CHEMICAL STUDIES OF *COMMIPHORATEREBINTHINA* AND *PYCNOSTACHYS ABYSSINICA* OCCURRING IN ETHIOPIA

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ABSTRACT

In the course of this study the resins of *Commiphora terebinthina* and the roots of *Pycnostachys abyssinica* were collected from Konso and Wolaita Sodo, Ethiopia, respectively. The essential oil of the resin of *Commiphora terebinthina* was isolated by hydro distillation and analyzed by GC and GC/MS. Identification of the constituents of the essential oil was performed by matching GC/MS of each constituent with Wiley, NIST and user generated mass spectral libraries. The main components identified from the oil of C. *terebinthina* were: geraniol (24%), aromadendrene (2l %), α-copaene (15%), α-humulene (3.2%) and α-cadinol (7.4%). The resins were also subjected to extraction by petroleum ether, ethyl acetate and methanol, sequentially resulting in the isolation of α-humulene, α-cadinol and T-cadinol using chromatographic techniques. The phytochemical screening of the petroleum ether, chloroform and methanol extracts from the root of *Pycnostachys abyssinica* revealed the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids, tannins, steroids and acidic compounds using the standard procedure. Chromatographic separation of the chloroform extract resulted in the isolation of β-sitosterol. Characterization of the isolated compounds was performed using the spectroscopic techniques Uv-Vis, IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135 and comparison with previously reported literature. *[African Journal of Chemical Education—AJCE 10(1), January 2020]*

INTRODUCTION

Plants used in traditional medicine have stood up to the test of time and contributed many novel compounds for preventive and curative medicine to modern science [1]. It has been confirmed by World Health Organization (WHO) that herbal medicines serve the healthcare needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries [2]. Natural products, from microorganisms, have been the primary source of antibiotics and with increasing acceptance of herbal medicine they serve as an alternative form of health care [3].

The *Burseraceae* is a large family with 17-20 genera and 500-600 species, widespread in tropical and subtropical regions [4,5]. Engler [6] 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 of North East Africa, where a large number of species are endemic to this area [7]. 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. *Commiphora myrrha* is the chief source of myrrh today. The plant grows wild in the North-Eastern province of Kenya and adjoining areas of Somalia and Ethiopia. This plant yields economically important gum exudate which has been collected for centuries as medicinal and perfumery substance [8].

Holmes [9,10] 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. *hildebrandtii,* C. *kataf,* C. *mukul,* and C. *schimperi* [11]. The odor of myrrh is described as warm-balsamic, sweet, and somewhat spicy aromatic, sharp and pungent when fresh [12].

The gifts presented by the Maji to the infant Christ symbolized His life: "gold for royalty, frank incense for divinity, and myrrh for suffering" [12]. 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 [13]. 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 [11].

Among local African traditional medicines, the resinous gums of C. *myrrha*and C. *guidottii;* which are locally known as "malmal" and "habak-hadi" in the Somali vernacular [14], respectively, are used on livestock against ticks [8]. The major use of myrrh is for burning as incense in religious ceremonies. Tucholka [15], in a thesis on the chemical composition of "bissabol", reported that "habakhadi" 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. It can be added that "habakhadi" is used in Somalia for the treatment of stomach complaints and diarrhea [16]. It is also used topically for the treatment of wounds. The resin is distilled to yield volatile oils and these have their own characteristic balsamic odour which finds use in perfumery [4].The resin of C. *guidottii* 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.

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 [4]. 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 [17]. Spectroscopic methods like UV, IR, ^{1}H and ^{13}C NMR are among the most powerful techniques for the characterization of isolated compounds.

The components of the essential oils obtained from few *Commiphora* species have been investigated and these include: C. *terebinthina* and C. *cyclophylla* [18], C. *myrrha* [19, 20] and C. *rostrata* [21]. The oils from C. *rostrata* are distinguished by the presence of the homologous ketones starting from 2-octanone, 2-nonanone, 2-decanone etc [21]. The other *Commiphora* species are rich both in the structures of monoterpenes and sesquiterpenes.Oxygenated terpenoids are the components of essential oils most often responsible fortheir distinctive aroma and flavor, even though they are often minor constituents of the oil [22]. 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.

