# Isolation, Characterisation, and biological activity evaluation of essential Oils of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy and *Hyparrhenia hirta* (L.) Stapf


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**A dissertation Submitted in Fulfillment for MSc. Degree in Organic Chemistry.**

**In the Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice**

**By**

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# DECLARATION

I declare that this dissertation that I here submit for the award of the degree of Masters of Science in Chemistry

is my original work apart from the acknowledged assistance from my supervisors. It has not been submitted to any university other than the University of Fort Hare (Alice).

Student signature…..…..... Date……

Supervisor's signature……. Date……

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# CONFERENCE AND PUBLICATIONS

## Conferences

1. PP Rungqu, OO Oyedeji, L Mlisa, V Hobololo, BN Chungag, SO Oluwafemi, SP Songca and AO Oyedeji: Use of *Cymbopogon* species in traditional medicine and its essential oils compositions for improved value chain. Poster presentation at the 41st National Convention of the South African Chemical Institute (SACI) Conference, 1-6th December, East London South Africa.

## Publications

1) P.P. Rungqu[1], O. Avoseh[1], K Aremu[2], B. N. Nkeh-Chungag[2], S. P. Songca[3], O.Oluwafemi[4], O. O. Oyedeji*[1] and A,O, Oyedeji. (2015). Anti-inflammatory activity of the essential oils from *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy from Eastern Cape, South Africa. Manuscript submitted to Journal of Natural Product Research.

2) P.P. Rungqu[1], O. Avoseh[1], K Aremu[2], B. N. Nkeh-Chungag[2], S. P. Songca[3], O. Oluwafemi[4], O. O. Oyedeji*[1] and A,O, Oyedeji. (2015). Anti-inflammatory activity of the essential oils from *Hyparrhenia hirta* (L.) Stapf from Eastern Cape, South Africa. Manuscript submitted to Journal of Essential Oil Research.

3) P. P. Rungqu, O. Avoseh, O. Oyedeji, A. Oyedeji (2015). *Cymbopogon* species, Ethnopharmacology, Phytochemistry and the Pharmacology importance. Manuscript submitted to Molecules.

# ABSTRACT

*Cymbopogon validus* and *Hyparrhenia hirta* belong to the Poaceae botanical family. Both plants are used as thatching material; *H. hirta* is also used for weaving mats and baskets. In this study, we investigated the anti-inflammatory effects of *C. validus* and *H. hirta* essential oils on fresh egg-albumin induced edema on Wistar rats. To fully understand the chemically induced anti-inflammatory properties of these plants, we first analyzed the chemical composition of the essential oils. The essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS). In *C. validus*, 13 compounds accounted for 74.3% of fresh leaves oil, 14 compounds 71.8% of dried leaves oil and 12 compounds 73.3% of flower oil were identified from the GC-MS Chromatogram. The percentage yields were as follows fresh leaves oil 2.2%, dried leaves oil 2.0% and flower oil 2.4% v/w respectively. Linalool (3.2-29.6%) and northujane (4.4-16.9%) were the dominant compounds found in the 3 oils analyzed. While, α-terpineol 37.5% and verbenone 13.5% was only found in the fresh leaf oil, this was absent in the dried leaves oil and the flowers oil of *C. validus*. In *H. hirta*, 25 compounds accounted for 68.1% of fresh leaves oil, 40 compounds 71.9% of dried leaves oil, 23 compounds 77.6% of fresh flowers oil and 18 compounds 80.1% of dried flowers oil were identified from the GC-MS Chromatogram. The percentage yields obtained from the different parts were 3.4% for fresh leaves oil, 2.8% for dried leaves oil, 2.8% for fresh flowers oil and 0.7% for dried flowers oil v/w respectively. Northujane (8.5-30.0%), diisooctyl phthalate (4.4-26.5%), phytone (1.1-10.4%) were the dominant compounds found in the 4 oils analyzed. While, dibutyl phthalate 26.9% was only found in the fresh flowers and was absent in the dried flowers oil. Moreover, caryophyllene oxide (1.7-9.6%) was found in fresh leaves and dried flowers oil of *H. hirta*. In *vivo* analysis revealed that the two essential oils displayed significant edema inhibition effect overtime. They displayed

strong anti-inflammatory properties when compared to control group. However, the *H. hirta* essential oil was more effective than that of *C. validus*. Linalool, α-terpineol, and northujane extracted from *C. validus* and *H. hirta* essential oils might have contributed to the anti-inflammatory effects observed in Wistar rats. This study, confirms the anti-inflammatory properties of *C. validus* and *H. hirta* suggesting that they may be used in diseases related to anti-inflammation.

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# CHAPTER ONE

## 1 INTRODUCTION AND LITERATURE REVIEW

### 1.1 History of Medicinal Plants

For thousands of years to present day, people have been using plants as medicine[1] due to their therapeutic values[2]. Plants are naturally gifted at the synthesis of medicinal compounds, and they are also considerably useful and economically essential. The usefulness of these plants was discovered due to the curiosity and imagination of man to nature as well as a necessity to feed[3-6]. Natural products engender from microbes, plants, or animal sources and about 25% of the drugs prescribed worldwide come from plants. It is also estimated that 60% of anti-tumor and anti-infectious drugs from plant sources are already on the market or are under clinical trial[7-8].

A number of active principles of medicinal plants are isolated and introduced as valuable drugs in modern medicine. Many medicinal plants are being investigated by pharmaceutical industries; some have been developed and proposed for antimicrobial and anti-inflammatory disease uses[3-9]. Medicinal plants are defined as plants that are commonly used in treating and preventing specific ailments and diseases which are usually considered to play a beneficial role in health care[10]. Crude drugs such as teas, powders, tinctures, poultices and other herbal formulations are also used as medicines[11]. Thai people have been using spices, vegetables, and fruits for flavours, as well as diverse herbs and condiments in cuisines. Several essential oils have been exploited as fragrances and flavours; some plants have the potential to be exploited templates for drugs and nutraceuticals[12].

## 1.2 Essential oils (Volatile oils)

Essential oils are known for their medicinal value as they are important natural plants[13]; they have a long history of use as natural antimicrobial agents with many industrial applications as they effectively inhibit the growth of a wide range of microorganisms and cause fewer side effects[14]. Essential oils are defined as aromatic oily liquids that have strong aromatic components that give distinctive odor, flavor and scent to a plant. They are obtained from plant materials such as flowers, seeds, leaves, twigs, bark, herbs, wood, fruits and roots merely by steam or hydrodistillation, expression, fermentation or extraction. In nature, these oils play a major part in the protection of plants as antibacterials, antivirals, antifungals, insecticides and reducing the appetite of herbivores. Essential oils, also known as ethereal oils or volatile oils, evaporate quickly when they are exposed to air at ordinary temperatures. In general, essential oils consist of mixtures of chemical compounds, only a few having a high percentage of a single component[18].

Essential oils are complex mixtures of volatile substances that are insoluble in water but soluble in organic solvents which may contribute to their characterization and isolation[19]. They are characterized by some major components at quite high concentration from 20-70 components as compared to other components present in trace amount; these major components at times, determine the biological properties of the essential oils[15]. Chemical constituents in essential oils can be divided into two broad classes: terpenes and phenylpropanoids, although certain amounts of several other substances with different chemical composition could be present[18,20]. Essential oil components can be classified as lipophilic terpenoids, phenylpropanoids, or short-chain aliphatic hydrocarbon derivatives of low molecular weight, with the first being the most frequent and characteristic constituent. Among these, allylic, mono-, bi-, tricyclic mono- and sesquiterpenoids are

different chemical classes that make up the major part of essential oils, such as hydrocarbons, ketones, alcohols, oxides, aldehydes, phenols, or esters[21].

## 1.3 Therapeutic values of essential oils

Essential oils are volatile aromatic liquids that come from natural products such as plants. They are regarded as the chemical weapons of the plant world, because their compounds protect plants against bacterial and fungal attack; they also act as plant pheromones in order to attract their pollinators. In most cases essential oils destroy infestation, aid growth and stimulate healing and that is due to the presence of the oxygenated molecules which act as chemical messengers to the cells that bring life to the plants. These oils have been largely employed for their therapeutic properties.

Essential oils are very important natural products because of their therapeutic value and beneficial properties which are supportive to the immune system, skin and muscles[22]. Among other qualities, they also possess biological properties; these oils are widely used for the prevention and treatment of diseases. The term “biological” comprises of all the activities that these volatile compounds (e.g. monoterpenes, sesquiterpenes, phenylpropanoids) exert on humans, animals and plants. So, because of their enormous therapeutic potential they have attracted the attention of researchers who use them to test for various medicinal properties including anticancer activities[23]. Roughly, 3000 essential oils are known and out of these, 300 are commercially important[24]. Besides the fact that these oils impart aroma and flavor to food, they also have important antioxidant activities hence they are further encouraged for their use[25]. Essential oils are known for their therapeutic properties hence they are used to treat different infections caused by both pathogenic and non-pathogenic diseases[26]. Some of the essential oil components are used in perfumes, make-up products, in sanitary products, in dentistry, agriculture, as food preservatives and additives and natural remedies[27]. In the medical field, inhalation

therapy of essential oils has been used to treat both acute and chronic bronchitis and acute sinusitis. Furthermore, inhalation vapors of essential oils or their individual volatile terpene has a significant role in controlling the central and nervous system[28]. There are quite large numbers of plants that are used because of their essential oils such as Lavender (*Lavandula angustifolia* Mill) (Lamiaceae), Clove (*Syzygium aromaticum* (Linn) Merrill & Perry) (Myrtaceae), Lemongrass (*Cymbopogon citratus* DC Stapf) (Poaceae), Tea tree (*Melaleuca alternifolia* Cheel) (Myrtaceae), to mention a few[29].

### 1.3.1 Lavender Oil:

Oil from lavender flowering tips has been used for centuries in perfumes and aromatherapy. Lavender is rich in compounds such as Linalyl acetate, geraniol and cineole. Its oils are natural antibiotic, antiseptic, sedative and anti-depressant; they are also used to treat scalds minor burns, inflammation, cuts, eczema, and rheumatism[30]. Lavender is known to reduce anxiety, stress and tension therefore it is used for calming, relaxation and soothing purposes[31].

### 1.3.2 Tea tree Oil:

During the first World War, Australian soldiers used tea tree oil as a disinfectant and in the second World War the oil was used for bites and infections. Aromatherapy chemists have proven this oil to be very effective in clearing colds and sore throat if its vapor is inhaled through steam. Tea tree oil is also known to possess anti-inflammatory properties, to also act as an antiseptic for treating rashes, cuts, insect bites as well as stings. It has also shown effectiveness against methicillin resistance Staphylococcus aureus (MRSA)[32].

### 1.3.3 Clove oil:

Oils from the clove buds are used as antiseptic and anaesthetic for mouth infections. European hospitals also use the oil for dental infection. The oil can also be used in thyroid dysfunction; the major constituent in clove bud oil is eugenol[33].

### 1.3.4 Lemongrass oil:

Lemongrass is widely used as a herb in tropical countries and its oil is also used as a component of ethnopharmaceuticals in tropical and subtropical countries. The compounds that are identified in lemon grass oil are α-citral, β-citral, citronellal, geranyl acetate, geraniol, myrcene to name a few. Studies also reveal that lemongrass essential oils possess antibacterial, anti-inflammatory, antifungal and antioxidant properties. The oils are also used in folk medicine for the treatment of fever, diabetes, digestive and nerve disorders[34-35].

## 1.4 Classes of essential oil compounds

### 1.4.1 Hydrocarbons

A great number of essential oils constituents are hydrocarbons. Hydrocarbons are organic compounds that consist of entirely hydrogens and carbons. They can either be acyclic, monocyclic or aromatic. Acyclic compounds are compounds that do not contain a ring for example hexane and myrcene (**1**) in (figure 1.1). Monocyclic compound e.g. Limonene (**2**), bicyclic e.g. α-pinene (**3**) are terpenes with closed system (ring). Aromatic hydrocarbons are hydrocarbons with alternating double bonds and single bonds between the carbons forming a ring, an example of an aromatic hydrocarbon is *p*-cymene (**4**)[37].

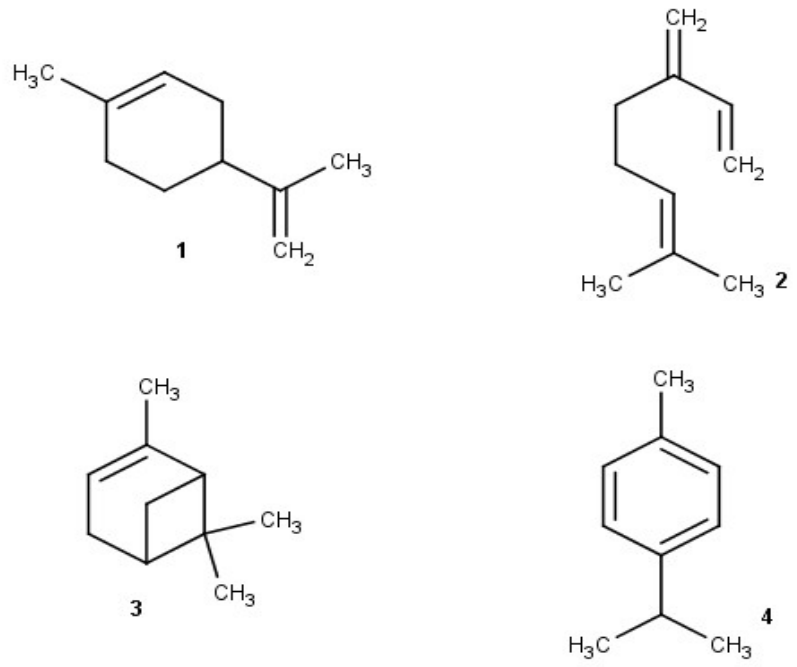


Figure 1-1: Structures of hydrocarbons commonly found in essential oils

### 1.4.2 Terpenes

Terpenes are a large and diverse class of organic compounds that are produced by a variety of plants, especially conifers[38], but some of the larger terpenes are more complex (for example squalene and lanosterol) and they occur in animals[39]. They are not only the largest group of natural products but also comprise of about 30 000 compounds which have a broad mixture of structural types[40]. Terpenes have a strong smell and because of that they tend to protect the plants that produce them by discouraging herbivores but attracting predators as well as parasites of herbivores[41]. These terpenes are largely distributed in secretory tissues such as oil glands or chambers, resin canals of higher plants, insects, fungi and marine organism[46]. Their structures are built up from 5-Carbon units often called isoprene units with the molecular formula [$C_5H_8$]. The basic molecular formulae for terpenes are multiples of [$C_5H_8$]n where n is the number of linked isoprene

units. The isoprene units are put together in a well-ordered way normally “head-to-tail” in terpenes, up to 25 carbons to form linear chains or they may be arranged such as to form rings[42-43]. Terpenes are classified according to the number of isoprene units they may contain; for example monoterpenes [$C_{10}H_{16}$] (two isoprene units), sesquiterpenes [$C_{15}H_{24}$] (three isoprene units), diterpenes [$C_{20}H_{32}$] (four isoprene units), sesterpenes [$C_{25}H_{40}$] (five isoprene units), triterpenes [$C_{30}H_{48}$] (six isoprene units), Tetraterpenes [$C_{40}H_{64}$] (eight isoprene units), polyterpenes [$C_5H_8$]n. The type of terpenes that form the majority of essential oils are monoterpenes, sesquiterpenes as well as Diterpenes; these terpenes are found in more than 2 000 plant species that belong to some 60 families like Rutaceae; Myrtaceae and Umbrellifereae to name a few[44].

#### *1.4.2.1 Biosynthesis of terpenoids (MEVALONATE PATHWAY)*

Biosynthesis of terpenoids involves the conversion of mevalonic acid to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Acetyl-CoA and acetoacetyl-CoA are condensated to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) which is then catalyzed by the HMG-CoA synthase enzyme. The next enzyme which is HMG-CoA reductase (HMGR) catalyzes reductive deacylation of (HMG-CoA) to mevalonate (MVA) via melvadate and then employs equivalents of NADPH as reductants. The first ATP-dependent phosphorylation of mevalonate is catalyzed by mevalonate kinase to yield mevalonate 5-phosphate. Mevalonate 5-diphosphate is produced by further action of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)[45].

