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Extraction and Analysis of Essential Oils from Cypress Species (*Cupressus lusitanica* and *Taxodium distichum*) from Selected Areas in Kenya

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Abstract:

The viability of extracting essential oils from cypress species Taxodium distichum and Cupressus lusitanica by Clevenger distillation has been examined. Reasonable quantities of essential oils are recovered from the leaves and bark. The extracted oil finds application in the cosmetic and medical fields. The first objective was to determine the yield of essential oil extracted from the bark and leaves of both cypress species. The study reveals a very low yield of essential oils recovered (0.05-3%). It is reported that the yield was higher from the leaves and bark of T. distichum (0.2252%) and (0.03125%) compared to C. lusitanica with a yield of (0.135%) and (0.0241%) over a duration of 6 hrs. Both species exhibited yellow essential oil from the leaves while bark yielded brown. Compounds present in the oil were determined using GC-MS. 46 compounds were isolated. The results further show that Totarol (2-phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl) -(4bS-trans) were the only compound present in the leaves and bark of both species. Research has found Totarol to have antimicrobial and therapeutic properties. Also, the following compounds were only present in the leaves of both species: α -terpinyl acetate, α -gurjunene, α -cadinol, β -cubebene, β -caryophyllene and sclareol. Androstan-17-one-3-ethyl-3-hydroxy was present in the bark only.

Keywords: Essential oil, cypress, Clevenger distillation, GC-MS, Totarol

1. Introduction

1.1. Background of Study

Cypress is the most common name given to plants in the cypress family *Cupressaceae*, a conifer of temperate regions. The word cypress is derived from Old French which was in tern adopted from Latin "*cupressus*". Both *Cupressus lusitanca* and *Taxodium distichum* belong to the *Cupressaceae* family.

1.2. Family Cupressaceae

This is a conifer family which has about 27-30 genera which include the junipers and redwoods. The leaves are arranged either in a spiral formation or in opposite pairs. These leaves are shaped like needles and the ones that are located on shoots develop into branches, which eventually fall off when the bark starts to flake. Adult leaves are usually narrow and appear to resemble scales. Awl like juvenile and transitional leaves is often present on mature leaves (Schultz *et al.*, 2005).

The bark of mature leaves is red-brown and of stringy texture, which often peels off in strips, but some species have been found to be smooth, scaly or hard and cracked. The seed cones are either have a leathery feel to them, woody or berry-like and fleshy which are green while the tree is young of age and upon aging the cones change colour to brown. The *Cupressaceae* is now widely regarded as including the *Taxodiaceae*, previously treated as a distinct family.

1.3. Taxodium Distichum

Taxodium distichum (bald cypress) is monoecious, that is, has both male and female reproductive organs on a single plant. Seeds are produced at intervals of 3 to 5 years. At maturity, cones drop to the ground. Such a drop is hastened by squirrels which are known to eat bald cypress seeds. This enhances the spread of these seeds which is a means of seed dissemination. Seedlings can endure partial shading but require overhead light for good growth, which often reach heights of up to 20 to 75 cm in the first year.

Bald-cypress is reputed to slow growing and very long-lived which appear to produce more than one ring of stem wood. These rings are a means to which botanists are able to estimate the ages of trees (Tolliver, 1990). Older, naturally seeded bald-cypress develop descending roots that provide anchorage as well as lateral roots from which conical structures known as 'knees' arise. Knees vary in height depending upon water levels of the site in which the tree is grown, especially in areas where flooding is a frequent occurrence.

However, small knees have been observed on quite a number of trees even in the absence of flooding. Nonetheless, it is quite common for knees to be produced by ornamental trees. They have an important role as aeration organs but are not crucial for survival. Also help in anchorage of trees because the masses of roots produced are extremely heavy or large. This extensive roots system makes bald-cypress firm during windy seasons; even hurricane winds are not strong enough to overturn them (Tolliver, 1990). Research has not found any physiological function for cypress knees.