Pycnostachys abyssinica is the family of *Lamiaceae*. *Lamiaceae* comprises about 3500 species distributed among more than 200 genera, represented by 41 genera in Ethiopia [23]. The genus *Pycnostachys* (*Lamiaceae*) is native to Tropical and Southern Africa with extension to Madagascar. There are about 40 species of woody stemmed perennials; they can grow about 3 m high with evergreen narrow leaves [1]. The genus name *Pycnostachys* is derived from the Greek words "Pyknos" meaning dense, and "stachys" an ear of corn. In botany a spike, refers to the inflorescence, which is a spike of many flowers densely and crowded together [24]. It is represented by about 37 species of *Pycnostachys* that occur in tropical Africa and Madagascar. Only three species occur in Southern Africa; *Pycnostachys reticulata*, *Pycnostachys urticifolia* and *Pycnostachys coerulea* [24]. Regardless of its endowment with medicinally important applications no phytochemical investigation of other than the essential oils obtained from its stems and roots was reported. The plant is, therefore a very good candidate to further investigate its chemical constituents.

EXPERIMENTAL

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 °C at a rate of 3 °C/min. The injector and detector temperatures were 220 °C and 270 °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 withsilica gel 60, size 0.063-0.200 mm (70-230 mesh ASTM) and thin layer chromatography (TLC) on aluminum sheets, silica gel 60 F_{254}, and 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 data was generated with 300 MHz for ^{l}H and 75 MHz for ^{13}C, for compounds isolated from *Commiphora terebinthina* and 400 MHz for ^{1}H and 100 MHz for ^{13}C, for the

compound isolated from *Pycnostachys abyssinica*, TMS as internal standard and $CDCl_3$ solvent. IR spectra were measured with Perkin-Elmer, 1600 series FTIR.

Plant materials

Plant materials of *Commiphora terebinthina* were collected from Gamo Gofa (Konso). Konso is located 587 km South of Addis Ababa, the capital of Ethiopia. The local name of the tree, at Konso, is "Qahatita". The tree is approximately 4-6 m, with bark that peels 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 for the study. Leaves, bark, and fruits were collected to aid in the botanical identification of the species. The species was identified by Kaj Vollesen (Kew Royal Botanical Garden, U.K.) and voucher specimen (Tegene 2/072770), have been deposited at the National Herbarium, Addis Ababa University.

The root of *Pycnostachys abyssinica* was collected from Wolaita Zone, Sodo Zurya Woreda, located 330 Km South of Addis Ababa. The plant specimen was authenticated by botanist Retta Regassa, Department of Biology, Hawassa College of Teacher Education.

Ethnobotany

Ethnobotanical information was gathered by interviewing the local people and through observation. Literatures are also consulted to get wider information.

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

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 [4]. The strongly aromatic oil was separated from the water layer by separatory funnel and dried by adding anhydrous Na_2SO_4 and then weighed.

Extraction

C. *terebinthina* resin (70 g) was crushed and extracted with petroleum ether (200 mL) using ultrasonic bath for 30 min. Filtration and removal of solvent yielded the petrol ether crude extract. The marc was soaked, likewise for 30 min in EtOAc (150 mL) to yield the EtOAc extract. Final extraction with MeOH (150 mL) yielded the methanolic crude extract [4].

Compound isolation

Five point five (5.5) gram 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 4^{th} (100 mL) and 5^{th} (100 mL) fractions (petrol: EtOAc, 99:1) showed the same TLC (petrol) pattern and were combined (50 mg) and applied on PTLC developing with petroleum ether resulted in the major compoundthat was coded **74-151A**.

