CHEMICAL STUDIES OF FOUR *COMMIPHORA* SPECIES OCCURRING IN GAMO GOFA REGION

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ABSTRACT

CHEMICAL STUDIES OF FOUR *COMMIPHORA* SPECIES OCCURRING IN GAMO GOFA REGION

BY

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The genus *Commiphora* is one of the 20 genera belonging to the Bnrseraceae family. In the course of this study leaves and barks of five *Commiphora* species, for botanical identification, and resins of four of these species (except C. *africana)*, for chemical analysis, were collected from Konso, Gamo Gofa. The species were identified by Dr. Kaj Vollesen (Kew Botanical Garden, U.K.) as *Commiphora kataf*, *C. lerebinlhina*, *C. schimperi*, C. *hahessenica* and *C. africana*.

The essential oils of the resins from the four species were isolated by hydro-distillation and some of the components identified by means of GC and GC/MS analysis. The main components were: *C. kataf* a-copaene (29%) and /raw-caryophyllene (33%); C. *lerebinlhina*: geraniol (24%), aromadendrenc (21%), a-copaene (15%), a-luimulene (3.2%) and a-cadinol (7.4%); *C. schimperi*: a-pinene (73%) and (3-pinene (17%); and C. *hahessenica*: a-copaene (27%) and a-cadinol (25%).

The resins were also subjected to extraction by petrol, ethyl acetate and methanol, and the components of *C. lerebinlhina* were isolated using chromatographic techniques and attempts were made to identify the isolated substances.

Analysis of the petrol extract of the resin of *C. schimperi* showed the resin to be an excellent source of the industrially important compounds, a- and (1-pinene.

1. INTRODUCTION

The Burseraceae is a large family with 17-20 genera and 500-600 species, widespread in tropical and subtropical regions [26, 12]. Engler [14] subdivided the Burseraceae into three tribes on the basis of fruit structure: Protieae (4 genera), Boswelliaea or Bursereae (eight genera which include *Boswellia* and *Commiphora*) and Canarieae (9 genera).

The genus *Commiphora* with 150-200 species is wide spread in the drier parts of tropical Africa and Madagascar, also from Arabia to India and with few species occurring in South America. The genus is a very conspicuous and dominant element in the dry bush lands ofNorth East Africa, where a large number of species are endemic to this area [19].

The resin is collected in such a way that the milky liquid exudate coming out from the tree hardens on exposure to air into droplets or “tears” which are then easily detached by a collector. Occasionally, some "tears" are produced by accidental injury or from splits which occur in the stems or branches of the tree.

1.1 Myrrh

Commiphora myrrha is the chief source of myrrh today. The plants grow wild in the North-Eastern province of Kenya and adjoining areas of Somalia and Ethiopia. These plants yield economically important gum exudates which have been collected for centuries as medicinal and perfumery substances [28].

Holmes [21, 22] apparently was the first to propose that the myrrh of the Bible was the perfumed myrrh or "bissabol" and not the medicinal myrrh or “heerabol” from *C. myrrha.*

Common myrrh (heerabol) is obtained from C. *myrrha*; this is the species from which "oil of myrrh", or stacte, was obtained. Other species sometimes passing as myrrh or bdellium include C. *africana*, C. *anglosomaliae*, C. *gileadensis* (C. *opobalsamum*), *C. hildebrandlii, C.*

kolaf, *C. mikiil*, and *C. schimperi* [35, 44]. The odor of myrrh is described as warm-balsamic, sweet, and some what spicy aromatic, sharp and pungent when fresh [42].

The gifts presented by the Maji to the infant Christ symbolized His life: "gold for royalty, frankincense for divinity, and myrrh for suffering" [42]. Myrrh was also in the final drink offered to Christ on the cross: "And they gave Him to drink wine mingled with myrrh; but He received it not " (Mark 15:23). Myrrh was in addition, used to embalm the body of Christ: "And there came Nicodemus, which at first came to Jesus by night, and brought a mixture of myrrh and aloes, about a hundred pound weight" (John 19:39). Myrrh was also included in the "oil of holy ointment" (Exodus 30 : 23-24).

Many herbalists recommend tincture of myrrh as an astringent for the mucous membranes of the mouth and throat [27]. Myrrh is found in salve used in treating bed sores, hemorrhoids, and wounds. Internally, myrrh is also used for indigestion, ulcers, and bronchial congestion and as an emmenagogue [35].

Among local African traditional medicines, the resinous gums of *C. myrrho* and *C. gukhlliv*, which are locally known as "malmal" and "habak-hadi" in the Somali vernacular, respectively, are used on livestock against ticks [28]. The major use of myrrh is for burning as incence in religious ceremonies. The resin is distilled to yield volatile oils and these have their own characteristic balsamic odour which find use in perfumery [12].

1.2. Bissabol (Opopanax or Scented myrrh)

The resin of *C. gitidollii* is the second major type of myrrh and it is commonly known in commerce as gum "bissabol" (Hindi) or "opopanax". Opopanax occurs also widely in Ethiopia from which the resin is collected for export to India, China and Europe.

Thulin *et al*. [39] suggested that the myrrh of the Bible and the incense of the ancient Egyptians and of Classical times [35, 44] was most likely the "perfumed myrrh" or "bissabol" from *C. guidottii* and not the medicinal myrrh from *C. myrrha*. "Bissabol", according to Holmes [20] meant buffalo myrrh, "as it is mixed with food given to milch cows and buffaloes to improve quality and quantity of their milk." Thulin and Claeson [39] recently confirmed that the tree called "hadi" in Somali is *C. guidottii* and produces the resin "habak hadi" also known by several other names including "opopanax", "bissabol", "scented myrrh", "abeked" (Amharic).

Tucholka [41], in a thesis on the chemical composition of "bissabol", reported that "habak hadi" was used during female circumcision, by bathing in water in which the resin was emulsified. A similar bath was also taken by Somali women after giving birth to a child. From more recent sources it can be added that "habak hadi" is used in Somalia for the treatment of stomach complaints and diarrhoea [9]. It is also used topically for the treatment of wounds.

1.3 Objective of the study

This study is part of an ongoing project on the chemistry of the resins of *Commiphora* species occurring in Ethiopia.

The main aim of the study was to first of all collect resins from *Commiphora* trees along with their corresponding botanical specimens in order to get the species identified. This was then to be followed by chemical studies on the resins. The results of the study are presented hereunder.

2. REVIEW OF THE CHEMISTRY AND PHARMACOLOGY OF THE RESINS OF THE GENUS *COMMIPHORA*

The Burseraceae comprises about 17-20 genera and 500-600 species [26]. *Commiphora,* a genus of the Burseraceae, has about 150-200 species, most of which are widespread in the drier parts of Tropical Africa and Madagascar, while a few are known form Arabia, India and South America [19].

2.1 Isolation and characterization methods

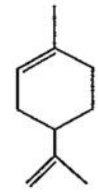
Extraction of the resins by organic solvents furnishes a "resinoid" or an "absolute." **The** "resinoid" is prepared by extraction of the crude resin with a hydrocarbon solvent such as hexane or petrol. The "resinoid" contains almost all the available essential oils of the resin. The "absolute" is prepared by extraction of the resins with alcohol [12]. Essential oils on the other hand are separated by steam or hydro-distillation at atmospheric pressure.

