including menthone, carvone, piperitone, pulegone and diosphenol. (2) Dicyclic ketones including camphor, fenchone and thujone.

2.2.5 PHENOL VOLATILE OILS

Two kinds of phenols occur in volatile oils: those that are present naturally and those that are produced as a result of destructive distillation of certain plant products. Eugenol, thymol and carvacrol are the most important phenols occurring in essential oils. Most phenols have very strong antibacterial properties (Buckle, 2003).

2.2.6 PHENOL ETHER VOLATILES

Anethole, safroles are some of phenol ethers commonly occurring in volatile oils. Studies of anthole biosynthesis in *foeniculum vulgare* revealed that formation take place from phenylalanine.

2.2.7 ESTER VOLATILE OILS

A wide variety of esters occur in volatile oils. The most common are the acetates of terpineol, borneol and geraniol. Terpene esters are generally formed from the respective alcohols by reaction with aliphatic acid moieties commonly acetic acids. Esters in essential oils and its hydrosol have been reported to posses antispasmodic, antifungal and calming properties (Buckle, 2003).

2.2.8 LACTONES

Lactones are present in most expressed oils and their percentage may be low but plays an important role as expectorants and mucolytics. Lactones have the same potential neurotoxic effect as ketones. Alantolactones is present in elecampane and is used to treat purulent bronchitis. Other examples of lactones found in essential oil include isolantolactone found in sweet inule, nepetalactone found in catnip and bicyclic lactone found in celery root (Buckle, 2000).

2.2.9 COUMARINS

Coumarin found in essential oils include visnagin, khellin, dicoumarol and Furanocoumarin present in citrus peel oils. Coumarins may be present in small amounts in essential oil but very potent. Coumarins have been reported to be a strong vasodilators, bronschodilator and antitumoral in vitro (Buckle, 2000).

2.2.10 PHENYLPROPANOIDS (PPs)

These compounds contain a benzene ring structure with an attached propane side chain. The most common precursor is cinnamic acid, a derivative of the shikimic acid pathway. They include some aldehydes, phenols and phenolic ethers. Examples of these molecules include Elemicin, Safrol, Eugenol and anethol. PPs belong to a large class of plant phenols produced through shikimic acid pathway. The synthesis of PPs has a common initial step deamination of phenylalanine to cinnamic acid catalyzed by phenylalanine ammonia lyase. Many of plant-derived phenolic compounds (flavonoids, isoflavonoids, coumarines, and lignans) are secondary products of PPs (Korkina, 2007). Phenylpropanoids (PPs) belong to the largest group of secondary metabolites produced by plants, mainly, in response to biotic or abiotic stresses such as infections, wounding, UV irradiation, and exposure to ozone, pollutants, and other hostile environmental conditions. It is thought that, the molecular basis for the protective action of phenylpropanoids in plants is their antioxidant and free radical scavenging properties. These numerous phenolic compounds are major biologically active components of human diet, spices, aromas, wines, beer, essential oils, propolis, and traditional medicine (Korkina, 2007).

The major constituents identified in the essential oil of the fruit *Piper guineense* were found to be β –pinene, β -caryophyllen, bicyclogermacrene, germacrene and Safrol (Oyedeji et al., 2005). Jazet et al. (2008) determined that the oils of *C. rigidus* and *C. citrinus* were dominated by the presence of 1, 8-cineole (79.1% and 73.8% respectively). Tenore et al. (2010) reported that a total of 67 compounds, representing 93.6% of the essential oil of aerial parts of *Salvia lanigera Poir.* (Lamiaceae) were identified and the major components were thymol (12.1%), hexadecanoic acid (6.0%), carvacrol and thujone (5.7%). Essential oils of aerial parts of *Hyptis suaveolens* (L)

Poit have been reported to be dominated by sabinene, β-caryophyllene, benzyl benzoate, 1, 8-cineole, germacrene, palustrol and a diterpene hydrocarbon (Tonzibo et al., 2009). Eugenol from cloves, *Eugenia cryophyllus*; 1,8- cineole from *Eucalyptus globules*; citronellal from lemon grass, *Cymbopogon nardus*; pulegone from *Mentha pulegium*, and thymol and carvacrol from *Thymus vulgaris* are among the most active constituents against insects (Koul et al., 2008)

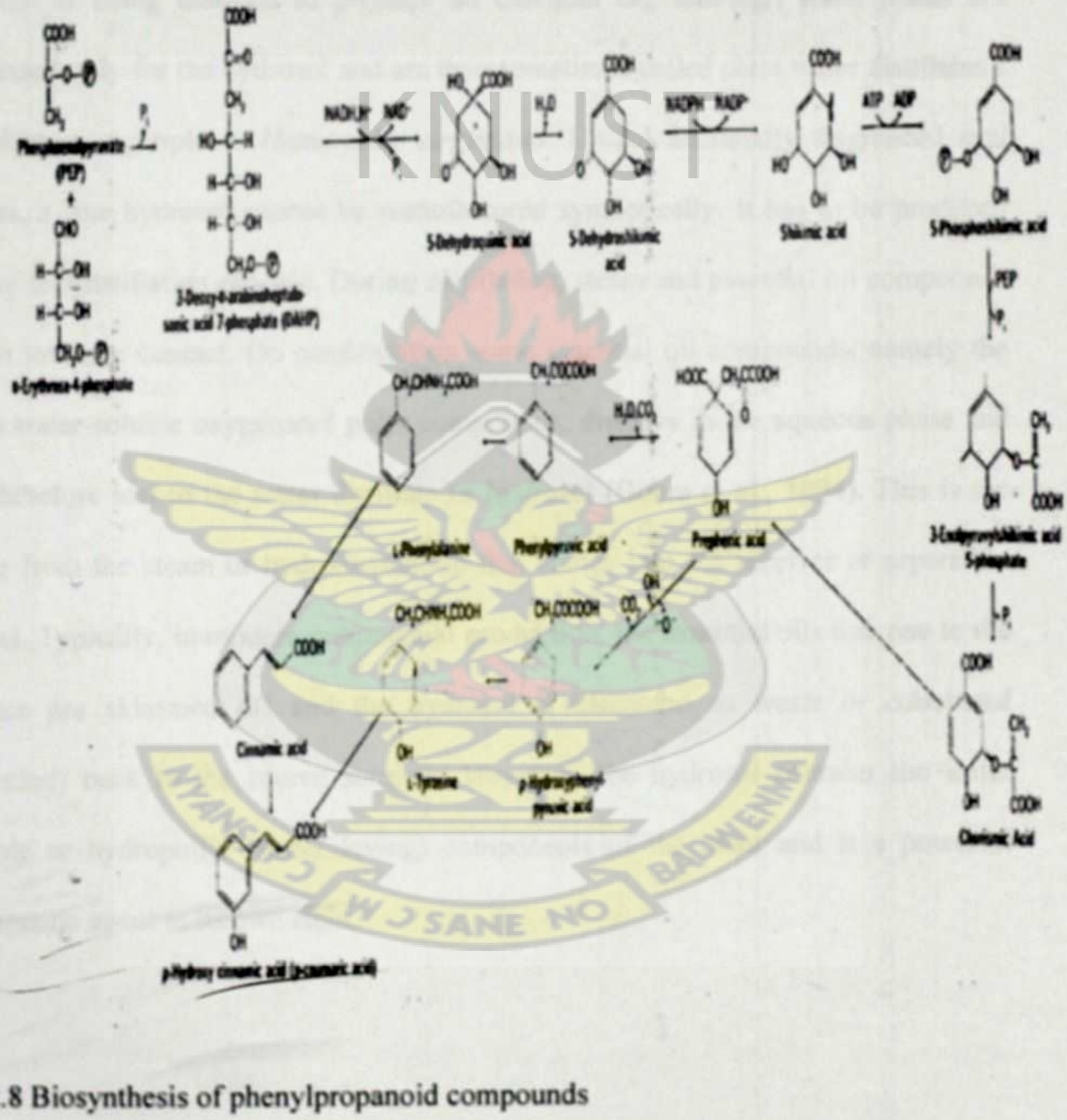


Fig 2.8 Biosynthesis of phenylpropanoid compounds

2.3 HYDROSOLS

Hydrosols, also known as floral water, distillate water or aromatic water, are the co-products or the by-products of hydro and steam distillation of plant material. Hydrosols are quite complex mixtures containing traces of the essential oils and, of course, several water- soluble components. Also known as hydrolats (a word derived from French), these are the products of the distillation of plant material. Usually the material is being distilled to produce an essential oil, although some plants are processed only for the hydrosol and are then sometimes called plant water distillates a well-known example is *Hamamelis virginiana*. Unlike artificially fragranced oral waters, a true hydrosol cannot be manufactured synthetically. It has to be produced during the distillation process. During distillation, steam and essential oil compounds are in intimate contact. On condensation some essential oil compounds, namely the more water-soluble oxygenated polar compounds, dissolve in the aqueous phase and are therefore lost to the water distillate or hydrosol (Bohra et al., 1994). This is the water from the steam or hydrodistillation that comes into the receiver or separation funnel. Typically, in modern commercial production, the essential oils that rise to the surface are skimmed off and the hydrosol is discarded as waste or cohobated (recycled) back to the source solution. However, the hydrosol contains the water soluble or hydrophilic (water loving) components of the plant and is a powerful therapeutic agent in its own right.

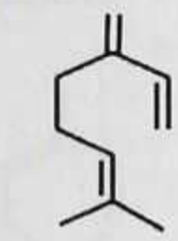
2.3.1 COMPOSITION OF HYDROSOLS

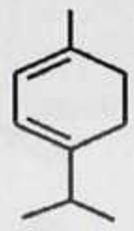
It would be incorrect to assume the same properties for hydrosols and their related essential oils, as their chemical properties are distinctly different. For example, the primary oil of geranium (Pelargonium ssp.) has been found to be richer in hydrocarbons compared to the distillate, which contains greater concentrations of the oxygenated compounds linalol, citronellol and geraniol (Rao et al., 1999). Hydrosols are either neutral or slightly acid and the pH greatly influences the therapeutic effects; the acidic nature of *Cistus ladaniferus* (rock rose) reputedly causes the constriction of tissues (an astringent action) while *Lavandula angustifolia* (lavender), with a pH close to neutral, lacks this effect (Catty, 2001). The low levels of terpene hydrocarbons means they are very well tolerated on the skin.

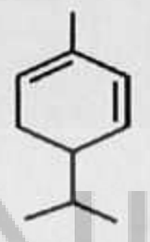
The presence of some components of essential oils gives the hydrosol its scent. Hydrosols are, therefore, quite fragrant, strongly flavoured and have a pH of 4.5 to 5.5 (Schorr, 2004; Paolini et al., 2008). During hydrodistillition the herbal material is exposed to temperatures close to 100°C, which can lead to changes in thermolabile components. Prolonged heating in contact with water can lead to hydrolysis of esters, polymerisation of aldehydes, or decomposition of other components. Hydrosols are known to contain water soluble components and micronized droplets of essential oil molecules (Suzanne, 2001) dissolved in the hydrosol solution. This indicates that the chemical composition of spice hydrosols differ from those of pure essential oils. Few reports on the volatile component analysis in hydrosols and respective pure essential oils revealed the presence of extremely low concentration of the major volatile component in hydrosols (Inouye et al., 2008). Volatile chemical group primarily hydrophilic acidic constituents are reported to occur in hydrosols. Essential oils of aromatic herbs and spices containing phenolic, aldehydes, ketones and alcohols

components have been reported to show stronger antimicrobial effect than the hydrocarbon terpenes (Vaghasiya *et al.*, 2011). Basil, cardamom, clove, thyme and cinnamon have been reported to contain such oxygenated constituents within their hydrosol (Inouye *et al.*, 2008).

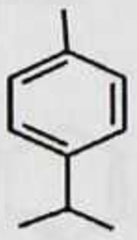
2.4 STRUCTURES OF SOME TERPENOIDS


Myrcene

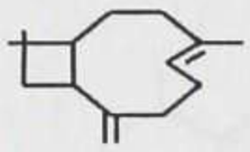

α-Terpinene

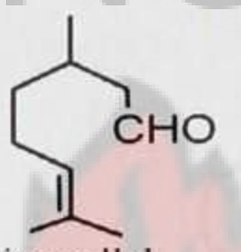

α -Phellandrene


Limonene

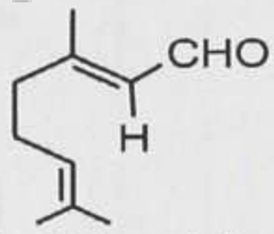

p- Cymene


α-Pinene

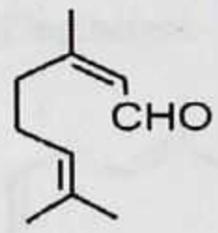

Caryophyllene



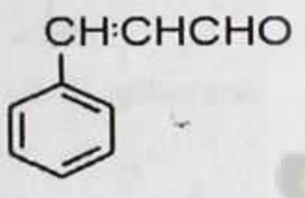
Citronellal



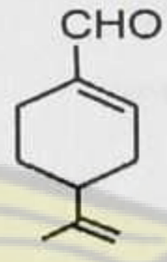
Citral (Geranial)



Citral (Neral)



Cinnamaldehyde



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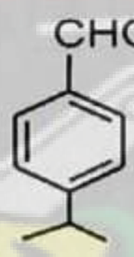
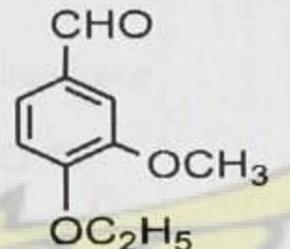
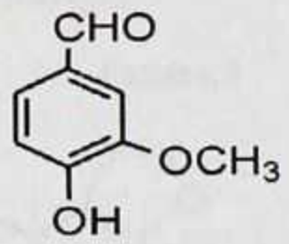


Cuminaldehyde



Ethyl vanillin



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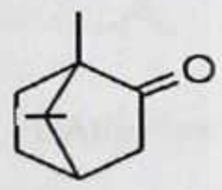
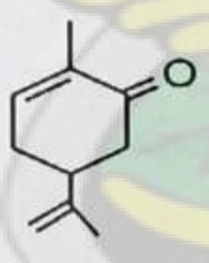


Camphor



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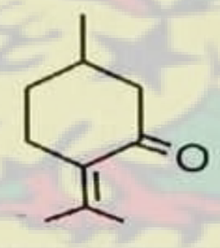
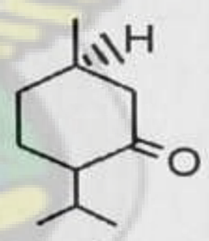
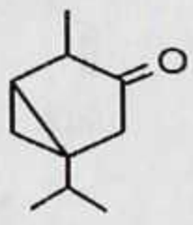


Pulegone



Menthone



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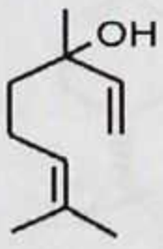

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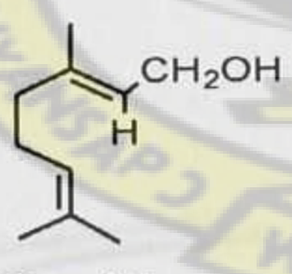

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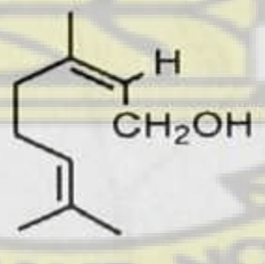
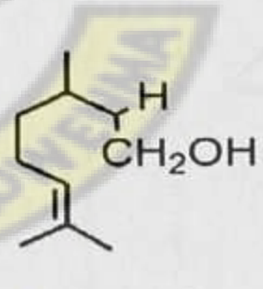


Nerol



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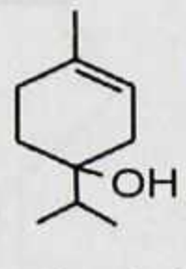
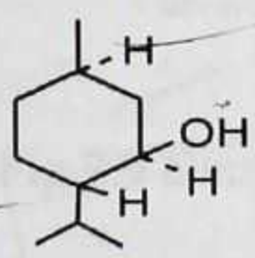


Terpine-4-ol



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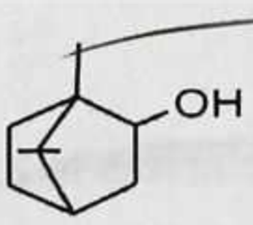
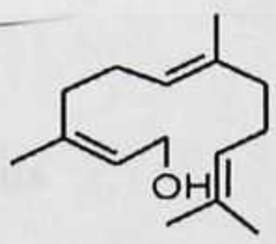