As the petrol, EtOAc, and MeOH extracts were similar (TLC), they 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. One hundred twenty milligram (120 mg) of this fraction was applied on PTLC and developed with petrol:EtOAc, 20:3 solvent system. The fourth band from the top yielded the major compound of the resin coded **74-169D** (TLC one spot, Rf = 0.2,

petrol:EtOAc, 25:3). The same fraction (850 mg) was applied on PTLC (petrol:EtOAc, 25:3) to get the pure compound coded **74-111A**. The remaining two bands were very close to each other and were found difficult to be separated.

Extraction, phytochemical screening and compound isolation of the root of *Pycnostachys abyssinica*

Extraction

The collected *Pycnostachys abyssinica* root was chopped into small pieces and air-dried under shade on a plastic material for days and milled to suitable size with a grinding machine. Five hundred grams of powdered root of *Pycnostachys abyssinica* was sequentially extracted with petroleum ether, chloroform and methanol (each 3 L) by maceration technique using an orbital shaker for 24 hrs through continuous shaking. The extract was filtered using Whatman filter paper No.1, and the residual solvent in each extract was removed using rotary evaporator under reduced pressure. The masses of the crude extracts were determined using analytical balance and stored in a refrigerator up until further analysis was performed.

Phytochemical screening

Phytochemical screening tests were carried out on the crude extracts (petroleum ether, chloroform and methanol) following the standard procedures of Ganesh and Vennila [25] and Prashant [26] in order to investigate the types of secondary metabolites present in the plant species under investigation.

Compound isolation

Ethyl acetate and n-hexane combination provided good TLC profile of the components in the extract. Silica gel (100 g) was activated in an oven at a temperature of 120 ^{o}C for one hour. The chloroform extract (6 g) was adsorbed on to silica gel (15 g) and subjected to column chromatography using EtOAc:n-hexane gradient. A total of 40 fractions were collected and each fraction was monitored by TLC to check its constituents. Spot detection on TLC was performed by using UV (254 nm and 365 nm) and iodine vapor. Fractions of the same TLC profile were combined and concentrated using rotary evaporator under reduced pressure. Fractions 25-30 revealed single spot (Rf = 0.65; EtOAc/n-hexane 12:1) and the compound was coded **LM-1**.

RESULTS AND DISCUSSION

Ethnobotany

Commiphora plant is widely grown in Arbaminch and Konso (low land areas of Southern Ethiopia) because it is suitable for hedge and fence. In Arbaminch the tree is known as "Tsedaki" (Amharic), the Konso people call it "Qahatita". 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. The leaves are,furthermore, used for cattle feed and the wood for building purposes. Interview made with several residents atArbaminch and Konso, however revealed that the residents have very little knowledge of the importance of the resins produced by the trees.The resins are not known for their use as incenselike the resins of C. *myrrha* is commonly used in other places in Ethiopia and Somalia.

Pycnostachys abyssinica Fresen is recognized as endemic species to Ethiopia. Ethnobotanical study showed that leaves of *Pycnostachys abyssinica* are boiled in water and the vapor is inhaled for the treatment of "Mitch*"*(a kind if inflammation on the lips due to exposure to sunlight after eating foods enriched with fats), headache, amoebiasis, diarrhea, stomach bloating, stomach ache, food poisoning and inflammatory disorder. After warming, the leaves are pressed and the juice is used in the treatment of skin and eye infections [27]. The leaves are also used to make a tea for the treatment of dysentery. The aerial parts of the plant are used as termiterepellant, as well. *Pycnostachys abyssinica* is known to produce essential oil capable of repelling insects, thus underscoring the plants' use as live fences. It has been reported by Kloose [28] as a useful remedy for tinea capitis and other skin diseases.

Extraction

The resin of *Commiphora terebinthina* was hydro-distilled and solvent extracted. The powdered root of *Pycnostachys abyssinica* was also solvent extracted and the results are presented hereunder (Tables 1 and 2).

Table 1 Physical data on the isolated oil of *Commiphora terebinthina*.