1.4. Uses of Taxodium Distichum

Bald-cypress seeds are eaten by wild turkey, squirrels, evening grosbeaks and wood ducks; and they were an important food for the now extinct Carolina parakeet. Large old bald-cypress trees are also used as habitat for some wildlife: bald eagles and ospreys build their nests at the top of the tree.

The wood from this tree is known especially for its ability to resist decay. This high decay resistance is attributed to the presence of Cupressene, a component found in the oil extracted from the tree. For this reason, the cypress wood is used in the building construction industry, furniture as well as in fencing. Nevertheless, this resistance to decay is only effective on old-growth bald cypress (Devall, 1998).

1.5. Cupressus Lusitanica

The name *Cupressus lusitanica* was established by Miller (1978), where a sample of the tree came from a tree beside the Chapel of San Jose at Bussaco, Portugal (Elwes and Henry, 1910). Franco (1945) asserted that many of the cypress trees cultivated in Portugal in that era were of the same species; *Cupressus Lusitania Mill*. Moreover, many modern European botanists including Franco believed *Cupressus lusitanica* must have been introduced from Mexico and Guatemala into Europe. This explains its common name Mexican cypress.

It was also believed that "*Cupressus*" is a Latin name that comes from the Greek name "*kuparissos*" which commemorates a youth of that name who was believed to have been turned into a cypress tree by Apollo. "*Lusitanica*" is also a Latin name, derived from *Lusitania*, a province in Portugal where the species was introduced from Mexico (Orwa *et al.*, 2009).

Naturally distributed in Mexico, El-Salvador, Nicaragua, Belize, Guatemala and Honduras, Mexican cypress was introduced into tropical Africa (Kenya, Tanzania, Zimbabwe, Madagascar and South Africa) in the 20th century as a plantation forest tree. An evergreen, medium-sized, monoecious tree which grows up to 35m tall, with a bore diameter of 2 to 3ft sometimes even reaching up to5ft. Its logs are well shaped, straight and cylindrical. Young trees have their barks smooth, red-brown in colour which exfoliate in large strips as they grow older.

The branches form a crown pyramidal shape spreading or ascending with the ends drooping (sagging). Leaves are arranged opposite each other, are scale-like, 1-2.5mm long and are green in colour. The male cones are yellow-green when young transforming into pale brown upon maturity whereas the female cones green or purple-glaucous when young and turn brown when mature. Each cone is reported to have about 8-12 seeds inside it which are angular, slightly flattened and yellow-brown in colour. The wood of Mexican cypress is characterized as being heartwood yellow, pale brown or pink, often streaked or variegated. Its texture is fine and uniform, luster is high and it is fragrantly scented (Chudnoff, 1984).

Initial growth is fast, with an annual increase in height of up to 1.5m (sometimes even 2m) in early years. Flowering occurs in the driest season of the year while the female cones take 2 years to mature. Trees normally start bearing fruit at 6-9 years of age. Occurs productively at altitudes of 1000m-4000m with annual temperature ranging between 12-30° Celsius; average rainfall of 800-1500mm and a dry season not exceeding 3 months. The tree is not damaged by frost or snow and prefers deep, moist, well-drained, fertile slightly acidic loamy soils. In prevention of soil erosion, *Cupressus lusitanica* is under-planted with other plants, but it is not suitable for intercropping with crops. (Brink *et al.*,2007).

1.6. Uses of Cupressus Lusitanica

Cupressus lusitanica is East Africa's most important exotic softwood tree where it is commonly grown as an ornamental tree for hedges and Christmas trees. Also, it has its importance as a shade tree and as windbreakers. The wood is used in construction, furniture, poles and posts attributed to the fact the wood saws well, it is easy to work with and hand and machine tools, finishes well and has good nail-holding properties as well as sustainability for moulding and peeling.