Borneol



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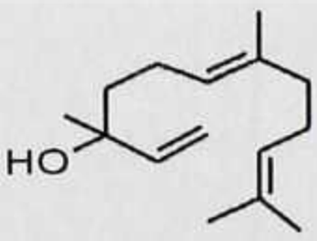

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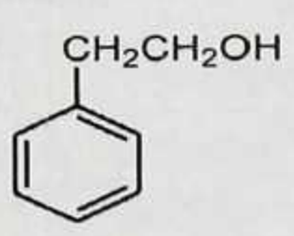

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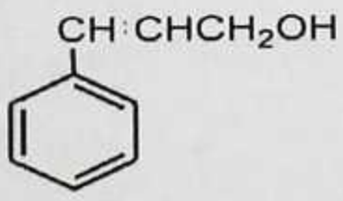
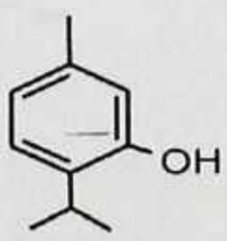


Cinnamic alcohol



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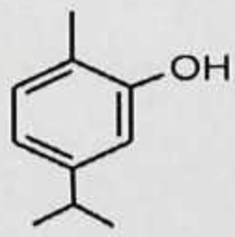

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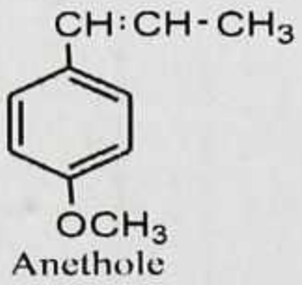
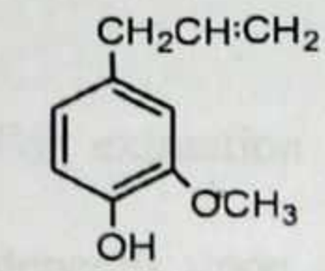


Anethole

Estragol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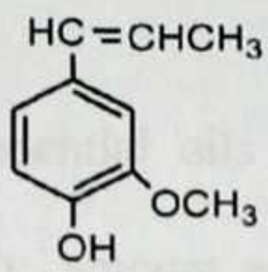
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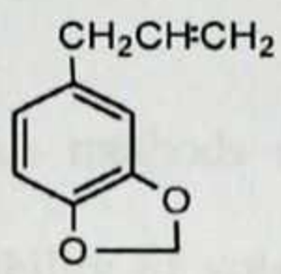
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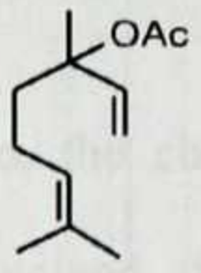
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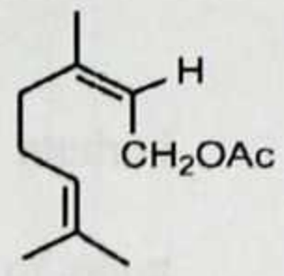
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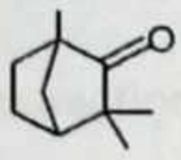
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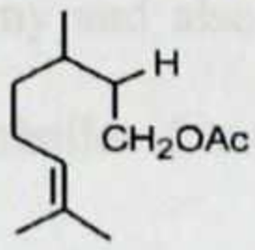
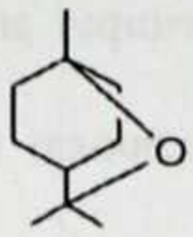
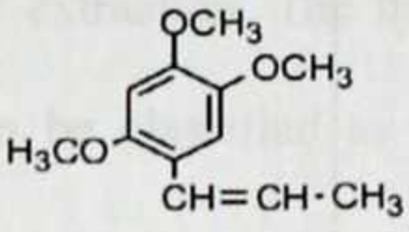

Citronellyl acetate


1,8 Cineole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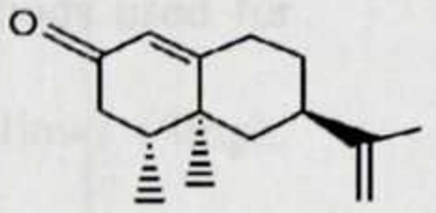
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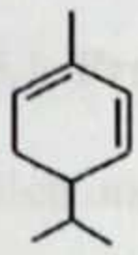
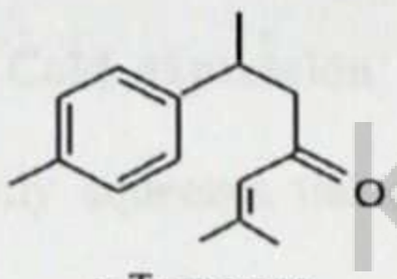

α-Phellandrene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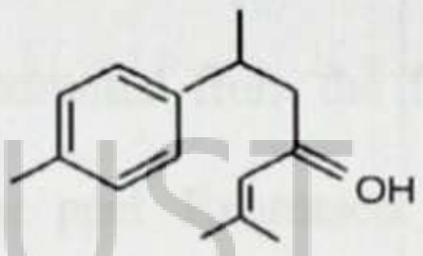
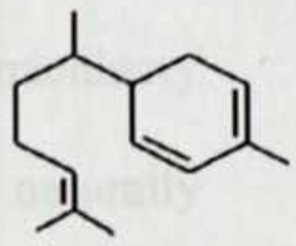

Turmerol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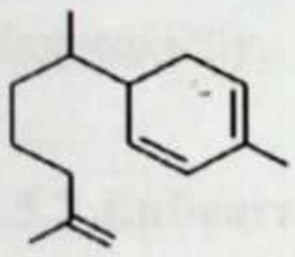
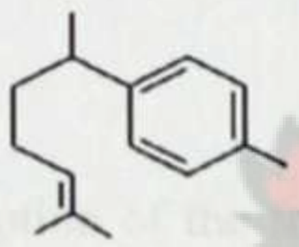

β-Zingiberene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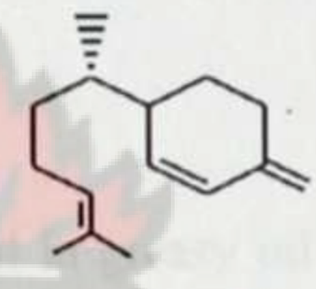
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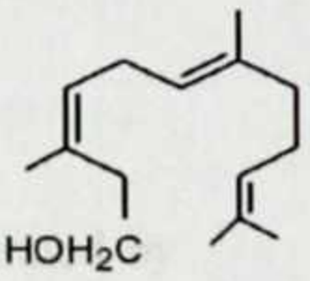
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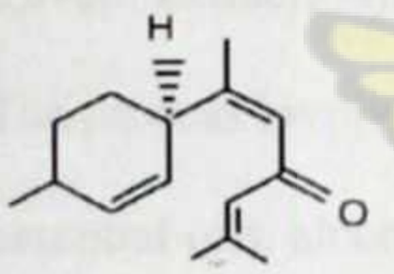
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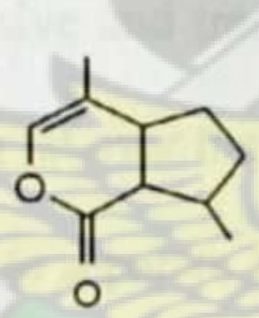
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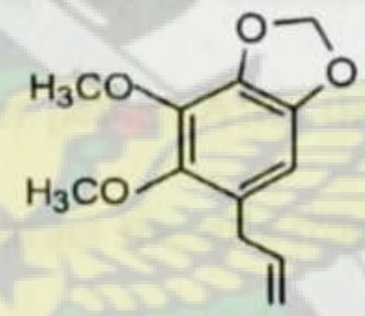
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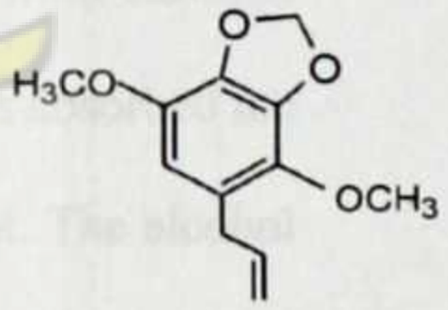
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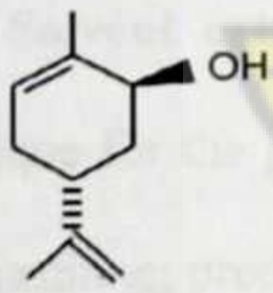
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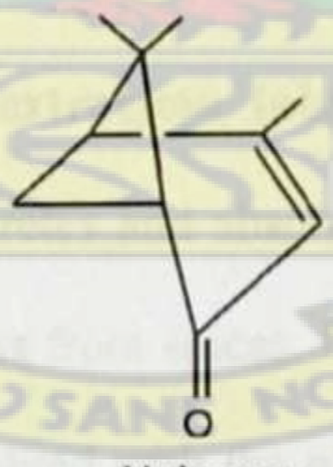
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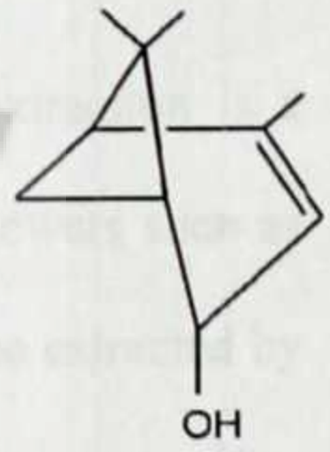
Verbenol

Fig 2.9 constituents of essential oils

2.5 EXTRACTION OF ESSENTIAL OILS

For extraction of essential oils various methods are used, the choice of method depends upon quality, amount and stability of volatiles obtained, the type of plant material, economy and also the time required for extraction. The methods used for extraction of volatiles from herbal resources can be classified as follows (Singh, 2001).

2.5.1 Pressing / Cold expression: oils are extracted from the fruit by machinery which mechanically squeezes the oil from the peel. Expressed oils will naturally contain a proportion of waxes and other nonsoluble components that may cause phototoxicity.

2.5.2 Enfleurage (absorption of the fragrant oil in greasy oil and then separated by solvent extraction): An intensive and traditional way of extracting oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. The alcohol is then evaporated and the essential oil collected.

2.5.3 Solvent extraction /Alcohol extraction (absolute): Solvent extraction is a technique for the production of concretes and absolutes of aromatic flowers such as rose, jasmine; production of oleoresins from spices. Essential oils can be extracted by using organic solvents and alcohol mixed with the plant material in order to produce an absolute.

2.5.4 Distillation: Distillation converts the essential oils into a vapor and then condenses the vapor back into a liquid. It is the most popular, and cost effective method in use today in producing essential oils. Under this method, the plant is

subjected to the distillation for approximately 3 h using a Clevenger- type apparatus (Tonzibo et al., 2009). The bulk of essential oils are produced by distillation. There are three systems of distillation namely hydro, hydro-steam and steam distillation. Hydrodistillation system, though the oldest, is still being widely practised for oil extraction. The plant material is in direct contact with boiling water in a crude metallic distillation outfit. Orange blossom and rose petal oil units employ this method. Hydro-steam distillation is employed where the perfumery material is vulnerable to direct steam. Consequently, the plant material is supported on a perforated grid or screen inserted at some distance above the bottom of the still. The lower part of the still contains water up to a level just below the grid. In a steam distillation system, live steam under pressure (up to 7 kg/cm2) is injected through steam tubes below the charge and the pressure within the distillation vessel is controlled according to the nature of the material being distilled (Joy et al., 2001). Essential oil after extraction is dried over anhydrous sodium sulphate to remove water from the oil (Dongmo et al., 2009). The isolated oil must be stored at temperature of 4°C until further analysis is performed (Alim et al., 2009).

2.5.5 Supercritical Fluid Extraction (SCFE)

This is emerging as a versatile and important tool to separate components that are susceptible to thermal degradation. It is employed for the extraction of flavours, fragrances and perfumes from a wide variety of plants. This method of extraction is superior and faster than distillation. Higher diffusiveness and lower viscosities of supercritical fluids enable better penetration and faster equilibration. Besides, the solvent power is manipulable, free from surface tension and wetting properties and easily adoptable to isolate highly thermolabile compounds. Carbon dioxide is the

favourite solvent by virtue of its cheapness, nontoxicity, noncorrosiveness, non-flammability, easy to handle, needing mild processing conditions during extraction, good solvent power for alcohols, aldehydes, esters and ketones (Joy et al., 2001).

2.6 ESSENTIAL OIL CONSTITUENT IDENTIFICATION

Separation and detection of different constituents in plants have been always complicated. While conventional research mainly focuses on determination of the active components, fingerprinting can offer characterization of a complex system with a degree of quantitative reliability, so it has gained increasing attention for quality control systems over the past years. Chromatography methods including TLC, HPLC, GC-MS and electromigration techniques such as capillary electrophoresis are mainly used for fingerprinting (Hajimehdipoor et al., 2009). TLC is a common, rapid and cost-efficient method used for fingerprinting plant extracts. It has been employed in essential oil works to establish fingerprint of the oils. Ashnagar et al., (2011) characterized *Thymus vulgaris* essential oil. Using TLC techniques, the oil was separated and put under successive TLC on silica gel with benzene:chloroform (3:1 v/v) as the mobile phase which resulted in the separation of two fractions with R_F =0.52 and R_F =0.36. α and β -pinene limonene, citronellol, and geraniol were the main compounds identified in *Ocimum basilicum* essential oil using TLC fingerprint technique (Soran et al., 2009).

TLC as a method of establishing a fingerprint of organic substance has been used as an authentication of various ginseng species essential oil and their stability preparation in China (Xie et al., 2006).

The Gas Chromatography –Mass Spectrometry (GC-MS) has long been the method of choice for identifying volatile compounds in complex mixtures. The combination of

gas chromatography and mass spectrometry became widely recognized in the 1960s as the most sensitive and versatile tool available for the identification of volatile or organic compounds. A GC-MS technique has been the main method used in the identification of chemical composition of essential oil from plants (Mazroa et al., 2011). The composition of the volatile oil from the fruits (berries) of Ashanti pepper (*piper guineense*) was investigated using gas chromatography-mass spectrometry techniques (Olonisakin et al., 2006). The work to identify the chemical composition of the volatile oil from T. fallax by Goze et al., (2009) was achieved using the gas chromatography-mass spectrometry techniques.

2.6.1 THIN LAYER CHROMATOGRAPHY (TLC)

Chromatography is a sophisticated method of separating mixtures of two or more compounds. The separation is accomplished by the distribution of the mixture between two phases: one that is stationary and one that is moving. Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases between which they are to be partitioned. Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase). Solids most commonly used in chromatography are silica gel (SiO_2 x H_2O) and alumina (Al_2O_3 x H_2O). Thin Layer Chromatography (TLC) is a sensitive, fast, simple and inexpensive analytical technique. In thin layer chromatography, a solid phase, the adsorbent, is coated onto a solid support as a thin layer (about 0.25 mm thick). In many cases, a small amount of a binder such as plaster of Paris is mixed with the absorbent to facilitate the coating. Many different solid supports are employed, including thin sheets of glass, plastic, and aluminum. The mixture (A plus B) to be separated is dissolved in a solvent and the resulting solution is spotted onto the thin layer plate near the bottom, next to the

reference substances. A solvent, or mixture of solvents, called the eluent, moves up the plate by capillary action. At all times, the solid will absorb a certain fraction of each component of the mixture and the remainder will be in solution. The more weakly a substance is adsorbed, the farther up the plate it will move. The more strongly a substance is adsorbed, the nearer it will stay to the origin. Several factors determine the efficiency of a chromatographic separation. The adsorbent should be as selective as possible towards the components of the mixture so that the differences in rate of elution will be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. The eluting solvent should also show a maximum of selectivity in its ability to dissolve or desorbs the substances being separated (Fried et al., 1999). The fact that one substance is relatively soluble in a solvent can result in its being eluted faster than another substance. If the solvent is more strongly adsorbed than the substances being separated, it can take their place on the adsorbent and all the substances will flow together. If the solvent is less strongly adsorbed than any of the components of the mixture, its contribution to different rates of elution will be only through its difference in solvent power toward them. If, however, it is more strongly adsorbed than some components of the mixture and less strongly than others, it will greatly speed the elution of those substances that it can replace on the absorbent, without speeding the elution of the others (Fried et al., 1999). Mixtures of solvents can be used and, since increasing eluting power results mostly from preferential adsorbtion of the solvent, addition of only a little (0.5-2%, by volume) of a more strongly adsorbed solvent will result in a large increase in the eluting power. Because water is among the most strongly adsorbed solvents, the presence of a little water in a solvent can greatly increase its eluting power. For this reason, solvents to be used in chromatography are quite dry. The particular

combination of adsorbent and eluting solvent that will result in the acceptable separation of a particular mixture can be determined only by trial. Because the distance travelled by a substance, relative to the distance travelled by the solvent front, depends upon the molecular structure of the substance, TLC can be used to identify substances as well as to separate them. The relationship between the distance travelled by the solvent front and the substance is usually expressed as the R_F value. The R_F values are strongly dependent upon the nature of the adsorbent and solvent. Therefore, experimental R_F values and literature values do not often agree very well (Fried et al., 1999).

$$R_F = \frac{\text{distance travelled by substance}}{\text{distance travelled by solvent front}}$$

2.7 PHYSICAL PARAMETERS OF ESSENTIAL OIL

2.7.1 REFRACTIVE INDEX

Is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of Sodium light passing from the air into the substance. It is a very important physical property of essential oil since it tells the purity of the oil.