Species	Resin (in g)	Oil (in mg)	% yield	$[\alpha]_D$	Ref. Ind.
C. *terebinthina*	10	139	1.4	-14.0	1.493

The solvent extract yield in *Commiphora terebinthina* (Table 2) reveals that the majority of its constituents are nonpolar compounds in the case of *Pycnostachys abyssinica*; however polar components predominate. The overall solvent extract yield for *Commiphora terebinthina* is substantial (nearly 50%) although the essential oil yield is not significant.

Table 2 Yield of crude extract obtained.

Species	Solvent	Sample (g)	Extract (g)	%Yield
Commiphoraterebinthina	Pet ether	70	18	26
	EtOAc		8	11
	MeOH		6	9
Pycnostachysabyssinica	Pet ether	500	2	0.4
	$CHCl_3$		7	1.4
	MeOH		6.5	1.3

Isolation and analysis of the essential oils

The essential oil of the resin of *Commiphora terebinthina* was obtained by hydro-distillation. GC and GC/MS analysis of the oil was undertaken and the result is presented below.

The total number of components in the essential oil was 19 (97.8%) and the chromatogram is shown below (Fig. 1).

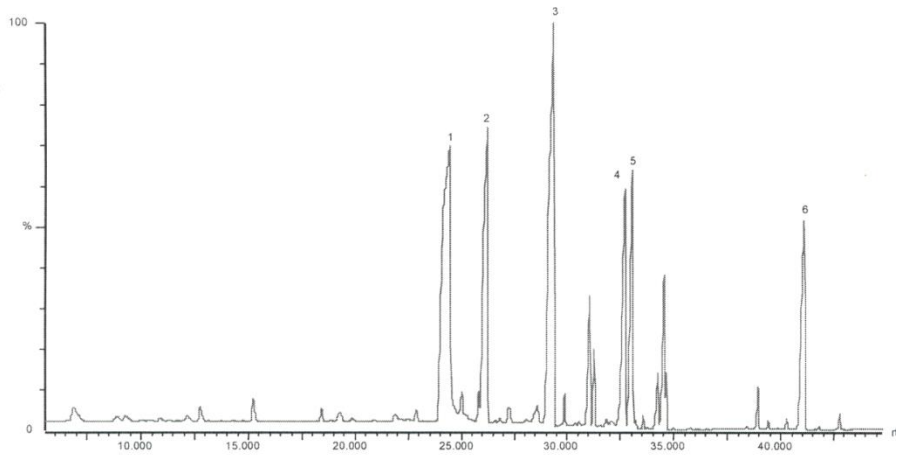


Fig.1. Gas chromatogram of hydro-distillate of C. *terebinthina*

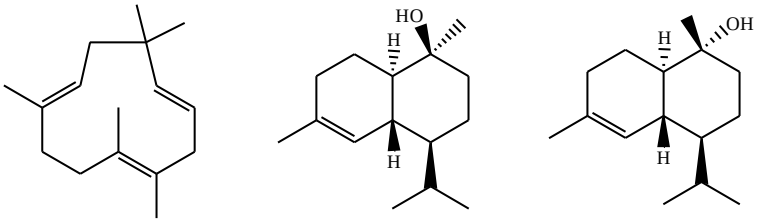
1: Geraniol (23.7%) 2: *α*-copaene (14.8%) 3: aromadendrene (21.3%) 4: *β*-selinene (8.6%) 5: *α*-selinene (7.9%) 6: *α*-cadinol (7.4%). These will make a total of 83.7%. The remaining 14.8% contains *α*-humulene, *α*-pinene, linalool, tagetone, *Z*-ocimenone, *E*-citral, *α*-cubebene, neryl acetate, alloaromadendrene, *β*-cubebene, 7-epi-*α*-selinene, *δ*-cadinene, and caryophyllene oxide.