Gas chromatography is an excellent tool for the separation, characterization, and quantitative analysis of essential oils. The combined gas chromatogram-mass spectrometer (GC/MS) method provides a facile, sensitive and convenient system for the separation and identification of complex mixtures [15]. Spectroscopic methods like UV, IR, ^{1}H and ^{13}C NMR are among the most powerful techniques for the characterization of isolated compounds.

2.2 Composition of Essential Oils of *Commiphora*

The components of the essential oils obtained from only few *Commiphora* species have been investigated and these include: C. *lerebinihina* and *C. cyclophylla* [1], *C. myrrha* 16. 18] and *C. roslrata* [29]. The oils from C. *rostrata* are distinguished by the presence of the homologous ketones starting from 2-octanone, 2-nonanone, 2-decanone etc [29]. The other

Commiphora species are rich both ih the structures of monoterpenes and sesquiterpenes and some of these compounds are shown below.

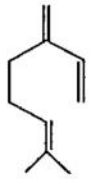


Limonene

1

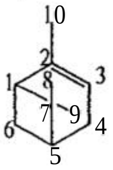
α-Thujene

2



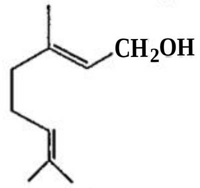
Myrcene

3



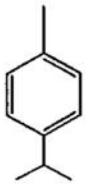
α-pinene

4



Geraniol

5

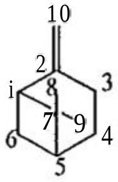


p-Cymene

6

Camphene

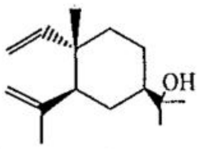
7



β-pinene

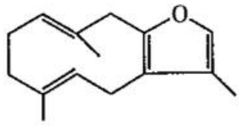
8

Some of the monoterpenes that are found in the essential oils of *Commiphora*



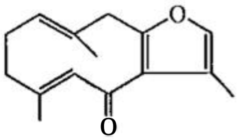
elemol

9



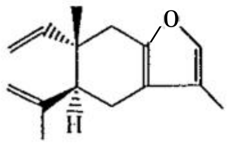
1(10)E,4E-furanodiene

10



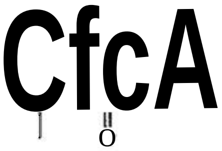
1(10)E,4E-furanodien-6-one

11



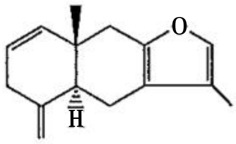
isofuranogermacrene

12



curzerenone

13



lindestrene

14

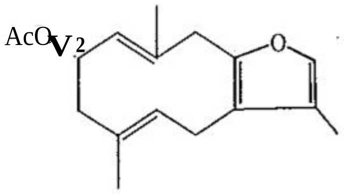
Sesquiterpenes reported from the essential oil of *Commiphora* species

Oxygenated terpenoids are the components of essential oils most often responsible for their distinctive aroma and flavor, even though they are often minor constituents of the oil [7].

It is interesting to note that as most of the previous reports on resin of *C. myrrha* are based on the study of materials obtained from commerce, it is highly likely that the resins are derived from different *Commiphora* species. This shows the significance of working on resins from properly identified trees.

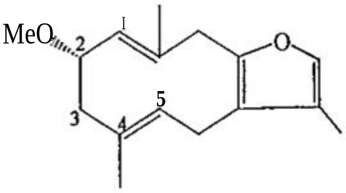
2.3 Compounds from the resinoid of *C. myrrha*

Maradufu [28] analyzed the hexane extract of resin obtained from an identified *C. myrrha* tree and characterized three furanosesquiterpenes. These are 2-O-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene (15) and 2-O-methyl-8,12-epoxygermacra-1(10),4,7,11-tetraene (16) which are reported for the first time and the furanodiene (1E, 4E)-8,12-epoxygermacra-1(10),4,7,11-tetraen-6-one (11). The new compounds (15, 16) were shown to be toxic to larvae of *Rhipicephalus apendiculatus* ticks responsible for transmitting organisms causing East Coast Fever in cattle.



2-O-acelyl-8,12-epoxygermacra-1(10),4,6,11-telraene

15

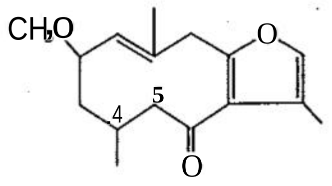


2-O-methyl-8,12-epoxygermacra-1(10),4,6,11-tetraene

16

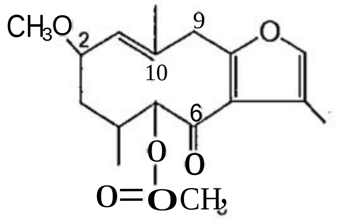
2.4 Compounds from the ethanol and ether extract of *Commiphora* resins

Three known and one novel furanogermacrenes were isolated from the ethanol extract of the resins of *C. holtziana*. The known compounds were 2-methoxy-4,5-dihydrofuranodiene-6-one (17), 5-acetoxy-2-methoxy-4,5-dihydrofuranodiene-6-one (18), and 3-methoxy-10-methylenefuranogermacra-1-ene-6-one (20), while the novel compound was 1,2-epoxyfurano-10(15)-germacren-6-one (19) [8].



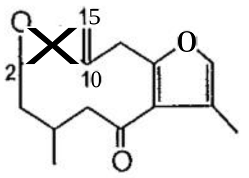
2-methoxy-4,5-dihydrofuranodiene-6-one

17

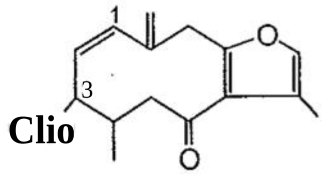


5-acetoxy-2-methoxy-4,5-dihydrofuranodiene-6-one

18



1,2-epoxyfurano-10(15)-germacren-6-one
19

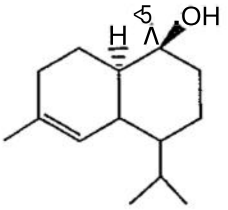


3-methoxy-10-methylenefuranogermacra-1-ene-6-one 20

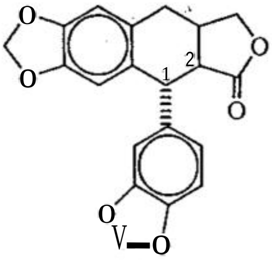
Bioassay guided fractionation of an ethyl acetate extract of scented myrrh (resin of C. *guidoUii)* using the guinea pig ileum test to monitor pharmacological activity, resulted in isolation of the sesquiterpene (+)-T-cadinol (21). T-cadinol exhibited a concentration-dependent smooth muscle relaxing effect [11]. This explains in part the traditional use of the resin to cure stomach complaints including diarrhoea [10].

Investigation of the diethyl ether extract of a sample of *C. kua* resin, collected in the Kota National Reserve, Tana River District, Kenya, yielded mansumbinone (23), the corresponding 3-hydroxy compound mansumbinol and the derivative 3,4-seco-mansumbinoic acid (24) in which the A-ring has been opened between C-3 and C-4. The trivial names of these compounds originate from the name of the locality where *C. kua* was collected from namely Mansumbi Hill within the Kora Reserve. A fourth compound was characterized as 16(S),20(R)-dihydroxydammar-24-en-3-one (26), a possible precursor of the mansumbinanes

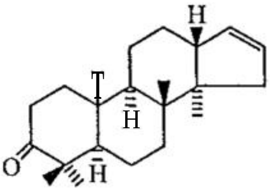
[36]. It was found that mansumbinoic acid (24) was 2.4 times more active in anti-inflammatory activity on molar basis than mansumbinone [13].