The wood also has its uses in the pulp and paper industries in Kenya and Ethiopia. In the pulping industry, the wood can be pulped by use of sulphate process, with yields of 38-51%. Unbleached pulp has moderate tensile and bursting strengths, although the tensile strength is too low for use in packaging paper. The bleached pulp is however suitable for printing and writing paper (Brink, 2007).

Distillation of leaves, twigs or cones yield 0.05-3% essential oil. Essential oil is used as an adjuvant and perfume in soaps, room sprays and deodorants. The tree is also used in making toothbrushes (due to its antiseptic property) and brooms. The leaves are used to treat catarrh and headaches, the sap of the tree to treat skin diseases. Essential oil from the leaves is used in treatment of hemorrhoids, rheumatism, whooping cough and as a styptic (Teke *et al.*,2013).

The vapour from the leaf immersed in boiling water is a treatment of the flu. The leaf juice is used to cure skin diseases and leaves used to protect stored grain against insect infestation in Cameroon (Teke *et al.*, 2013).

1.7. History of Essential Oils

Essential oils at some point in time were also referred as aromatic oils. They have been used worldwide for thousands of years for different purposes ranging from medicinal purposes to religious ones. The Egyptians have been known to use aromatic oils as early as

4500 B.C. They have been recorded to be knowledgeable in cosmetology, ointments and aromatics where they have used these oils to manufacture incense, perfumes, ointments and medicine.

The method used by the Egyptians was the effleurage extraction method which they had access to via the Mesopotamians which have been discovered by archaeologists at Tepe Gawa dating back to 3500 B.C. High priests, the most powerful authority in the early Egypt rule, were the only ones allowed to use essentials oils, as they were regarded necessary to be at one with their gods. Specific fragrances were dedicated to specific gods of which their statues were anointed with these oils by the high priests.

The Chinese used aromatic plants for medicinal purposes between 2697-2897 B.C. during the reign of Huang Ti, the legendary Yellow Emperor.

"Ayur Verda", a traditional Indian medicine has been discovered to consist of essential oils of different plants consisting: ginger, cinnamon, myrrh and sandalwood. This medicine was believed to replace ineffective antibiotics during the outbreak of the Bubonic plague.

Among the Greeks, the most famous physician, Hippocrates, also known as the "Father of Medicine" believed in the medicinal significance of essential oils of over 300 plants some of which include: thyme, marjoram, cumin and peppermint. He found that the Indian medicine "Ayur Verda" was harmonious with some of their own medicinal practices. Hippocrates also included aromatherapy as a form of treatment.

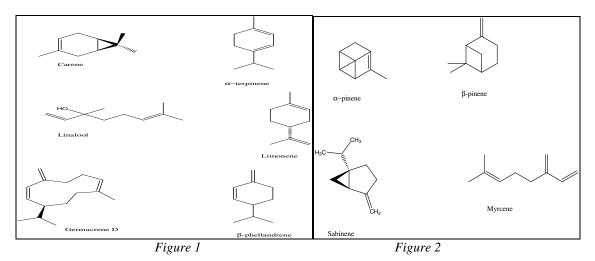
The Romans were famously known for applying perfumed oils to their clothing and bodies which were essential oils extracted from aromatic plants. Some believed that it was the Romans who discovered perfumes in their quest to be outstanding in scent as well as curiosity. They were great believers in hygiene making it customary to use oils in baths and massages and believed essential oils to promote good health.

1.8. Chemical Composition of Essential Oils

Most essential oils are primarily comprised of terpenes (monoterpenes and sesquiterpenes) and their oxygenated derivatives: alcohols, aldehydes, ketones, esters, acids and ethers. These components originate from secondary metabolism of the plants (Chamorro *et al.*, 2012). Secondary metabolites occur only in such species and nit in others, often classified into terpenoids, shikimates, polyketides and alkaloids (Sell, Chapter 5 "Chemistry of essential oils").