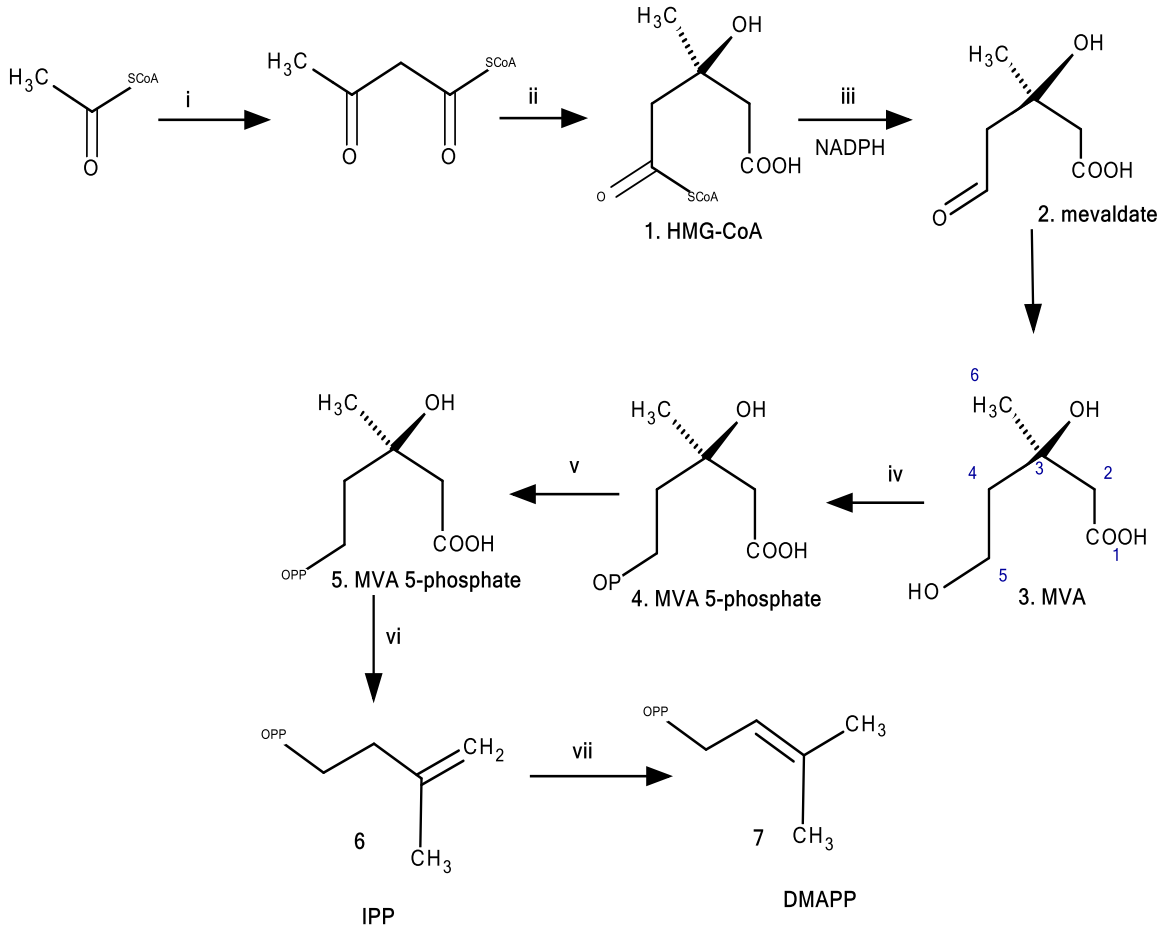


Figure 1-2: Biosynthesis of Terpenoids via the Mevalonate pathway

**Key:** Enzymes: i-acetoacetyl-CoA thiolase (AACT); ii- HMG-CoA synthase; iii-HMG-CoA reductase (HMGR); iv-mevalonate kinase; v- phosphomevalonate kinase; vi-mevalonate 5-diphosphate decarboxylase; vii- IPP isomerase

### 1.4.2.2 *Monoterpenes*

Monoterpenes, $C_{10}$ are formed from the joining of two isoprene units following the Ruzicka rule of head-tail connection as shown in fig. 1-3.

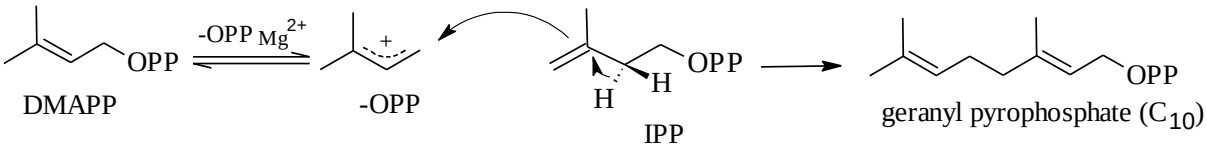


Figure 1-3: Formation of monoterpenes.

Monoterpenes are naturally occurring compounds, which consist of two isoprene units with the molecular formula of $[C_{10}H_{16}]$[46]. The branched chain $C_{10}$ hydrocarbons (nerol (**5**), citral (**6**) and terpineol (**7**)) (fig. 1-4) are widely distributed in nature with more than 400 naturally occurring monoterpenes that have been identified[47]. Geraniol (**8**) and citronellol (**9**) are some of the monoterpene examples [47-48].

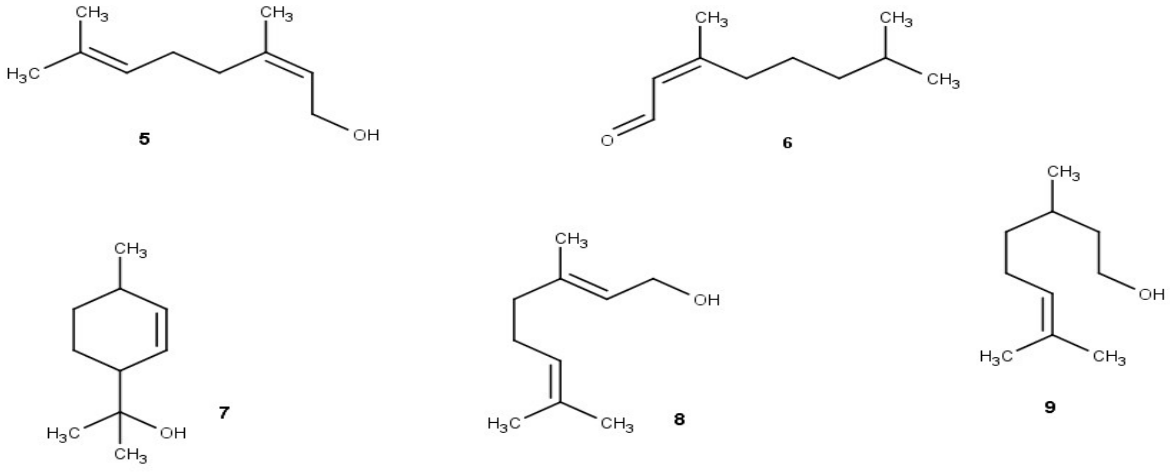


Figure 1-4: Structures of monoterpenes commonly found in essential oils

### *1.4.2.3 Oxygenated monoterpenes*

Monoterpene ketones are stable molecules that are not usually found in great numbers in essential oils; although they have a very distinguishable fragrance, they are not that important in fragrance and flavour substances but are very calming and sedative. They are involved in the promotion of new tissue growth, liquefying mucous and stimulating cell growth. Some therapeutic effects of these compounds are antiviral, analgesic and have digestive effects. Common monoterpene ketones found in essential oils include carvone (**10**), menthone (**11**) and pulegone (**12**) (fig. 1-5)[67-69].

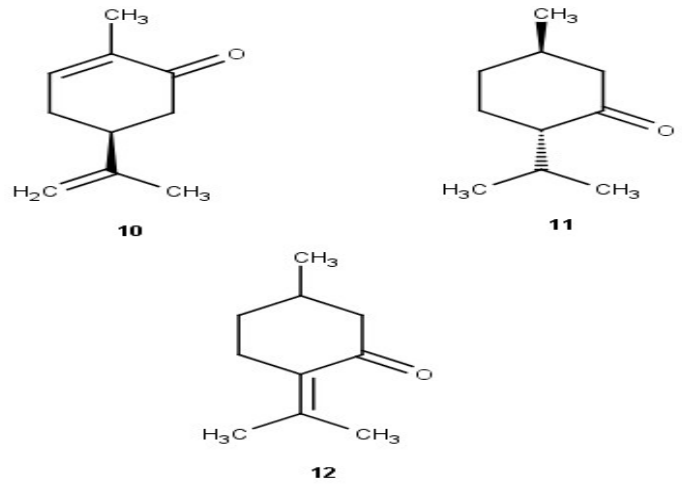


Figure 1-5: Structures of oxygenated monoterpenes (ketones) commonly found in essential oils

### *1.4.2.4 Monoterpene alcohols*

Monoterpene alcohols are a group of organic compounds in which a hydroxyl group (-OH) is bonded to a carbon atom, which in turn is bonded to other hydrogen atoms. Monoterpene alcohols are known for their pleasant fragrance and they are the most therapeutical beneficial of essential oil components. Oil containing these types of organic compounds has good antiseptic, antiviral, antimicrobial, antifungal properties with few effects such as skin irritation or toxicity. Monoterpene alcohols also have an

uplifting energizing effect. Examples of essential oil alcohols are linalool (**13**), borneol (**14**) and menthol (**15**) (fig. 1.6)[67-70].

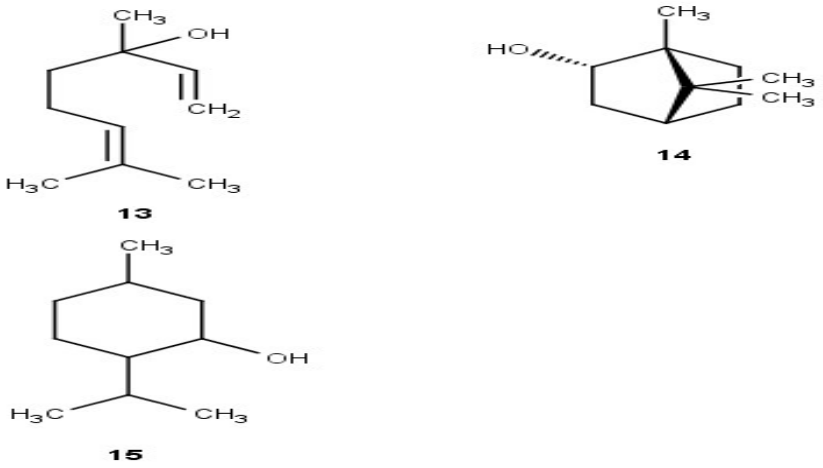


Figure 1-6: Structures of monoterpene alcohols commonly found in essential oils

### *1.4.2.5 Monoterpenoid oxides*

Monoterpene oxides are compounds in which an oxygen atom is bonded to two alkyl or aryl groups an ether or an epoxide. They are the strongest odorants and some of their therapeutic effects are expectorants and stimulants of the nervous system. The most known oxide is 1,8-cineole (**16**) which is ubiquitous in essential oils. Another example of a monoterpene oxide is linalool oxide (**17**) (fig. 1-7) [67,71-72].

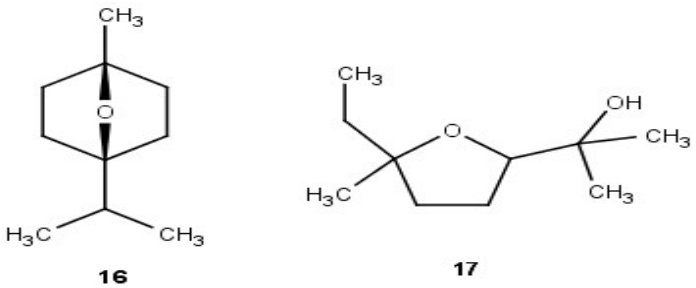


Figure 1-7: Structures of monoterpene oxides commonly found in essential oils

### *1.4.2.6 Monoterpenoid aldehydes*

Monoterpene aldehydes are compounds that contain a formyl group with the structure R-CHO, which consist of a carbonyl center that is a carbon bonded to an oxygen atom, that is also bonded to hydrogen atom as well as the R group where R can be any generic alkyl group or side chain. These compounds are very common in essential oils; they are unstable and are easily oxidized in the presence of oxygen and low heat. Monoterpene aldehydes have a distinguishable sweet, pleasant fruity smell and are often found in culinary herbs such as cumin and cinnamon. They sensitize the skin, are mucous membrane irritants, they also have antiviral, anti-inflammatory, anti-fungal, antimicrobial, antipyretic properties. Essential oils containing aldehydes are also good disinfectants. The most known aldehydes in essential oils are citral (geranial and neral) (**18**), citronellal (**19**), Benzaldehyde (**20**) and cinnamaldehyde (**21**) (fig. 1-8)[67-73,74].

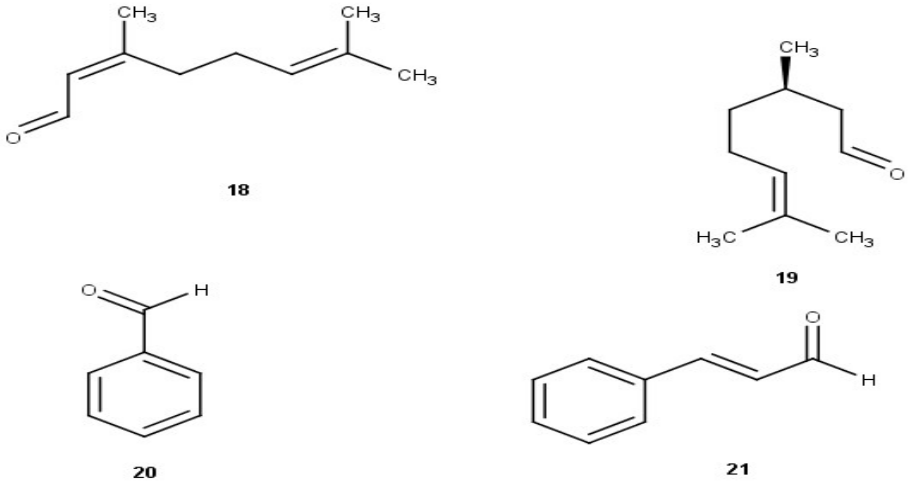


Figure 1-8: Structures of monoterpene aldehydes commonly found in essential oils

### *1.4.2.7 Monoterpenoid phenols*

Monoterpene phenols are organic compounds where one hydrogen atom attached to a benzene ring is replaced with a hydroxyl group. These compounds are most reactive, they can be toxic if they are used over a long period of time, are irritant to the skin and mucous membrane. They also have good antiseptic qualities though they can also cause severe skin reactions. Monoterpene phenols have similar properties to those of alcohols but are highly distinctive; these compounds possess antibacterial, antimalarial, antiseptic and disinfectant properties. They also stimulate the immune system and the nervous system and they may also reduce blood cholesterol levels. Some monoterpene phenol examples include thymol (**22**), eugenol (**23**), chavicol (**24**) and carvacrol (**25**) (fig. 1-9) [67, 72, 75].

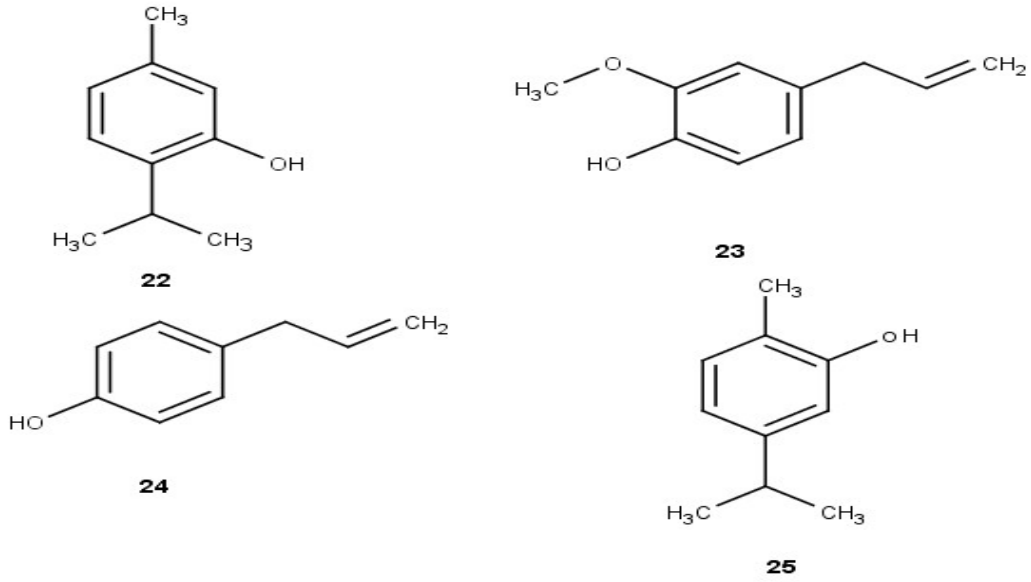


Figure 1-9: Structures of monoterpene phenols commonly found in essential oils

### *1.4.2.8 Monoterpenoid esters*

Monoterpene esters are commonly found in a very large number of essential oils and they tend to be fragrant and fruity. Linalyl acetate (**26**) is one of the ester examples found in lavender. Some other ester examples include geraniol acetate (**27**), eugenol acetate (**28**), bornyl acetate (**29**), clary sage and petitgrain. These compounds are usually used with great ease and are gentle in their actions. They also have great qualities such as anti-inflammatory, sedative, antifungal, antispasmodic etc (fig. 1-10)[67,72,76-77].

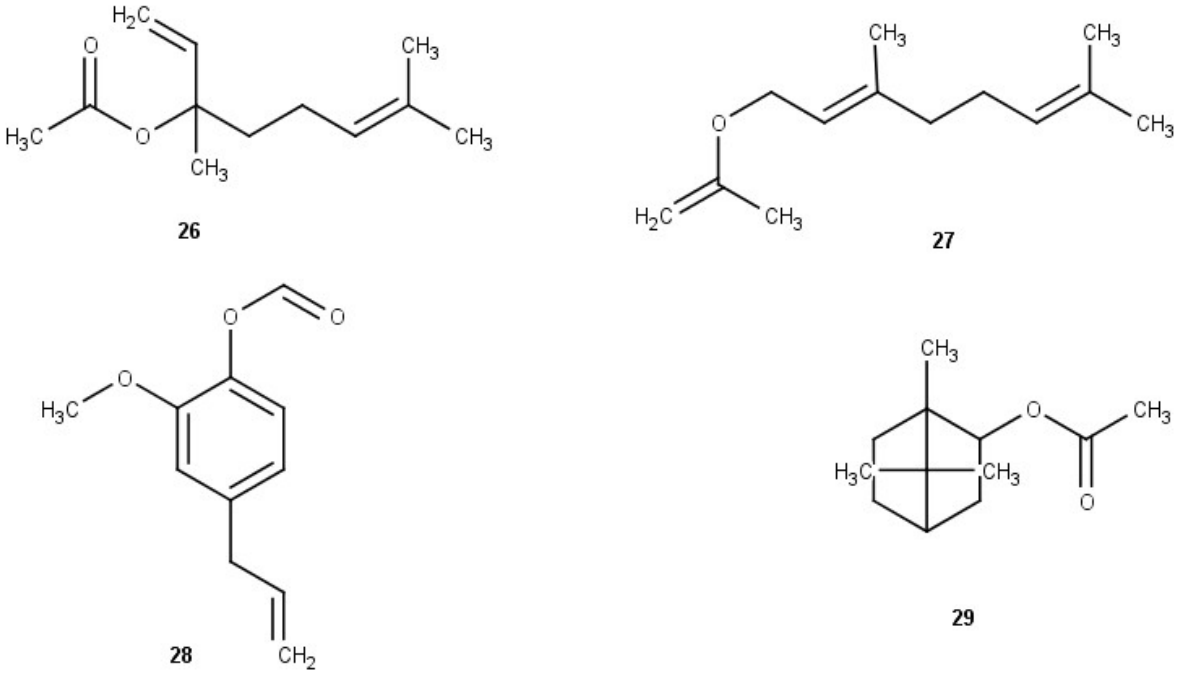


Figure 1-10: Structures of monoterpene esters commonly found in essential oils

### 1.4.3 Sesquiterpenes

Sesquiterpenes are a class of terpenes that is obtained when three isoprene units combine to form a compound. General molecular formula of sesquiterpenes is [$C_{15}H_{24}$] (fig. 1-11).