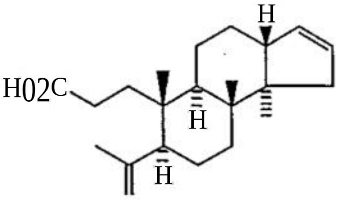
21



22a C-2a
22b C-2p



23



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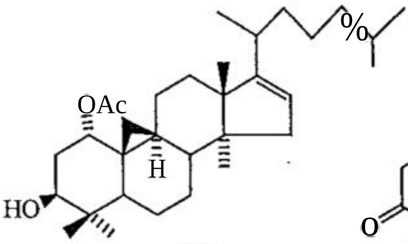

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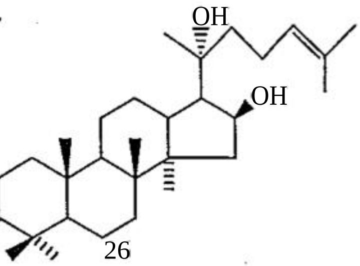


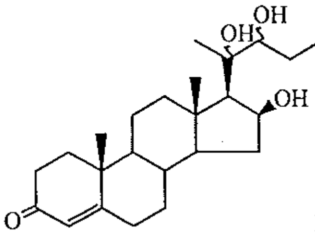
26

The ether extract of the resin of *C. incisa* has yielded two epimeric aryltetralin lignans which have been identified as the known polygamain (1R, 2R, 3R) (22a) and its novel C-2 epimer to which the trivial name picropolygamain (1R, 2S, 3R) (22b) has been assigned [35]. It has also yielded two novel triterpenes which have been identified as 1a-acetoxy-9,19-cyclolanost-24-en-3(3-ol (25) and 29-norlanost-8,24-dien-1a,2a,3p-triol [37]. C. *incisa* showed a marginally greater anti-inflammatory activity than C. *mukul*.

2.5 Anti-inflammatory compounds from resins of *Commiphora mukul*

The gum-resin of *C. mukul* is known to furnish an essential oil (*ca*, 0.4%) consisting chiefly of myrcene and "dimyrcene" (camphorene). "Guggulu", the gum-resin exudate from the tree C. *mukul* is a complex mixture of steroids, diterpenoids, aliphatic esters, carbohydrates and a variety of inorganic ions, besides minor amounts of sesamin and other unidentified constituents [33].

The steroidal constituents are obtained from the petrol fraction and include, cholesterol, 4,17(20)-(*trans*)-pregnadiene-3,16-dione, 4,17(20)-(*cis*)-pregnadiene-3,16 –dione, α-camphorene and cembrene (**30**). The ethyl acetate fraction yielded the sterols-guggulsterol-I (**27**), guggulsterol-II (**28**) and guggulsterol-III (**29**).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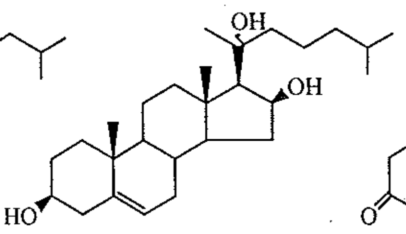
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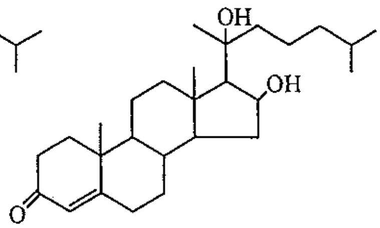
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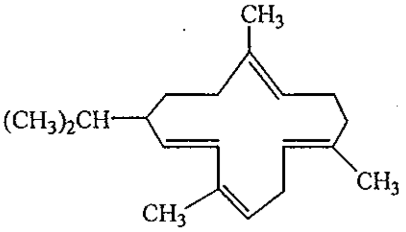
The diterpene alcohol allyl cembrol (2-hydroxy-4,8,12-trimethyl-1-isopropyl-3,7,11-cyclodecatriene) (**31**) was isolated from the over ground parts [38].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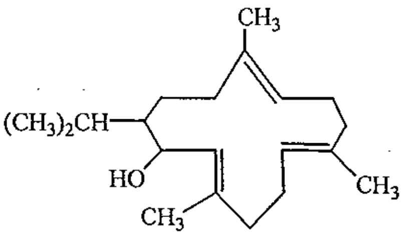
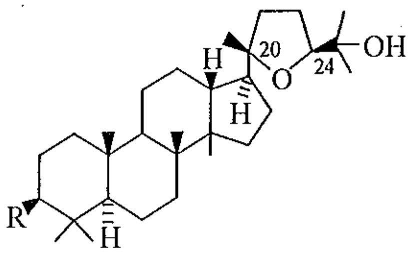

31

Two long-chain aliphatic tetrols, now formulated as octadecan-1,2,3,4-tetrol and eicosan-1,2,3,4-tetrol are isolated from the EtOAc extract of the resin. There is evidence for the occurrence of nonadecan-1,2,3,4-tetrol, in the resin [34]. 20α-Hydroxy-4-pregnen-3-one (**32**), 20β-Hydroxy-4-pregnen-3-one (**33**),16β-Hydroxy-4,17(20)Z-pregnadien-3-one (**34**) and 16α-hydroxy-4-pregnen-3-one (**35**) were also reported as new steroidal components from the petrol fraction [4].



37 R = H
38 R = O

3. RESULTS AND DISCUSSION

3.1 Specimen collection and identification

In the course of this study plant specimens (leaves, barks and fruits) were collected for botanical identification and resins for chemical analysis. These plants were identified by Dr. Kaj Vollesen (Kew Royal Botanical Garden, U.K.) and specimens were deposited at the National Herbarium (see Table 3.1).

Table 3.1 List of identified *Commiphora* species.

Collectors No	Herbarium No	Species identified
Tegene 1	072769	*C. kataf* (*C. erythreae*) (Ehrenb.) Engl. (1883)
Tegene 2	072770	*C. terebinthina* Vollesen (1985)
Tegene 3	072773, 072774	*C. schimperi* (Berg) Engl. (1883)
Tegene 4	072771	*C. habessinica* (Berg) Engl. (1883)
Tegene 5	072772	*C. africana* (A. Rich.) Engl. (1883)

3.2 Ethnobotany

The *Commiphora* plant is widely grown in Arbaminch and Konso because it is suitable for hedge and fence. In Arbaminch the tree is known as "Tsedaki" (Amharic), the Konso people call it "Qahatita" or "Kokomarritta". However, the name "Kokomarritta" is used specifically for one species, *C. kataf*. The name "Qahatita" indicates that the plant drives away wild animals that destroy vegetables in the garden.

The ease with which the plant is propagated from cutting accounts for its wide use for fences and hedges in Konso and Arbaminch. Furthermore the leaves are used for cattle feed and the wood for building purposes. However, interview of several residents in Arbaminch and Konso revealed that the residents have very little knowledge of the resins produced by the trees. The resins are not collected for use as incense. The resin and plant specimens used in this study were collected from Konso.