Monoterpenes or monoterpenoid hydrocarbons are usually defined as substances composed of isoprene (2-methylbutadiene) units; whose skeleton is discernable in these terpenoids. Hydrocarbons occurring in essential oils with the molecular formula $C_{10}H_{16}$, were named terpenes by Kekule due to their occurrence in turpentine oil. Similarly, constituents with the molecular formula $C_{10}H_{16}O$ and $C_{10}H_{16}O$ were classified under the name camphor (Wallach, 1914).

Essential oils extracted from cypress generally contain the following components:



1.9. Pests and Diseases

Accidental introduction of pests and diseases poses as the greatest hazard of pest and disease damage to forest plantations. In the absence of natural enemies, these pests and diseases are provided with suitable host material causing devastating losses.

The cypress aphid *Cinara cupressivora* is prone to attacking *Cupressus lusitanica*, causing branches to turn yellow and eventually dry out. It was first encountered in Africa in Malawi in 1866. It first appeared in Kenya in 1990 and Ethiopia in 2004. The damage caused to cypress trees include: browning and defoliation, which in some cases has been known to cause dieback and tree death. Another problem caused by this aphid is the deposition of numerous quantities of honeydew which encourages the growth of sooty mould. The cypress aphid has also been detected in other various member of the *Cupressaceae* family (FAO, 2001). *Seiridium unicorne* is a pathogen that caused small, annual cankers on bald-cypress (*Taxodium distichum*) in Kansas landscape plantings. A symptom of the canker disease is the appearance of small areas of dead tissue. The severity of this tissue on bald-cypress was investigated and shown to increase during spring and summer, after winter characterized by prolonged extremely cold temperatures. The cankers were found on twigs, branches and stem of the bald-cypress. These girdling cankers caused a rapid death of foliage (Tisserat *et al.*, 1991).

1.10. Methods Used in the Extraction of Essential Oils

Ibn Sina, a notable Arab physician and naturalist, known also as Avicenna, used the herbal distillation techniques early as the1st century A.D and around 1000 A.D to extract rose oil from rose petals. The ancient Arabs and Europeans began conducting studies on the chemical properties of essential oils, extracted via distillation as early as the 12th century.

The most common and traditional methods used globally are: water distillation, water and steam distillation, steam distillation and cohobation. Solvent extraction is another technique that may be used but mainly for delicate, expensive and thermally unstable plants such as hyacinth, jasmine and tuberose.

1.10.1. Distillation

Distillation is a process in which a liquid mixture or vapour mixture of two or more different entities is separated into its individual component fractions, of specified purity, by application and removal of heat. Distillation of aromatic plants involves liberating oils from the plant under study, specifically from the cell membrane or trichomes, in the presence of water (moisture) and at high temperature then applying a cooling mechanism for the vapour mixture to condense to liquid so as to separate out the oil from water. Studies have shown the boiling point of most essential oils exceeds that of water (100°C) and ranges between 150-300°C. There are some factors that govern the extraction of essential oils via distillation methods. These include:

- Sensitivity of essential oils to the action of heat and water.
- Oils which are highly soluble in water cannot be distilled using steam. Also, essential oils which can be damaged, through decomposition, by action of heat cannot be steam distilled.
- Volatility of the essential oil.
- The oil must be volatile for any distillation method to be successful.
- Water solubility of the essential oil.
 - Most essential oils are practically insoluble in water and hence suitable for processing via distillation techniques.

1.10.2. Techniques of Distillation

Hydro distillation

To isolate the essential oils, the aromatic plant is packed in a still (vessel) and a sufficient amount of water added (enough to cover the entire plant) to the still and brought to a boil. Due to the influence of boiling hot water and steam, the essential oils are released from the glands of the plant and the vapour mixture of the oil and water carried over to a condenser and cooled. The distillate flows into a collecting vessel and the oil is separated from the water distillate.