Comparison of the GC/MS data of essential oil of C. *terebinthina* of literature [18] with the current analysis showed the presence of, *α*-pinene, *α*-cubebene, aromadendrene, *α*-humulene, *β*-cubebene, caryophylleneoxide and *δ*-cadinene in both cases. 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, ego *Cannabis sativa, Humulus lupulus, Anthocephalus cadamba* and it is also present in liverworts. In the essential oil obtained from the resins, Hanus and co-workers [29] reported the presence of: (E)-*β*-ocimene (33%) and *α*-santalene (15.8%) in C. *guidottii*; *β*-elemene (8.7%), furanodiene (19.7%), furanoeudesma-1,3-diene (34%) and lindestrene (12%) in C. *myrrha*; *α*-selinene (11%), curzerenone/furanodienone (13%), T-cadinol (7%), *β*-selinene (8%) and *α*-gurjunene (7%) in C. *sphaerocarpa*; germacrene D (23%), (1E)-8,12-epoxygermacra-1,7,10,11-tetraen-6-one (11.4%), germacrene-B (7.2%) and *β*-selinene (7%) in C. *holtziana*; and germacrene D (9%), germacrene-B (7.1%), (1E)-8,12-epoxygermacra-1,7,10,11-tetraen-6-one (22%), (1(10)E,2R, 4R)-2-methoxy-8,12-epozygermacra-1(10),7,11-trien-6-one (13%) in C. *kataf*; and *α*-pinene (5.7%), sabinene (4.7%), limonene (50.4%) and *β*-cubebene (11.1%) in C. *terebinthina*. Compared to this report the only two components found in the current investigation were *α*-pinene and *β*-cubebene.

Compounds isolated from the resin of *Commiphora terebinthina*

The resin of C. *terebinthina* was extracted with petrol (26%), EtOAc (11%) and MeOH (9%). The petrol, EtOAc and MeOH extracts were combined and subjected to column chromatography resulting in the isolation of three compounds. The three isolated compounds were coded: **74-151A**, **74-111A**, and **74-169D**.

Compound **74-151A**: Colorless oil 32 mg; 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%). This compound (99% by GC) is colorless oil and is also found in the hydro-distillate (3.2%). The GC-MS analysis of the compound using Wiley, NIST and user generated MS libraries, resulted in α-humulene (l,4,8-cycloundecatriene-2,6,6,9-tetramethyl-(*E*,*E*,*E*) **1** as the best match. α-Humulene was also found in C. *myrrha* [29].



1 **2** **3**

Caryophyllene-6,7-episulfide (3), humulene-9,10- (4) and -6, 7-episulfide (5) found in hop oil, were prepared by heating caryophyllene or α-humulene with sulfur at 120 °C. Examination of various types of olefins showed that this peculiar reaction occurred only to the medium ring olefins [30].

Compound **74-111A**: Yellowish oil; Rf = 0.6 (pet:EtOAc, 25:3); UV, IR, GC and GC/MS data are used to identify this compound.

UV: This was run twice and showed an intense peak at λ_{max} 231 and 230 nm respectively. An intense peak in the region 200-230 nm shows the presence of a double bond.

IR: ν_{max}(cm^{-1}) 3422 (*s*, broad) shows an O-H str., 3000 (*s*) alkene (sp^2) =C-H str., 2970 (*s*) and 2859 (*s*) alkane -C-H str., 1650 (*w*) C=C str., the doublet at 1454 (*s*) and 1384 (*s*) -C-H deformation of branched saturated compounds.

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 (**2**). Loss of H_2O from the molecular ion (m/z; 222 – 204 = 18) confirms presence of a tertiary OH group.

Compound **74-169D**: Amorphous solid 60 mg, Rf = 0.2 (pertrol:EtOAc 25:3); UV, IR, ^1H and ^{13}C NMR data, and comparison with literature is used to identify this compound.

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

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

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

^{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, and 15.2. The ^{13}C NMR shows that the total number of carbons is 15.

Therefore the compound is a sesquiterpene. Two peaks at δ 134.3 and 122.6 reveal the presence of an alkene (C=C) and 70.6 shows an oxygenated carbon R_3-C-O. The ^{l3}C NMR spectral data of T-cadinol (**3**) reported in the literature [31] exactly matches with ^{l3}C NMR data of **74-169D**. T-cadinol was also found in C. *sphaerocarpa* [29].