2.7.2 OPTICAL ROTATION

Is the turning of the plane of linearly polarized light about the direction of motion as the light travels through certain materials. It occurs in solutions of chiral molecules such as sucrose (sugar), solids with rotated crystal planes such as quartz, and spin polarized gases of atoms or molecules. To determine the rotation of polarized light through a liquid to establish its optical activity whether dextrorotatory (bends light to the right) or laevorotatory (bends light to the left). The degree of rotation and its

direction are important in determining the purity of substances. The specific rotation that is observed by an essential oil tells how pure the oil is and so a very important physical property of the oil (Buckle, 2000).

2.8 MOSQUITO REPELLENCY ASSAY

In 2008, there were 247 million cases of malaria and nearly one million deaths mostly among children living in Africa. In Africa, a child dies every 45 seconds of Malaria, the disease accounts for 20% of all childhood deaths. Malaria is caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected *Anopheles* mosquitoes, called "malaria vectors", which bite mainly between dusk and dawn. About 20 different *Anopheles* species are locally important around the world. They breed in shallow collections of freshwater like puddles, rice fields, and hoof prints (WHO, 2011). The mosquito *Anopheles* contains seven species, of which *A. gambiae (s.s), A. arabiensis and A. melas* are three of the major vectors of lymphatic filariasis (LF) and malaria caused by *Wuchereria bancrofti*, and *Plasmodium falciparum* respectively in West Africa. In Ghana, previous studies have found the *An. gambiae s.s* and the *An. funestus* to be the major vectors in the southern coastal zone and in the northern region of the country (Souza et al., 2010). *Anopheles* mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting. The African malaria mosquito, *Anopheles gambiae sensu stricto* (*s.s.*), is an especially important vector because it is highly anthropophilic and a very efficient carrier of the most potent malaria parasite, *Plasmodium falciparum.* Only female adult mosquitoes transmit the malaria parasite

into the human body. *An. gambiae s.s.* shows a highly selective, anthropophilic biting pattern even when humans are outnumbered by other potential hosts (Li et al., 2005). The female obtains the protein she needs for the developmentof her eggs by feeding on vertebrate blood. The ecology, development, behaviour, and survival of mosquitoes and the transmission dynamics of the diseases they transmit are strongly influenced by climatic factors (Reiter, 2001). Although most malaria transmission occurs during the rainy season and in humid habitats, some mosquitoes are capable of surviving during the dry season and in dry savannah areas of Africa (Gray et al., 2005). Current Ghana policy on vector control against Anopheles vectors prioritizes the use of insecticide treated materials and indoor residual spraying (Souza et al., 2010). The discovery, development and use of synthetic insecticides have reduced the interest in plant origin products. However, widespread use of these insecticides in public health and agriculture for the control of vector and pest species have created different problems, such as the development of physiological resistance in major vector species, environmental pollution and toxic hazards to human and other non-target organisms due to their broad spectrum of activity. Synthetic chemicals and insecticides used for the control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature. Some repellents of synthetic origin may cause skin irritation and affect the dermis. Majority of commercial repellents are prepared by using chemicals like allethrin, N-N-diethyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use. As a result, there has been an increased interest in developing potential alternative or additional control methods that are effective against the target vector species, environmentally safe, biodegradable, with low cost, and can be used by individuals

and communities in specific situations. One of these potential alternatives or additional control methods is the use of selected botanical derivatives against the target mosquito species (Massebo et al., 2009). Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable (Das *et al*., 2003). The monoterpenes, pinene, limonene, terpinolene, citronellol, citronellal and camphor which are common constituents of some oils have been reported to possess high repellent properties against various insects (Jantan et al., 1998). The mosquito repellent activities of 38 essential oils have effectively been screened against the mosquito *A. aegypti* under laboratory conditions using the arm-in-cage method (Trongtokit *et al*., 2005). When the tested oils were applied at a 10% or 50% concentration, none of them prevented mosquito bites for as long as 2 h, but the undiluted oils of *Cymbopogon nardus* (citronella), *Pogostemon cablin* (patchuli), *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* were the most effective and provided 2 h of complete repellency.This same method has been used to evaluate some commercial repellents of their efficacy using a low density *A.aegypti* mosquito (Fradin et al., 2002).

2.9 ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND PLANT HYDROSOLS

The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multidrug resistant bacteria has spurred scientists on the research for plant based antimicrobial of therapeutic potentials. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles. The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of

consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives (Reische et al., 1998). Essential oils and their by- products have been widely used in folkloric medicine and as such, they have been screened for their potential uses as alternative remedies for the treatment of infectious diseases (Afolayan et al., 2009). Essential oils from plant edible parts which are eco friendly in nature have been used by several workers for controlling fungi, bacteria, viruses and insect pests (Singh, 1996). The main reasons for using essential oils as antifungal agents are their natural origin and low chance of pathogens developing resistance. Antifungal properties of plant essential oils have been reported by researchers throughout the world (Bouchra et al., 2003; Daferera et al., 2003). Six essential oils Clove, Cedar wood, Lemon grass, Peppermint, Citronella and Nutmeg oils have been tested for *in vitro* antifungal activity on *Phomopsis azadirachtae* and all showed a significant antifungal activity against the tested pathogen. The results indicated that the citronella and lemongrass showed 100% inhibition of mycelial growth at 2,500 ppm (Prasad et al., 2010). Generally, the extent of inhibition of the oils could be attributed to the presence of an aromatic nucleus containing a polar functional group (Singh et al., 2003). In addition the differences in composition related to variety, agronomic practices and processing are also likely to influence their properties since these factors contribute to both the profile and relative concentrations of active ingredients (Singh et al., 2003).

Inhibitory effects of the hydrosols of thyme, black cumin, sage, rosemary and bay leaf have been investigated against *Salmonella Typhimurium* and *Escherichia.* Bay leaf hydrosol treatments for 60 min reduced significantly ($P < 0.05$) *E. coli* population on apple and carrot samples, thyme hydrosol showed the highest antibacterial effect on both *S. typhimurium* and *E.coli* counts. Inhibitory effect of thyme hydrosol on *S.*

typhimurium was higher than that for *E. coli*.(Fatih et al., 2011). In vitro antimicrobial effects of the hydrosols of basil (*O. basilicum)*, thyme (*T. schimperi*), cardamon (*E. cardamom)*, cinnamon (*C. Zeylanicum*), mustard (*B. nigra*) and clove (*S. aromaticum*) have been evaluated against *S. aureus*, *E. coli*, *S. typhi*, *P. aerugenosa* and *Candida albicans*. The percent inhibition of the hydrosols were found to range from 20 to 100% (against *S. aureus,* p = 0.005), 10 to 100% (against *E. coli*, p = 0.005), 0 to 35% (against *P. aerugenosa*, p = 0.069) and 15 to 100% against *S. typhi*, p = 0.00). Complete (100%) growth inhibition was demonstrated at 15% hydrosol concentration of cardamom and thyme (against *E. coli*), cardamon and cinnamon (against *S. aureus*) and cardamom, thyme and cinnamon (against *S. typhi*) with *Candida albicans* were inactive to the test hydrosols (Hussien et al., 2011).

It is known that the compositions of hydrosols and their antimicrobial effects depend on plant species and regional conditions (Sağdç et al., 2003). The in vitro antibacterial activity of the hydrosols of (distilled plant water) twenty plant samples (Thyme, sumach, clove, nettle, angelica, acacia, oak, sage, juniper, rosemary, echinacea, green tea, basil, myrtle, walnut, laurel, mint, strawflower, daisy, hypericum) were tested on *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* which play role especially at spoilage of freshwater fish. Thyme and clove were effective against all bacteria (Oral et al., 2008). The antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrosols, widely used in food products as drinks and food additives, have been tested for their inhibitory effects against *Bacillus subtilis* and *Salmonella enteritidis*, obtained data showed that all of the tested herbal hydrosols demonstrated antibacterial activities against all of tested bacteria. Garlic hydrosols demonstrated stronger antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with thyme, peppermint, sage and black

pepper. Combined extracts of thyme, mint and sage (1: 1 mixing ratio) had slightly higher antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with single plant hydrosols. Thyme hydrosols demonstrated higher antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with sage, peppermint and black pepper (Al-Turki, 2007).

Hydrosol of leaves obtained from *Lantana camara* as waste of essential oil have been studied for antibacterial activities against Gram-negative and Gram-positive bacteria by zone of inhibition and MIC by Dilution method. Leaf hydrosol exhibited considerable antibacterial activities against the Gram-positive bacteria used where the value of zone of inhibition ranged from 10-11 mm, respectively (Dubey et al., 2001).

2.10 PLANT CONSIDERED FOR THE STUDIES

2.10.1 Cymbopogon citratus (DC.) Stapf

It is cultivated in parts of Ghana and all over west tropical Africa. It is a perennial tufted grass 60-90 cm high, rhizome short and annulate from which pale green leaves in dense fascicles arise, basal sheaths wide, hollow, cinnamon-coloured on inner side, about 30 cm long, bearing blades over 90 cm long, 20-25 mm broad. It is cultivated in parts of Ghana as a decorative grass on compounds and along roadsides (Dokosi, 1998). Chemical compounds present in varying concentrations in lemon grass have a great demand due to their use in perfumery, flavour and pharmaceutical industry. The oil extracted from leaves of lemon grass is used for its spasmolytic, analgesic, anti-inflammatory, antipyretic, diuretic and tranquilizing properties in treating various digestive disorders, inflammation, diabetes, nervous disorders and fever as well as other health problems (Kumari et al., 2009). The oil yield from fresh grass ranges from 0.26 to 0.52 per cent with a specific gravity of 0.85 to 0.900 and a citral content

of 78 to 85.5 per cent. The plant has been reported to poses insecticidal property (Dokosi, 1998). Essential oil distilled from the leaves is used as flavouring agent and in medicines. The essential oil of the lemongrass and the plant itself is characterized by its lemony aroma. The important constituent of the oil is citral, a monoterpene aldehyde, which is mainly used for the manufacture of vitamin A (Kumari et al., 2009). As a medicinal herb, lemon grass has been considered as carminative, insect repellent and widely used as herbal tea. Lemongrass oil is one of the most important essential oils produced in the world. India is a major producer of this oil. Composition of volatile constituents of lemongrass has been reported. The essential oil of lemongrass is characterized by a high content of citral (>45%) and its quality is generally determined by the amount of citral present in the oil. Essential oil of *C. citratus* is mainly composed of citral (30–93.74%) with general predominance of geranial (Kumari et al., 2009). Essential oils extracted by steam distillation from *Cymbopogan citratus* have been evaluated for larvicidal, ovicidal and repellent activities against the filarial mosquito *Culex quinquefasciatus*. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm2 concentration of *C. citratus* gave protection up to 3.00, 4.00 and 5.00 hours respectively. The total percentage protection of the essential oil was 49.64% at 1.0 mg/cm2, 62.19% at 2.5 mg/cm2 and 74.03% at 5.0 mg/cm2 for 12 hour (Pushpanathan et al., 2006).

2.10.2 *Azadirachta indica* A. Juss

The tree is part of the mahogany family Meliaceae and it is one of two species in the genus Azadirachta, native to India, Sri Lanka, Malaysia, Bangladesh and Pakistan. It is classified under the order Sapindales, from the family Meliaceae, with the genus Azadirachta and the specie A. indica. The Neem tree is a fast growing, long-life tree popular in the tropics and is grown for its ornamental value, as well as for its

therapeutic value. The seed yields Margosa oil and is non-drying oil with insecticidal and antiseptic properties. It is a bitter tonic herb that is used for clearing toxins, reducing inflammation, lowering fever, promoting healing and in general promoting and improving body functions. It destroys a wide range of parasitic organisms and is also an insecticidal compound. The oil comprises mainly of triglycerides and large amounts of triterpenoid compounds. It furthermore contains steroids (campesterol, β - sitosterol, stigmasterol) and triterpenoids of which Azadirachtin is the most well known and studied. The oil from the plant can be used as a household pesticide for ant, bedbug, cockroach, housefly, sand fly, snail, termite and mosquitoes both as repellent and larvicides (Puri, 1999). Neem is widely advertised as a natural alternative to DEET and it has been tested for repellency against range of arthropods of medical importance, with variable results. Several field studies from India have shown very high efficacy of Neem-based preparation. Neem oil, obtained by cold-pressing seeds, can be effective against soft-bodied insects and mites but is also useful in the management of phytopathogens. Apart from the physical effects of neem oil on pests and fungi, disulfides in the oil contributes to its bioactivity. Neem seeds actually contain more than a dozen azadirachtin analogs, but the major form is azadirachtin and the remaining minor analogs likely contribute little to overall efficacy of the extract. Seed extracts include considerable quantities of other triterpenoids, notably salannin, nimbin, and derivatives. Azadirachtin has two profound effects on insects. At the physiological level, azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects, a similar mechanism of action leads to sterility. In addition, azadirachtin is a potent antifeedant to many insects (Isman, 2006). Topical application of 2% neem oil mixed in coconut oil produced varying

degrees of protection against different vector species and the repellent effect was more pronounced against Anopheles than against C. Quinquefaciatus. A complete protection for 12 h from the bites of all the anopheline mosquitoes species was reported by using 2% neem oil in coconut oil on the exposed part of the body. The application of neem oil and cream has been found safe and hence can be used as a personal protection measure against mosquito bites particularly against malaria vectors (Mittal et al., 2003).

2.10.3 Ocimum gratissimum Linn (Labiatae)

Ocimum gratissimum is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite; petiole 2-4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5-16 cm x 1-8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate-serrate, apex acute, puberulent or pubescent. O. gratissimum is a variable polymorphic complex species, often subdivided into subspecies, varieties and forms, mainly based on differences in chemical content, the morphology of the fruiting calyx, and on different degrees of hairiness, but the variation forms a continuum (Orwa et al., 2009). *Ocimum gratissimum* is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties. The whole plant and the essential oil are used in traditional medicine especially in Africa and India. The essential oil is also an important insect repellent (Akinmoladun et al., 2007).The fresh above ground parts of O. gratissimum contain 0.8-1.2% essential oil. The chemical composition of the oil is variable and at least 6 chemotypes have been reported, characterized by the main component of the

essential oil: eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool (Orwa et al., 2009).

2.10.4 *Citrus sinensis (L.)*, *Citrus aurantifolia* Christm.

The genus *Citrus*, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulate* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus*. *Citrus* are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major *Citrus* producers. *Citrus* fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics and folk medicine.

Citrus peel essential oils are reported to be one of the rich sources of bioactive compounds, namely coumarins, flavonoids, carotenes, terpenes and linalool etc. (Mondello *et al.*, 2005). Recently, *Citrus* peel essential oils have also been searched for their natural antioxidant and antimicrobial properties. It is widely accepted that biological activities of plant materials are strongly linked with their specific chemical composition, mainly the secondary metabolites such as plant phenolics and flavonoids. Furthermore, studies revealed that drying the plant materials under different conditions can exert significant effect on the chemical profile and biological attributes of the essential oils derived (Kamal et al., 2011).

C. sinensis oil yield has been reported to be in range of 0.24-1.07% with a refractive index of 1.4636 for air dried and 1.4631 for fresh extract at a temperature of 25 ^{0}C (Kamal et al., 2011). A total of 18-22 compounds have been identified in the *C.*

sinensis peel essential oils, Limonene (80.9, 72.7 and 66.8%), β-myrcene (4.19, 3.76 and 4.41%), has the main constituents in the oils from fresh, ambient-dried and oven-dried peels of *C. sinensis*. Oxygenated monoterpenes, mainly comprising of linalool and isophorone with amounts varying from 1.52-2.10% and 1.09-2.92%, respectively constituted 5.78-18.49% of the oils. Sesquiterpenoides and oxygenated sesquiterpenes constituted 0.41-1.90% and 1.32-5.70% of the oils of *C. sinensis* (Kamal et al., 2011).