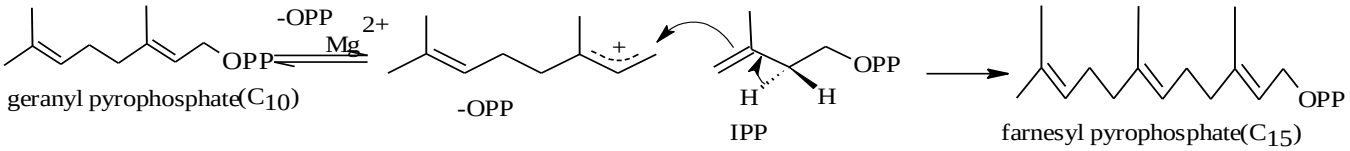


Figure 1-11: Formation of sesquiterpenes

They are found mainly in higher plants and invertebrates; sesquiterpenes just like monoterpenes are important constituents of essential oils in plants[60]. They are biogenetically derived from farnesyl pyrosphosphate and in structure they may be linear (farnesol (**30**) and nerolidol (**31**)), bicyclic (cadinene (**32**)), tricyclic (capnellene (**33**)) or

even tetracyclic (avarol (**34**)) (fig. 1-12) systems[61]. Some of the most known sesquiterpenes are farnesol (**30**) and nerolidol (**31**) and they are usually isolated from essential oils of various sources. Farnesol (**30**) is normally found in essential oils extracted from citronella, lemon grass, rose, neroli to mention a few; this sesquiterpene is used in perfume industries as to enhance odors of perfume, it is a good pesticide for mites and a pheromone for some insects and mammals[62]. The therapeutic qualities of nerolidol (**31**) include anti-inflammatory, antiseptic, antibacterial and antiviral; they also work as a liver and gland stimulant[63]. Nerolidol is also reported to have antinociceptive activities[64]. Furthermore, there are reports that nerolidol has antimalarial and antileishmanial activities[65-66].

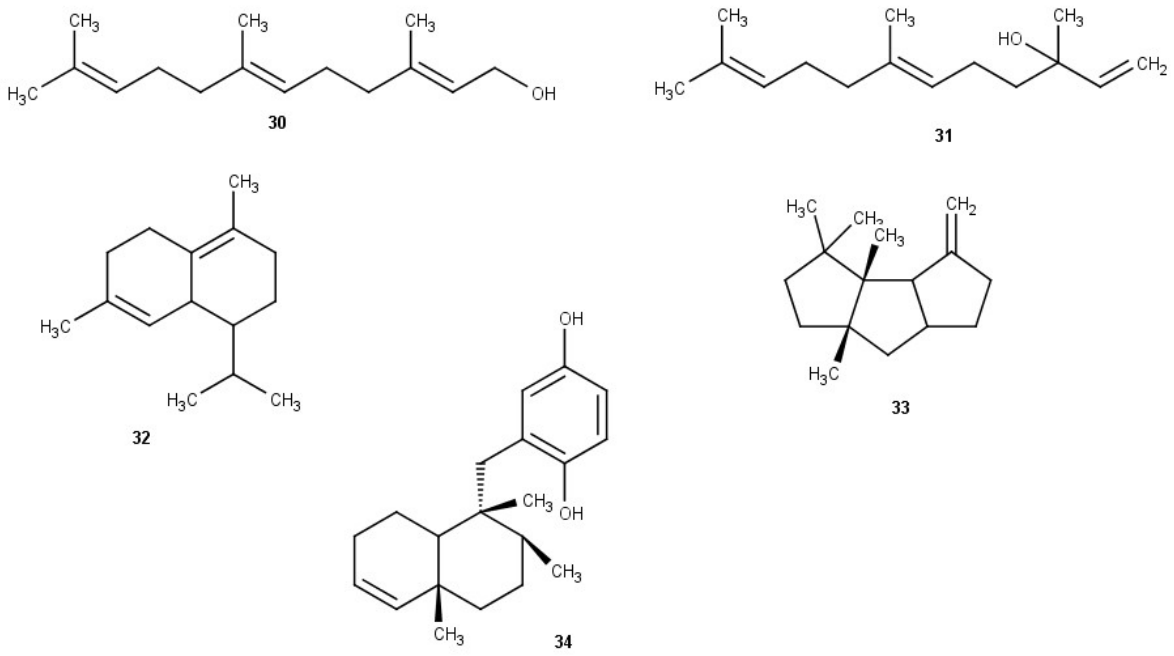


Figure 1-12: Structures of sesquiterpenes commonly found in essential oils

## 1.5 Extraction methods of essential oils

Since ancient times, humans have developed a way of extracting aroma and flavour components from fresh and dried plant materials. The preparation of tea and coffee is a good example of extracting aroma components including the stimulant caffeine with hot water[78]. The term "extraction" is used pharmaceutically and it involves the separation of medicinally active portions of plants or animal tissues from inactive or inert components by using selective solvents in standard extraction procedures[79]. In most cases, the type of plant material determines which method will be used to obtain essential oils: essential oils are highly concentrated essences of aromatic plants, which easily evaporate and are insoluble in water[80]. These oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process[81]. The oil droplets contained in the oil glands or sacs can be removed by either accelerated diffusion through cell wall or by crushing the cell walls. The techniques used to extract these oils also depend on the part of the plant where the oil is to be extracted from whether the oil is able to withstand heat and the sensitivity of the oil constituents to chemical reactions. Some of the common techniques used to extract essential oils are: hydrodistillation, solvent extraction, steam distillation and supercritical carbon dioxide extraction[82].

### 1.5.1 Hydrodistillation

Hydrodistillation has been the main method for extracting essential oils from plant material such as wood, flowers, leaves or seeds with high boiling water[83]. This technique involves the complete immersion of a plant material (either fresh or dried) in water followed by boiling. The plant material is usually cut into small pieces or grinded before it is transferred into the Clevenger set up. This method protects the extracted oils to a certain degree since the surrounding water acts as a barrier preventing it from

overheating. As the water heats up, the steam rises and gets in contact with the plant material, the distillation vessel (round bottom flask) is heated over a heating mantle. The volatile content of the plant evaporates and condenses to an aqueous fraction that is collected in a receiving container which is the Clevenger apparatus arm[84].

### 1.5.2 Solvent extraction

This method involves the extraction of the oils from the oil bearing materials with the use of a solvent. It is mostly used when plant material cannot be expressed or the essential oil components are so delicate that they can be denatured by heat. In this technique solvents used depend on the part of the plant material to be extracted. For example, plant materials like leaves, roots and fruits are extracted with benzene mixed with the mixture of acetone or petroleum ether or with just benzene without being mixed with the mixture of acetone or petroleum ether in cold or at boiling point. Flowers are extracted with ethers; non-polar organic solvents such as hexane can also be used to extract essential oils, which then can be dried off leaving the oil content behind. In most cases the chosen solvents do not mix with the compound in which the substance of interest is currently left undissolved, so that when left undisturbed they will form two separate layers just like oil and water[85].

The solvent then enters the plant to dissolve the oil waxes and color, after extraction, the solvent is then removed by distillation under reduced pressure leaving behind the semisolid concentrate which is then extracted with absolute ethanol. The second extract is cooled off so that it can precipitate the waxes and is then filtered; the alcoholic solution that does not contain wax is distilled under reduced pressure to remove alcohol and finally leaving behind the essential oil[82-86].

### 1.5.3 Steam distillation

Distillation is the method of separating components based on differences in volatile constituents in a heated mixture. Steam distillation involves bubbling steam through the plant material[87]. It is the most common method of extracting essential oils from plant materials; this method involves the steaming of the plant material of choice for a determined period of time under specific pressure and certain temperature as to release the oil. In this technique, the desired plant material (fresh or dried) is first added into the vessel. The steam then passes through the plant material that has aromatic molecules or oils. Once the plant releases these aromatic plant molecules, they travel within a closed system and towards the cooling device. The vapors are then cooled off with cool water, as the vapor cools off, it condenses and then transforms into a liquid which is then collected as an essential oil and the remaining water is known as hydrosol[82-88].

### 1.5.4 Supercritical fluid: carbon dioxide extraction

Carbon dioxide is a supercritical fluid extraction which works by separating solutions that involve the use of supercritical fluid. These fluids are then brought to a state of high heat and pressure and they both act like a gas and a liquid and they no longer undergo phase changes. In this type of extraction, the plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel, the carbon dioxide then turns into liquid and acts as a solvent that extracts the essential oil from the plant material. When the pressure decreases, the carbon dioxide changes back into gas thereby releasing the oil which is then collected as an essential oil[82-85].

## 1.6 Research problem

The South African society is plagued by numerous life threatening and communicable diseases. The spread of these diseases is made even more serious due to inadequate medical facilities allowing the use of medicinal plants to be prevalent. *Cymbopogon validus* and *Hyparrhenia hirta* are valuable source of anti-inflammation, antimicrobial and versatile traditional brooms in South Africa. A well detailed research on these plant species are still limited, therefore, a well detailed and comprehensive evaluation of their terpenoids and consequent anti-inflammatory studies are required to ascertain their use.

## 1.7 Justification

*Cymbopogon validus* and *Hyparrhenia hirta* are invasive plants distributed in South Africa. They are considered as useless and without major economic importance. This research will henceforth entail an exhaustive review of these species and then evaluate their chemical and biological significance.

## 1.8 Aims

The study is aimed at extracting essential oils from both fresh and dry parts of the *Cymbopogon validus* and *Hyparrhenia hirta* (flowers and leaves), to determine their chemical compositions and then evaluate the biological potential of the oils as anti-inflammatory agents.

## 1.9 Specific Objectives

- To extract essential oils of *Cymbopogon validus* and *Hyparrhenia hirta* using hydro-distillation apparatus.
- Characterization of the essential oils using GC-MS
- To determine the suitability of the essential oils for medicinal and potential uses
- To evaluate the essential oils as anti-inflammatory agent

## 1.10 Hypothesis

It is hypothesized that since *Cymbopogon validus* and *Hyparrhenia hirta* essential oils offer outstanding anti-inflammatory activities, they can be used as lead candidates for preparation of anti-inflammatory and analgesic therapy.

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# CHAPTER TWO

## 2 Extraction and Chemical composition of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy

### 2.1 *Cymbopogon* genus

Grasses are an important group of plants in the life of human beings and animals; Essential oil yielding grasses mostly belong to the Poaceae family of which *Cymbopogon* forms an important genus[1]. *Cymbopogon* comes from the Greek word "kumb" meaning nacelle (boat) and "pogon" meaning bores or beard. It is said that it appeared in the composition of holy oil (e.g. olive, grooves, myrrh and citronella)[2]. This genus comprises of about 180 species, subspecies, varieties, and sub varieties. It is native to warm temperate and tropical regions of the Old World and Oceania[3-4]. *Cymbopogon* species are scattered all around the world and more than 52 species are said to be found in Africa, 45 in India, 6 in Australia and South America, 4 in Europe, 2 in North America and the remaining species are dispersed in South Asia. These *cymbopogon* species are distributed from mountains and grasslands to arid zones[5-8].

The essential oils from *Cymbopogon* species contain a wide variety of terpenoids, some of which are geraniol and its ester, citronellol and citronellal, which are important perfume materials, in cosmetics and pharmaceutical applications. Citral is one of the major components of the oil present in several species of *Cymbopogon* with wide industrial uses such as raw material for perfumery, confectionery and vitamin A as well as ionone synthesis. Several *Cymbopogon* species possess significant anthelmintic, anti-inflammatory, analgesic, antiaging, pesticidal, antimicrobial, mosquito repellent and

larvicidal activities and thus, are used in native medicine for curing a number of diseases[5,9,10]

### 2.1.1 Ethnopharmacology of some *Cymbopogon* species

The genus comprises of about 140 to 180 species of which most of them are aromatic and they yield essential oils once their aerial parts are steam distilled. *Cymbopogon* essential oils have commercial importance in perfumery, cosmetic and pharmaceutical applications[35]. The common economic species include *C. winterianius*, *C. flexuosus*, *C. martini*, *C. nardus*, *C. citratus*, *C. pendulus*, *C. jwarancusa* and *C. khasianus*. These species produce different types of essential oils such as palmarosa oil, lemongrass oil, citronella oil, ginger grass or rusa oil which are of commercial importance[15]. Three out of the above mentioned species namely *C. winterianus*, (java citronella), *C. martinii* (palmarosa), lemongrass (*C. flexuosus* and *C. pendulus*) are cultivated for their essential oils simply because they are of commercial importance and are used in perfumes, soaps, cosmetics, toiletry, tobacco, products and some other industrial products. The uniqueness of these aromatic grasses is that they are able to adapt and grow in different types of soil regardless of agroclimation conditions and crop sequence[3,16-18].

*C. winterianus* (Jowitt ex Bor) is an aromatic grass that yields essential oils. It is cultivated in India and Brazil but mainly cultivated in India. Essential oils extracted from the leaves of this *Cymbopogon* species are used in perfumery, cosmetics, pharmaceuticals and flavouring industries. It has been reported that *C. winterianus* possess anticonvulsant and antinociceptive properties in rodents. It can also be used traditionally as a repellent; its repellent properties can also be used to protect cartons containing muesli and wheat germ from beetles. The main constituent of the plant is citronella[19].

*C. jwarancusa* (Jones) Schult is an important herb that is found all around India as a weed, the whole plant is used in traditional systems of medicine but the roots and shoots are the most important parts of the plant which are medicinally used. Literature reveals that quite a large number of constituents have been isolated from this plant and are used to treat several diseases like blood impurities, skin problems, abdominal pain, vomiting, unconsciousness and fever. Ethanol extracts of *C. jwarancusa* showed higher antioxidant properties, this plant is also known to possess antimicrobial properties. The plant's main constituents are piperitone (58.6%), elemal (18.6%) and agarospirol (9.5%)[20]. *C. citratus* (Stapf) is commonly known as the West Indian lemongrass and is native to India and Sri Lanka [21]; it has been cultivated in many countries for its citral-rich oils, however, the essential oils of the Ethiopian grown *C. citratus* have been reported as containing geraniol (40%) as the main component and only consisting 14% of citral as the second main component which makes it geraniol-rich[22]. Studies have demonstrated that the main constituents of *C. citratus* are neral (cis-citral, citral B), geranial (trans-citral, citral A) and myrcene[23]. This *Cymbopogon* species is used in folk medicine as a sedative, a diuretic and to treat nervous and gastrointestinal disturbances. Its leaves are used in Zambia for flavouring. The plant also possesses biological activities such as antimicrobial, anti-inflammatory, analgesic, antipyretic, antispasmodic and antifungal properties[22,24].

*C. martinii* (Roxb) wats. var. *motia* Burk also known as palmarosa is an essential oil yielding grass that is rich in geraniol which is then used in high grade of perfumes and cosmetics. Geraniol constitutes about 80% of palmarosa oil which is derived from pyrophosphorylysis of GPP, whilst in immature inflorescence a considerable amount of geraniol undergoes acetylation to form geranyl acetate which then gets hydrolyzed to produce geraniol as the inflorescence matures. Palmarosa oil is used in cosmetic

products such as soaps, toiletry and tobacco products. These oils are also considered to have pharmaceutical significance because of their antiseptic, mosquito repellent and pain relieving properties[25].

*C. schoenanthus* L. Spreng is an aromatic herb whose fresh leaves are consumed in salads in Tunisia and also used to prepare traditional meat recipes. It is also used to make an aromatic tea because of its pleasant aroma and taste. Apart from its culinary use, *C. schoenanthus* is also used in folk medicine for treating rheumatism and fever, as diuretic, insecticide and a poultice to cure dromedary wounds. In North Africa, it is used for anorexia, in Alger (Djanet area) is known for bringing back appetite and in South Tunisia is used for diminishing fever and treating rheumatism. Its infusion which are taken as a diuretic are known to cure intestinal troubles and acts against food poisoning and also helps in digestion when it is taken in the form of decoction. *C. schoenanthus* also possesses antioxidant and antiacetylcholinesterase activities[26]; geraniol (59%) is the main constituent with geranial (13%) as the second main constituent[27]. *C. schoenanthus* is also rich in piperitone (68%), 2-carene (16.48%), cis-p-menth-2-en-1-ol (18.6%), trans-p-menth-2-en-1-ol (9.5%), elemol (7.4%), cis-piperitol (7.2%) and limonene (7.0%)[28].

*C. flexuosus* is an East India lemongrass that grows in all types of soil. Its essential oils contain mixtures of complex volatile constituents which are used in perfumery, flavor, fragrance, food and fine chemicals. These oils also contain biological activities which include anticancer, analgesic, anti-inflammatory, antibacterial and antiviral properties. Since ancient time in Asia, *C. flexuosus* has been used in culinary flavoring where its leaves were cooked with foods; *C. flexuosus* stems can be found in local markets of these countries. In India, its fresh leaves are crushed in water and are used to wash hair or as a detergent, Ganjewala (2009) reported isointermedeol as the main constituents in *C.*

*flexuosus* and it also has anticancer properties. According to Vinutha and Saraswathi (2013) in the aerial parts of *C. flexuosus* the major compounds are citral (64.98%), 1,7-octadien-3-ol (10.97%), dimethyl oxatricyclononanone (9.44%), nerol (2.88%), verbenol (1.77%) and caryophyllene oxide (0.71%); and in sub-aerial parts, the major constituent was citral (30.47%), with eudesmol (17.82%), elemol (14.16%), dihydro isopropyl methyl azulene (11.08%) as the other compounds found in sub-aerial parts of *C. flexuosus* [29-30].