3.3 Isolation and analysis of the essential oils

The essential oils of the resins of the four *Commiphora* species were obtained by hydro-distillation. GC and GC/MS analyses of these oils were undertaken and the results are presented below.

C. terebinthina: The total number of components was 19 (97.8%) in the essential oil and the chromatogram is shown in Fig 3.1.

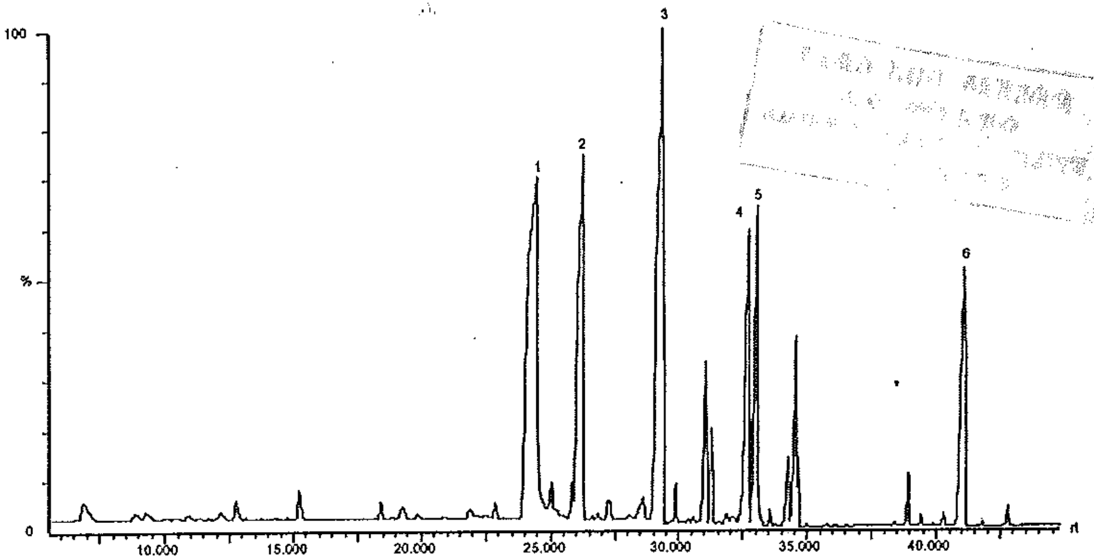


Fig. 3.1 Gas chromatogram of hydro-distillate of *C. terebinthina*

1: geraniol (23.7%) 2: α-copaene (14.8%) 3: aromadendrene (21.3%) 4: β-selinene (8.6%) 5: α-selinene (7.9%) 6: α-cadinol (7.4%).

Geraniol, the major component of this oil, is found in free state and esters in many essential oils including geranium oil and palmarosa oil (70-85%). It is extensively used in perfumery and as flavoring agent. α-copaene is constituent of oils of African *Copaiva balsam* and *Sindora wallichii*. Also it is obtained from various other higher plant oils and from brown alga *Dictyopteris divaricata*. Aromadendrene is constituent of *Agathis australis*, *Artemisia vestita* and Eucalyptus oils. α-selinene occurs in celery oil and in various other essential oils, eg. *Cannabis sativa*, *Humulus lupulus*, *Anthocephalus cadamba* and it is also present in liverworts.

C. schimperi: The total number of components is 15 (98.6%) with α-pinene and β-pinene accounting for 89% of the oil, making this resin an excellent source of these industrially important chemicals..

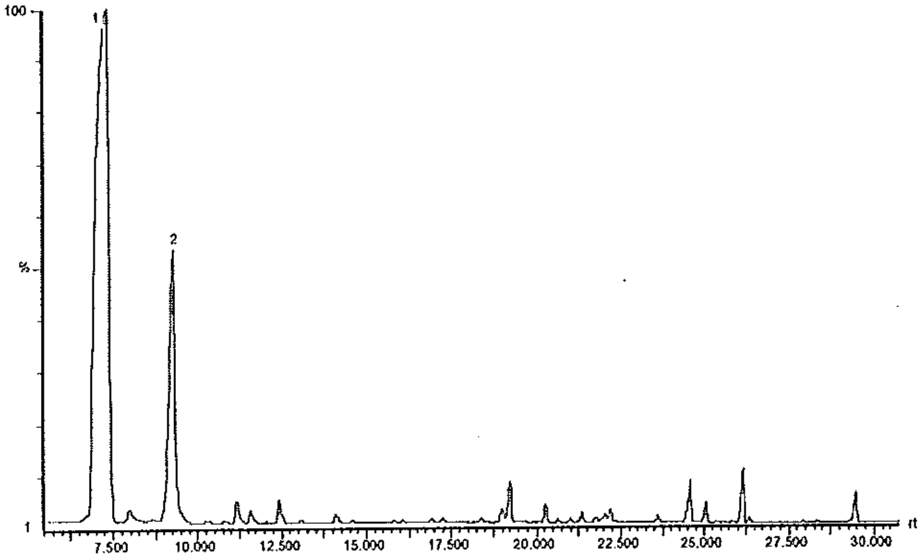


Fig.3.2 Gas chromatogram of hydro-distillate of *C. schimperi*

1: α-pinene (72.5%) 2: β-pinene (16.8%)

GC-MS, ^1H and ^{13}C NMR of the essential oil confirmed that the major components are α-pinene and β-pinene. Also fractionation of the petrol extract of the resin resulted in isolation of α-pinene and a mixture of α-pinene (69%) and β-pinene (20%).

α-Pinene is the main constituent of turpentine, widely distributed in conifers and other plants. It is important intermediate in the manufacture of synthetic aroma compounds and also used as a flavoring ingredient. It undergoes cationic polymerization to give terpene resins used in adhesives. In addition to its use in perfumery and as flavoring agent β-pinene is used in the manufacture of 7-methyl-3-methylene-1,6-octadiene.

C. habessinica **:** The total number of components in the essential oil is 21 (98.3%) being rich in sesquiterpenes.

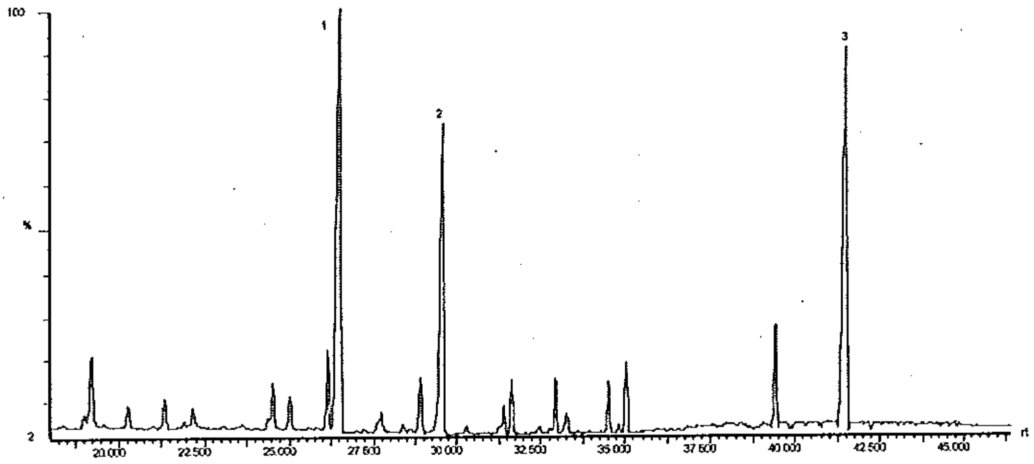


Fig.3.3 Gas chromatogram of hydro-distillate of *C. habessinica*.