Citrus aurantifolia essential oil is a complex mixtures of chemical compounds like limonene, γ-terpinene, citral, linalool and β-caryophyllene among others, which can be represented by three main classes: terpenes, oxygenates and sesquiterpenes. The most significant flavor compound is citral, while linalool possesses highly distinctive organoleptic characteristics. In addition, limonene, myrcene, octanal, and γ-terpene among others contribute to high aromatic flavour. The oil is employed in perfumes, toilet waters, eaux de Cologne, and in cosmetics to which it imparts a refreshing top note. GC analysis identified about 10 main substances, being limonene, p-cymene, myrcene and β-bisabolene the most significant compounds (Gamarra et al., 2005).

2.10.5 *Chromolaena odorata* (L.) King and H.E Robins.

Chromolaena odorata is a species of flowering shrub in the sunflower family, Asteraceae. It is native to North America, from Florida and Texasto Mexico and the Caribbean, and has been introduced to tropical Asia, West Africa, and parts of Australia. Common names include Siam Weed, Christmas Bush, and Common Floss Flower. It is sometimes grown as a medicinal and ornamental plant. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds. It was earlier taxonomically classified under the genus *Eupatorium*, but is now considered more closely related to other genera in the tribe Eupatorieae. *Chromolaena odorata* is

considered invasive weed of field crops in its introduced range, and has been reported to be the most problematic invasive species within protected rainforests in Africa. It forms dense stands that prevent the establishment of other plant species (Chakraborty et al., 2011). *Chromolaena odorata* is an herbaceous perennial that forms dense tangled bushes 1.5-2.0m in height. Its stem branches freely, with lateral branches developing in pairs from the axillary buds. The older stems are brown and woody near the base; tips and young shoots are green and succulent. The root system is fibrous and does not penetrate beyond 20-30cm in most soils. The flowers are white or pale bluish-lilac, and form masses covering the whole surface of the bush. Essential oil extracts from their leaves have been tested for efficacy on the mortality of the maize grain weevil, *Sitophilus zeamais* (Coleoptera, Curculionidae). Significant insect mortality was obtained with essential oil extract of *C. odorata* (LD_{50}=6.78%). These results show that the essential oils of the leaves of C. Odorata may be exploited for insect control (Chakraborty et al., 2011). Thirty-three components have been identified from the volatile oil of *C. odorata*. Terpenoid compounds are major components of the volatile oil. The main terpenic components identified were trans-caryophyllene(16.22%), cadinene (15.53%), copaene(11.32%), caryophyllene oxide(9.42%), germacrene-D(4.86%) and humulene (Chakraborty et al., 2011).

The extracts of fresh leaves of *C. odorata* have been used in the treatment of malaria in Ghana and Benin. The major constituents presented in the oil of *C. odoarata*, presented a broad antimicrobial spectrum and had better antimicrobial activity against yeast and gram-positive bacteria (Bedi et al., 2010).

2.10.6 *Cymbopogon nardus* (L.) Rendle.

Citronella (*Cymbopogon nardus*) essential oil has been used for over fifty years both as an insect repellent and an animal repellent. Citronella oil has been mainly attributed to its major monoterpenic constituent citronellal (Koul et al., 2008). *Cymbopogon nardus* (Linn.) commonly known as citronella grass or Ceylon citronella, is a tall herb, 1.5–2.1 m high, copiously branched above and forming a large decomposed nodding panicle and distributed throughout the hotter parts of India, Burma, Malay Peninsula, Sri Lanka and the Seychelles (Vijender et al., 2001). The grass contains about 0.4% oil, and the yield is 30.6 kg acre per annum. The infusion of the leaves is used as a stomachic and carminative. The oil is stimulant, carminative, antispasmodic and diaphoretic. In Cambodia, the flowers are considered bechic and diaphoretic. Citronella oil is chiefly used as a soap perfume and as a source of geraniol and citronellal (Vijender et al., 2001). Essential oil from plants grown in Bangladesh contained predominantly citronellal (32%), citronellol (14.4%) and geraniol (21.1%). Elemol and methyl isoeugenol have been identified for larvicidal activity in the Ceylon citronella (Vijender et al., 2001). Essential oils and extracts belonging to plants in the citronella genus (Poaceae) are commonly used as ingredients of plant-based mosquito repellents. Citronella is one of the most widely used natural repellents on the market, used at concentrations of 5-10% (Maia et al., 2011).

2.10.7 *Cinnamomum Zeylanicum Breyn.*

C. zeylanicum, the source of cinnamon bark and leaf oils, is a tree indigenous to Sri Lanka. Major compounds present in stem-bark oil and root bark oil are cinnamaldehyde (75%) and camphor (56%), respectively (Jayaprakasha et al., 2002). Cinnamon bark oil possesses the delicate aroma of the spice and a sweet and pungent taste. It is employed mainly in the flavouring industry where it is used in meat and

fast food seasonings, sauces, pickles, baked goods, confectionery, cola-type drinks, tobacco flavours and in dental and pharmaceutical preparations. Thirty-four compounds have been previously identified in cinnamon oil with *(E)*-cinnamyl acetate (54%) and caryophyllene (14%) as the major component. Twenty-six compounds constitutes 97% of the volatile oil from cinnamon flowers were characterized with *(E)*-cinnamyl acetate (42%), *trans*-α-bergamotene (8%) and caryophyllene oxide (7%) as the major compounds (Jayaprakasha et al., 2002). Fresh buds (100 g) subjected to hydro-distillation in a Clevenger-type apparatus for 4 h yielded 0.2% (v/w) of volatile oil (Jayaprakasha et al., 2002).

CHAPTER THREE

MATERIALS, EQUIPMENT AND METHODOLOGY

This chapter deals with materials, equipment and experimental procedure employed in the research.

3.1 APPARATUS/ EQUIPMENT AND CHEMICALS USED

The apparatus used were

- Separating funnel
- Clevenger type apparatus
- Repellency test cage
- TLC chamber
- Refractometer

Chemicals

- B.D.H. AnalaR Ethanol
- B.D.H Sodium sulphate (anhydrous)
- Merick HPLC Choroform
- B.D.H Pet- ether (40^0C to 60^0C)
- Concentrated AnalaR Hydrochloric acid
- Anisaldehyde sulphric acid

3.2 SAMPLE COLLECTION

Plants for the work were obtained from the Department of Pharmacognosy Herbarium and the Botanic gardens all of K.N.U.S.T Kumasi whilst the essential oil from *Cinnamomum zeylanicum* and *Azadirachta indica* were provided by Mr. G.K Tuani of K.N.U.S.T Chemistry department. Plants sample were collected between the months of May to August 2011 for the distillation.

Scientific name	Family	Common name	Plant part used
Cymbopogon nardus	Poaceae	Citronella	leaves
Chromolaena odorata	Asteraceae	Siam Weed	leaves
Citrus sinensis	Rutaceae	Orange	fruit peels
Citrus aurantifolia	Rutaceae	lime	fruit peels
Ocimum gratissimum	Labiatae	Basil	Leaves
Cymbopogon citratus	Poaceae	Lemon grass	Leaves
Azadirachta indica	Meliaceae	Neem	seed
Cinnamomum zeylanicum	Lauraceae	cinnamon	leave

3.3 EXTRACTION OF THE OIL

Between 80 and 450g of plant samples were hydrodistilled freshly and also after seven days air drying for their essential oil using the Clevenger type apparatus for over 3 hours. Leaves from *Cymbopogon nardus, Chromolaena odorata,* Ocimum gratissimum and *Cymbopogon citrates* whiles peels from *Citrus sinensis* and *Citrus aurantifolia* were the parts of the plants used for the oil extraction. The extracted oil was dried over anhydrous sodium sulphate and stored in a refrigerator at a temperature of -4^0C until further analysis was performed. The percentage oil yield was calculated as

Percentage oil yield (v/w) = $\frac{\text{Volume of oil obtained}}{\text{Weight of plant used}} \times 100$

3.4 REFRACTIVE INDEX

Refractometer was used for the determination of the refractive index of oil at a temperature of 25^0 C.

3.5 SMELL OF THE OIL

The smell of the oil was evaluated by 3 people selected to grade the smell of the oil from 0 to 10 as described by Buckle (2003). Oils were graded as follows, 10 corresponding to excellent, 7 to 9 ranked as very good, 5 to 6 as good, 4 as acceptable and below 4 as offensive.

3.6 MOSQUITO REPELLENCY TEST

Mosquito larvae's were bred under laboratory conditions of temperature 27^0C, a 12h light and 12h dark at the Department of Theoretical and Applied Biology, K.N.U.S.T, Kumasi. The repellence of the essential oils was evaluated by using an arm-in-cage test. The oil was tested undiluted and also diluted with 70% alcohol to 10% and 50% concentration. 0.1 mL of the 10%, 50% concentrations and undiluted oil were applied to about 30cm of the tested arms (from the elbow to the wrist). The treated arm was then exposed for 1 min to 25 blood starved (hungry) female mosquitoes, 4–5 days old. The treated arm was re-exposed at an interval of 30 min to mosquitoes and the time recorded for the first bite. The duration (min) of repellence after application was used as a measure of the repellence of the essential oils. The arm treated with 70% ethanol was used as the control. The control arm was first exposed to the mosquitoes to evaluate their readiness to bite.

3.7 ANTIMICROBIAL SCREENING OF THE HYDROSOLS

Materials: Nutrient Agar, Microbial Test culture, Thermostatic water bath and Bacteriological Incubator.

Microbial Test Cultures: *Candida albicans, Bacillus Sub, Eschericia Faeculis, Staphylococus aureus, Eschericia coli*

Method: Agar-well diffusion assay was used to evaluate the antimicrobial of the hydrosols.

20ml of Nutrient Agar was melted on a water bath. The molten agar was then stabilized at 45°C for 15 minutes in thermostatic water bath. The agar containing tubes were then inoculated separately with 0.1ml of the cultured test organisms. The tubes were then rolled in between the palms to ensure thorough mixing. The contents were then poured into separate sterilized Petri dishes. The dishes were then allowed to set on a laminar horizontal flow chamber after which holes were punched in each dish using 10mm sterilized diameter cork borer. The hydrosols were then poured into the labeled holes made in the dishes. The dishes were then allowed to pre- incubate for one hour before incubating them in a bacteriological incubator at temperature of 37°C for a period of 24 hours.

3.8 PREPARATION OF ANISALDEHYDE SULPHRIC ACID

85 ml of methanol was poured into a 100ml conical flask after which 5ml concentrated sulphric acid was added. 10ml glacial acetic acid was then added, followed by the addition of 0.5 ml anisaldehyde to the resulting solution.

3.9 TLC ANALYSIS

TLC analysis was performed on a Silica Gel F_{254} Pre-coated plates as the stationary phase. Oils were dissolved in Choroform before spotting. A mixture of petroleum ether in combination with Choroform (85:15 v/v), petroleum ether and ethyl acetate in the ratio of 92: 8 and Petroleum ether in combination with Choroform in the ratio of 80:20 were the solvents used as the mobile phase. The spotted plates were immersed into a solvent saturated TLC chamber for development. The developed plates were sprayed with anisaldehyde spraying agent and then heated at 110°C for the appearance of red-bluish bands. The plates were inspected in daylight and also at 366 nm in UV range using a UV lamp. Distance moved by samples and mobile phase were measured and values used to calculate R_F values.

3.10 CONDUCTIVITY AND pH

Pc 300 cyber scan conductivity pH meter was used to determine the conductivity and pH of the plants hydrosols at a room temperature of 25°C. The instrument was calibrated using a buffer of pH 4.00 and 7.00 before the determination.

3.11 DATA ANALYSIS

Data obtained from the work was analysed using Microsoft office excel 2007 edition.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter deals with the data gathered, their analysis and discussion.

4.1 ESSENTIAL OIL CONTENT

The results for the oil contents obtained from the extraction of both seven days air dried and freshly extracted essential oil obtained by hydrodistillation of the plants are shown in the tables 4.1.1 to 4.1.6 below.

***Ocimum gratissimum* essential oil content**

Table 4.1.1(a) Fresh leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
450	4.70	1.04
448	4.90	1.09
213	2.90	1.36

Table 4.1.1 (b) Dried leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
82.00	3.4	4.15
83.00	3.4	4.09
82.00	3.2	3.90

***Cymbopogon citratus* essential oil content**

Table 4.1.2 (a) fresh leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
457.80	2.60	0.57
459.71	2.50	0.54
461.14	2.30	0.49

Table 4.1.2 (b) dried leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
140.06	2.70	1.92
144.00	2.80	1.94
144.04	2.70	1.87

***Cymbopogon nardus* essential oil content**

Table 4.1.3 (a) fresh leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
458.17	5.10	1.11
460.04	5.00	1.08

Table 4.1.3 (b) dried leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
135.00	3.70	2.74
135.03	3.60	2.66

***Citrus sinensis* essential oil content**

Table 4.1.4 (a) fresh peels

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
335.01	2.10	0.63
400	3.90	0.97

Table 4.1.4 (b) dried peels

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
420.00	15.40	3.66
418.09	15.20	3.63

***Chromolaena odorata* essential oil content**

Table 4.1.5 (a) fresh leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
400.00	1.00	0.25
403.02	1.10	0.27

Table 4.1.5 (b) dried leaves

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
274	0.8	0.29
280	0.7	0.25

***Citrus aurantifolia* essential oil content**

Table 4.1.6 (a) fresh peels

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
487.16	6.70	1.37
480.00	6.70	1.40

Table 4.1.6 (b) dried peels

Weight (g)	Volume of oil obtained (ml)	Percentage oil (v/w)
101.61	2.00	1.97
100.09	2.00	1.99

Table 4.1.7 Average oil content of the plant materials.

Plant material	**Fresh**	**Dried**
C. aurantifolia	1.38	1.98
O. gratissimum	1.16	4.05
C. citrates	0.53	1.91
C. nardus	1.09	2.70
C. sinensis	0.80	3.64
C. odorata	0.26	0.27

From the average oil content obtained for each plant, seven days air dried plant materials yielded higher amount of oil than the fresh plant materials. This might be due to the fact that the air dried plant material lost most of its water content thereby concentrating the oil as compared to the fresh materials. For the dried materials, *O. gratissimum* plant showed the highest average oil content of 4.05% followed by *C. sinensis* (3.64%), *C. nardus* (2.7%), *C. aurantifolia* (1.98%), *C. citrates* (1.19%) and dried *Chromolaena odorata* having the lowest oil of 0.27%. For the fresh materials, *C. aurantifolia* had the highest average oil content of 1.38% followed by *O. gratissimum* with oil content of 1.16% which was in agreement with the value obtained by Orwa et al., 2009. Freshly extracted oil from *C. nardus* had a content of 1.09% followed by *C.sinensis* with an oil content of 0.80% with value in agreement with Kamal et al., 2011. Freshly extracted essential oil from *C. citrates* yielded a percentage oil of 0.53% with value in agreement with that reported by Dokosi, 1998.

Freshly extracted essential oil from *Chromolaena odorata* had the lowest oil content of 0.26%.