Table 2-1: *Cymbopogon* species common names, countries or regions they are found, parts used, major constituents, how they are applied and references

| Species | Common name | Country/ Region found | Part used | Major components | Traditional uses | Biological properties | References |
|---|---|---|---|---|---|---|---|
| *C. jwarancusa* (Jones) Schult | Lwarancusa grass | India | Leaves and Roots | Piperitone (58.6%), Elemal (18.6%), Agarospirol (9.5%) | Vomiting, Fever, unconsciousness, skin disorders, | Antimicrobial, Antioxidant | 20 |
| *C. nardus* (L.) Rendle | Citronella | | Leaves | Citronella (29.6%) | Repellent | Antimicrobial, Antifungal, Antibiotics for aquaculture use | 31, 32 |
| *C. giganteus* (Hochst.) | Tsauri grass | Cameroon | Flowers, Leaves, Stems | Cis-p-mentha-1(7), 8-dien-2-ol (22.8%), trans-p-mentha-1(7), 8-dien-2-ol (24.9%), trans-p-mentha-2,8-dien-1-ol (17.3%), cis- p-mentha-2,8-dien-1-ol (8.3%) | Rheumatics, Fever, cough, skin disorders, arterial hypertension, food flavoring | Antimalarial, Antioxidant, Anti-inflammatory, Analgesic, Antiradical, Antimicrobial | 33 |
| *C. winterianus* (Jowitt ex Bor) | Java citronella grass | Brazil | Leaves | Citronella (36.19%), geraniol (32.82%), citronellol (11.37%) | Repellent, pain disorders, anxiety, sedative | Hypertension, anticonvulsant, antinociceptive | 19 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *C. flexuosus* (NEES ex Steud) Wats | Indian lemongrass | India | Aerial parts & Sub-aerial parts | Citral (30.47-64.98%), 1,7-octadien-3-ol (10.97%), dimethyl oxatricyclo nonanone (9.44%), nerol (2.88%), eudesmol (17.82%), elemol (14.16%), dihydro isopropyl methyl azulene (11.08%) | Treatment for fever | Anticancer, Analgesic, Anti-inflammatory, Antibacterial, Antiseptic | 30, 38 |
| *C. martini* (Roxb) Wats. Var. *motia* Burk | Palmarosa | India | Whole plant | Geraniol (80.0%), geranyl acetate (9.0%), geraniol (65.0%), geranyl acetate (20.0%) | Mosquito repellent, flavourant | Pain relieving properties, Antibacterial, Antifungal, Antiseptic, Antimicrobial | 25, 39 |
| *C. Olivier* (Boiss.) Bor | Kah-makki or Putar | Iran | Aerial parts | Piperitone (53.3%), elemol (7.7%), β-eudesmol (4.4%), torreyol (3.3%), limonene (2.9%), α-cadinol (2.1%) | Stomachic | Antiseptic | 40 |

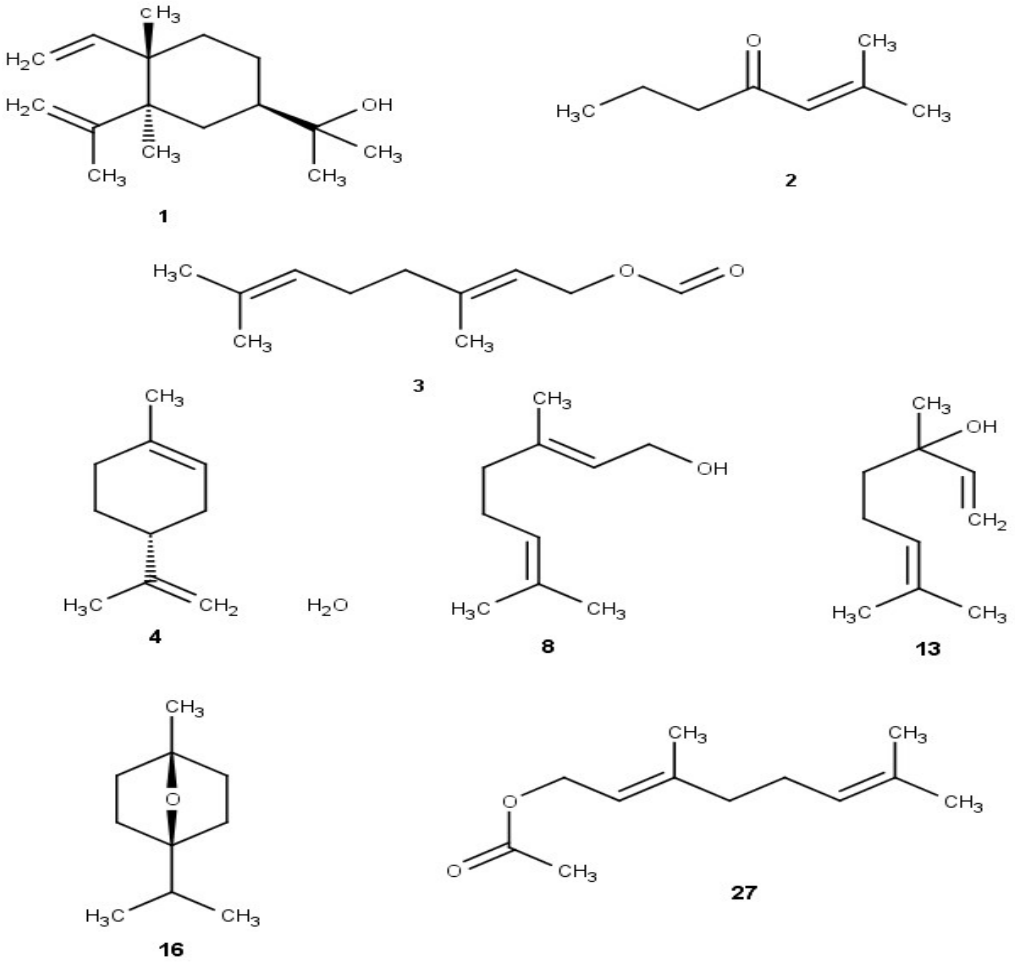


Figure 2-1: Structures of compounds commonly found in *Cymbopogon* species essential oils.

Elemol (**1**), methyl heptenone (**2**), geranyl formate (**3**) limonene (**4**), geraniol (**8**), linalool (**13**), 1,8-cineole (**16**) and geranyl acetate (**27**)

## 2.2 Ethnobotany of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy

### 2.2.1 Botanical description and distribution

*Cymbopogon validus* (Stapf) Stapf ex Burtt Davy is commonly known as a giant turpentine grass or the African Blue Grass in South Africa. The Afrikaner people call it

the “Reuse Terpentynegras”. This plant species belongs to the Poaceae family and has been described as a robust, aromatic, tufted perennial and largely unpalatable grass with culms that grow up to 2.4 m tall; it is inflorescence and has a false panicle that consists of paired groups of racemes which are each surrounded by a leaf-like spathe; spikelets are in pairs with one member sessile and the other pedicellate which are slightly shorter and awnless. This plant also has glabrous leaf blades with a clear midrib and rough leaf margin. Their leaf sheaths are short and hairy with a conspicuous membrane called the ligule. Normally, *C. validus* is found in mountainous grasslands and also in the high-rainfall areas of South Africa. It is known to grow in wet sites, along roads and on the margins of tree communities; it is widespread in the Eastern Cape and is often used as a durable thatch[11-12]

### 2.2.2 Therapeutic values of *Cymbopogon validus*

*C. validus* oil is pure therapeutic quality aromatherapy essential oil that is produced by using wild-crafted plants and traditional methods from South Africa[13]. These essential oils are used as an astringent, skin toner and in anti-ageing preparation for men; has anti-fungal, antiseptic, as well as anti-viral properties. The oils are also popularly used as a soothing foot bath. *C. validus* volatile oil and decoction are also used traditionally as an anti-rodent, fermifuge, emetic, anti-infective, and anti-plasmodic as well as to treat morning sickness[2,14].


Figure 2-2: *Cymbopogon validus* flowers B: *Cymbopogon validus plant*

### 2.2.3 Literature report on *Cymbopogon validus*

The only work carried out on the chemical composition of *Cymbopogon validus* essential oils is that of Naidoo (2007) and Chagonda *et al*. (2000). Chagonda reported on the chemical composition of the essential oils from the aerial parts of both wild and cultivated *C. validus* found in Zimbabwe. Myrcene (23.1-35.6%), (E)-β-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalool (3.2-3.7%), and camphene (5.2-6.0%) were the major constituents found in essential oils of wild *C. validus* whilst myrcene (11.6-20.2%), (E)-β-ocimene (6.0-12.2%), borneol (3.9-9.5%), geraniol (1.7-5.0%) and camphene (3.3-8.3%) dominated the oils of cultivated *C. validus*[14].

Seven years later, Naidoo (2007) reported on the chemical composition of essential oils of flower heads, leaves, culms and rhizomes from *C validus* collected in Durban, South Africa in which she revealed that flower-head essential oils constituted of 58 constituents with 18.17% as monoterpenes, 20.69% as sesquiterpenes, 12.07% as oxygenated monoterpenes and 6.90% as oxygenated sesquiterpenes. The monoterpenes constituents

present were allo-ocimene, γ-terpinene, citronellal, geraniol, isoterpenolene, limonene, linalool and sabinene. The essential oil extracted from the leaves had fifty-six constituents of which 19.64% constituted of monoterpenes, 14.29% sesquiterpenes, 10.71% oxygenated monoterpenes, 3.57% oxygenated sesquiterpenes and 1.79% diterpenes. Monoterpenes constituents present in significant amount were α-pinene, β-myrcene, camphene, citronellal, citronellol, p-cymene and tricyclene. In culms (stem of the plant) essential oils, fifty-six constituents were present consisting of 19.64% monoterpenes, 10.71% oxygenated monoterpenes, 12.50% sesquiterpenes, 7.14% oxygenated sesquiterpenes, 1.79% diterpenes and 1.79% oxygenated diterpenes; predominant monoterpenoid constituents were (+)-2-carene, α-pinene, camphene, decyl aldehyde, geraniol, limonene & sabinene. Forty-six constituents were found to be present in rhizome essential oils with monoterpenes consisting of 31.11%, sesquiterpenes 13.33%, oxygenated monoterpenes and 4.44% oxygenated sesquiterpenes; present monoterpenes were α-pinene, α-terpipene, β-myrcene, camphene, citronellal, geraniol, and trans-β-ocimene[41].

## 2.3 Methodology

### 2.3.1 Plant collection

*C. validus* plant material was collected in the month of April 2013 at the Komga road, near King William's Town. The plant was taxonomically identified by Dr T. Dold; and the voucher specimen was deposited in Selmar Schonland Herbarium Grahamstown (GRA) at Rhodes University and the voucher number was PR/PL02.

### 2.3.2 Extraction of essential oils

Fresh and dry (leaves and flowers) of the *C. validus* were cut into small pieces; 300g of the plant material was subjected to hydro-distillation for approximately 5 hours using the Clevenger apparatus, (see figure 2.5). The extracted essential oils were stored in sealed sample vials and kept inside a refrigerator at a controlled temperature until the moment of use.


Figure 2-3: Clevenger apparatus set-up for hydrodistillation of *C. validus*
1: condenser, 2: Clevenger arm, 3: round bottom flask with plant material, 4: heating mantle. 5: volatile oil extracted from the plant material.

Figure 2-4: Volatile oils extracted from *C. validus*

## 2.4 Analysis of essential oils

### 2.4.1 Quantitative analysis

The essential oil yield was calculated on the basis of fresh/ dried weight of the plant material (v/w) using the formula

Essential oil content (v/w) = a*100/b

Where a = volume of the oil in mL collected through hydro-distillation

b= weight in grams of the plant sample used

### 2.4.2 Qualitative analysis

Chemical analysis was performed using Gas Chromatography Mass Spectrometry (GC-MS).

#### *2.4.2.1 GC-MS analysis*

**GC-MS:** The oils were analyzed by EI on a Hewlett Packard-6890 system equipped with a HP-5MS fused capillary column (30 m x 0.25 mm; 0.25 μm film thickness), directly coupled to a selective mass detector Hewllet Packard-5973. Helium (1mL/min.)

was used as carrier gas; oven temperature program: 60°C-240°C at 3°C/min; splitless during 1.50 min; sample volume 2 μL of the oil solution in hexane (2:1000). Injector and detector temperature was 250°C. EIMS: electron energy, 70 eV; ion source temperature and connection parts: 180°C.

## 2.5 Identification of compounds

Identifications of compounds were made by matching of their mass spectra and retention times with those recorded in the MS library and by comparison of retention indices and mass spectra with literature values[42-43].

## 2.6 Results

### 2.6.1 Chemical composition of essential oils

The oils were extracted by hydro-distillation having a pale yellow color with turpentine odour; the yields were 2.20% v/w for fresh leaves, 2.0% v/w for dried leaves and 2.43% v/w for flowers of the plant, respectively (Table 2.2). Table 2.3 displays the chemical constituents identified by the GC-MS analysis as well as the percentage composition, extracted from fresh, dried leaves; and flowers of *C. validus*. A total of 39 constituents were present in all the oils extracted (as indicated by Table 2.3); in which 13 constituents accounted for (74.3%) in fresh leaves, 14 constituents for (71.8%) in dried leaves and 12 constituents for (73.3%) in flowers. The *C. validus* essential oils constituted of terpenoids, monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids and aromatics. The oils were found to be highly rich in monoterpenoids (99.7%), monoterpenes (43.6%), sesquiterpenes (29.9%) and sesquiterpenoids (29.8%) as they contained the highest percentage composition, aromatics were also present but with a low percentage composition of (2.2%). α-terpineol (37.5%) was the most predominant monoterpenoid in all the oils found in the different parts of the plant. Linalool (29.6%)

was the second major component in monoterpenoids of dried leaves oil; Monoterpenes (43.6%) were the second group to be dominant with northujane (16.9%) as the major constituent followed by β-pinene (4.0%) as the second major constituent; The third group to be dominant were the sesquiterpenes (29.9%) with α-cadinene (8.1%) as the major constituent; the oils also contained sesquiterpenoids (29.8%) with α-eudesmol (6.4%) as the major constituent in flower oil and β-eudesmol (5.4%) as the second major constituent in dried leaves oil.

Table 2-2: (Extraction results) percentage yield for different parts of *C. validus*

| **Species** | **Part of the plant** | **% yield (v/w)** | **Physical appearance** |
|---|---|---|---|
| ***C. validus*** | Fresh Leaves | 2.20 | Pale yellow colour, turpentine odour |
| | Dried Leaves | 2.0 | Pale yellow colour, turpentine odour |
| | Flowers | 2.43 | Pale yellow colour, turpentine odour |

## 2.7 Discussion

The *C. validus* essential oils that were analyzed in this study varied greatly in oil content according to the part of the plant. The number of compounds present in the plant parts also varied with 39 components identified, representing 74.3% in fresh leaf oil, 71.8% in dried leaf oil and 73.3% in flower oil respectively (table 2.3). The major constituents

being α-terpineol (37.5%) and verbenone (13.5%) in fresh leaf oil, linalool (29.6%) and northujane (12.3%) in dried leaf oil and linalool (28.1%) and northujane (16.5%) in flower oil. Although the chemical composition of *C. validus* essential oils has been reported before, comparing our results to those of *C. validus* essential oils reported by Chagonda *et al*., (2000) and Naidoo (2007) we observed that our *C. validus* essential oils showed a very distinct composition which was mainly characterized by α-terpineol (37.5%), linalool (28.0-29.6%), northujane (12.3-16.9%) and verbenone (13.5%). We also observed that our *C. validus* essential oils and that from Zimbabwe both had monoterpenes as their major oil constituents, while the essential oils of *C. validus* from Durban identified monoterpenes, sesquiterpenes and carboxylic acid as their major oil constituents. We then concluded that the two plants from different provinces are 2 different chemotype of the same plant species. This could be due to climatic and soil variation, stage of vegetative cycle and seasonal variation[45]. Literature does support the identification of different chemotypes of plant species based on variation of their chemical constituents. Therefore, our results support the fact that plant species from the same genus can differ in composition because of their geographical location.

Table 2-3: Chemical constituents from different parts of *C. validus* essential oil extracts

| **Constituents** | **$KI_a$** | **$KI_b$** | **Fresh Leaf (%)** | **Dried Leaf (%)** | **Flower (%)** |
|---|---|---|---|---|---|
| **Terpenoids** | | | | | |
| Verbenone | 1204 | 1204 | 13.5 | - | - |
| Carvone | 1242 | 1239 | 0.7 | - | - |
| **Total** | | | **14.2** | **0.0** | **0.0** |
| **Monoterpenes** | | | | | |
| Northujane | 859 | - | 4.4 | 12.3 | 16.9 |
| Santolinatriene | 908 | 906 | - | 0.6 | - |
| β-pinene | 980 | 980 | 4.0 | 1.3 | 1.9 |
| α – phellandrene | 1005 | 1002 | 2.2 | - | - |
| **Total** | | | **10.6** | **14.2** | **18.8** |
| **Monoterpenoids** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Cis-linalool oxide | 1074 | 1088 | - | 1.4 | - |
| Linalool | 1098 | 1099 | 3.2 | 29.6 | 28.0 |
| α –terpineol | 1189 | 1190 | 37.5 | - | - |
| **Total** | | | **40.7** | **31.0** | **28.0** |
| **Sesquiterpenes** | | | | | |
| Cyclosativene | 1368 | 1369 | - | 0.9 | - |
| β –elemene | 1375 | 1392 | - | 1.2 | 1.5 |
| α-copaene | 1376 | 1376 | 0.7 | - | - |
| β –caryophyllene | 1418 | 1418 | 1.9 | - | - |
| Germacrene D | 1480 | - | 2.3 | - | - |
| α-muurolene | 1499 | 1500 | - | 1.0 | 1.2 |
| β – dihydroagarofuran | 1504 | - | - | 2.4 | 6.5 |
| δ-cadinene | 1524 | 1524 | 0.5 | 8.1 | 1.7 |
| **Total** | | | **5.4** | **13.6** | **10.9** |
| **Sesquiterpenoids** | | | | | |
| Elemol | 1547 | 1548 | - | 2.2 | 1.8 |
| Globulol | 1576 | 1590 | - | - | 1.7 |
| Caryophyllene oxide | 1581 | 1582 | 1.2 | 1.9 | - |
| Torreyol | 1645 | 1644 | - | 3.5 | - |
| β-eudesmol | 1649 | 1649 | - | 5.4 | 1.6 |
| α-eudesmol | 1652 | 1652 | - | - | 6.4 |
| α-cadinol | 1653 | 1652 | - | - | 4.1 |
| **Total** | | | **1.2** | **13.0** | **15.6** |
| **Aromatics** | | | | | |
| 1-ethyl-2,4-Dimethylbenzene | 1087 | - | 2.2 | - | - |
| **Total** | | | **2.2** | **0.0** | **0.0** |
| **Grand total** | | | **74.3** | **71.8** | **73.3** |

[a]Kovat Retention indices on HP-5MS capillary column; [b]Literature retention indices[44]

## 2.8 Conclusion

Essential oils from *C. validus* fresh leaves, dried leaves and flowers had both qualitative and quantitative difference. A total of 40 constituents were present in all the oils extracted; with 13 constituents in fresh leaves, 14 constituents in dried leaves and dried flowers. This was different from what has been reported in literature. We also noticed that our yields were higher than those reported in literature[14,41], this could be due to the climatic and soil variation, vegetative cycle and seasonal variation[45].