1: α-copaene (26.6%) 2: *trans*-caryophyllene (15.3%) 3: α-cadinol (25.1%)

trans-Caryophyllene is constituent of clove, cinnamon and many other oils (*Artemisia*, *Caryopteris*, *Cinnamomum*, *Citrus*, etc.). It is used as the fragrance ingredient and active principle of extract of lemon balm, used for its sedative and antibacterial properties.

α-Cadinol is constituent of *Chamaecyparis lawsoniana* and *Juniperus communis*.

Caryophyllene oxide a major product of the epoxidation of caryophyllene, is found in cloves (*Eugenia caryophyllata*), *Betula alba, Mentha piperita.*

C. kataf **:** In the case of *C. kataf* the total number of components in the essential oil is 13 (98.6%) (Fig. 3.4) and the major components are indicated below.

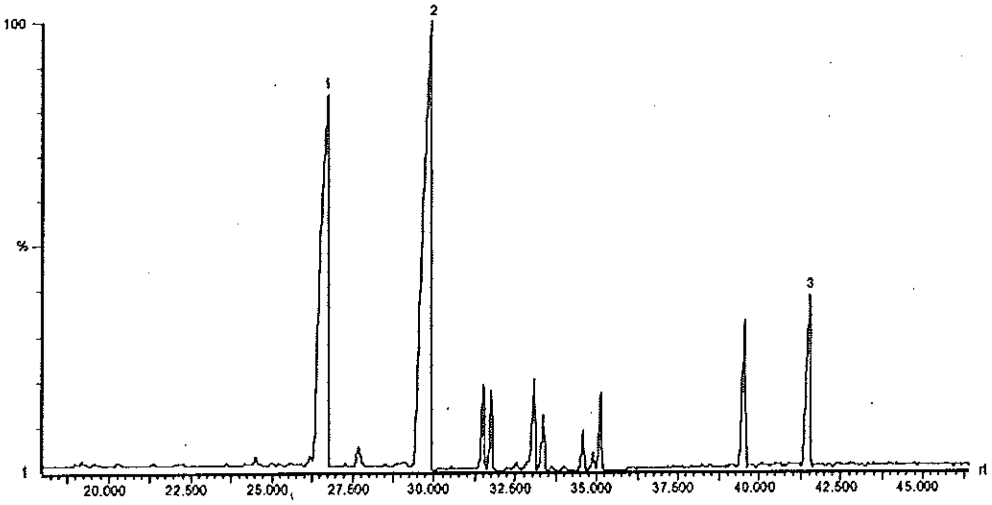


Fig.3.4 Gas chromatogram of hydro-distillate of *C. kataf*

1: α-copaene (28.5%) 2: *trans*-caryophyllene (33.4%) 3: β-cadinene (6.7%)

The Table below shows the compositions and percentages (w/w) of each oil (hydro-distillate). The components are listed according to increasing retention time.

Table 3.2. Constituents of hydro-distillates of the four *Commiphora* species

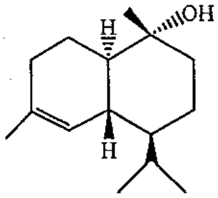
Compound	*C. schimperi*	*C. habessinica*	*C. kataf*	*C. terebinthina*
α-pinene	72.5	1.2	6.2	0.8*
camphene	0.6	-	-	-
β-pinene	16.6	-	7.4	-
dl-limonene	0.9	-	-	-
β-phelandrene	0.5	-	-	-
p-cymene	0.9	-	-	-
linalool	-	-	-	0.7
pinocarveole	0.5	0.5	-	-
t-verbenol	0.3	3.4	-	-
tagetone	-	-	-	0.4
p-menth-1-en-4-ol	0.5	0.9	-	-

linalyl propionate	-	1.3	-	-
myrtenol	0.2	0.6	-	-
geraniol	-	-	-	23.7
Z-citral	1.4	1.5	-	-
Z-ocimenone	-	-	-	0.6
2-pinene-4-one	0.6	1.5	-	-
E-citral	1.5	2.7	-	0.6
α-copaene	-	26.6	28.5	14.8
α-cubebene	-	-	-	0.6*
germacrene	-	-	0.7	-
thymol	-	1.3	-	-
α-bergamotene	-	2.5	-	-
aromadendrene	-	-	-	21.3*
t-caryophyllene	0.8	15.3	33.4	-*
nerylacetate	-	-	-	0.5
α-humulene	-	0.8	2.3	3.2*
alloaromadendrene	-	2.0	1.9	1.3
β-selinene	-	1.0	2.4	8.6
valencene	-	1.8	-	-
α-selinene	-	-	1.6	7.9
β-cubebene	-	-	-	1.1*
γ-cadinene	-	1.8	0.9	-
7-epi-α-selinene	-	-	-	2.7
δ-cadinene	-	2.4	1.8	0.8*
caryophyllene oxide	0.8	4.0	4.7	0.8
α-cadinol	-	25.1	-	7.4
β-cadinene	-	-	6.7	-
Total %	98.9	98.3	98.6	98.5

* is a compound reported in the previous literature [1].

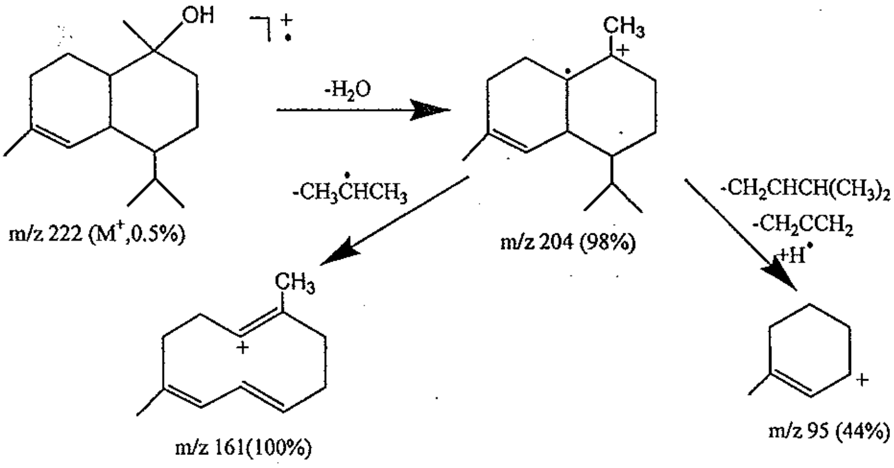
GC: This compound which was only 7.4% in the hydro-distillate was enriched to 89% after separation as described above.

GC/MS: The MS m/z 222 (M^+, 0.5%), 204 (98%), 161 (100%), 105 (67%), 95 (44%) and 81 (66%). Matching the MS with libraries (Wiley, NIST and others) best match is α-cadinol (**40**). Loss of H_2O from the molecular ion confirms presence of a tertiary OH group.



α-cadinol
40

The proposed mass fragmentation pattern is shown below.



Scheme 3.1. Proposed EIMS fragmentation pattern of α-cadinol (**40**)

However this assignment is not conclusive without ^1H and ^{13}C NMR data.

Compound 74-141B: IR, ^1H and ^{13}C NMR comparison with literature [16] are used for the partial characterization of this compound.