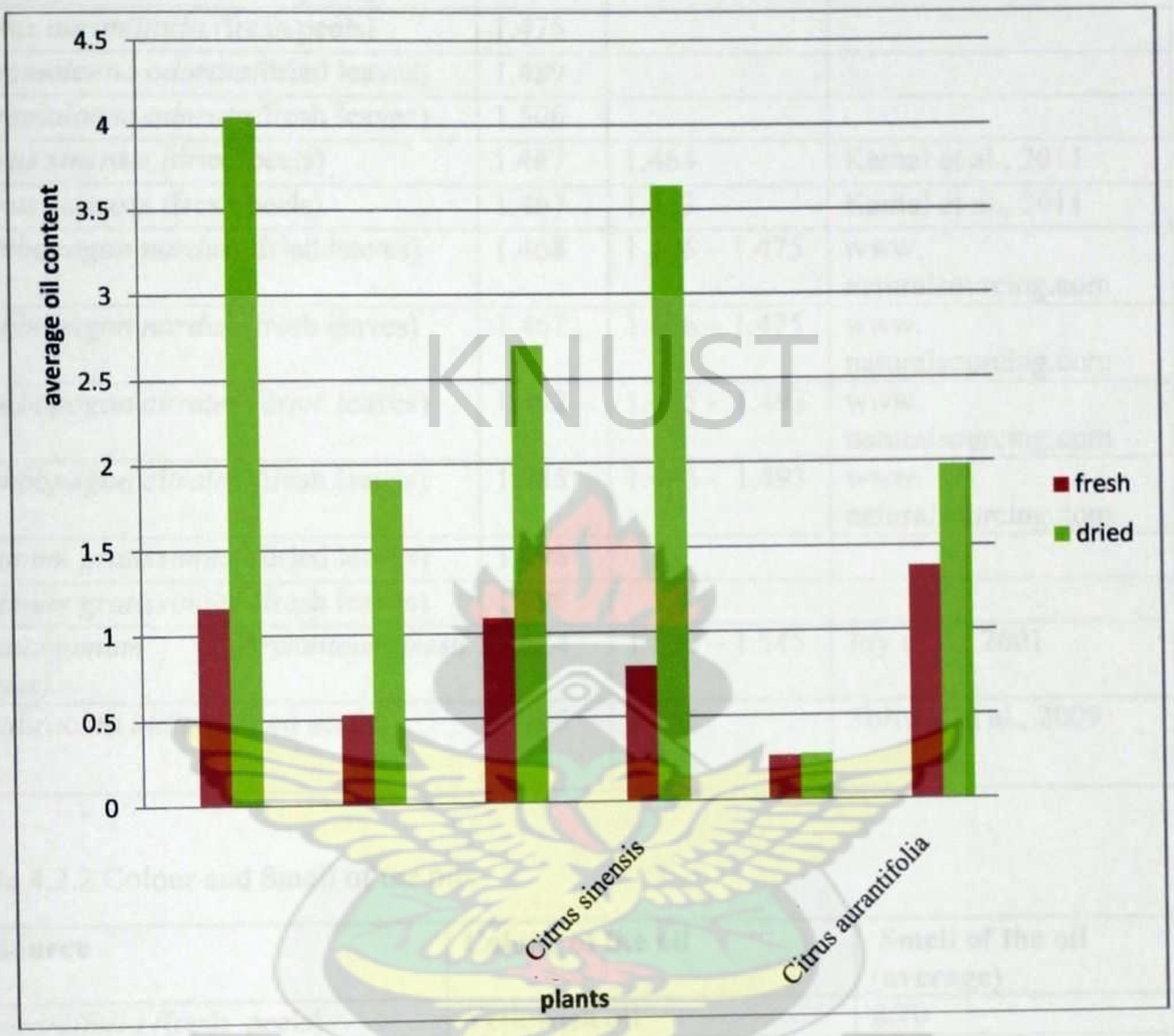

Fig 4.1 Bar chart showing average oil content for both fresh and air dried plant materials.

4.2 PHYSICAL PROPERTIES OF THE OIL

Table 4.2.1 Refractive Index of the oils

Oil source	**(RI)**	**Literature (RI)**	**reference**
*Citrus aurantifolia (*dried peels)	1.475		
*Citrus aurantifolia (*fresh peels)	1.476		
*Chromolaena odorata(*dried leaves)	1.489		
*Chromolaena odorata(*fresh leaves)	1.506		
*Citrus sinensis (*dried peels)	1.467	1.464	Kamal et al., 2011
*Citrus sinensis (*fresh peels)	1.467	1.463	Kamal et al., 2011
*Cymbopogon nardus (*dried leaves)	1.468	1.466 – 1.475	www. naturalsourcing.com
*Cymbopogon nardus (*fresh leaves)	1.467	1.466 – 1.475	www. naturalsourcing.com
*Cymbopogon citrates (*dried leaves)	1.492	1.485 - 1.493	www. naturalsourcing.com
*Cymbopogon citrates (*fresh leaves)	1.485	1.485 - 1.493	www. naturalsourcing.com
*Ocimum gratissimum (*dried leaves)	1.498		
*Ocimum gratissimum (*fresh leaves)	1.496		
Cinnamomum Zeylanicum(fresh leaves)	1.524	1.530 – 1.545	Joy et al., 2001
*Azadirachta indica (*dried seeds)	1.466	1.465	Toliwal et al., 2009

Table 4.2.2 Colour and Smell of the oils

Oil source	**Colour of the oil**	**Smell of the oil (average)**
*C. aurantifolia (*fresh peels)	Yellowish oil	8.70
*C. aurantifolia (*dried peels)	Yellowish oil	8.70
*O. gratissimum (*fresh leaves)	whitish oil	2.00
O. gratissimum (dried leaves)	whitish oil	2.30
*C. citrates (*fresh leaves)	Yellowish oil	7.33
*C. citrates (*dried leaves)	Yellowish oil	6.67
*C. nardus (*fresh leaves)	whitish oil	7.30
*C. nardus (*dried leaves)	whitish oil	7.70
*C. sinensis (*fresh peels)	whitish oil	6.33
*C. sinensis (*dried peels)	Yellowish oil	5.33
*C. odorata (*dried leaves)	Dark green oil	0.00
*C. odorata (*fresh leaves)	Yellowish oil	0.00
C. Zeylanicum (fresh leaves)	Reddish yellow	4.67
A. indica (dried seeds)	Yellowish oil	0.66

The refractive index value obtained for all the oil showed that, with exception of both fresh and dried *Citrus sinensis* oil which had the same RI value of 1.467 the rest showed differences in RI value for both freshly and air dried material of that same plant. Refractive index values obtained were all in agreement with literature values. Also, with the exception of *Citrus sinensis* and *C. Odorata,* all the plants oils had the same colour for both dried and fresh plant materials.

4.3 MOSQUITO REPELLENCY

Table 4.3 Repellence of the oils (min)

OIL source	**10%**	**50%**	**undiluted**	**control**
*C. aurantifolia (*fresh peels)	0, 0, 0	30, 0, 30 (20)	30, 60, 30 (40)	0
*C. aurantifolia (*dried peels)	0, 0, 0	30, 30, 30 (30)	30, 30, 30 (30)	0
A. indica (dried seeds)	0, 0, 30 (10)	60, 30, 30 (40)	60, 60, 30 (50)	0
*O. gratissimum (*fresh leaves)	0, 0, 0	0, 0, 30 (10)	30, 30, 30 (30)	0
O. gratissimum (dried leaves)	0, 0, 0	30, 0, 30 (20)	30, 30, 30 (30)	0
*C. citrates (*fresh leaves)	0, 0, 0	30, 30, 0 (20)	30, 30, 30 (30)	0
*C. citrates (*dried leaves)	0, 0, 30 (10)	30, 30, 30 (30)	30, 30, 30 (30)	0
*C. nardus (*fresh leaves)	0, 0, 30 (10)	60, 90, 60 (70)	120,90,120 (110)	0
*C. nardus (*dried leaves)	0, 0, 0	60, 60, 60 (60)	120,120,120 (120)	0
*C. sinensis (*fresh peels)	0, 0, 0	30, 30, 0 (20)	30, 30, 30 (30)	0
*C. sinensis (*dried peels)	0, 0, 0	30, 0, 30 (20)	90, 30, 30 (50)	0
*C. odorata (*fresh leaves)	0, 0, 0	0, 0, 30 (10)	30, 0, 0 (10)	0
*C. odorata (*dried leaves)	0, 0, 0	0, 0, 0	0, 0, 30 (10)	0
C. Zeylanicum	0,0,30 (10)	3 0, 30, 30 (30)	30, 30, 30 (30)	0

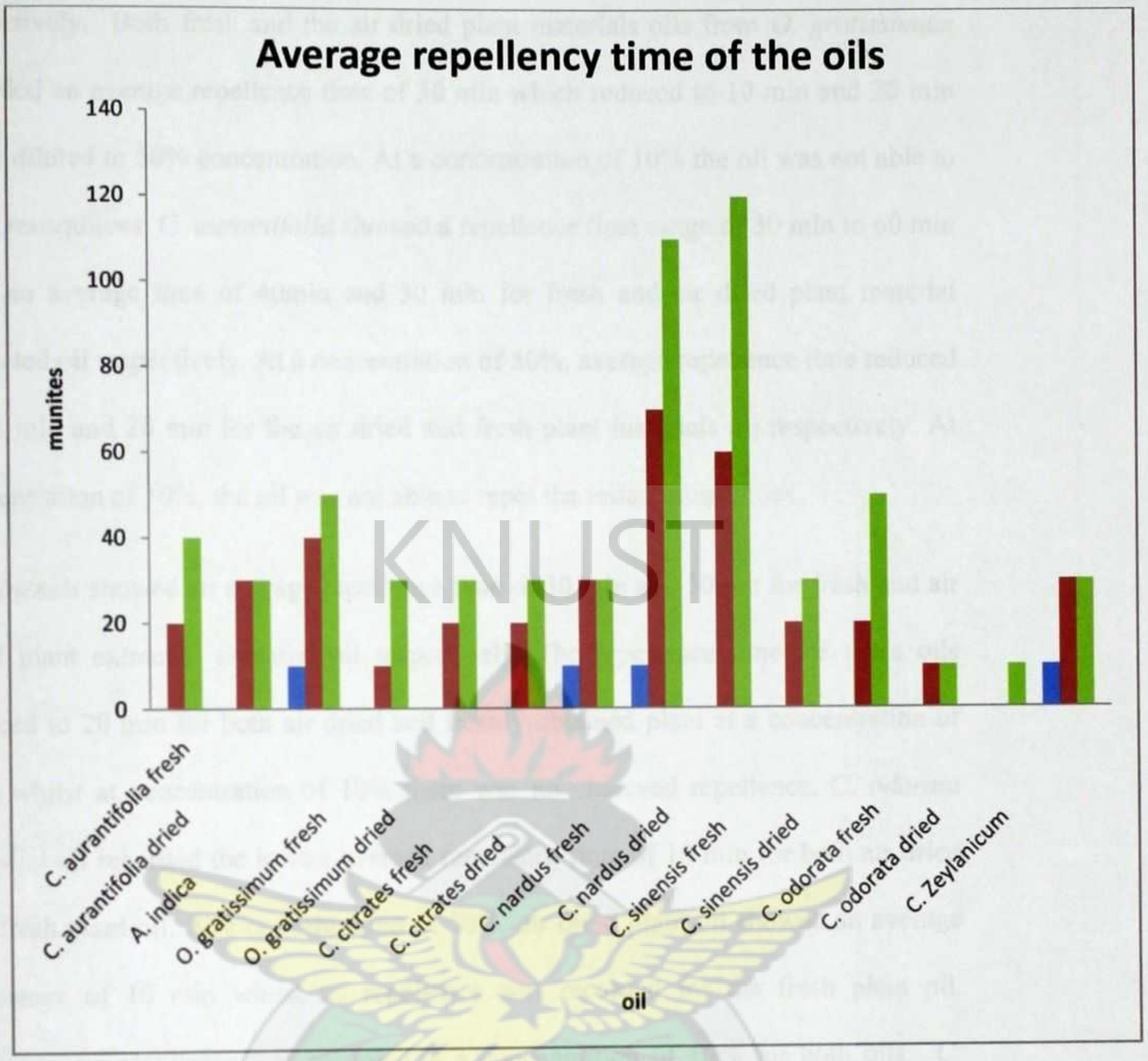

Fig 4.2 Bar chart showing repellency time for oils from both fresh and air dried plants materials (Red indicates 50% oil in ethanol, blue indicates 10% of oil in ethanol and green indicates undiluted oil).

Of all the plant oils tested, *C. nardus* essential oil showed the highest repellence within the range of 90 to 120 minutes against the tested mosquitoes. The repellence reduced when the oil was diluted to a concentration of 50% with an average repellence of 70min and 60min for fresh and air dried plant materials oil respectively. *A. indica* showed a repellence time range of 30 to 60 minutes with an average time of 50 min which reduced to 40 min and 10 min when diluted to 50% and 10%

respectively. Both fresh and the air dried plant materials oils from *O. gratissimum* recorded an average repellence time of 30 min which reduced to 10 min and 20 min when diluted to 50% concentration. At a concentration of 10% the oil was not able to repel mosquitoes. *C. aurantifolia* showed a repellence time range of 30 min to 60 min with an average time of 40min and 30 min for fresh and air dried plant material extracted oil respectively. At a concentration of 50%, average repellence time reduced to 30 min and 20 min for the air dried and fresh plant materials oil respectively. At concentration of 10%, the oil was not able to repel the tested mosquitoes.

C. sinensis showed an average repellence time of 30 min and 50min for fresh and air dried plant extracted essential oil respectively. The repellence time for these oils reduced to 20 min for both air dried and freshly obtained plant at a concentration of 50% whilst at concentration of 10% there was no observed repellence. *C. odorata* essential oil recorded the lowest average repellence time of 10 min for both air dried and fresh plant oil. At a concentration of 50%, air dried plant oil showed an average repellence of 10 min whilst no repellence was recorded for the fresh plant oil. However, no repellence was recorded at a concentration of 10% for both oils. *C. zeylanicum* essential oil recorded an average repellence of 30min for both 50% concentration and undiluted. At concentration of 10% an average repellence time of 10min was recorded for the oil. The undiluted oil showed the highest repellence in each case whilst the 10% concentration also showed the lowest repellence time for the tested essential oil. *C. citrates* oil was able to repel the tested mosquitoes for 30min which was recorded for both air dried and the fresh plants oil with results in agreement with the work of Trongtokit *et al*., 2005.

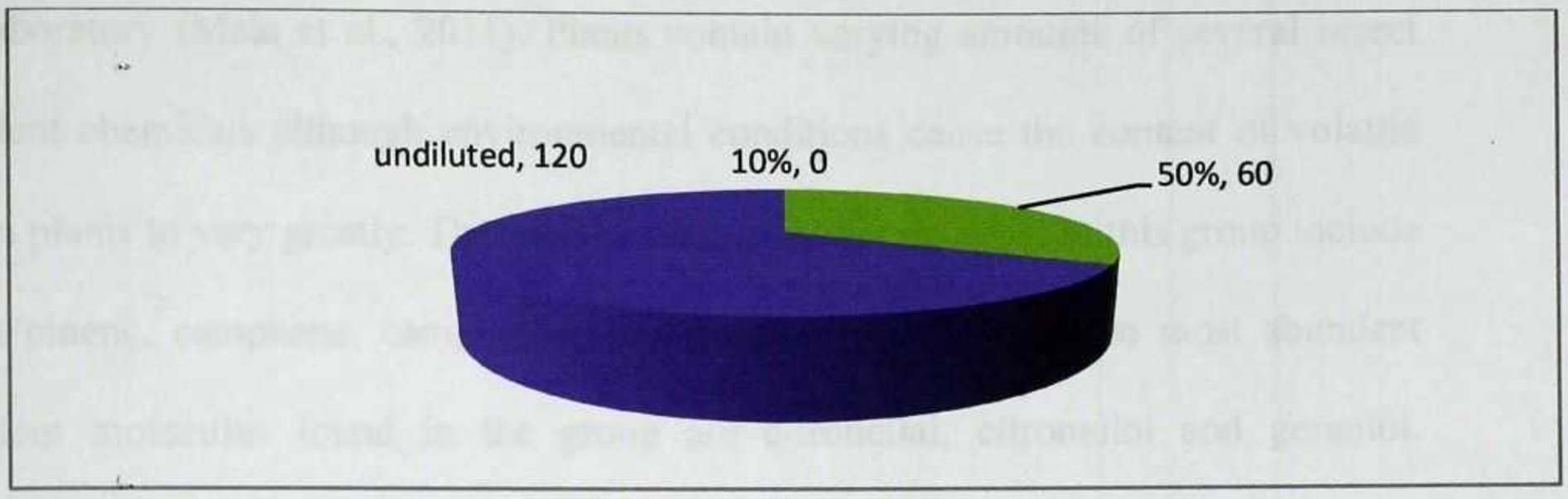

Fig 4.3 Pie chart showing mosquitoes repellence of undiluted air dried *C. nardus* oil

Repellency evaluation is preferably carried out using human subjects, as testing repellents on animals or artificial membranes may not give representative data of how the repellent will perform when applied to a human skin (Trongtokit *et al.*, 2005). The studies evaluated the repellent activities of 14 essential oils against the African malaria mosquito, *Anopheles gambiae* which is an especially important vector because of it's highly anthropophilic and a very efficient carrier of the most potent malariă parasite, *Plasmodium falciparum.* The test showed that out of the 14 undiluted essential oils, the most effective oils were from fresh leaves of *C. nardus,* air dried leaves of *C. nardus,* air dried oil peel of *C. sinensis* and dried seeds of *A. indica.* For all the oils tested, their repellence activity was concentration dependent and this is in agreement with Trongtokit et al., 2005. The results obtained from the repellence test indicated that there was not much difference between the repellence time recorded for both oils from fresh and air dried plant materials. In South Africa, *C. excavatus* gave 100% repellency for 2 h, when it was evaluated in the laboratory against *A. arabiensis* and its repellency decreased to 59.3% after 4 h (Govere *et al.*, 2000*). C. nardus* essential oil applied topically gave a 100% protection against *A.aegypti* for 120 min, 100% protection against *C. quinquefasciatus* for 100 min and a 100% protection against *A.dirus* for 70 minutes when its repellency was evaluated in

the laboratory (Maia et al., 2011). Plants contain varying amounts of several insect repellent chemicals although environmental conditions cause the content of volatile oils in plants to vary greatly. The repellent compounds contained in this group include alpha pinene, camphene, camphor, geraniol and terpenen-4-ol. The most abundant repellent molecules found in the group are citronellal, citronellol and geraniol. Derivative of citronella (a mono-terpene aldehyde), is the main constituent of citronella oil and has been used as the active ingredient of commercial repellents (Trongtokit *et al*., 2005). Citronella is one of the most widely used natural repellents on the market, used at concentrations of 5-10% (Maia et al., 2011). Neem is widely advertised as a natural alternative to DEET and it has been tested for repellency against range of arthropods of medical importance, with variable results. Direct burning of the leaves of the plant gave a 76% protection against mosquitoes' for 2 hours (Palsson et al., 1999) also a thermal expulsion of leaves gave a 24% against *A. gambiae* (Maia et al., 2011). Topical application of 2% neem oil mixed in coconut oil produced varying degree of protection against different vector species and the repellent effect was more pronounced against Anopheles spp than against *Cx. Quinquefaciatus*. A complete protection for 12 h from the bites of all the anopheline mosquitoes species was reported by using 2% neem oil in coconut oil on the exposed part of the body (Mittal et al., 2003). DEET has been reported to offer up to 12 hours protection for 100 % concentration and also 3-6 hours protection at a concentration of 20 % - 30 % against *Anopheles gambia* (williamson, 2002) this suggest that the repellents abilities of the tested essential oils were below that of DEET.