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# CHAPTER THREE

## 3 Extraction and Chemical composition of *Hyparrhenia hirta* (L.) Stapf

### 3.1 *Hyparrhenia* genus

*Hyparrhenia* is a type of genus that belongs to the Poaceae family. It consists of about 55 species that are dispensed all over the Mediterranean and African continent[1]; some species can be found in hot landmasses of Europe and Asia. Species from this genus are commonly known as thatching grass, they are annual and perennial bunch of grasses with inflorescence that branches into twin spikes of paired spikelets[2].

#### 3.1.1 *Hyparrhenia hirta* (L.) Stapf

##### *3.1.1.1 Botanical Description and Distribution*

*Hyparrhenia hirta* is a species of grass that is known by the common names such as "common thatching grass" and "coolataigrass"[3]. This species is a wiry tufted perennial that grows up to 1m in height with rhizomatous and slender culms. The leaf blade is 20-150x 1-2 (-4) mm. The panicle is scanty consisting of 2-10 raceme pairs which are 20-40 mm long, they are never deflexed; bases are unequal, cylindrical, 8-14 awns, 10-35 mm long, with hairs up to 0.3 mm long. The base of the upper raceme has 0-1 homogamous pairs of spikelets. A sessile spikelet is 4.0-6.5mm long, yellowish to violet, white – villous and callus is acute. The pedicelled spikelet is white villous and awnless[4]. It is very common in South Africa and occurs in open grassland, on rocky slopes and along rivers on moist soil types. *H. hirta* is used for thatching of roofs, for making mats and baskets and as food for grazing livestock[4-5].

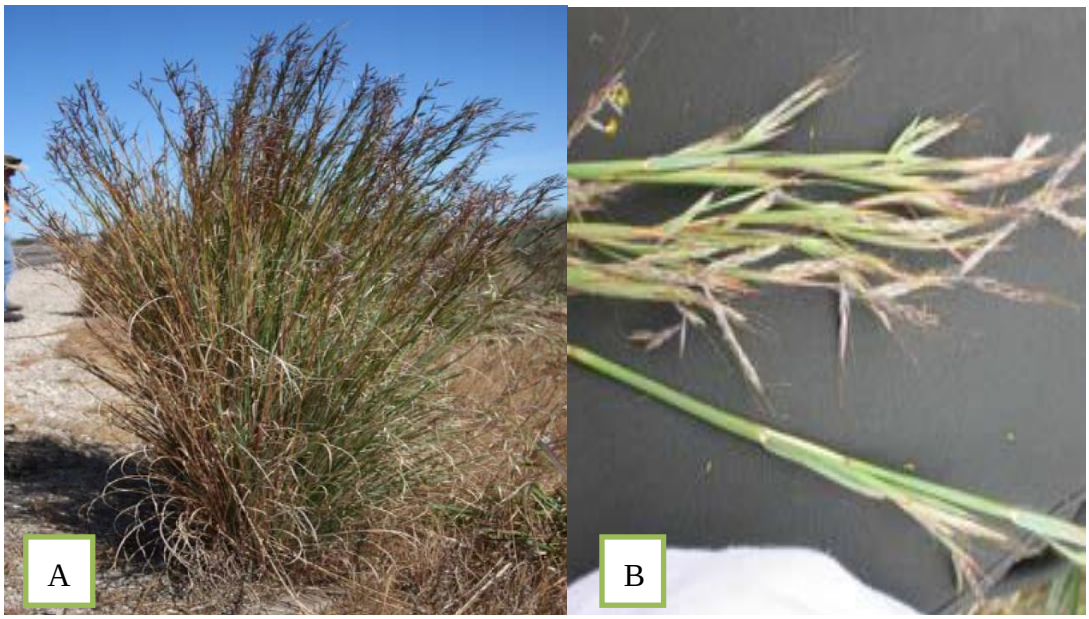


Figure 3-1: A: *Hyparrhenia hirta* plant B: *Hyparrhenia hirta* flowers

#### *3.1.1.2 Literature report on Hyparrhenia hirta (L.) Stapf*

Studies reveal that *H. hirta* is a perennial tussock forming grass which invades pasture and nature vegetation[6], however in Tunisia it is used for diuretic properties[7]. A number of compounds have been isolated from *H. hirta* namely β-ketone, triterpenes and phenolic derivatives[8]. (Bouaziz *et al*, 2002)[8] also reported that leaves of *H. hirta* contained two rare diastereoisomeric flavonolignans tricin (figure 3.2) which were 4'-O-(erythro-β-guaiacylglyceryl) ether (**1**) and tricin 4'-O-(threo-β-guaiacylglyceryl) ether (**2**) together with their 7-O-glucosides were the first flavonolignans glycosides to be isolated as natural products[9]. Four flavonoids were isolated from the methanol extract of *H. hirta* which were tricin (**3**), tricin-7-*O*-glycoside (**4**), luteolin (**5**) and isoorientin (**6**) (figure 3.2). The methanol extracts had some biological activities and that might be due to the isolated flavonoids[10] isoorientin (**6**) and tricin-7-*O*-glucoside (**4**) had anti-inflammatory activity; luteolin (**5**) had anti-oxidant activities[11], Studies also revealed that *H. hirta* methanol extracts appeared to be very effective against hematotoxic and

genotoxic changes induced by nitrate[12]. Additionally, *H. hirta* ethyl acetate extract showed high in vitro antioxidant activity[13].

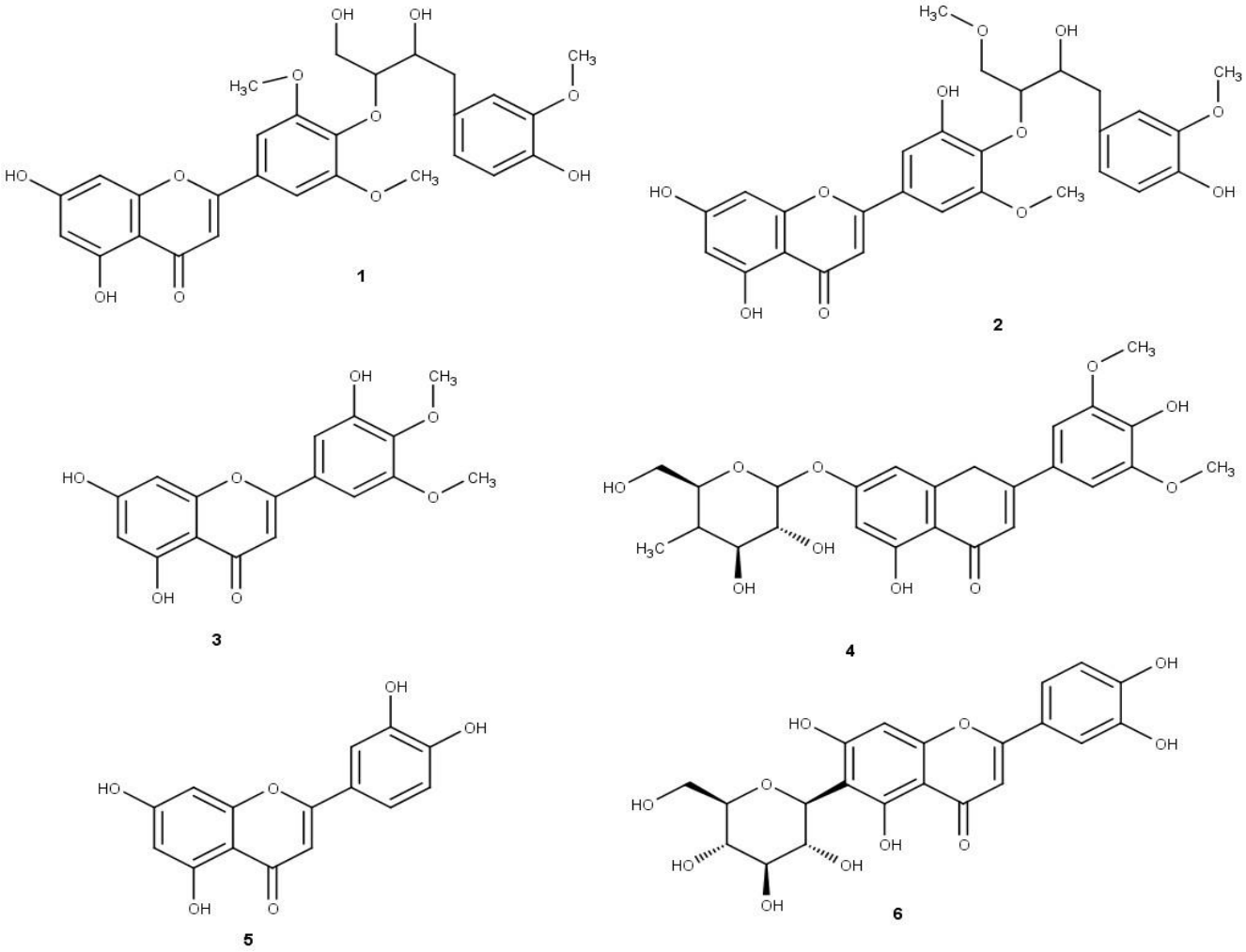


Figure 3-2: Compounds isolated from *H. hirta*

## 3.2 Methodology

### 3.2.1 Plant collection

The plant material was collected in the month of April 2013 at the Komga road, near King William's Town. The taxonomic identification of the plant samples was performed by Dr T. Dold and the voucher specimen was deposited in Selmar Schonland Herbarium Grahamstown (GRA) at Rhodes University; the voucher number for *Hyparrhenia hirta* was PP/PL01.

### 3.2.2 Extraction of essential oils

Fresh and dry (leaves and flowers) of the *H. hirta* were cut into small pieces. 300g of the plant sample was subjected to hydro-distillation for approximately 5 hours using the Clevenger apparatus, (see fig.3-3). The extracted essential oils were stored in sealed sample vials and kept inside a refrigerator at a controlled temperature until the time of analysis and bioassay.


Figure 3-3: A: Clevenger apparatus for hydrodistillation of *H. hirta*
1: condenser, 2: Clevenger arm, 3: round bottom flask with the plant material, 4: heating mantle 5:

Figure 3-4: Volatile oil extracted from *H. hirta*

## 3.3 Analysis of essential oils

### 3.3.1 Quantitative analysis

The essential oil yield was calculated on the basis of fresh/ dried weight of *Hyparrhenia hirta* (v/w) using the formula

Essential oil content (v/w) = a*100/b

Where a = volume of the oil in ml collected through hydro-distillation

b= weight in grams of the plant sample used

### 3.3.2 Qualitative analysis

Chemical analysis was performed using Gas Chromatography Mass Spectroscopy (GC-MS)

#### *3.3.2.1 GC-MS analysis*

The oils were analyzed by EI on a Hewlett Packard-6890 system equipped with a HP-5MS fused capillary column (30 m x 0.25 mm; 0.25 μm film thickness), directly coupled to a selective mass detector Hewllet Packard-5973. Helium (1mL/min.) was used as carrier gas; oven temperature program: 60°C-240°C at 3°C/min; splitless during 1.50 min; sample volume 2 μL of the oil solution in hexane (2:1000). Injector and detector temperature was 250°C. EIMS: electron energy, 70 eV; ion source temperature and connection parts: 180°C.

#### *3.3.2.2 Identification of compounds*

Identifications were made by matching of their mass spectra and retention times with those recorded in the MS library and by comparison of retention indices and mass spectra with literature values[14-16].

## 3.4 Results

### 3.4.1 Chemical composition of essential oils

Hydro-distillation of fresh and dried (leaves and flowers) of *H. hirta* produced a pale yellow oil with a characteristic unpleasant odor. The percentage yield was 3.4% v/w from fresh leaves, 2.8% v/w from dried leaves, 2.8% v/w from fresh flowers and 0.7% v/w from dried flowers respectively (Table 3.1). The constituents identified by the GC-MC analysis and their percentage composition are displayed on (Table 3.2). A total of 106 constituents were present in all the oils extracted. The GC-MS showed that 25 constituents accounted for (68.1%) in fresh leaves oil, 40 constituents for (71.9%) in dried leaves oil, 23 constituents for (77.6%) in fresh flowers oil and 18 constituents for (80.1%) in dried flower oil. GC-MS results also showed that the oils were dominated by a carbonyl group (70.2%) with phytone (10.4%) and 2-nonadecanone (9.0%) as the major constituents. Phthalates (65.7%) were the second dominant group with dibutyl phthalate (26.9%) as the major constituents and diisooctyl phthalate (26.5%) being the second as the major constituent. *H. hirta* oils were also rich in monoterpenes (58.2%); northujane (30.0%) was the major constituent followed by β-pinene (2.9%) as the second major constituent.

Table 3-1: (Extraction results) percentage yield for different parts of *H. hirta*

| Species | Part of the plant | % yield (v/w) | Physical appearance |
|---|---|---|---|
| *H. hirta* | Fresh Leaves | 3.4 | Pale yellow with an unpleasant odor |
| | Dried Leaves | 2.8 | Pale yellow with an unpleasant odor |
| | Fresh Flowers | 2.7 | Pale yellow with an unpleasant odor |
| | Dried Flowers | 0.71 | Pale yellow with an unpleasant odor |

## 3.5 Discussion

The present study is the first report on essential oil composition of *H. hirta* with northujane (30.0%), dibutyl phthalates (26.9%), phytone (10.4%) and caryophyllene oxide (9.66%) as the major constituents. However, there is quite a lot of work that has been done on the non-volatile constituents of *H. hirta* with a number of compounds being isolated which include β-ketone, triterpenes and phenolic derivatives[8]. Moreover, (Isiaka *et al*, 2004)[18] revealed that the main compounds from the volatile oils of *Hyparrhenia rufa* were found to be τ-cadinol (17.43%) and β-selinene (11.64%). These authors also reported that there were four uncommon infrequently occurring terpenoids esters which were (E,E)-methyl farnesoate (1.01%), (E,E)-methyl-10,11-epoxyfarnesoate (12.17%), methyl (2E,6E)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate (4.3%) and methyl (2E, 6E)-3,7,11-trimethyl-10-oxodecadienoate (2.25%)[18]. When comparing these results with our own results we found that the major constituents of *H. rufa* were different from the *H. hirta* major constituents. This was not surprising since these plants are different species of *Hyparrhenia*. But when looking at other constituents found in these plants we found that both species had common constituents, for example, both plants' essential oils contained β-pinene, α-phellandrene, terpinene-4-ol, linalool and

globulol; with β-pinene having the percentage composition of (2.90%), α-phellandrene (0.40%), terpinene-4-ol (0.70%), linalool (2.30%) and globulol (0.50%) in *H. hirta* whilst in *H. fura*, the percentage composition of β-pinene was (0.50%), terpinene-4-ol (0.33%), linalool (4.10%), globulol (1.31%) while α-phellandrene was in trace amounts. Essential oil composition of *H. hirta* is almost identical to that of *H. hirta* since both essential oils of the plants contained β-pinene, α-phellandrene, terpinene-4-ol, linalool and globulol. However, their major constituents were different. Composition of the essential oils varies among species as the essential oil content and composition are greatly influenced by climate, season and daily effects[19-20].