Hydro distillation of aromatic plants involves 3 main physicochemical processes:

Hydrodiffusion

This process follows the principle of diffusion and works by allowing steam to penetrate the top of the plant charge and diffuse through it by force of gravity. This process also obeys the principle of osmotic pressure to diffuse oil from the oil glands. At the temperature of boiling water, some of the volatile oil from the plant dissolves in water within the glands and this oil-water mixture permeates the swollen membranes of the plant cells, via osmosis, and reaches the outer layer where the oil is finally vapourised by passing steam. Notably, the degree of solubility in the water is an influential factor in determining the speed of oil vapourisation and not the volatility of oil components.

> Hydrolysis

Defined as a chemical reaction between water and the constituents of essential oils. Some of the constituents, especially esters, in the presence of water and at high temperatures, react leading to the production of acids and alcohols. The relationship of the molar concentrations of the reactants and products at equilibrium is given by:

$K = \frac{(alcohol) \times (acid)}{(ester) \times (water)}$

The larger the quantity of water used, the larger the amounts of alcohol and acids produced, leading to decreased yield of the essential oil. The extent of hydrolysis is dependent on the time of contact between the oil and water; this is a disadvantage.

➢ Effect of heat

Constituents of essential oils are very volatile therefore unstable at high temperature. To obtain the most efficient yield and quality of oils, this is done by:

- \rightarrow Maintaining the temperature as low as possible.
- \rightarrow Use of little amounts of water.
- \rightarrow Comminuting the plant material, that is, reduce to small, fine particles or making into powder, and packing it uniformly before distillation.

Disadvantages of hydro distillation:

- 1. This is a slow process thereby more fuel consumption which makes it very expensive thus uneconomical.
- 2. Variation in the rates of distillation because of the difficulty in controlling the heat.
- 3. Extraction of the oil from the plant material under study may not always be complete.

4. Prolonged application of heat may cause hydrolysis of the esters present in the oil, which react with water producing acids and alcohols.

• Steam and water distillation

This technique is sometimes referred to as wet steam distillation. The equipment used is similar to that used in hydro distillation. The difference lies in the fact that the plant material is placed on a perforated grid or plate below which water is boiled.

The water in the still (vessel) below the perforated plate with the plant is heated by open fire producing wet steam which rises through the plant material vapourising the essential oil from it. This technique ensures better quality of the oil extracted as compared to hydro distillation. If the amount of water is inadequate to allow complete distillation, a cohobation tube is attached and the condensate water is directed back to the still ensuring the water (which is the steam source) does not run out as the distillation is on progress. Also, distillation with cohobation may also work by moving the condenser above the distillation still so that the condensate water can flow by means of gravity to the still. This also controls the loss of oxygenated constituents from the oils that dissolve in the condensate water because the recycled water will allow saturation of the dissolved constituents, thus more oil.

Advantages of water and steam distillation over hydro distillation:

- \rightarrow Higher oil yield.
- \rightarrow The components of the volatile essential oils are not likely to undergo hydrolysis and polymerization because the plant material is not in direct contact with water.
- \rightarrow The essential oil quality is more reproducible.
- \rightarrow A faster process, hence more energy efficient (economical).

Disadvantages are as follows:

- \rightarrow A baffle is used to prevent water from boiling vigorously and that the plant material does not get into direct contact with the water, which would make the plant waterlogged.
- \rightarrow Time consuming. Due to the low pressure of rising stem, essential oils that have high boiling points require a larger amount of steam to vaporize thus longer hours of distillation.
- Direct Steam Distillation

This technique is sometimes referred to as dry steam distillation. Direct steam distillation is the process of distilling a plant material with steam generated from an outside source, that is, a boiler. The plant material is supported on a perforated grid or plate above the steam inlet. The amount of steam can be controlled and the maximum temperature is 100°C, thus thermal degradation is prevented. Use of high pressure steam enhances rapid and complete distillation of essential oils. Advantages are:

- \rightarrow Control of the amount of steam during distillation.
- \rightarrow Thermal decomposition of oil is prevented.
- \rightarrow Widely used for large-scale or commercial oil production.