Phytochemical screening test of the root extracts of *Pycnostachys abyssinica*

Phytochemical screening tests were performed for preliminary examination of secondary metabolites on the petroleum ether, chloroform and methanolic root extracts of *Pycnostachys abyssinica.* The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, steroids and acidic compounds. Recently Getahun and Masresha [32] investigated the secondary metabolites in the methanolic extract of the leaf of P. *abyssinica* and reported the presence of: alkaloids, flavonoids, phenols, terpenoids, saponins and tannins. Cardiac glycosides were absent in the methanolic leaf extract. They [32] investigated the antibacterial activity of the methanolic leaf extract and found it to have positive effect, as well. The plant material extracts exhibited antimicrobial activities against one of the most common bacterial pathogens, namely *Staphylococcus aureus* [33]. The ethno-medicinal use of the plant is therefore due to the presence of these biologically active secondary metabolites. Flavonoids have broad biological and pharmacological activities such as antimicrobial, cytotoxicity, anti-inflammatory, antitumor, oestrogenic, anti-allergic, and antioxidant. The anti-inflammatory effect is associated to the traditional use of the plant for treatment of the disease known as “Mitch” (Amharic). Tannins are used as astringents against diarrhea, as diuretics against stomach and duodenal tumors, as anti-inflammatory, as antiseptic, as antioxidant and haemostatic pharmaceuticals. The antiseptic activity of tannins is associated to

the traditional use of the leaf for treating eye infection. Alkaloids have significant role in pharmacological activities including antihypertensive, antimalarial and anticancer. Terpenoids have medicinal properties such as anti-carcinogenic, anti-malarial, anti-ulcer, hepaticidal, anti-microbial or diuretic [34].

Isolated compound from the chloroform extract of root of *Pycnostachys abyssinica*

Compound **LM-1:** A white crystal 45 mg; mp: 134-135 °C; Rf = 0.65 (EtOAc/n-hexane 92:8). The structure of the compound was illustrated using IR and NMR data and comparing these data with literature.

IR: ν_{max}(cm^{-1}) 3499 indicates the presence of O-H stretching of alcohol. The strong sharp band at 2936 and 2821indicates symmetric and asymmetric C-H stretching of CH_3 and CH_2. The medium band at 1520 indicates the C=C stretching. The medium band ~ 1250 indicates a C-O stretching.

^{1}H NMR: δ 0.5-2.5 a total of 46 hydrogens (6CH, 11CH_2 and 6 CH_3); δ 3.5 (1H) is a hydrogen atom bonded to a carbon atom which in turn is bonded to oxygen i.e. O-CH; δ 5.2 (broad 1H) is alcohol OH; δ 5.35 (1H) olefinic proton.

^{13}C NMR: δ12.2, 22.7, 23.1, 24.3, 26.1, 28.2, 29.4, 31.5, 31.9, 34.3, 36.5, 37.3, 39.8, 42.3, 45.8, 50.1, 56.1, 56.8, 56.9, 71.7, 121.6, 140.7.

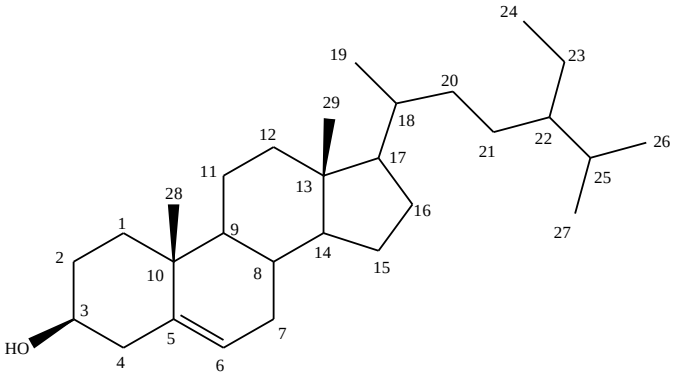
DEPT-135: δ12.2, 22.7, 23.1, 24.3, 26.1, 28.2, 29.4, 31.5, 31.9, 34.3, 37.3, 39.8, 42.3, 45.8, 50.1, 56.1, 56.8, 56.9, 71.7, 121.6.