4.4 ANTIMICROBIAL SCREENING OF THE HYDROSOLS

Table 4.4.1 pH and conductivity of plants hydrosols

	Fresh		Dried	
Plant hydrosols	**pH**	**Conductivity (µs)**	**pH**	**Conductivity (µs)**
C. aurantifolia	2.34	132.8	2.63	119.5
O. gratissimum	2.74	160.0	7.38	614.0
C. citrates	2.54	233.0	2.41	730.0
C. nardus	3.53	25.2	5.35	173.9
C. sinensis	3.69	27.8	3.24	62.1
C. odorata	2.46	106.1	2.19	80.6

Table 4.4.2 Result of antimicrobial screening of hydrosols (inhibition zone in mm)

Oil source	***E. Coli***	***S. aureus***	***C. albicans***	***B. sub***	***E. faeculis***
C. nardus (dried leaves)	-	-	-	-	-
C. nardus (fresh leaves)	-	-	-	-	-
C. sinensis (dried peels)	-	-	-	-	-
C. aurantifolia (dried leaves)	-	1	-	-	-
C. odorata (fresh leaves)	-	1	-	3	-
C. odorata (dried leaves)	-	-	-	-	-
C. aurantifolia (fresh peels)	4	5	2	4	1
C. sinensis (fresh peels)	-	-	-	-	-
C. citrates (dried leaves)	3	3	2	6	-
O. gratissimum (dried leaves)	2	1	1	-	1
C. citrates fresh (leaves)	-	-	-	-	-
O. gratissimum (fresh leaves)	2	2	1.5	2	-

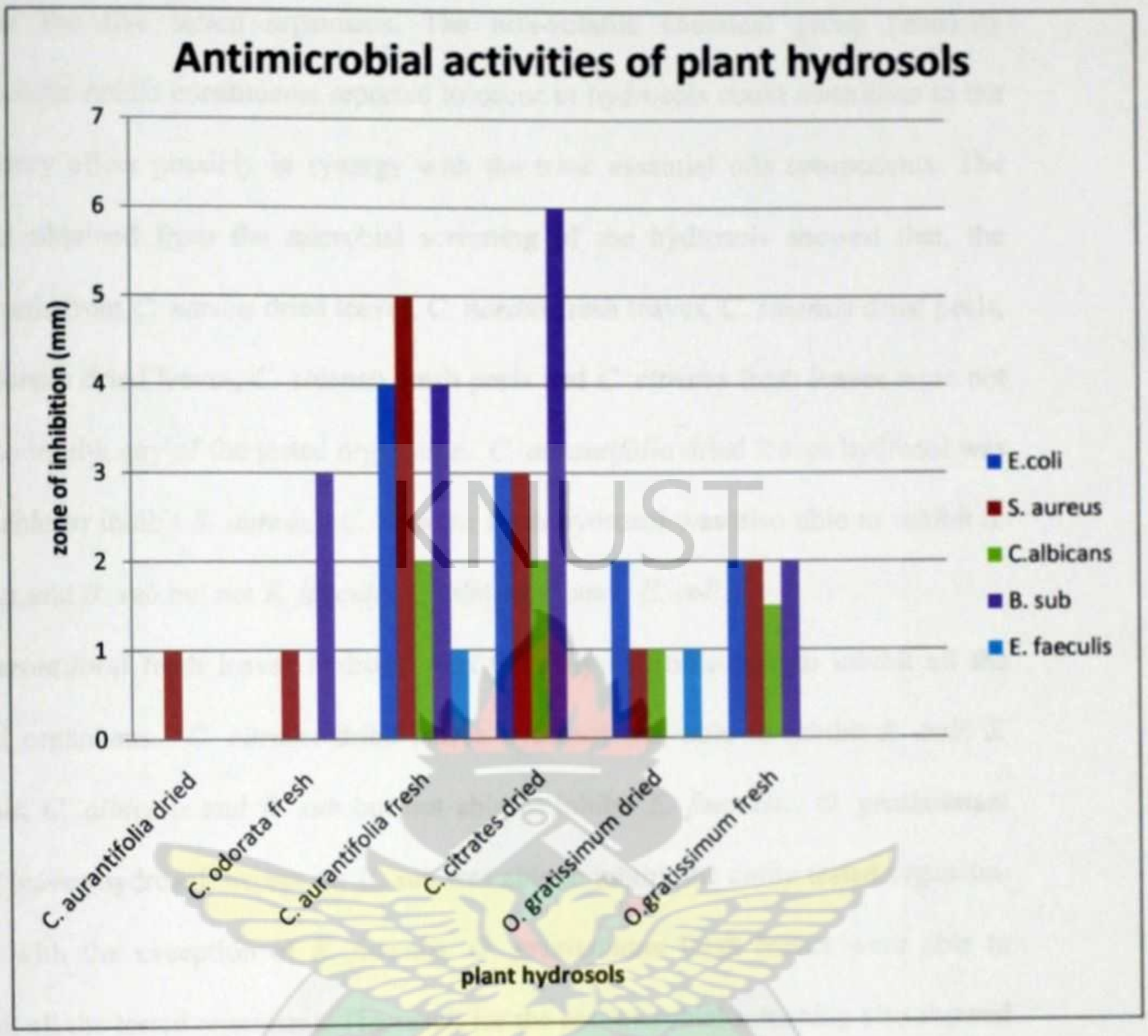

Fig 4.4 Antimicrobial activities of the plant hydrosols

Many naturally occurring extracts like essential oils have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Oral et al., 2008). It is known that the compositions of hydrosols and their antimicrobial effects depend on plant species and regional conditions (Oral et al., 2008). Several studies have been conducted on the antimicrobial properties of herbs, spices and their derivatives such as essential oils, extracts and decoctions but attention has not been focused intensively on studying antimicrobial effect of plant hydrosols. The results obtained from this work shows

that hydrosols from the plants studied exerted varying levels of antimicrobial effect against the five tested organisms. The non-volatile chemical group primarily hydrophilic acidic constituents reported to occur in hydrosols could contribute to the inhibitory effect possibly in synergy with the trace essential oils components. The results obtained from the microbial screening of the hydrosols showed that, the hydrosols from *C. nardus* dried leaves, C. *nardus* fresh leaves, *C. sinensis* dried peels, *C. odorata* dried leaves, *C. sinensis* fresh peels and *C. citrates* fresh leaves were not able to inhibit any of the tested organisms. *C. aurantifolia* dried leaves hydrosol was only able to inhibit *S. aureus*. *C. odorata* fresh hydrosol was also able to inhibit *S. aureus* and *B. sub* but not *E. faeculis*, *C. albicans* and *E. coli*. *C. aurantifolia* fresh leaves hydrosol was the only hydrosol able to inhibit all the tested organisms. *C. citrates* dried leaves hydrosol was able to inhibit *E. coli*, *S. aureus*, *C. albicans* and *B. sub* but not able to inhibit *E. faeculis*. *O. gratissimum* dried leaves hydrosol, except for B. sub was able to inhibit the entire tested organism. Also with the exception of *E. faeculis*, *O. gratissimum* fresh leaves were able to inhibit all the tested organisms. The result for the antimicrobial screening also showed differences in levels of inhibition between fresh and dried material hydrosol of the same plant. Fresh *C. aurantifolia* was able to inhibit the entire tested organism as compared to the dried *C. aurantifolia* which was only able to inhibit *S. aureus*.

Nascimento et al. investigated inhibitory effect of plant extracts on antibiotic resistant bacteria and reported that thyme (*Thymus vulgaris*), clove (*Caryophyllus aromaticus*) and basil (*Ocimum basilicum*) were effective against *P.aeruginosa* but they did not affect *E. coli*. It has been demonstrated that the antimicrobial effects of the essential oils act by causing structural and functional damages to the bacterial cell membrane (Goni et al., 2009). Hydrosols are either neutral or slightly acid and the pH greatly

influences the therapeutic effects. The pH values obtained for the plant hydrosols indicated that the hydrosols were acidic which was in agreement with Catty, 2001. There was an observable difference in pH and conductivity between the fresh plant hydrosol and that of the dried hydrosol of the same plant species which is indicative of difference in therapeutic effects.

4.5 TLC FINGER PRINT OF THE OILS

Chromatography involves the separation of mixture of substances flowing across or through absorbing medium. Conditions are chosen such that components of the fluid phase interact reversibly with the stationary phase to different events, thus effecting a separation. A number of solvent systems were used in this work to resolve the constituents of the oil. Three of the solvent systems which gave the best resolution and their corresponding chromatogram R_F are shown in tables 4.5.1 to 4.5.8.

With exception of *C. odorata* which showed a difference between the fresh and dried oil, there were no observable differences between the TLC chromatograms of both fresh and dried extracted oil from the plants indicating the similarity in chemical compositions between the two. Pet-ether in combination with ethyl acetate (92:8) was the most appropriate solvent used since it was able to give a distinct resolution followed by pet-ether in combination with Choroform in the ration 80:20. The various R_F values for each solvent system for the oils have been calculated and are indicated in the tables below.

4.5.1 TLC finger print of *Cinnamomum Zeylanicum*

Mobile phases

Pet-ether/ Choroform 85: 15 **pet-ether/ Choroform 80:20** **pet-ether/ ethyl acetate 92:8**

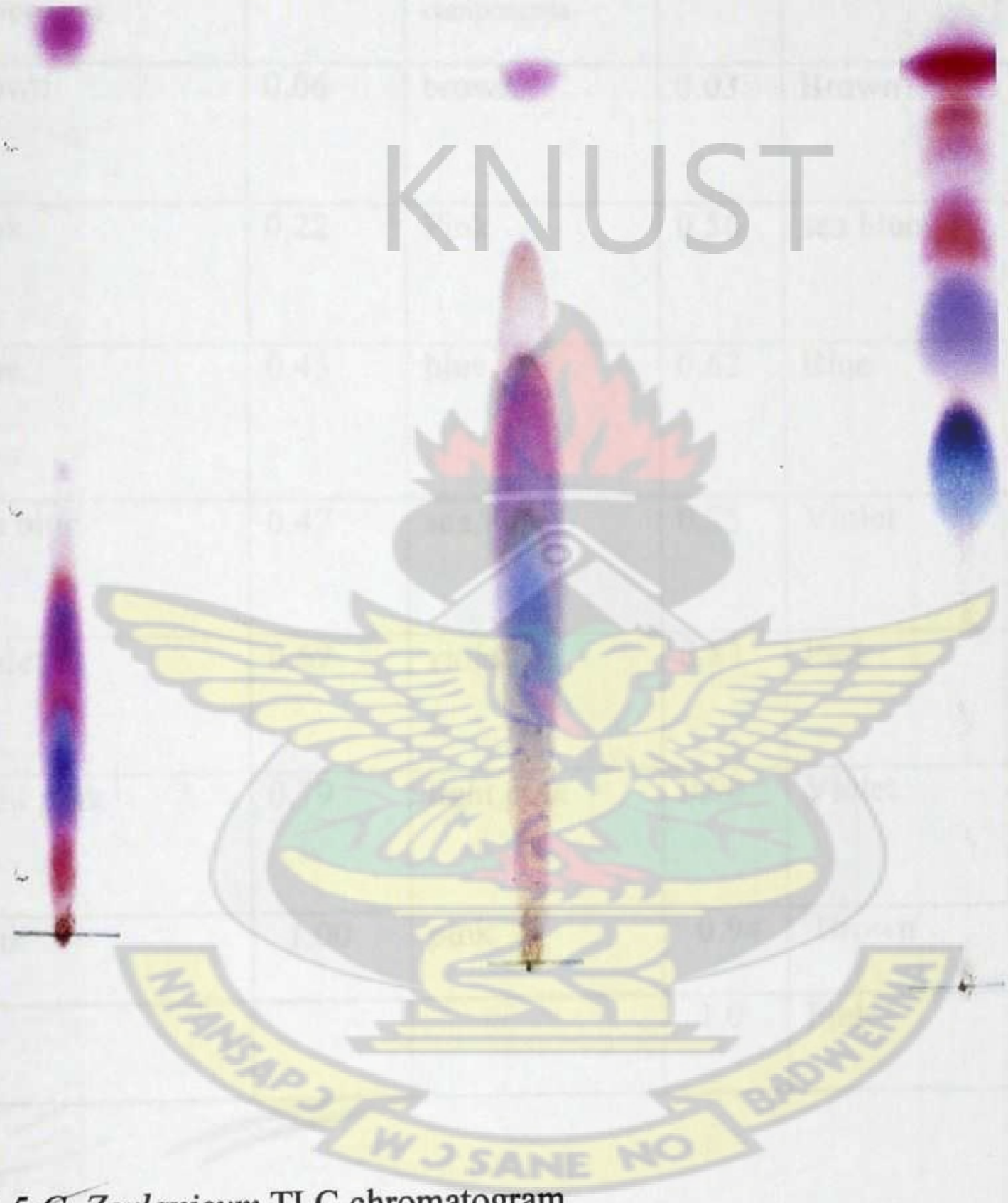

Fig 4.5 *C. Zeylanicum* TLC chromatogram

Table 4.5.1 R_F values for *C. Zeylanicum*

pet-ether/Choroform (85: 15)		pet-ether/Choroform (80: 20)		pet-ether/ethyl acetate (92:8)	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.03	brown	0.06	brown	0.03	Brown
0.11	Pink	0.22	Pink	0.56	sea blue
0.20	blue	0.45	blue	0.62	Blue
0.24	sea blue	0.47	sea blue	0.75	Violet
0.36	violet	0.67	violet	0.83	Pink
0.49	light pink	0.79	light pink	0.90	Violet
1.00	pink	1.00	pink	0.94	Brown
				1.0	Pink

4.5.2 TLC finger print of *Citratus aurantifolia*

Mobile phases

Pet-ether/Choroform 85:15 **pet-ether/Choroform 80:20** **pet-ether/ ethyl acetate 92:8**