The major disadvantage of direct steam distillation is that the capital expenditure is very high to establish a facility for the production of essential oils.

2. Methodology

2.1. Plant Material

Samples of *Cupressus lusitanica* leaves and barks were collected from a specific location in a garden in Chiromo campus while samples of the *Taxodium distichum* were collected from a single location in a homestead in Kisii.

The fresh plant materials were taken to the herbarium at the University of Nairobi, Chiromo campus for identification where a voucher specimen was deposited under the codes: NKW 2016/01 for *Cupressus lusitanica* and NKW 2016/02 for *Taxodium distichum*.

The plant materials were thereafter taken to the laboratory in the Department of Chemistry. The leaves were plucked off the stalks, sealed in zip-lock bags to prevent escape of the volatile oils and stored in a cool, dry place away from direct sunlight. The bark was diced into small pieces and stored as mentioned for the leaves.

2.2. Extraction of the Oil from Plant Materials

50g of the *Cupressus lusitanica* leaves were added into a clean, dry round bottomed flask. 500ml of distilled water was added to the flask containing the leaves. The round bottomed flask with its contents was placed in a heating mantle (that was an exact fit for the flask). The round bottomed flask was connected to the corresponding apparatus: Clevenger apparatus and Liebig's condenser. The temperature on the heating mantle was adjusted to a maximum and the water in the round bottomed flask was heated to boiling. Once the boiling begun, the temperature on the mantle was regulated to lower value to a point where the boiling was controlled.

50g of the diced *Cupressus lusitanica* bark were added into a clean dry round bottomed flask. 500ml of distilled water was added to the flask containing the leaves. The round bottomed flask with its contents was placed in a heating mantle (that was an exact fit for the flask). The round bottomed flask was connected to the corresponding apparatus: Clevenger apparatus and Liebig's condenser. The temperature on the heating mantle was adjusted to a maximum and the water in the round bottomed flask was heated to boiling. Once the boiling begun, the temperature on the mantle was regulated to lower value to a point where the boiling was controlled.

The same procedure was followed for the leaves and bark of the Taxodium distichum respectively in the separate experiments.



Figure 3: Clevenger setup

3. Results and Discussion

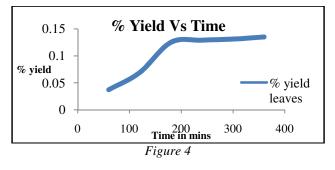
3.1. Results

The results for the analysis are listed below. Yield of the extracted essential oil for *Cupressus lusitanica*

	Leaves		Bark	
Duration/ Time of	Mass of oil extracted per amount of	% Yield	Mass of oil extracted per amount	% Yield of
extraction (min)	leaves used(g)	of oil	of bark used(g)	oil
60	0.019/51.073	0.037	Nil	Nil
120	0.0344/50.0958	0.069	0.0054/50.4888	0.0107
180	0.063/50.106	0.125	0.0103/50.3854	0.0204
240	0.0649/50.1235	0.129	0.0294/50.3954	0.0583
300	0.066/50.2929	0.131	0.012/50.6136	0.0237
360	0.0681/50.2820	0.135	0.0122/50.5553	0.0241

Table 1: Variation of oil yield with extraction time

A graph of percentage oil yield versus time (min) was plotted and is shown below.



Yield of the extracted essential oil for Taxodium distichum

	Mass of extracted oil per amount of plant material used (g)	% Yield of oil
Leaves	0.1131/50.2277	0.2252
Bark	0.0158/50.5595	0.03125

Table 2: Oil yield of extraction time of 360 min

The colour and odour of the oils from the leaves and bark was found to differ. The oil was yellow from the leaves with a spicy fragrance while that from the bark was brown with a woody scent. These findings were similar in both species under study.