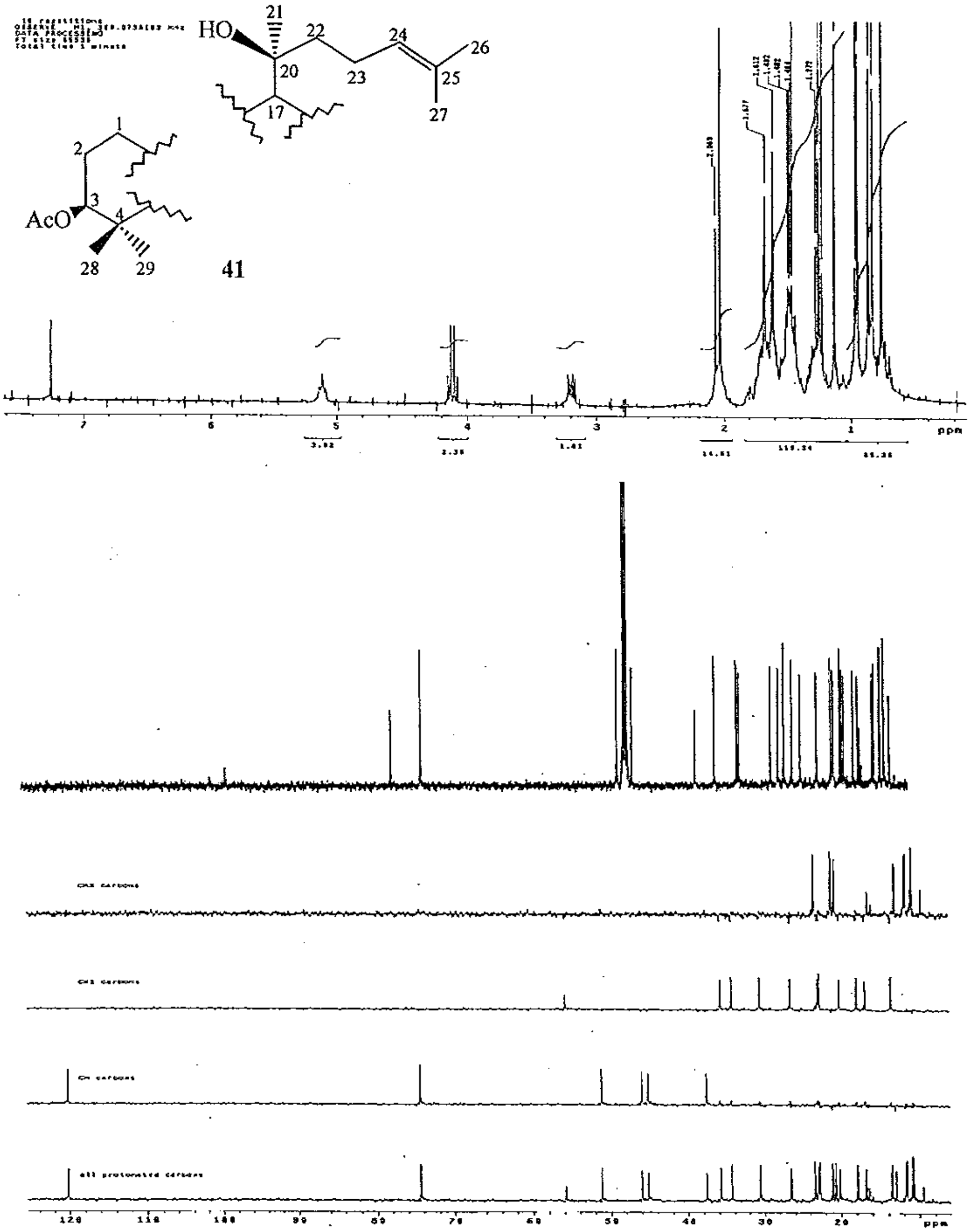


Fig.3.5 ^{1}H and ^{13}C NMR and DEPT spectra of 74-141B (41).

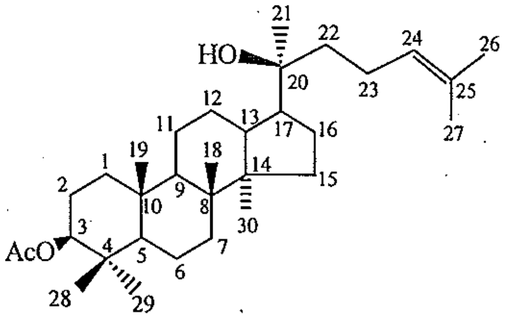
IR: ν_{max} cm^{-1} 3439 (*b*) shows an -OH group, 2998 and 2855 (both *s*) -C-H str. of saturated compounds, 1706 (*s*) ester carbonyl str., 1653 (*w*) non conjugated C=C str.

^{1}H NMR: δ 5.11 (1H, *t*) is terminal vinyl proton $-CH_2-CH=C(CH_3)_2$, 3.20 (1H, *dd*) carbinolic proton, 2.03 (3H, *s*) methyl attached to carbonyl CH_3CO-, 1.67 (3H, *s*) and 1.60 (3H, *s*) are methyls on vinyl carbon, 1.24 (3H, *s*) methyl on an oxygen bearing carbon atom, 0.76 (3H, *s*) and 0.96 (3H, *s*) are methyls on quaternary carbon (C-4).

^{13}C-NMR: This shows that the total number of carbons is 31 indicating the compound is clearly a triterpene. DEPT spectrum shows 8 of the carbons to be methyls (CH_3), 10 methylenes (CH_2), 6 methines (CH) and 7 quaternary carbons.

The signal at 124.8 ppm can be attributed to a carbon of the type $-CH=CR_2$ (C-24), 75.6 implies a carbon atom bonded to oxygen and two alkyl groups like $O-CHR_2$ (C-3), 171.4 is a carbonyl (ester), 131.8 imply an olefin carbon attached to two methyl groups $R_2C=CH-$ (C-25), 76.7 imply an oxygenated carbon $O-CR_3$ (C-20).

The dammarane type triterpene (**42**) which is isolated from *Boswellia freerana* (3β-acetoxy-20(R)-hydroxydammar-24-ene) [16] has some features that fit the data above, indicating that compound 74-141B (**41**) maybe closely related to it.



3β-acetoxy-20(R)-hydroxydammar-24-ene

42

The features that are in common are: an ester group (C-3), an alcohol functional group bonded to tertiary carbon HO-CR_3 (C-20), vinylic carbon attached to proton and vinyl quaternary carbon which is terminal -CH=CR_2 (C-24, C-25).

Compound 74-169D:

UV: The UV was run twice (for 7 min each) and the spectra show an intense peak at λ_{max} 229 nm and 230 nm respectively. Therefore this shows the presence of double bond in the compound.

IR: ν_{max} cm^{-1} 3314 (*s*, broad) shows the presence of -OH group, 2986 (*s*) and 2848 (*s*) show alkane -C-H stretching, 1646 (*w*) imply C=C str. (non-conjugated), the doublet at 1454 (*s*) and 1374 (*s*) imply -C-H deformation of branched saturated compounds.

NMR: Both ^1H and ^{13}C NMR are performed with 300 MHz (for ^1H) and 75 MHz (for ^{13}C) spectrometer with TMS as internal standard.