Table 3-2: Chemical components from different parts of *H. hirta* essential oil extracts

| **Constituents** | **KI[a]** | **KI[b]** | **Fresh Leaf (%)** | **Dried Leaf (%)** | **Fresh Flower (%)** | **Dried Flower (%)** |
|---|---|---|---|---|---|---|
| **Monoterpenes** | | | | | | |
| Northujane | 859 | - | 8.5 | - | 16.4 | 30.0 |
| β-pinene | 980 | 980 | - | - | 2.9 | - |
| α-phellandrene | 1005 | 1002 | - | - | 0.4 | - |
| **Total** | | | **8.5** | **0.0** | **19.7** | **30.0** |
| **Monoterpenoids** | | | | | | |
| Artemisia ketone | 1062 | 1056 | - | - | - | 0.4 |
| Linalool | 1098 | 1099 | 1.4 | 2.3 | 0.9 | - |
| Terpinen-4-ol | 1177 | 1174 | - | 0.7 | - | - |
| α-terpineol | 1189 | 1190 | - | - | - | 0.4 |
| Geranyl acetone | 1453 | 1454 | - | 0.5 | - | - |
| **Total** | | | **1.4** | **3.5** | **0.9** | **0.8** |
| **Sesquiterpenes** | | | | | | |
| β-elemene | 1375 | 1392 | 0.8 | - | - | - |
| α-copaene | 1376 | 1376 | - | - | 0.4 | - |
| β-caryophyllene | 1418 | 1418 | 0.3 | - | 0.3 | - |
| Alloaromadendrene | 1461 | - | 0.4 | - | - | - |
| α-muurolene | 1499 | 1500 | 1.0 | - | - | - |
| **Total** | | | **2.5** | **0.0** | **0.7** | **0.0** |
| **Sesquiterpenoids** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| β-ionol | 1406 | 1412 | - | - | - | 1.6 |
| Cubedol | 1484 | - | 7.4 | - | - | - |
| Cis-nerolidol | 1534 | - | 0.2 | - | - | - |
| Elemol | 1547 | 1548 | 4.2 | 0.6 | - | - |
| Spathulenol | 1576 | 1577 | 0.5 | - | - | 1.7 |
| Caryophyllene oxide | 1581 | 1581 | 1.7 | - | - | 9.6 |
| Globulol | 1604 | 1590 | - | 0.5 | - | - |
| γ-eudesmol | 1630 | 1630 | - | 0.4 | - | 0.7 |
| Agarospirol | 1631 | 1646 | - | 0.6 | - | - |
| Hinesol | 1638 | 1640 | - | 0.5 | - | - |
| Cubenol | 1642 | 1645 | - | - | - | 1.6 |
| β-eudesmol | 1649 | 1649 | 6.2 | 0.8 | - | - |
| Bulnesol | 1666 | 1670 | 3.1 | - | - | - |
| Hedycaryol | 1694 | 1546 | 7.0 | - | - | 0.4 |
| **Total** | | | **30.3** | **3.4** | **0.0** | **15.4** |
| **Carbonyls** | | | | | | |
| Heptanal | 899 | 901 | - | 0.5 | - | - |
| δ-Octen-3-ol | 978 | 978 | - | 0.4 | | |
| Sulactone | 985 | - | - | 0.3 | - | - |
| 2,4-Dimethylcyclohexanol | 1032 | - | - | 0.8 | - | - |
| 2,2,6-Trimethylcyclohexanone | 1036 | - | - | 0.3 | - | - |
| 1,2-phenylacetaldehyde | 1043 | - | - | 0.9 | - | - |
| Hydrocinnamaldehyde | 1160 | 1599 | - | 0.5 | - | - |
| Nonanol | 1171 | 1173 | - | 0.3 | - | - |
| Decanal | 1204 | 1204 | - | 0.4 | - | - |
| Undecan-1-ol | 1372 | - | - | 0.4 | - | - |
| 6,10-Dimethylundecan-2-one | 1385 | - | - | 0.4 | - | 1.0 |
| 2-tridecanone | 1481 | - | 2.6 | 4.2 | 2.5 | - |
| Pentadecanal | 1513 | 1717 | - | 0.9 | 0.9 | - |
| Hexadecanal | 1819 | 1830 | 1.5 | 1.6 | 3.1 | 0.5 |
| Methyl 14-methylpentadecanoate | 1884 | - | - | - | 0.9 | - |
| Phytone | 1845 | - | 7.8 | 10.4 | 1.1 | 2.2 |
| E,E-farnesyl acetone | 1918 | 1927 | 0.5 | 0.9 | - | - |
| Methyl oleate | 2103 | - | - | - | 5.5 | - |
| 2-nonadecanone | 2106 | - | 6.8 | 9.0 | - | 0.5 |
| Methyl-octadecanoate | 2128 | 2124 | - | - | 0.6 | - |
| **Total** | | | **19.2** | **32.2** | **14.6** | **4.2** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Phthalates** | | | | | | |
| Dibutyl phthalate | 1922 | - | 4.4 | 7.5 | 0.4 | 26.9 |
| Diisooctyl phthalate | 2704 | - | - | - | 26.5 | - |
| **Total** | | | **4.4** | **7.5** | **26.9** | **26.9** |
| **Aromatics** | | | | | | |
| Toluene | 762 | - | - | 6.5 | - | - |
| Ethylbenzene | 878 | - | - | 0.7 | - | - |
| p-xylene | 883 | - | - | 2.4 | - | - |
| Benzaldehyde | 961 | 962 | - | 3.4 | - | - |
| 2-pentylfuran | 992 | 986 | - | 0.3 | - | - |
| m-cymene | 1082 | 1082 | - | - | 1.3 | - |
| **Total** | | | **0.0** | **13.3** | **1.3** | **0.0** |
| **Hydrocarbons** | | | | | | |
| 2,3-Dimethylhexane | 758 | - | - | 8.1 | - | - |
| 3-Methylheptane | 771 | - | - | 0.4 | - | - |
| Octane | 800 | 800 | - | 0.8 | - | - |
| Hexadecane | 1600 | 1600 | 0.2 | - | - | - |
| Eicosane | 2000 | 2000 | 0.2 | 0.3 | 0.3 | 0.4 |
| Heneicosane | 2100 | 2100 | 0.2 | - | - | - |
| Tetracosane | 2400 | 2400 | - | 0.3 | 0.5 | 0.8 |
| Nonacosane | 2900 | 2900 | - | - | 3.7 | - |
| Triacontane | 3000 | 3000 | 0.3 | 0.9 | 2.9 | 1.0 |
| Tritriacontane | 3300 | 3300 | - | - | 2.7 | - |
| Tetratriacontane | 3400 | 3400 | 0.9 | 0.4 | 1.9 | 0.4 |
| Hexatriacontane | 3600 | - | - | 0.4 | 1.5 | - |
| **Total** | | | **1.8** | **11.6** | **13.5** | **2.6** |
| **Grand total** | | | **68.1** | **71.9** | **77.6** | **80.1** |

[a] Kovat Retention indices on HP-5MS capillary column; [b]Literature kovat retention indices[17].

## 3.6 Conclusion

As stated before that there is no work that has been done on the chemical composition of *H. hirta* volatile oils in literature. Therefore, the present study presents the first comprehensive analysis of the chemical constituents of the essential oils of *H. hirta*.

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# CHAPTER FOUR

## 4 Anti-inflammatory properties of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy and *Hyparrhenia hirta* (L.) Stapf

### 4.1 Introduction

Inflammation is a body's response to injury induced by tissue injury or infection; it functions as to replace injured tissues or damaged host cells[1]. Inflammation can be classified as acute inflammation or chronic inflammation depending on whether the response is short lived or long lived, respectively[2]. Acute inflammation is an instantaneous response to trauma that is an injury or surgery, this type of inflammation is short lived. On the other hand, chronic inflammation reflects a prolonged response; this type of inflammation is associated with a large number of chronic human disorders which include autoimmune diseases, arthritis, cancer, allergy and atherosclerosis[3].

Many drugs such as steroids and antihistamines have been used in the treatment of inflammatory diseases, but they also exist for the treatment of those inflammatory diseases such as asthma rheumatoid arthritis, systematic vasculitis and psoriatic arthritis to name a few[4]. In the preceding decades, researchers have been targeting the medicinal prospective of essential oils as well as their constituents in order to search for novel drugs of plant origin, especially those showing anti-inflammatory effects, so that they can be used to prevent and to treat diseases[5].

Chemical constituents of plant essential oils mostly belong to terpenoid compounds such as monoterpenes, sesquiterpenes as well as their oxygenated derivatives. These low molecular weight compounds (< 300g/mol) diffuse across cell membranes to induce biological reactions. In recent years, there has been a tendency for applied studies of essential oils to focus on antimicrobial and the mosquito larvicidal activities as well as

anti-inflammatory bioactivity. Previous studies found that essential oils of some Lamiaceae can reduce carrageenan induced hind paw inflammation in the rat[6]. Components of essential oils such as α-pinene and β-caryophyllene have been found to have anti-inflammation actions. α-pinene has been found to have anti-inflammatory effect in a rat model while β-caryophyllene has anti-inflammatory effects both *in vitro* and *in vivo*[7-9]. 1,8-cineole which is a major component of Eucalyptus and some other essential oils, has been found to have anti-inflammatory activity *in vitro*[10] and *in vivo* in an animal model[11].

Some essential oils possess anti-inflammatory activity, for example essential oils from chamomile have been used for centuries for their anti-inflammatory effect and also for alleviating the symptoms associated with eczema, dermatitis and other pronounced irritations[12]. Essential oils from Eucalyptus, Rosemary, Lavender and Millefolia along with other plants such as pine and myrrh have been used as mixed formulations as anti-inflammatory agents[13]. For example, lavender oil and eugenol (a major compound of clove oil) have been found to inhibit allergic responses *in vitro* and *in vivo* in animals[14-15] while ginger essential oil and eugenol have been found to have significant anti-inflammatory effects in rats[16].

## 4.2 Methodology

### 4.2.1 Experimental animals

Both female and male wistar rats weighing 195-240g were used. The rats were obtained from the South African Vaccine Producers (SAVP) and were housed in the animal holding facility at the Zoology Department of Walter Sisulu University (WSU) in Mthatha. Ethical clearance for the study was obtained from the Walter Sisulu Research Ethics Committee DVC (AA&R) DRD/SREC: Reference No: 31. The animals were kept under standard conditions with each cage housing 5 rats; room temperature was maintained at 24$^{o}$C and lighting was by daylight only. Animals had free access to food and water throughout, except 8 hrs before experimentation, animals were given only water. Rats were administered 2% essential oil of *C. validus* orally

**Group 1 –** treated with 1 ml 0.09% NaCl (control group)

**Group 2-** treated with 100mg/kg Aspirin (standard)

**Group 3**- 1 ml of 2% essential oil from fresh leaves of *C. validus* (C.V.F.L)

**Group 4**- 1 ml of 2% essential oil from dried leaves of *C. validus* (C.V.D.L)

**Group 5**- 1 ml of 2% essential oil from dried flowers of *C. validus* (C.V.D.F)

**Group 6**-1 ml of 2% essential oil from fresh leaves of *H. hirta* (H.H.F.L)

**Group 7**-1 ml of 2% essential oil from dried leaves of *H. hirta* (H.H.D.L)

**Group 8**-1 ml of 2% essential oil from fresh flowers of *H. hirta* (H.H.F.F)

**Group 9**-1 ml of 2% essential oil from dried flowers of *H. hirta* (H.H.D.F)

## 4.3 Drug used

Aspirin was procured from Reckitt Benckiser Pharmaceutical (PTY) LTD/ (EDMS) BPK Elansfontein –South Africa.

## 4.4 Anti-inflammatory activity: Fresh egg albumin – induced right hind paw edema

Animals were randomly distributed to one of the 6 groups as indicated earlier. Rats were given saline, Aspirin (100 mg/kg) or 1 ml of 2% essential oil preparations after determining baseline right hind paw diameter (size) for each rat using a pair of YATO digital calipers[17]. 30 minutes later the right hind paw of each rat was injected with 1 ml of 50% (v/v) fresh egg albumin. Paw sizes were again measured 30, 60, 90 and 120 minutes after albumin injection. Change in paw size was calculated as: paw size after albumin injection at selected times - paw size before albumin injection.

### 4.4.1 Statistical Analysis

One way Analysis of variance (ANOVA) with Turkey-Kramer Multiple Comparisons Test was performed using GraphPad Instat to determine the difference between treatment groups. Results were expressed as mean ± standard error of the mean (SEM) and $p<0.05$ was considered significant.

## 4.5 Results for *Cymbopogon validus* essential oils

### *4.5.1* Anti-inflammatory effect of *C. validus*

Figure 4-1 illustrates the anti-inflammatory effects of essential oils of *C. validus* on fresh egg albumin - induced inflammation: measured 30, 60, 90 and 120 minutes after injection of the phlogistic agent. All the essential oils showed significant ($p<0.01$) anti-inflammatory effects from 30 to 120 minutes. Results obtained with essential oils were better than those obtained with aspirin during the experimental period. On the other hand, figure 4-2 shows a comparison of the anti-inflammatory effect of aspirin with oils from fresh and dried leaves and dried flowers of *C. validus.* The fresh leaves oil showed

significantly ($p$<0.05 and $p$<0.01) greater anti-inflammatory effect compared to aspirin throughout the experimental period. Essential oil from the dried flowers also showed significantly (p<0.05) greater anti-inflammatory effects compared to aspirin during the 60 and 120 minutes while the anti-inflammatory effects of oil from dried leaves was not different from results obtained with aspirin.

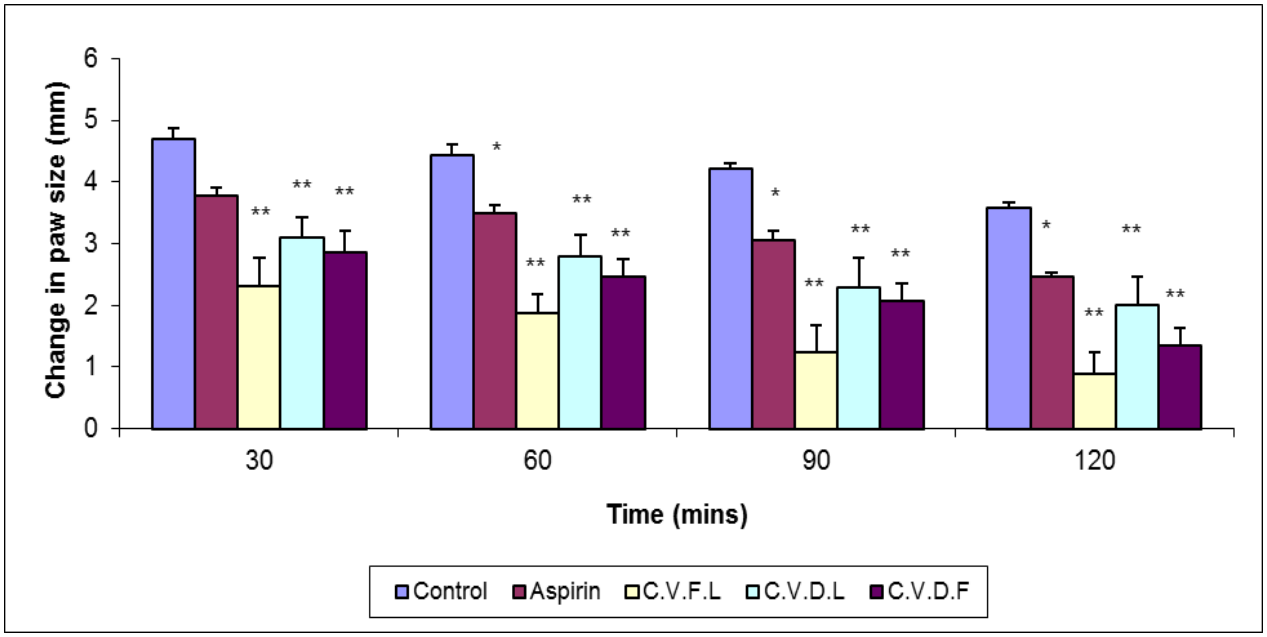


Figure 4-1: Anti-inflammatory effects of *C validus* oil (fresh and dry leaves and dry flowers) on albumin-induced paw edema, at different times of inhibition.

C.V.F.L = *C. validus* fresh leaves; C.V.D.L = *C. validus* dried leaves; C.V.D.F = *C. validus* dried flowers; each bar represents mean ± S.E.M for 5 rats per group. *$p$ <0.05, **$p$ <0.01 compared to control animals.

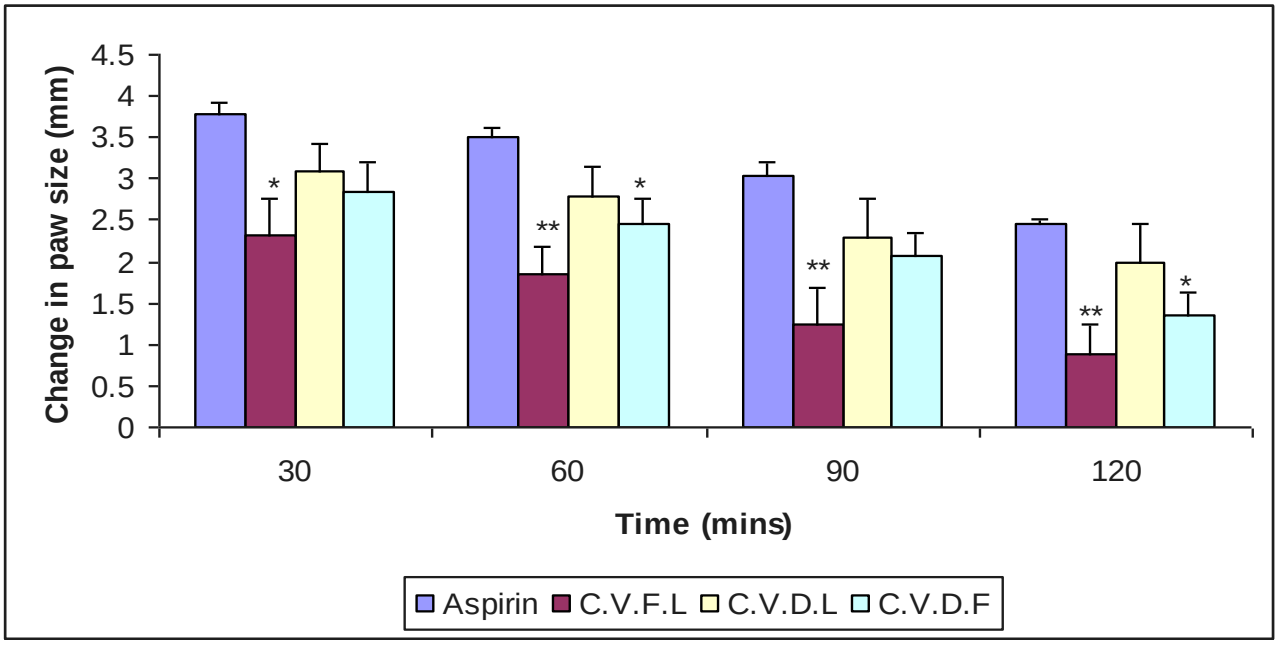


Figure 4-2: Anti-inflammatory effects of *C. validus* oil (fresh and dried leaves; and fresh buds and dried buds) on fresh egg albumin-induced paw edema at different time of inhibition.

Results compared the effects of essential oils from *C. validus* with aspirin, a known NSAID. C.V.F.L = *C. validus* fresh leaves; C.V.D.L = *C. validus* dry leaves; C.V.D.F = *C. validus* dry flowers; each bar represents mean ± S.E.M for 5 rats per group. *$p$ <0.05, **$p$ <0.01 compared to aspirin treated animals.