3.2. Significant Chemical Compounds Present in Both Species

Compound	CL: % Composition		TD: % Composition	
	Leaves	Bark	Leaves	Bark
α-Terpinyl acetate	4.94	-	4.61	-
β-Cubebene	2.16	-	3.04	-
α-Gurjunene	4.34	-	4.22	-
β-Caryophyllene oxide	2.11	-	4.17	-
α-Cadinol	6.58	-	6.28	-
Sclareol	3.45	-	7.10	-
Totarol	3.65	18.71	1.72	3.04
Androstan-17-one-3-ethyl-3-hydroxy	-	51.57	-	31.88

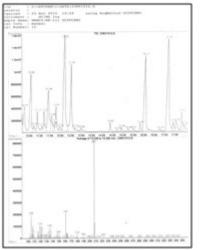
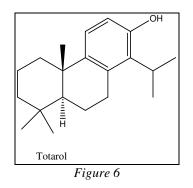


Figure 5: GC-MS Chromatogram (Bark)

4. Discussion

The optimum extraction time of 360 min was achieved through experiments using *Cupressus lusitanica* as it was readily available. It was observed that the yield of the oil increased with increasing time. Between 300 to 360 min, the yield was almost constant with only a slight increase thus the optimum time was set as 360 min.

46 compounds were identified upon analysis as listed above. Similarities and differences were identified in the various compounds found in both species. 10 were found in the oil of the species *Taxodium distichum* while 36 in the oil of *Cupressus lusitanica*. The key components found in both species include:



Totarol is classified as a meroterpene, defined as a chemical compound that is both a terpene plus a phenol. It has anti-microbial activity due to its ability to inhibit microorganisms. For this reason, Totarol is mostly used in cosmetics especially in products that are used to treat acne and other skin diseases. It also has therapeutic properties.

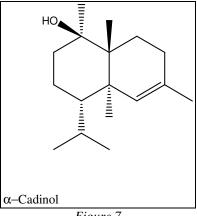
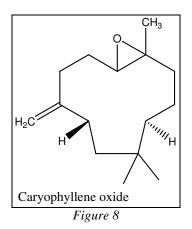
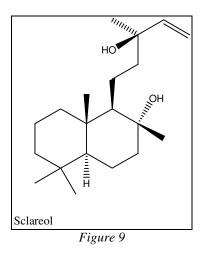


Figure 7

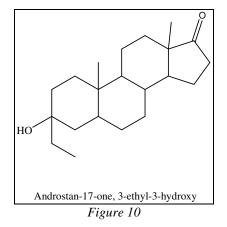
This compound is believed to have anti-fungal activity. Also, it acts as a hepatoprotective. Hepatoprotection is referred to as the ability to protect the liver from damage. Studies have shown that α -Cadinol has anti-termitic activity as well as insecticidal activity against yellow fever mosquito larvae.



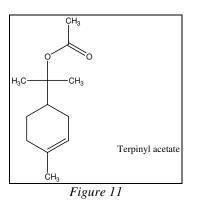
B- Caryophyllene and its oxides have the following biological activities: insecticidal, antimicrobial, local anesthetic and antiinflammatory.



Sclareol is a chemical compound which is a fragrant and is classified as a bicyclic diterpene alcohol. It is commonly used in the cosmetic and perfume industries as a fragrant and also in the food industry as a flavoring additive. Also, it has antibacterial and antifungal properties.



Androstan-17-one, 3-ethyl-3-hydroxy is a steroid in nature; an organic compound which gas four ring systems arranged in a specific order or configuration. It is also neuroative (the ability of a drug or substance to interact with or act in the central nervous tissue), analgesic (any drug or group of drugs used to achieve pain relief) and anesthetic (a drug that causes the reversible loss of sensation or consciousness).



Terpinyl acetate is used in the perfume industry due to its herbal, citrus, woody, spicy and floral odor. The results from the compositional analysis have shown some similar medicinal importance of the oil from previous literature done on essential oils.

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