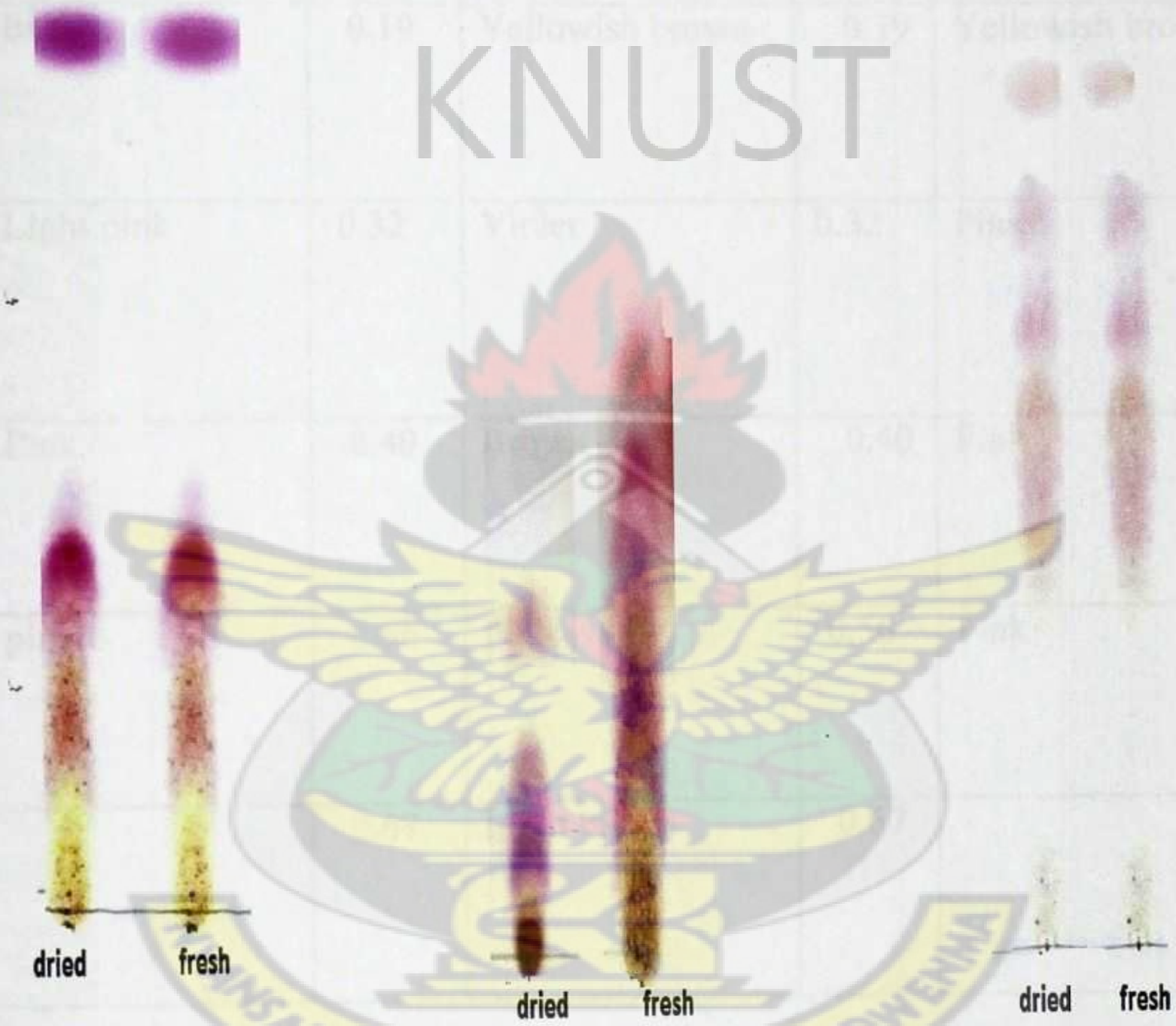

Fig 4.6 *C. aurantifolia* TLC chromatogram

Table 4.5.2 R_F values for *C. aurantifolia*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.14	Orange	0.01	Brown	0.10	Orange
0.23	Brown	0.19	Yellowish brown	0.19	Yellowish brown
0.35	Light pink	0.32	Violet	0.32	Pink
0.42	Pink	0.40	Brown	0.40	Pink
1.00	pink	0.56	Pink	0.56	Pink
		0.67	Light brown	0.67	

4.5.3 TLC finger print of *O. gratissimum*

Fig 4.7 *O. gratissimum* TLC chromatogram

Table 4.5.3 R_F values for *O. gratissimum*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.04	Pink	0.04	Brown	0.03	Pink
0.25	Pink	0.40	Violet	0.43	Pink
0.46	Pink	0.65	Pink	0.56	Pink
1	Violet	0.71	Red	0.88	Red
		1	violet	0.95	Pink
				1	Violet

4.5.4 TLC finger print of *C. nardus*

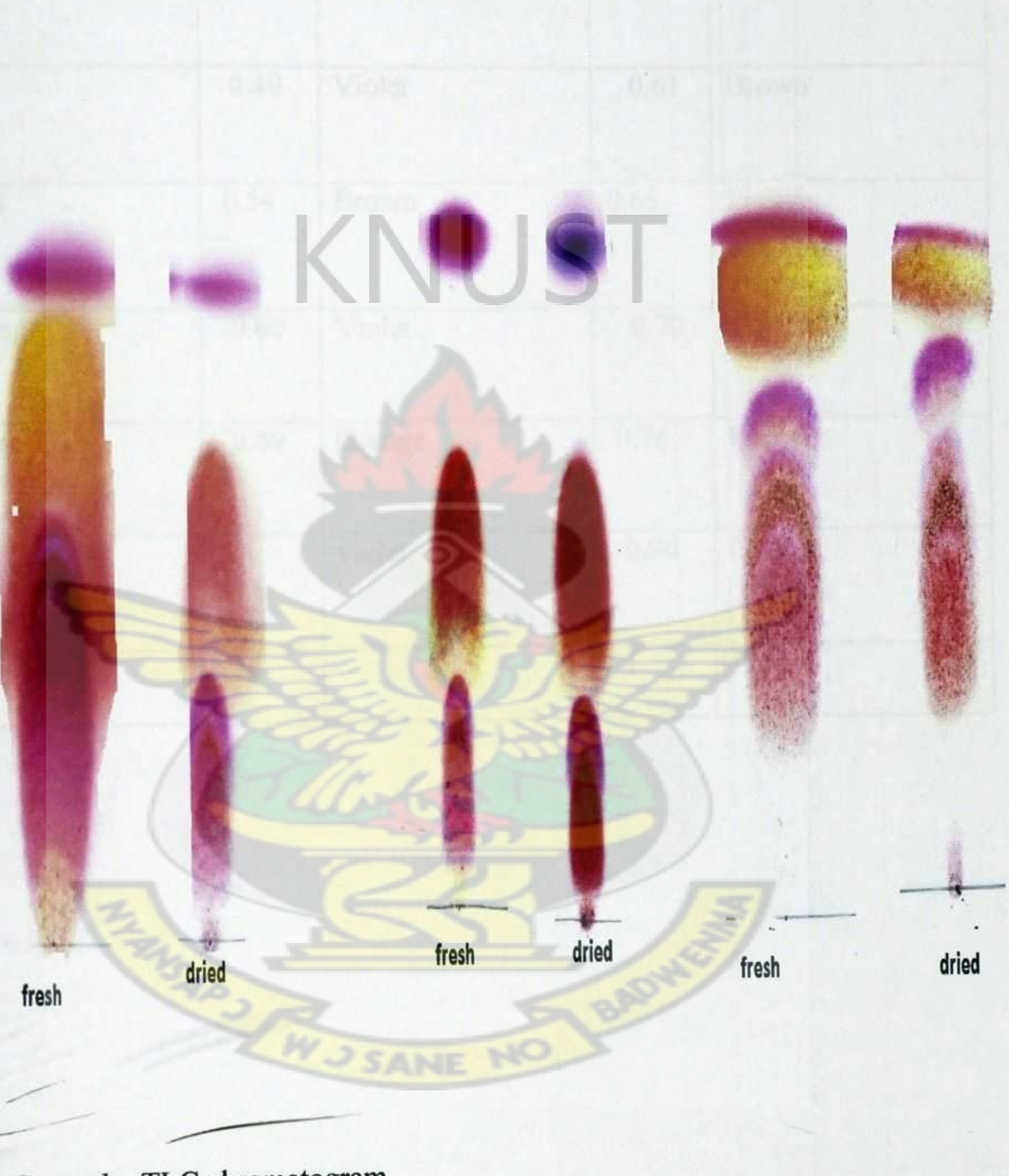

Fig 4.8 *C. nardus* TLC chromatogram

Table 4.5.4 R_F values for *C. nardus*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.25	Pink	0.13	Brown	0.54	Pink
0.28	Red	0.49	Violet	0.61	Brown
0.33	Pink	0.54	Brown	0.65	Pink
0.67	Orange	0.60	Violet	0.70	Orange
1	pink	0.89	Orange	0.76	Pink
		1	violet	0.96	Orange
				1	Pink

4.5.5 TLC finger print of *C.citrates*

Mobile phases		
Pet-ether/Choroform 85:15	**Pet-ether/Choroform 80:20**	**pet-ether/ ethyl acetate 92:8**

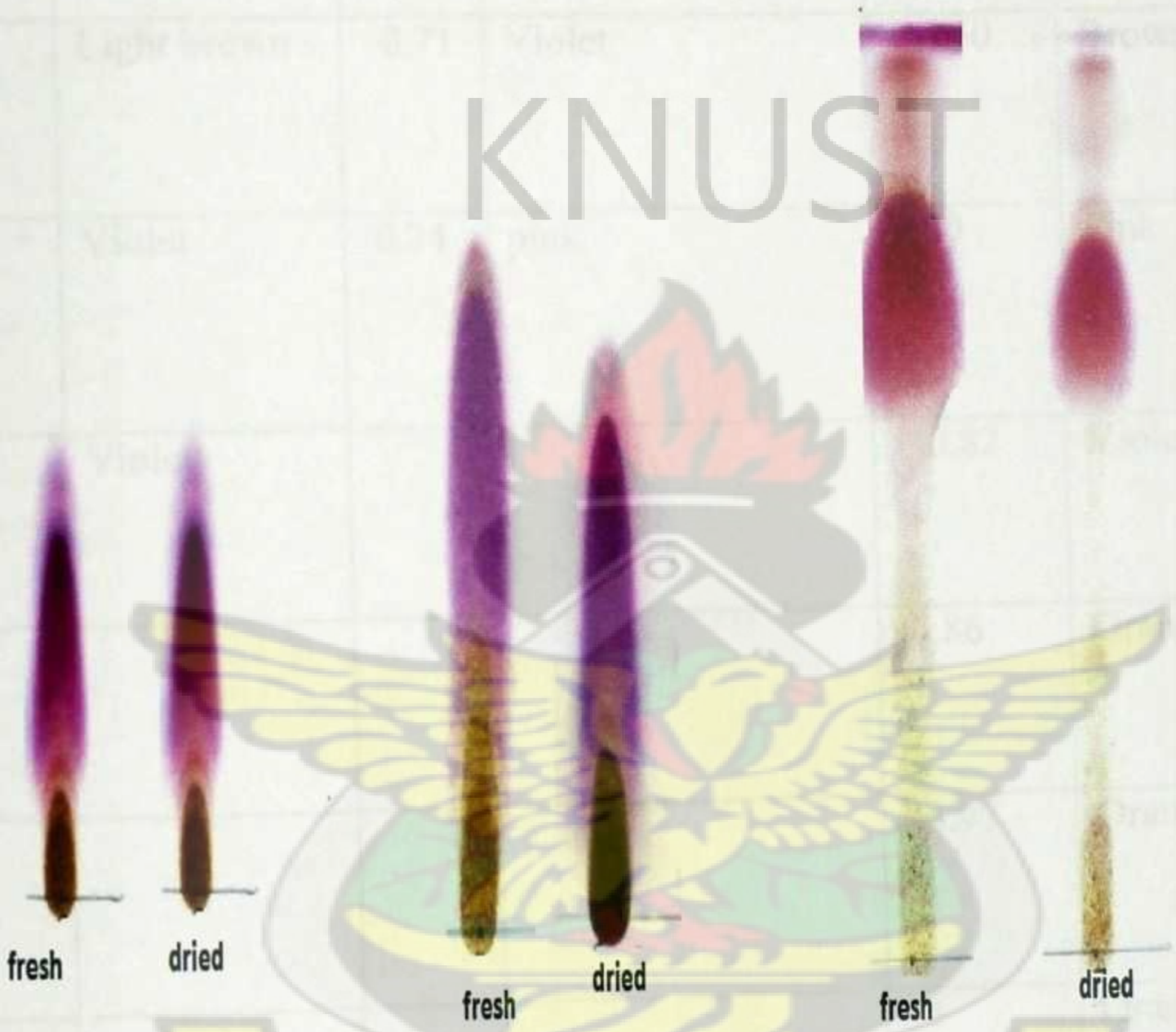

Fig 4.9 *C.citrates* TLC chromatogram

Table 4.5.5 R_F values for *C.citrates*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.12	Brown	0.31	Brown	0.15	Brown
0.14	Light brown	0.71	Violet	0.40	Brown
0.43	Violet	0.74	pink	0.60	Pink
0.49	Violet			0.82	Violet
				0.86	Orange
				0.97	Orange
				1	Orange

4.5.6 TLC finger print of *C. odorata*

Mobile phases

Pet-ether/Choroform 85:15 **Pet-ether/Choroform 80:20** **pet-ether/ ethyl acetate 92:8**

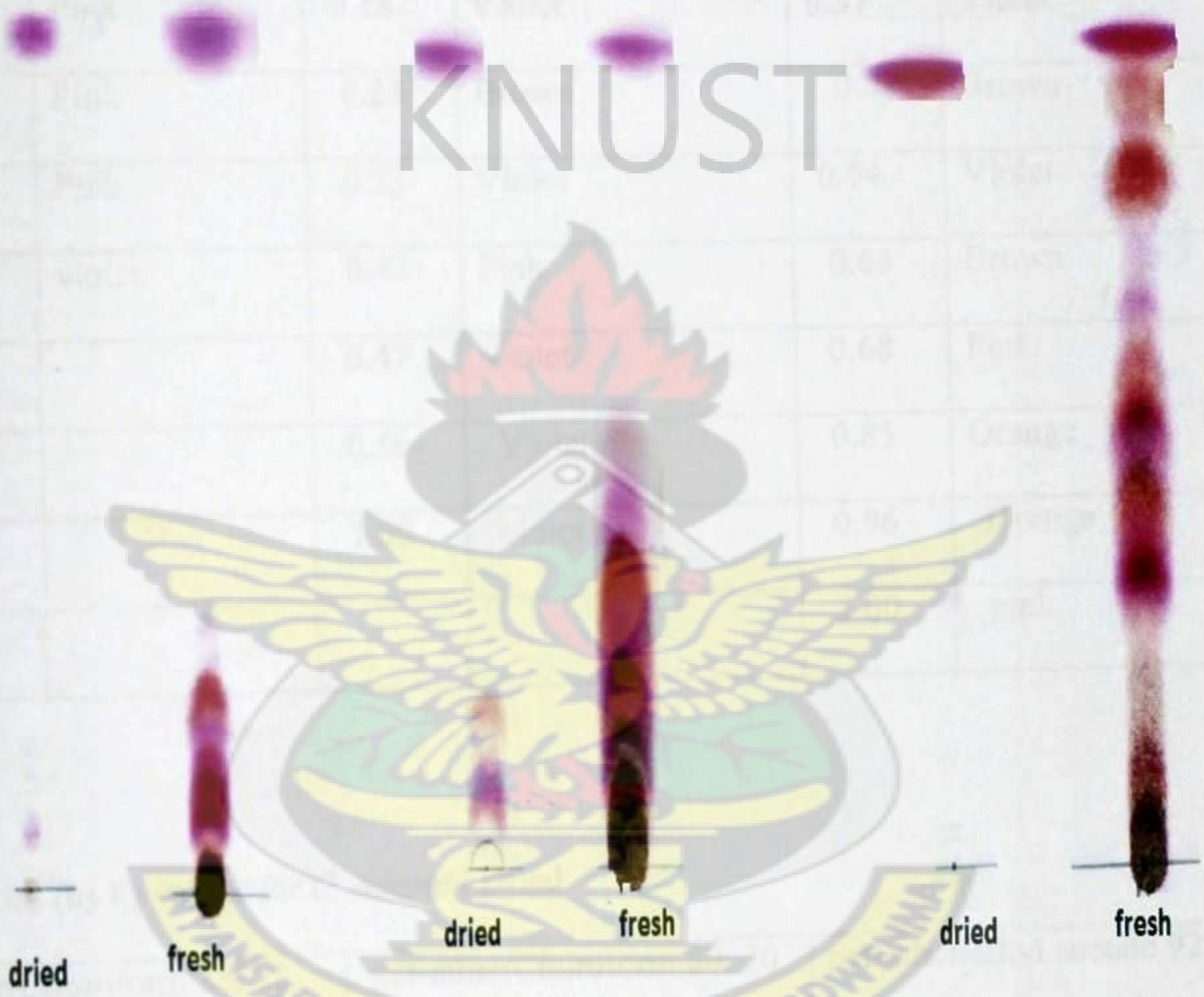

Fig 4.10 *C. odorata* TLC chromatogram

Table 4.5.6 (a) R_F values for fresh *C. odorata*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.05	Deep brown	0.09	Deep brown	0.06	Deep brown
0.10	Violet	0.15	Brown	0.14	Brown
0.14	Pink	0.18	Violet	0.37	Violet
0.17	Pink	0.23	Brown	0.48	Brown
0.26	Pink	0.33	Violet	0.54	Violet
1.00	violet	0.42	Pink	0.63	Brown
		0.47	Violet	0.68	Pink
		0.56	Violet	0.85	Orange
		1.00	violet	0.96	Orange
				1.00	pink

Table 4.5.6 (b) R_F values for *C. odorata* Dried

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.06	pink	0.12	Pink	0.03	pink
1.00	Pink	0.20	Pink	0.36	Pink
		1.00	pink	1.00	Pink