## 4.6 Discussion

### 4.6.1 Anti-inflammatory activity

We evaluated the anti-inflammatory effect of *C. validus* essential oils in wistar rats using the fresh egg albumin-induced rat paw edema model. The essential oils from *C. validus* (i.e. fresh and dried leaves and dried flowers) showed anti-inflammatory action by reducing the paw volume significantly. Fresh egg albumin induced inflammation occurs in 3 phases. An initial phase during the first 1.5-2h which is caused by the release of histamine and

serotonin[18]. The second phase involves the release of bradykinin from 1.5 h to 2.5 h, whilst the third phase involves the release of prostaglandins and that occurs from 2.5 h to 6 h after albumin injection[19]. The current study lasted for only 120 minutes due to rapid resolution of inflammation. This corresponds to the first phase of inflammation, indicating that the essential oils from the fresh and dried leaves and dried flowers of *C. validus* exerted their anti-inflammatory properties by inhibiting the release of histamine and serotonin. Linalool, a major constituent in the oil could have contributed to the observed anti-inflammatory effects. Indeed a few studies have revealed an anti-inflammatory effect of (-) - Linalool on chronic inflammation, which significantly reduced Complete Freund's Adjuvant induced paw edema at a dose of 200mg/kg[20-22]. α-terpineol which is also a major constituent in *C. validus* essential oil has anti-inflammatory effects as it was found to be an effective inhibitor of superoxide production[23]. Contents of essential oils from *C. validus* may exert their effects by inhibiting the release of mediators such as histamine and serotonin during the first phase.

## 4.7 Results for *Hyparrhenia hirta* essential oils

### 4.7.1 Anti-inflammatory effect for *Hyparrhenia hirta*

Figure 4.3 illustrates the anti-inflammation effects of essential oils of *H. hirta* on fresh egg albumin - induced inflammation: measured as a factor of time for 30, 60, 90 and 120 minutes. All the essential oils showed significant ($p$<0.01) anti-inflammatory effects from 30 to 120 minutes. Results obtained with essential oils were better than those obtained with aspirin during the experimental period. A comparison of the anti-inflammatory effect of aspirin with oils from (fresh; dried leaves and fresh; dried flowers) of *H. hirta* revealed that

the oils were able to inhibit inflammation ($p<0.01$), during the 60, 90 and 120 minutes post albumin injection. However, during the first 30 minutes inhibition occurred poorly and only the fresh bud oil was able to inhibit inflammation ($p<0.05$) (Figure 4.4.)

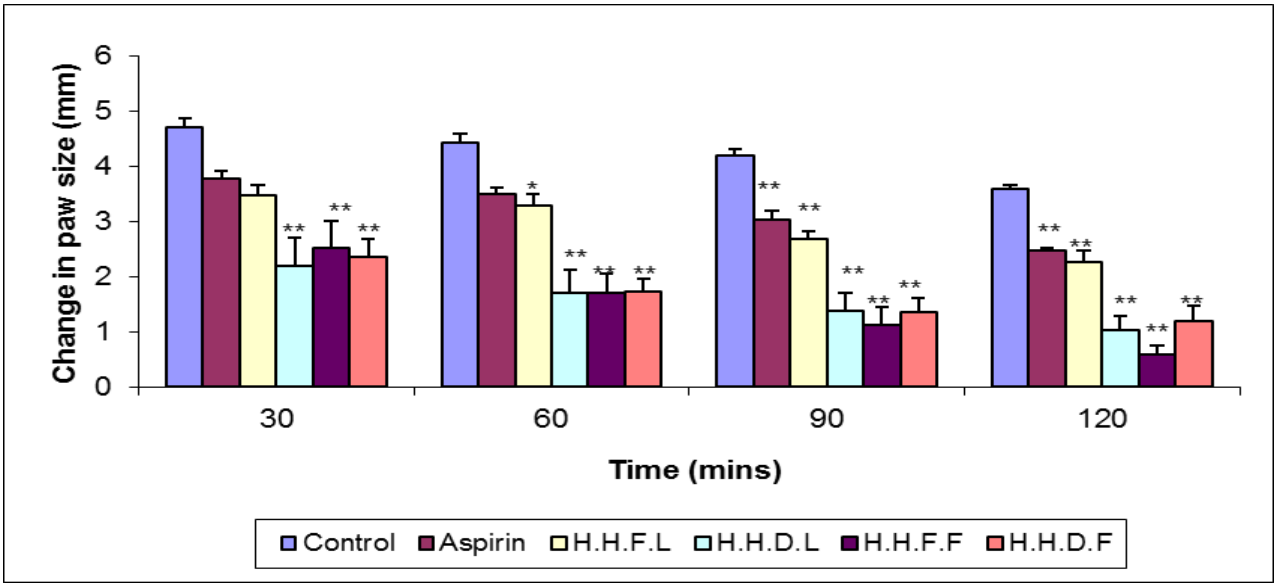


Figure 4-3: Anti-inflammatory effects of essential oils of *Hyparrhenia hirta* for (fresh; dried leaves; and fresh; dried flowers) on egg albumin- induced paw edema, at different times of inhibition.

H.H.F.L = *H. hirta* fresh leaves; H.H.D.L = *H. hirta* dried leaves; H.H.F.F = *H. hirta* fresh flowers; H.H.D.F = *H. hirta* dried flowers; each bar signifies mean ± S.E.M for 5 rats per group. *=$p$ <0.05, **=$p$ <0.01 compared to control animals.

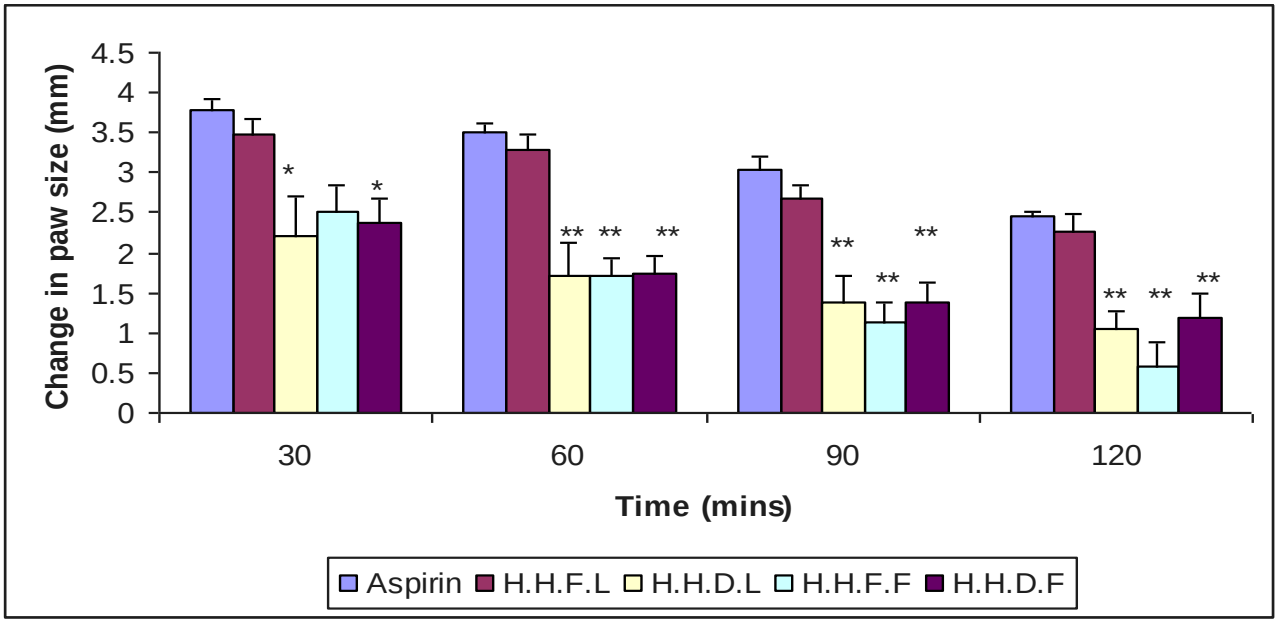


Figure 4-4 :Anti-inflammation effects of *H. hirta* oil (fresh and dried leaves; and fresh buds and dried buds) on fresh egg albumin- induced paw edema, at different time of inhibition.

Results compare effects of essential oils from *H. hirta* with aspirin, a known NSAID. H.H.F.L = *H. hirta* fresh leaves; H.H.D.L = *H. hirta* dried leaves; H.H.F.F = *H. hirta* fresh flowers; H.H.D.F = *H. hirta* dried flowers; each bar represents mean ± S.E.M for 5 rats per group. *=p <0.05, **=p <0.01 compared to aspirin treated animals.

## 4.8 Discussion

### 4.8.1 Anti-inflammatory activity

In the present study, anti-inflammatory effects of the essential oils of *H. hirta* were investigated after subplantar injection of fresh egg albumin- induced right hind paw edema of the rat. The inflammatory mediators released in this model include histamine, serotonin which is released during the first 1.5 h, bradykinin mediator which released within the 2.5 h after albumin injection[23]. As for prostaglandins it occurs from 2.5 h to 6 h post albumin injection and this phase is known as the second phase. These mediators are able to promote vasodilation as well as increasing vascular permeability and synergistically producing

edema[24-26]. The experimental findings from the fresh egg albumin induced rat paw edema showed that essential oils from (fresh; dried leaves and fresh; dried flowers) of *H. hirta* reduced the paw volume significantly ($p<0.01$) from 30 to 120 minutes. However, aspirin showed no effectiveness during the first 30 minutes, but only a low effect ($p<0.05$) at 60 minutes, highest effect ($p<0.01$) for aspirin was observed during the 90 and 120 minutes when it was compared to control group. A comparison between aspirin with *H. hirta* essential oils showed inhibitory effect ($p<0.05$ and $p<0.01$) from 30 to 120 minutes. Moreover, dried leaf oil, fresh flower oil and dried flower oil were found to be the most active in anti-inflammatory effect ($p<0.01$) during 60, 90 and 120 minutes. However, during the first 30 minutes dried leaf oil and dried flower oil showed low inhibition ($p<0.05$). Fresh leaf oil was inactive throughout the 120 minutes of the experiment. These results tend to suggest that the significant activity of the oils observed in the first phase of egg-albumin induced inflammation may be due to inhibition of early mediators, such as histamine and serotonin. Additionally, both fresh and dried parts of the plant were proved to have anti-inflammatory properties. Thus, both the fresh and dried parts of the plant may be used as they exhibit high anti-inflammation activities.

## 4.9 Conclusion

In conclusion, both *C. validus* and *H. hirta* essential oils have anti-inflammatory effects when they are orally administered to rats. Therefore, our study indicates that *C. validus* and *H. hirta* can be used as anti-inflammatory agents.

## 4.10 References


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# APPENDIX

APPENDIX A- 1: **GC-MS chromatogram of *C. validus* Fresh Leaves (C.V.F.L.)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-12 09:42:08 AM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract1 |
| Sample ID | : Plant extract1 |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 2 |
| Injection Volume | : 1.00 |
| Data File | : C:\GCMSsolution\Data\Dr Oyedeji\Plant extract1.qgd |
| Org Data File | : C:\GCMSsolution\Data\Dr Oyedeji\Plant extract1.qgd |
| Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Org Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Report File | : |
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| Modified by | : Neal |
| Modified | : 2014-01-13 10:51:12 AM |

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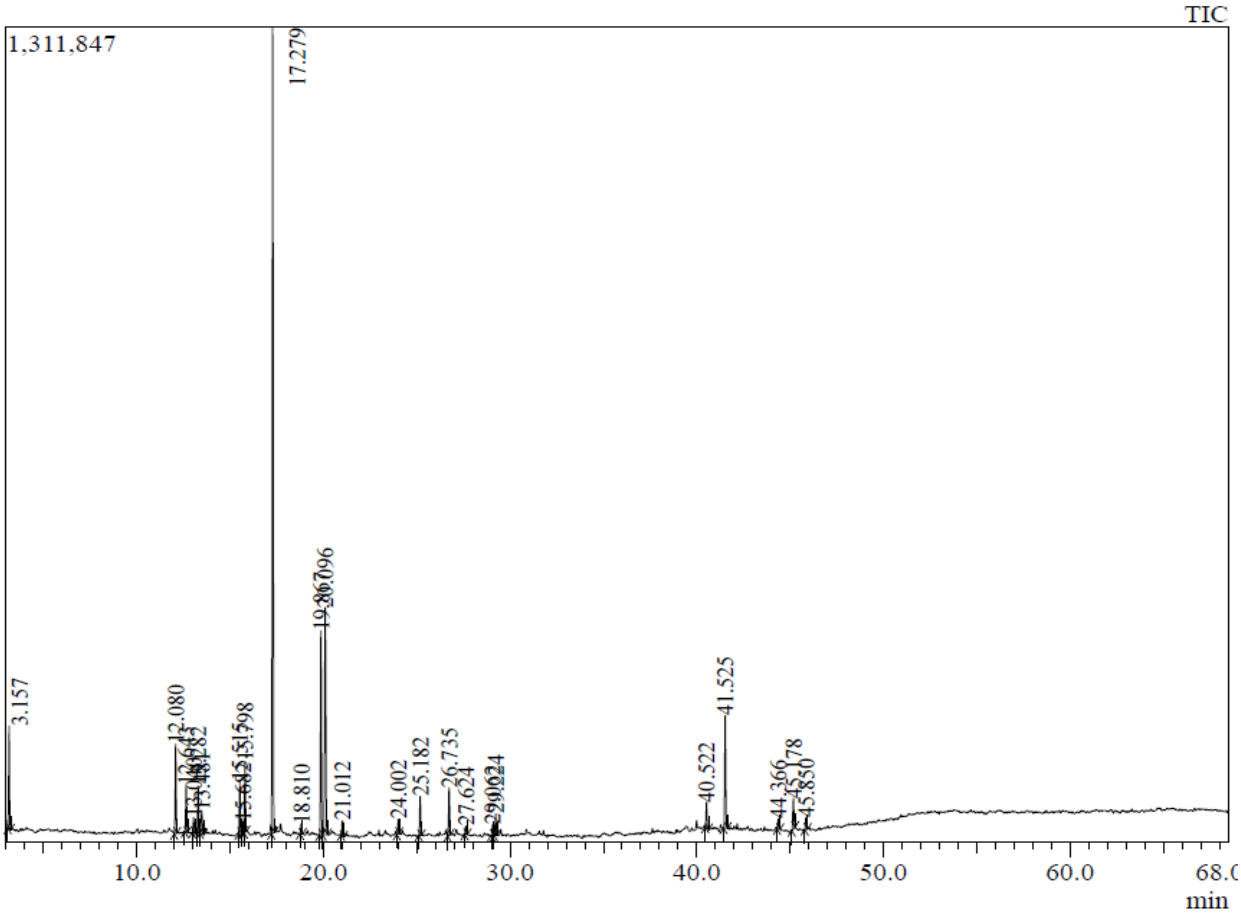

APPENDIX A- 2: **GC-MS chromatogram of *C. validus* dried leaves (C.V.D.L.)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-12 12:46:41 PM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract2 |
| Sample ID | : Plant extract2 |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 3 |
| Injection Volume | : 1.00 |
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| Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Org Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Report File | : |
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| Modified by | : Neal |
| Modified | : 2013-12-12 01:58:23 PM |

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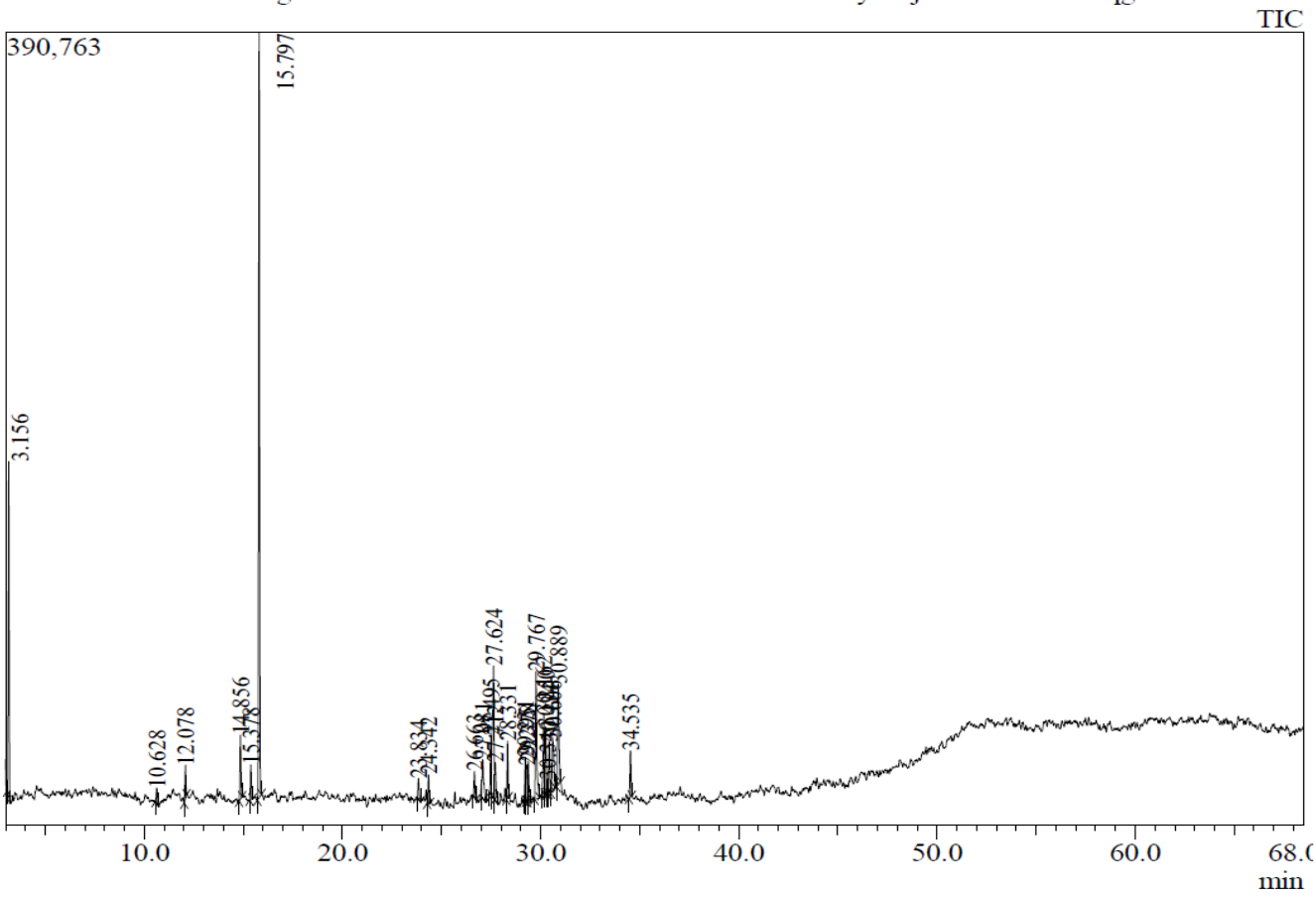