^1H NMR: δ 5.03 (1H, *t*) is vinyl proton, 0.79 (3H, *d*), 0.91 (3H, *d*), 1.56 (3H, *s*) and 1.67 (3H, *s*) are methyl protons.

^{13}C NMR shows that the total number of carbons is 15. Therefore the compound is a sesquiterpene. Two peaks at δ 134.4 and 122.6 reveal the presence of an alkene (C=C) and 70.1 shows an oxygenated carbon R_3-C-O.

Comparison of the above ^{13}C NMR with literature [11] shows that this compound is closely related to T-cadinol. The ^{13}C NMR data of T-cadinol reported in the literature matches with ^{13}C NMR data of 74-169D . But we need extra data to confirm this.

3.5 Compounds isolated from the resin of *Commiphora schimperi*

The resin of *C. schimperi* was first extracted with petrol (48%) and then with MeOH (6%). Fractionation of the petrol extract resulted in the isolation of the two major constituents of the essential oil which make up 90%. They are coded as 82-1A and 82-1B.

Characterization: IR, ^1H and ^{13}C NMR values are compared with literature [17] for characterization.

Compound 82-1A (4):

IR: ν_{max} cm^{-1} 3024 (*w*) H-C str. of unsaturated compounds, 2918 (*s*) H-C- str. of saturated compounds, 1380 and 1355 (both *m*) H-C- def. of branched saturated compounds.

^1H NMR: δ 5.15 (1H, *t*) imply vinyl proton, 1.63 (3H, *s*), 1.23 (3H, *s*), and 0.79 (3H, *s*) are methyl protons at carbon number 8, 9 and 10, respectively.

^{13}C NMR: The total number of carbons is 10. Therefore the compound is a monoterpene. Comparing NMR data with literature [17], all the δ values match with that of α-pinene (4). Therefore the compound is α-pinene as indicated by its retention time in GC.

Compound 82-1B (4, 8): ^1H and ^{13}C NMR data comparison with literature [17], GC, and GC/MS data are used for characterization.

GC and GC/MS: The gas chromatogram and GC/MS clearly showed that, fraction 82-1B is a mixture of α-pinene (4, 69%) and β-pinene (**8**, 20%).

^1H NMR: δ 4.58 (1H, *td*) and 4.52 (1H, *td*) are vinyl protons of β-pinene. In addition to these all the chemical shifts of α-pinene are found in the spectrum.

^{13}C NMR: Signals whose δ values match with α-pinene and β-pinene, when compared to literature [17], are found in the spectrum. Therefore, 82-1B is clearly mixture of α-pinene and β-pinene.

3.6 Conclusion and recommendations

Analysis of the essential oils of the four *Commiphora* species shows that, the main components were: *C. kataf*, α-copaene (29%) and t-caryophyllene (33%); *C. terebinthina*, geraniol (24%), aromadendrene (21%), α-copaene (15%), α-humulene (3%) and α-cadinol

(7%); *C. schimperi*, α-pinene (73%) and β-pinene (17%); and *C. habessinica*, α-copaene (27%) and α-cadinol (25%).

Comparison of the GC/MS data of essential oil of *C. terebinthina* of literature [1] with that separated here showed that, α-pinene, α-cubebene, aromadendrene, α-humulene, β-cubebene, *trans*-caryophyllene and δ-cadinene are found in both cases.

There is no previous report on the analysis of the essential oils from resins of the other three species, namely *C. kataf, C. schimperi and C. habessinica.*

Fractionation of the petrol extract of the resin of *C. schimperi* resulted in the isolation and characterization of α-pinene and β-pinene, which together make up 90% of the essential oils.

Due to limitation of time, resin material and facilities some of the isolated compounds have not been fully characterized. Further work is therefore necessary.

4. EXPERIMENTAL

4.1 General

Hydro-distillation of resin was done at atmospheric pressure using 4 L round bottom flask fitted with Clevenger apparatus and glass condenser. Optical rotation of the hydro-distillate was measured with Perkin-Elmer 241, Polarimeter, at room temperature using sodium D line.

GC was run using Hewlett-Packard 6890 GC series equipped with FID and HP-5 capillary column (cross linked 5% diPh, 95% dimethylpolysiloxane, 30 m x 0.32 mm i.d. x 0.25 μm film thickness). The column temperature was programmed at 50-210 ^{0}C at a rate of 3 ^{0}C/min. The injector and detector temperatures were 220 ^{0}C and 270 ^{0}C, respectively. Samples (0.5 μL of the oil solutions in $CHCl_3$, 2 mg/mL) were injected by the splitless technique. Nitrogen was used as carrier gas (10 Psi or 2.3 mL/min).

GC/MS was performed on a Fisons GC model 8000 series coupled to a mass spectrometer, MD 800 quadrupole analyzer operating at 70 eV. The capillary column type was DB-17 (50% Ph, 50% methylpolysiloxane, 30 m x 0.25 mm i.d. x 0.25 μm film thickness) with helium as the carrier gas (5 Psi or 1.15 mL/min). Samples (0.6 μL of the oil solutions in $CHCl_3$, 5 mg/mL) were injected by the split technique.

Identification of the constituents of the essential oils was performed by matching MS data of each constituent with Wiley, NIST and user generated mass spectral libraries.

Refractive index was measured at room temperature using Atago Abbe refractometer, No 99996, Japan.

Column chromatography (CC) was done with column size 3 cm x 30 cm packed with silica gel 60, size 0.063-0.200 mm (70-230 mesh ASTM) and thin layer chromatography (TLC) on aluminium sheets, silica gel 60 F_{254}, layer thickness 0.2 mm (Merck).

Preparative thin layer chromatography (PTLC) plates were prepared on 20 cm x 20 cm glass, silica gel 60 $PF_{254+366}$, 7748 (Merck) layer thickness 0.5 mm.

Spot detection on TLC was performed by using UV (254 nm, 365 nm) and spray reagent 1% vanillin in H_2SO_4.

NMR was done with 300 MHz for ^1H and 75 MHz for ^{13}C, TMS as internal standard, with $CDCl_3$. IR spectra were measured with Perkin-Elmer, 1600 series FTIR.

4.2 Plant materials

Plant materials used in this study were collected on two occasions in January, and April, 1998 from Gamo Gofa (Konso). Konso is located 587 km South of Addis Ababa. The local name of all the trees at Konso is "Qahatita" except for *C. kataf* which is known as "Kokomarritta". The trees are approximately 4-6 m, with bark that peel into flakes. When bark is incised milky exudate flows out and solidifies within 30 min and becomes dark-brown after one day. Naturally exuded resins were collected in all cases except in the case for *C. terebinthina*, where the resin was also induced to exude by incision and *C. africana* (absence of resin during collection). Leaves, bark (for all) and fruits (for *C. kataf* and *C. habessinica*) were collected to aid in the botanical identification of the species.

The five species were identified by Dr. Kaj Vollesen (Kew Royal Botanical Garden, U.K.) and are listed in Table 3.1. Voucher specimens have been deposited at the National Herbarium, Addis Ababa University with numbers given in Table 3.1.

4.3 Hydro-distillation

The dry resin was first crushed as much as possible into smaller particles and placed in a round bottom flask fitted with Clevenger type apparatus and was hydro-distilled for 3 h at atmospheric pressure. The strongly aromatic oil was separated from the water layer by separatory funnel and dried by adding anhydrous Na_2SO_4 and then weighed.