^{13}C-NMR and DEPT-135spectra showed 22 and 20 signals (one signal 42.3 is the same for C4 –CH_2 and C13), respectively, that can be assigned to 6 methyl, 11 methylene, 9 methine and 3 quaternary carbon atoms.

Reported [35] ^{13}C NMR: δ 12.0, 12.2, 19.0, 19.2, 19.6, 20.1, 21.3, 23.3, 26.3, 28.5, 29.4, 31.9, 32.1, 34.2, 36.7, 37.5, 39.9, 42.5, 42.6, 46.1, 50.3, 56.3, 56.9, 72.0, 121.9, 140.9.

Reported [35] ^{1}H NMR: δ 5.36 (*t*, 1H, *J* = 6.4 Hz, C5), 3.53 (*tdd*, 1H, *J* = 4.5, 4.2, 3.8 Hz, C3), 0.93 (*d*, 3H, *J* = 6.5 Hz, C19), 0.84 (*t*, 3H, *J* = 7.2 Hz, C24), 0.83 (*d*, 3H, *J* = 6.4 Hz, C26), 0.81 (*d*, 3H, *J* = 6.4 Hz, C27), 0.68 (*s*, 3H, C28), 1.01 (*s*, 3H, C29).

The ^{1}H &^{13}C NMR spectra of **LM-1** and that of *β*-sitosterol from literature [35] are very similar. It is, therefore, possible to conclude that **LM-1** is *β*-sitosterol (**4**).



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CONCLUSIONS

The constituents of the essential oils of *C. terebinthina,* revealed the presence of geraniol (24%), aromadendrene (21%), *α*-copaene (15%), *α*-cadinol (7%), and *α*-humulene (3%) as the major components. The remaining 14.8% contains *α*-humulene, *α*-pinene, linalool, tagetone, *Z*-ocimenone, *E*-citral, *α*-cubebene, neryl acetate, alloaromadendrene, *β*-cubebene, 7-epi-*α*-

selinene, δ-cadinene, and caryophyllene oxide. Chromatographic separation of the mixture of the petrol, EtOAc and MeOH extract of the resin of *Commiphora terebinthina* resulted in α-humulene, α-cadinol and T-cadinol. Chromatographic separation of the chloroform extract of the root of *Pycnostachys abyssinica* resulted in the steroid β-sitosterol. The results of the phytochemical screening of the root extract of *Pycnostachys abyssinica* revealed the presence of the medicinally important secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, terpenoids, tannins, steroids and acidic compounds.

IMPLICATIONS TO CHEMICAL EDUCATION

Africa in general and Ethiopia in particular are endowed with hundreds of thousands of medicinal plants. The chemical knowledge of the traditionally used medicinal plants, however, is in its infancy due to the little work that has been done so far, in this regard. There exists tremendous endogenous knowledge of using traditional medicinal plants as remedy for various diseases in Africa. This practice, however, have drawbacks due to the application of inaccurate dosage, unknown mechanism of action of the chemical constituents in the specific disease and lack of scientific knowledge of the active ingredients in the plant materials.

In the Ethiopian Higher Learning Institutions, students take Natural Product Chemistry courses. The courses, however, focus only on the theoretical aspects of Natural Products, their use as remedy for different diseases, and the methods employed during isolation and characterization of the chemical constituents. It is, therefore, imperative to apply the theoretical knowledge they acquired, in the chemical laboratories to make sure that they have gained the necessary experimental skills and laboratory techniques. This paper will help them show the practice of the various techniques they have educated with regard to isolation and

characterization of the chemical constituents from different medicinal plants of African origin. In doing so, they will develop the skills and upgrade the knowledge with regard to the chemical constituents of medicinal plants of African origin in general and Ethiopian origin in particular.

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