APPENDIX A- 3: **GC-MS chromatogram of *C. validus* flowers (C.V.F)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-12 02:02:49 PM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract4 |
| Sample ID | : Plant extract4 |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 4 |
| Injection Volume | : 1.00 |
| Data File | : C:\GCMSsolution\Data\Dr Oyedeji\Plant extract4.qgd |
| Org Data File | : C:\GCMSsolution\Data\Dr Oyedeji\Plant extract4.qgd |
| Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Org Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
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| Modified | : 2013-12-12 03:14:32 PM |

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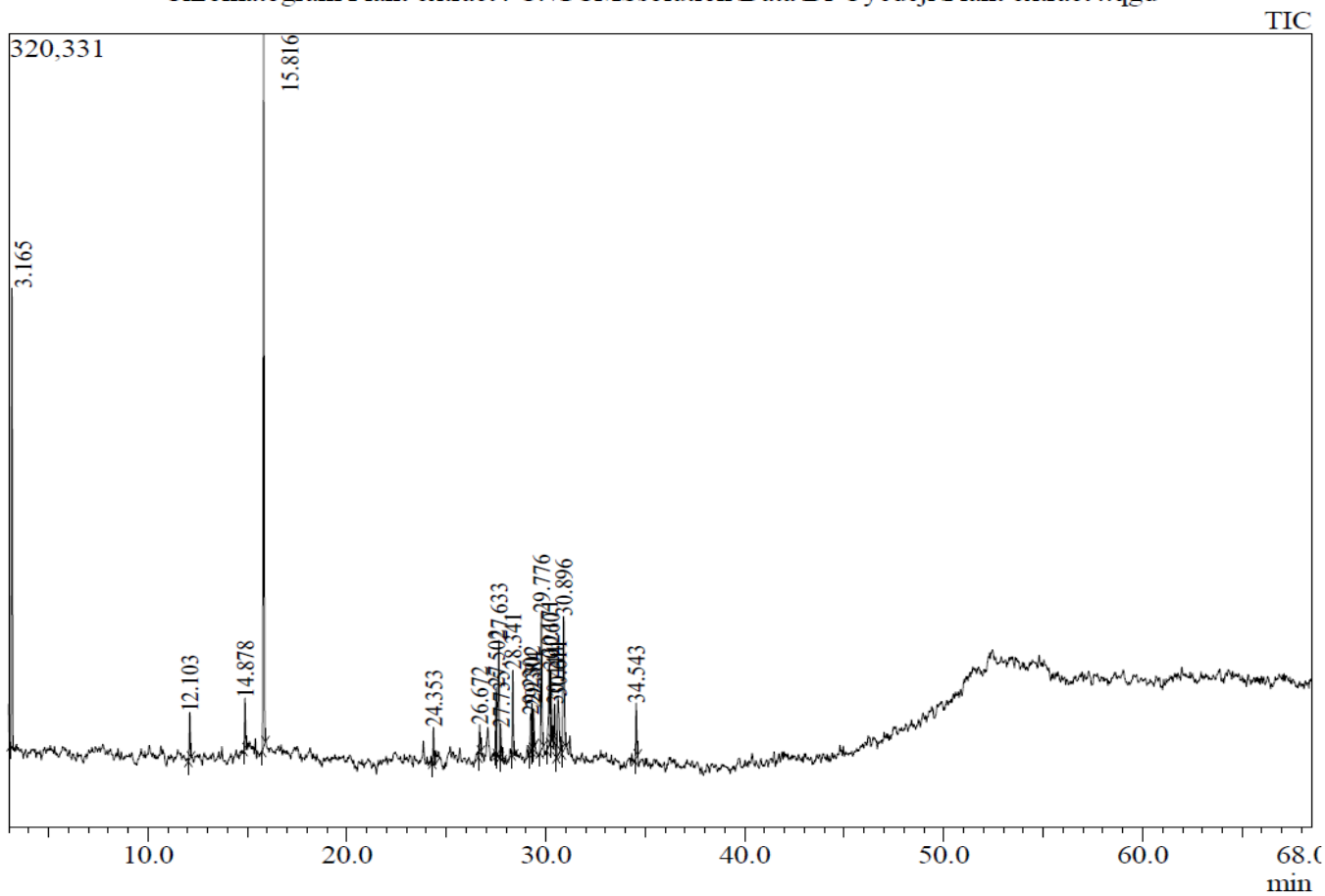

APPENDIX B- 1: **GC-MS chromatogram of *Hyparrhenia hirta* fresh leaves (H.H.F.F)**

Sample Information

| | |
|---|---|
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| Analyzed | : 2013-12-13 03:41:46 PM |
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| Level # | : 1 |
| Sample Name | : Plant extract5x10dil |
| Sample ID | : Plant extract5x10dil |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 5 |
| Injection Volume | : 3.00 |
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| Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Org Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
| Report File | : |
| Tuning File | : C:\GCMSsolution\System\Tune1\12Dec2013 Normal.qgt |
| Modified by | : Neal |
| Modified | : 2013-12-13 04:53:33 PM |

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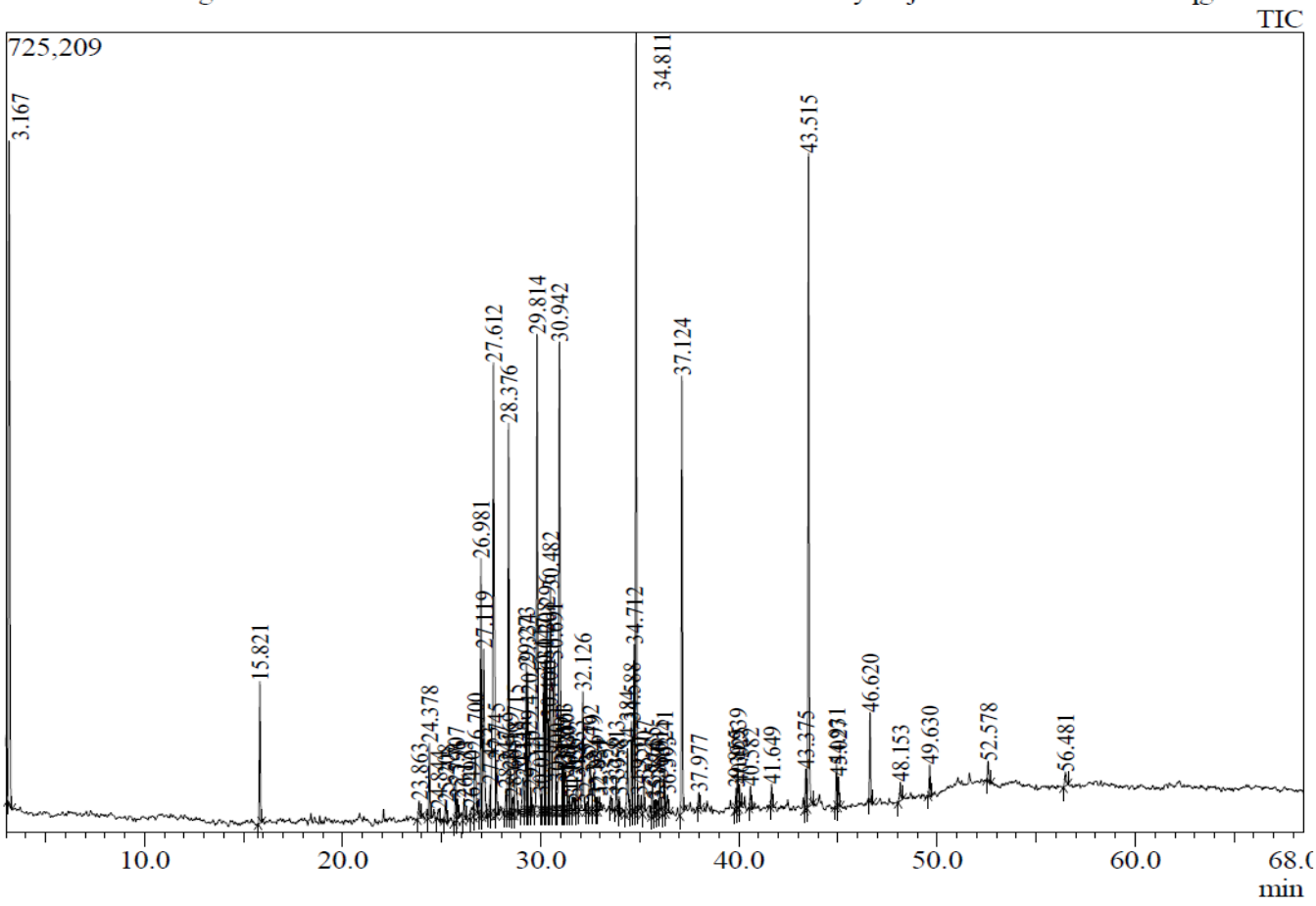

APPENDIX B- 2: **GC-MS chromatogram of *Hyparrhenia hirta* dried leaves (H.H.D.L)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-13 04:58:04 PM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract6x10dil |
| Sample ID | : Plant extract6x10dil |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 6 |
| Injection Volume | : 3.00 |
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| Org Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
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| Modified by | : Neal |
| Modified | : 2013-12-13 06:09:43 PM |

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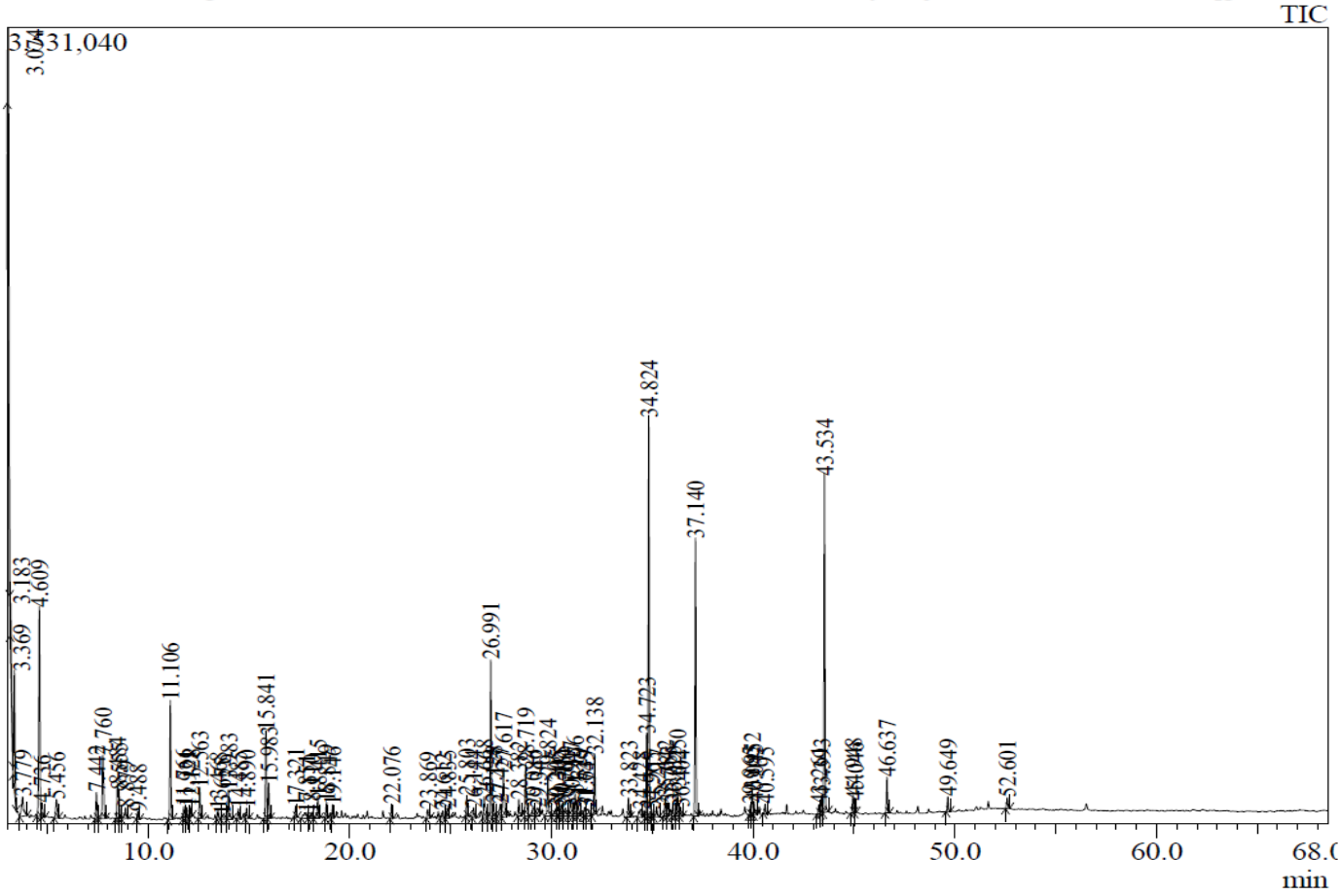

APPENDIX B- 3: **GC-MS chromatogram of *Hyparrhenia hirta* fresh flowers (H.H.F.F)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-13 06:14:11 PM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract7x10dil |
| Sample ID | : Plant extract7x10dil |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
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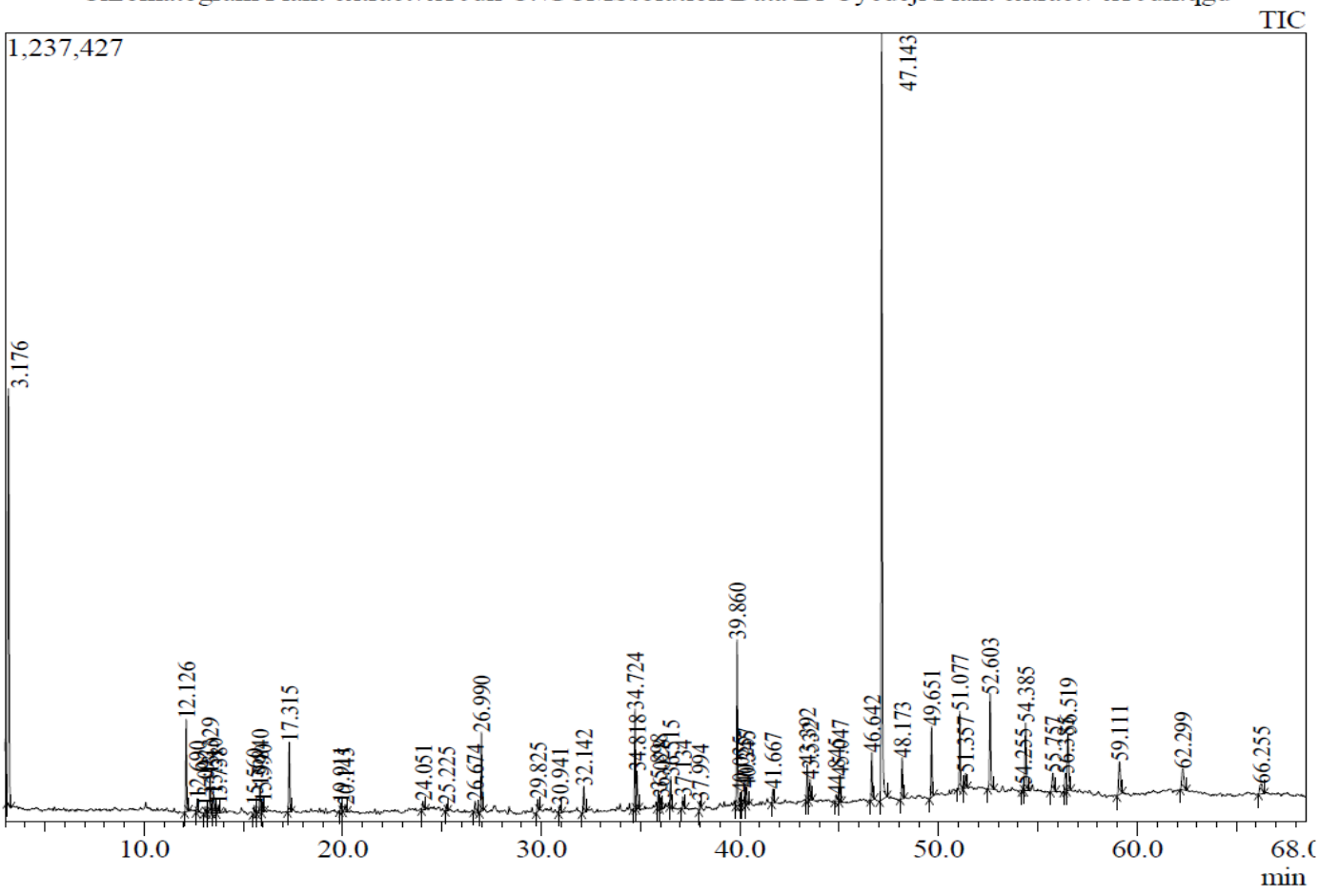

APPENDIX B- 4: **GC-MS chromatogram of *Hyparrhenia hirta* dried flowers (H.H.D.F)**

Sample Information

| | |
|---|---|
| Analyzed by | : Neal |
| Analyzed | : 2013-12-13 07:30:33 PM |
| Sample Type | : Unknown |
| Level # | : 1 |
| Sample Name | : Plant extract8x10dil |
| Sample ID | : Plant extract8x10dil |
| IS Amount | : [1]=1 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Vial # | : 8 |
| Injection Volume | : 3.00 |
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| Method File | : C:\GCMSsolution\Methods\Oyedeji plant extracts.qgm |
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| Report File | : |
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| Modified by | : Neal |
| Modified | : 2013-12-13 08:42:11 PM |

Chromatogram Plant extract8x10dil C:\GCMSsolution\Data\Dr Oyedeji\Plant extract8 x10dil.qgd