4.5.7 TLC finger print of *A. indica*

Mobile phases		
Pet-ether/Choroform 85:15	**Pet-ether/Choroform 80:20**	**pet-ether/ ethyl acetate 92:8**

Fig 4.11 *A. Indica* TLC chromatogram

Table 4.5.7 R_F values for *A. Indica*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.02	Brown	0.02	Brown	0.26	Pink
0.34	Violet	0.08	Pink	0.95	Pink
1.00	pink	0.55	Pink	1.00	pink
		0.58	Pink		
		0.73	Pink		
		1.00	Pink		

4.5.8 TLC finger print of *C.sinensis*

Mobile phases

Pet-ether/Choroform 85:15 **Pet-ether/Choroform 80:20** **pet-ether/ ethyl acetate 92:8**

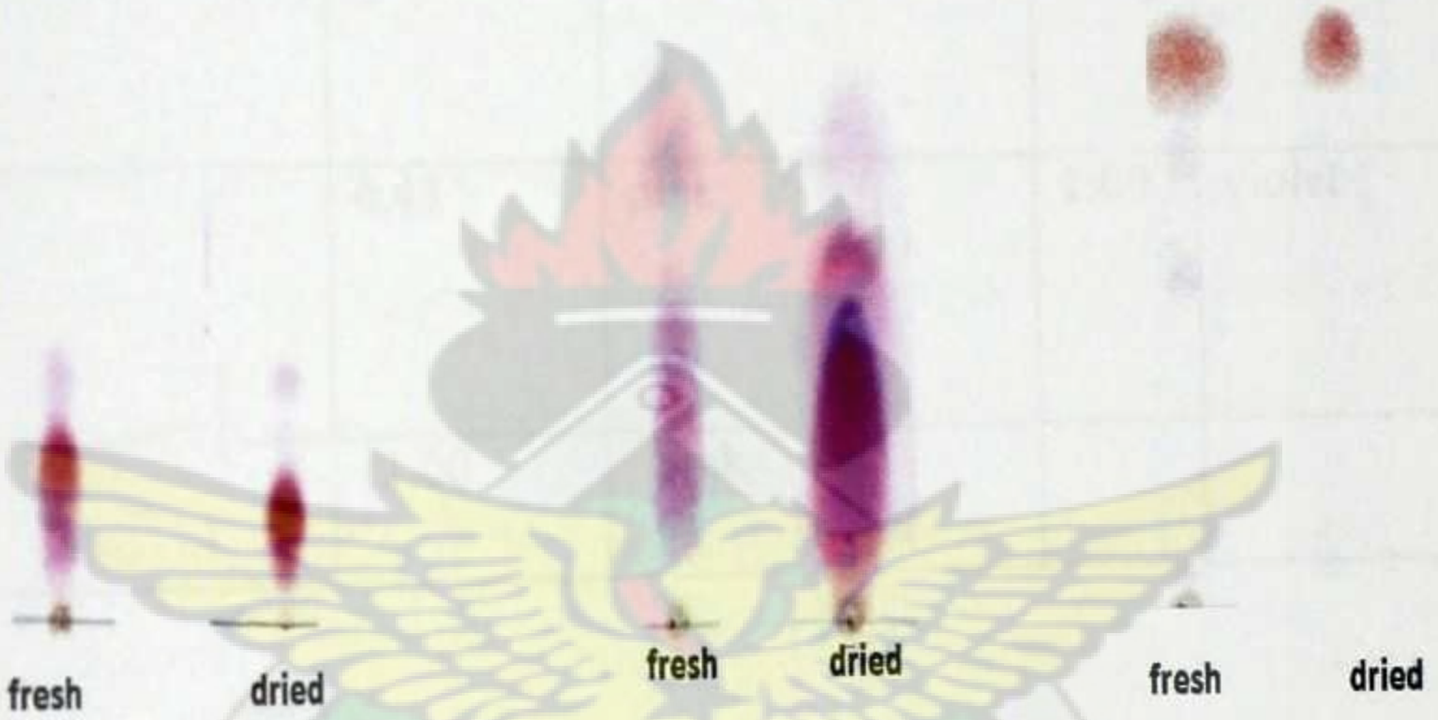

Fig 4.12 *C.sinensis* TLC chromatogram

Table 4.5.8 R_F values for *C.sinensis*

pet-ether/Choroform 85: 15		pet-ether/Choroform 80: 20		pet-ether/ethyl acetate 92:8	
R_F	Colour of components	R_F	Colour of components	R_F	Colour of components
0.13	Pink	0.32	Brown	0.56	Brown
1.00	pink	0.35	Violet	0.94	Pink
		0.43	pink	1.00	violet
		1.00	pink		

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The percentage essential oil content obtained for each plant showed that dried plant material yielded higher amount of oil as compared to the fresh material of the same plant species. Among the eight plants species considered for the work, peels of dried *C. sinensis* yielded the highest oil content of 3.64% whereas the fresh leaves of *C. odorata* was the plant that recorded the lowest yield of 0.26%.

The repellency test conducted on the essential oils from the eight local plants revealed *C. nardus* oil having the longest repellence time of 120 minutes. C. odorata was also the plant that showed the lowest repellence of 10 minutes. There was however, no significant difference in the repellence time between the fresh and dried plant material of the same plant species.

The plant hydrosols exhibited varying levels of inhibition against five tested microbes; *E. Faeculis, B. sub, C. albicans, S. aureus and E. coli.* Hydrosol from fresh leaves of *C. aurantifolia* was the only one which inhibited the entire tested organisms.

The TLC profile of the oils suggested that among all the solvent systems employed petroleum ether in combination with ethyl acetate in the ratio of 92: 8 was the best system for the resolution of the component of the oils. The various R_F values calculated revealed that with the exception of dried and fresh C. odorata, there was no significant difference in the TLC chromatogram of the rest (comparing the fresh and dried plant material) an indication of similarities in chemical compositions.

5.2 RECOMMENDATION

Based on the findings of the study, it is recommended that

1. GC-MS or HPLC is used in the identification of component of the various essential oils used in the work.
2. Chemical analysis should be conducted on the plants hydrosols in order to establish their chemical composition and properties.
3. Oils from *C. nardus*, *C.sinensis* and *A.indica* should be formulated into ointment, lotion, aerosols etc and their repellence determined for commercial purposes.

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APPENDICE

Appendix 1: table of distance moved by oil components

Appendix 1.1 *Cinnamomum Zeylanicum*

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
0.3	9.8
0.11	9.8
0.20	9.8
0.24	9.8
0.36	9.8
0.49	9.8
1	9.8

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
0.6	9.7
2.1	9.7
4.4	9.7
4.6	9.7
6.5	9.7
7.7	9.7
9.7	9.7

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
0.3	10.0
5.7	10.0
6.3	10.0
7.6	10.0
8.4	10.0
9.1	10.0
9.5	10.0
10.0	10.0

Appendix 1.2 *Citrus aurantifolia*

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
1.4	10.0
2.3	10.0
3.5	10.0
4.2	10.0
10.0	10.0

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
1.0	10.0
6.5	10.0
7.4	10.0
8.5	10.0
9.9	10.0

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
0.4	10.0
0.6	10.0
1.5	10.0
2.4	10.0
3.9	10.0
5.9	10.0

Appendix 1.3 *Ocimum gratissimum*

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
0.4	10.3
2.6	10.3
4.7	10.3
10.3	10.3

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
0.4	10.0
4.0	10.0
6.5	10.0
7.1	10.0
10.0	10.0

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
0.3	10.5
4.5	10.5
5.9	10.5
9.3	10.5
10.0	10.5
10.5	10.5

Appendix 1.4 *Cymbopogon nardus*

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
1.3	9.9
4.9	9.9
5.4	9.9
6.0	9.9
8.9	9.9
9.9	9.9

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
2.5	10.0
2.8	10.0
3.3	10.0
6.7	10.0
10.0	10.0

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
5.5	10.1
6.2	10.1
6.6	10.1
7.1	10.1
7.7	10.1
9.7	10.1
10.1	10.1

Appendix 1.5 *Cymbopogon citrates*

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
1.2	10.0
1.4	10.0
4.3	10.0
4.9	10.0

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
3.2	10.0
7.2	10.0
7.5	10.0

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
1.6	10.5
4.2	10.5
6.3	10.5
8.6	10.5
9.0	10.5
10.2	10.5
10.5	10.5

Appendix 1.6 *Azadirachta indica*

Mobile phase: Pet-ether/Choroform 85: 15

Distance move by component	Distance move by solvent
0.2	9.6
3.3	9.6
9.6	9.6

Mobile phase: Pet-ether/Choroform 80: 20

Distance move by component	Distance move by solvent
0.2	9.6
0.8	9.6
5.3	9.6
5.6	9.6
7.1	9.6
9.6	9.6

Mobile phase: Pet-ether/ethyl acetate 92:8

Distance move by component	Distance move by solvent
2.5	9.6
9.1	9.6
9.6	9.6

Appendix 1.7 *Chromolaena odorata*

Mobile phase: Pet-ether/Choroform 85: 15

Fresh plant		Dried plant	
Distance move by component	Distance move by solvent	Distance move by component	Distance move by solvent
0.5	10.6	0.6	10.6
1.1	10.6	10.6	10.6
1.5	10.6		
1.8	10.6		
2.8	10.6		
10.6	10.6		

Mobile phase: Pet-ether/Choroform 80: 20

Fresh plant		Dried plant	
Distance move by component	Distance move by solvent	Distance move by component	Distance move by solvent
0.9	10.1	1.3	10.1
1.5	10.1	2.1	10.1
1.8	10.1	10.1	10.1
2.3	10.1		
3.3	10.1		
4.2	10.1		
4.7	10.1		
5.6	10.1		
10.1	10.1		

Mobile phase: Pet-ether/ethyl acetate 92:8

Fresh plant		Dried plant	
Distance move by component	Distance move by solvent	Distance move by component	Distance move by solvent
0.6	10.4	0.3	10.0
1.5	10.4	3.6	10.0
3.9	10.4	10.0	
5.0	10.4		
5.6	10.4		
6.6	10.4		
7.1	10.4		
8.9	10.4		
10.0	10.4		
10.4	10.4		

APPENDIX 2: Smell grades of the oils

OIL	0	1	2	3	4	5	6	7	8	9	10
Fresh *Citrus aurantifolia*								++	+		
Dried *Citrus aurantifolia*								+	++		
Fresh *Chromolaena odorata*	+++										
Dried *Chromolaena odorata*	+++										
Fresh *Citrus sinensis*						+	+		+		
Dried *Citrus sinensis*					+	+		+			
Fresh *Cymbopogon nardus*						+			+	+	
Dried *Cymbopogon nardus*						+	+			+	
Fresh *Ocimum gratissimum*		+	+	+							
Dried *Ocimum gratissimum*			++	+							
Fresh *Cymbopogon nardus*								++	+		
Dried *Cymbopogon nardus*								+	++		
Cinnamomum Zeylanicum					+ +		+				
Azadirachta indica	+	++									

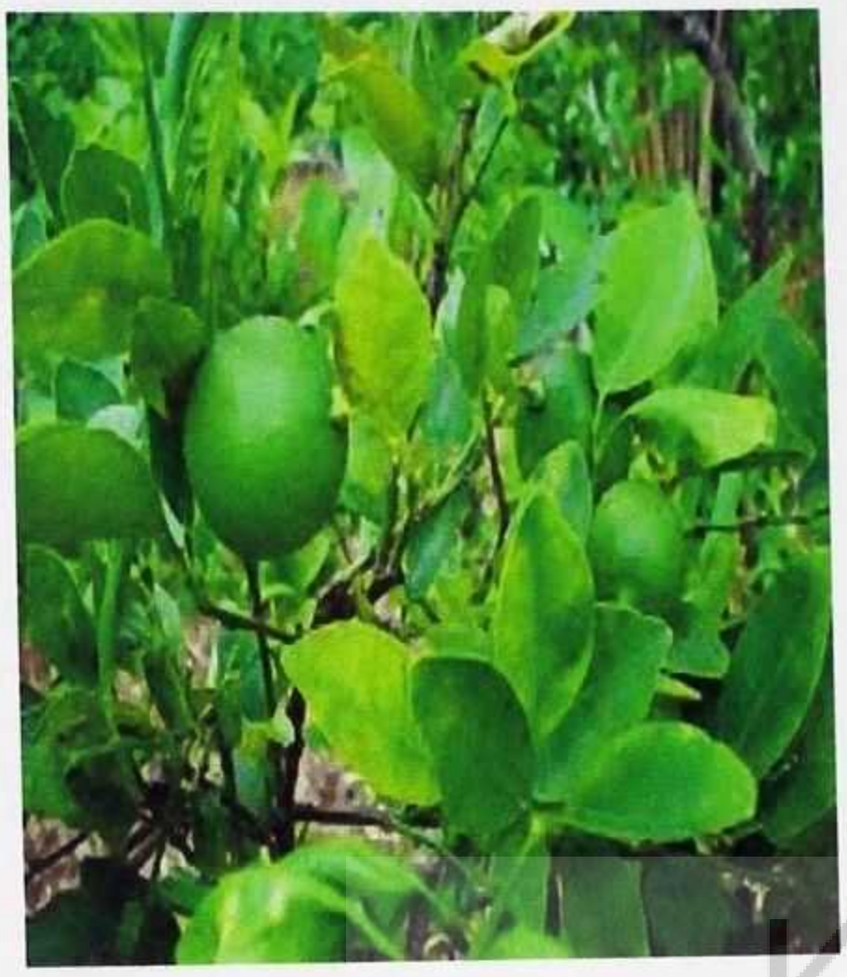
APPENDIX 3: Pictures of Plants used

Cymbopogon citrates

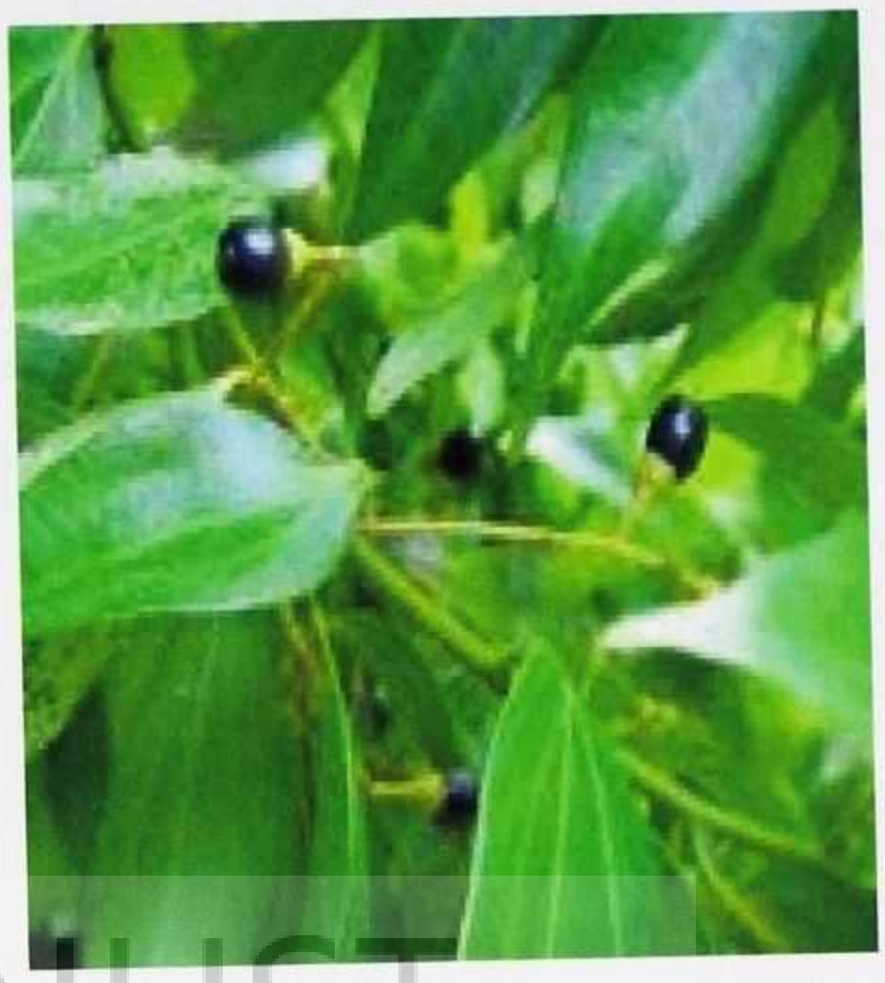
Azadirachta indica

Cymbopogon nardus

Ocimum gratissimum



Citrus aurantifolia



Cinnamomum Zeylanicum

Citrus sinensis

chromolaena odorata