Table 4.2 Physical data on the isolated oils.

Species	Resin (in g)	Oil (in mg)	% yield	$[\alpha]_D$	Ref. Ind.
C. terebinthina	10	139	1.4	-14.0	1.493
C. schimperi	15	662	5.0	- 41.6	1.472
C. habessinica	15	8.0	0.05	-	-
C. kataf	15	35	0.2	- 10.0	1.472

Optical rotation and refractive index of *Commiphora habessinica* were not measured due to small amount of oil.

4.4 Extraction and compound isolation from the resin of *C. terebinthina*

C. terebinthina resin (70 g) was crushed and extracted with petrol (200 mL) using the sonic bath for 30 min. Filtration and removal of solvent yielded the crude extract (18 g, 26%). The marc was soaked likewise for 30 min in EtOAc (150 mL) to yield 8 g (11%) of extract. Final extraction with MeOH (150 mL) yielded 6 g (9%) of the crude extract bringing the total amount of extract to 32 g (46%).

Isolation: 5.5 g of the petrol extract was allowed to pass through a column packed with silica gel (petrol) and eluted with petrol-EtOAc gradient to collect a total of 9 fractions. The 4th (100 mL) and 5th (100 mL) fractions (petrol:EtOAc, 99:1) showed the same TLC (petrol) pattern and were combined (50 mg) and applied on PTLC developing with petrol. The major compound (Rf = 0.7) was isolated (32 mg) and coded 74-151A.

As the petrol, EtOAc, and MeOH extracts were similar (TLC), these were combined and 18 g applied on a column of silica gel and eluted with petrol-$CHCl_3$ gradient to get a total of 23 fractions. Fractions 11-15 from CC (petrol:$CHCl_3$, 1:1) of the petrol extract yielded (8 g), which revealed a mixture of four components. 120 mg of this fraction was applied on PTLC and developed with solvent system petrol:EtOAc, 20:3. The fourth band from the top yielded the major compound of the resin, 74-169D (60 mg, TLC one spot, Rf = 0.2, petrol:EtOAc, 25:3). 850 mg of the same fraction was applied on PTLC (petrol:EtOAc, 25:3) to get 50 mg of the pure compound 74-111A (Rf = 0.6). The remaining two bands were very close to each other and were found to be difficult to separate them.

The MeOH extract (2 g) was applied in column packed with silica (petrol). 16 Fractions were collected by eluting with petrol-EtOAc gradient. The 11th fraction (petrol:EtOAc, 3:2) was collected and found to be 180 mg after removing the solvent with rotary evaporator under reduced pressure. This was applied on PTLC and developed with petrol:EtOAc, 5:2 for further purification. The second band from top was collected. The TLC developed with the same solvent system as that of PTLC showed one spot. Finally a pasty nearly pure compound (74-141B) was obtained (85 mg, Rf = 0.7).

α-Humulene (39) (74-151A): Colorless oil

Rf = 0.7 (petrol)

GC (0.5 μL, 2 mg/mL, $CHCl_3$) 99% pure.

GC/MS (0.6 μL, 5 mg/mL, $CHCl_3$): m/z 204 (M^+, 17%), 147 (46%), 121 (57%), 93 (100%), 80 (40%).

α-Cadinol (40) (74-111A): Yellowish oil

Rf = 0.6 (pet:EtOAc, 25:3)

UV (1 mg in 2 mL, $CHCl_3$): λ_{max} nm 230, 231.

IR (1 mg in 50 mg KBr): ν_{max} cm^{-1} 3422 (*s*, broad, OH), 3000 (*s*, H-C= str.), 2970 (*s*, H-C- str.), 2859 (*s*, H-C- str.), 1650 (*w*, C=C str.), 1454 (*s*, -C-H def.) and 1384 (*s*, -C-H def.).

GC (0.5 μL, 2 mg/mL, $CHCl_3$): 90% pure.

GC/MS (0.6 μL, 5 mg/mL, $CHCl_3$): m/z 222 (M^+, 0.5%), 204 (98%), 161 (100%), 105 (67%), 95 (44%), 81 (66%).

Dammarane type triterpene (41) (74-141B): Amorphous solid

Rf = 0.7 (pet:EtOAc, 5:2)

IR (1 mg in 50 mg KBr): ν_{max} cm^{-1} 3439 (*b*, -OH), 2998 and 2855 (both *s*, -C-H str.), 1706 (*s*, ester carbonyl str.), 1653 (*w*, C=C str.).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.11 (1H, *t*), 3.20 (1H, *dd*), 2.03 (3H, *s*), 1.67 (3H, *s*), 1.60 (3H, *s*), 1.24 (3H, *s*), 0.75 (3H, *s*), 0.95 (3H, *s*).

^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4 (ester carbonyl), 131.7 (C-25), 124.8 (C-24), 76.6 (C-20), 75.5 (C-3).

An unknown compound (74-169D): Amorphous solid

Rf = 0.2 (pet:EtOAc, 25:3)

UV (1 mg in 2 mL, $CHCl_3$): λ_{max} nm, 229, 230.

IR (1 mg in 50 mg KBr): ν_{max} cm^{-1} 3314 (*b*, OH), 2986 and 2848 (*s*, C-H str.), 1716 (*w*, C=C str.), 1454 and 1374 (*s*, *d*, C-H def.).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.03 (1H, *t*), 0.79 (3H, *d*), 0.91 (3H, *d*), 1.56 (3H, *s*), 1.67 (3H, *s*).

^{13}C NMR (75 MHz, $CDCl_3$): δ 134.3, 122.6, 70.6, 47.9, 46.6, 40.3, 37.7, 30.9, 28.5, 26.2, 23.8, 22.6, 21.4, 19.8, 15.2.

4.5 Extraction and compound isolation from the resin of *C. schimperi*

Resin of *C. schimperi* (62 g) was first crushed and extracted with petrol (200 mL) to give crude extract (30 g, 48%). The marc was soaked with MeOH (150 mL), filtered, and the solvent removed to give 3.5 g (6%). The total amount of extract obtained was 33.5 g (54%).

The petrol extract (28 g) of the resin of *C. schimperi* was applied on column packed with silica gel (petrol) and eluted with petrol-$CHCl_3$ gradients. Out of the 31 fractions collected, fractions number 4 and 5 [petrol:$CHCl_3$, 49:1] gave nearly pure α-pinene (1 g). Subsequent fractions gave mixtures of α- and β-pinene.

α-Pinene (4) (82-1A): Colorless oil.

IR (1 mg in 50 mg KBr): v_{max} cm^{-1} 3024 (*w*, H-C= str.), 2918 (*s*, H-C- str.), 1380 and 1355 (both *m*, H-C- def.).

^1H NMR (300 MHz, $CDCl_3$): δ 5.15 (1H, *t*), 1.63 (3H, *s*), 1.23 (3H, *s*), 0.79 (3H, *s*), 1.13 (2H, *dd*),2.30 (1H, *m*), 2.15 (2H, *dd*), 1.90 (1H, *t*).

^{13}C NMR (75 MHz, $CDCl_3$): δ 144.5 (C-2), 116.0 (C-3), 47.0 (C-1), 40.7 (C-5), 37.9 (C-7), 31.4 (C-4), 31.2 (C-6), 26.3 (C-8), 22.9 (C-9), 20.7 (C-10).

5. REFERENCES

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