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Phytochemical investigation of *Withania somnifera* and *Commiphora kataf*

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Abstract
This study was conducted to investigate the phytochemical constituents of the root of *Withania somnifera* and the resin of *Commiphora kataf*. Investigation of the phytochemicals on the root of *Withania somnifera* was first performed with phytochemical screening of the secondary metabolites using the standard procedures. This was followed by chromatographic isolation of compound and structure elucidation. Phytochemical screening of the crude extract revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and glycosides. One compound was isolated from the chloroform extract and its structure was characterized by using the spectroscopic techniques (UV, IR, and NMR) and comparison with reported literature. The root extract of *Withania somnifera* yielded the pharmacologically active compound (1R, 3aR, 5aR, 5bR, 7aR, 9S, 11aR, 11bR, 13aR, 13bR)-3a, 5a, 5b, 8, 8, 11a-hexamethyl-1-prop-1-en-2-yl-1, 2, 3, 4, 5, 6, 7, 7a, 9, 10, 11, 11b, 12, 13, 13a, 13b-hexadecahydrocyclopenta[a]chrysen-9-ol commonly known as lupeol, clerodol, monogynol B, or farganasterol. The essential oil of the resin from *Commiphora kataf* was isolated by hydro-distillation and the components were identified by GC and GC/MS analysis. The main components of the essential oil were the industrially important constituents: *α*-pinene (6.2%), *β*-cadinene (6.7%), *α*-copaene (29%) and *trans*-caryophyllene (33%).

Keywords: *Commiphora kataf*, Ethiopia, phytochemical, investigation, *Withania somnifera*

Introduction
Medicinal plants have been used for centuries to cure a number of human and animal diseases. Natural products of higher plants became a source of antimicrobial agents with possibly novel mechanism of action [1-4]. In many parts of the world, medicinal plants are used against bacterial, viral and fungal infections. Although the use of modern synthetic drugs became the predominant curing agent in the developed countries, not least numbers of developing countries still depend on natural products. Originally teas or decoctions (aqueous extracts) or tinctures or elixirs action protocols and applied and coupled with modern isolation techniques were used to prepare and administer herbal remedies. These were usually the starting points for isolation work. These days, various extraction protocols are applied and coupled with modern isolation techniques which include chromatography, often guided by bioassay, to isolate the active compounds.

The primary benefits of using plant-derived medicines are that: they are relatively safer than synthetic drugs, contribute wide therapeutic benefits, and more reasonable treatment [5]. A large number of plants are claimed to possess antibiotic properties in the traditional system and are also used broadly worldwide. Plants have been known to reduce various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda.

Twenty-three known *Withania* species are widely distributed in the drier parts of tropical and subtropical zones, ranging from the Canary Islands, the Mediterranean region and northern Africa to Southwest Asia [6]. Among them, only two species, *Withania somnifera* and *Withania coagulans*, are cultivated in several regions due to their economical and medicinal significance [7]. *Withania somnifera* also known as *Ashwagandha* or winter cherry is one of the most important plants in the traditional systems of Indian medicine. This plant is used in more than 100 formulations in Ayurveda [8]. In Ayurveda, *Withania* is widely claimed to have strong aphrodisiac, sedative, rejuvenative and life prolonging properties [9]. *Withania somnifera* usually known in Sanskrit as *Ashwagandha* is a plant belonging to the family Solanaceae. The name "somnifera" in Latin means "sleep-inducer" which probably refers to its general use as a remedy against stress [10]. *Withania somnifera*is found in the dry part of low land area of Arsi, North Shewa, Gojam, Wollo, and Tigray region in Ethiopia. Although the root of *Withania somnifera* was investigated worldwide, phytochemical investigation of its methanolic extract

in four different climatic regions in India: Lucknow (WsL), Karnataka (WsK), Mumbai (WsM), and Nimuch (WsN) resulted in different chemical compositions using the same solvent system [11]. To our knowledge there was no phytochemical investigation that has been done on the root of *Withania somnifera* of Ethiopian origin. The current study is therefore, aimed at phytochemical investigation of the root of *Withania somnifera*, found in Ethiopia (Limunabilbilo Wereda) which would add to the knowledge of the phytochemicals of the species under investigation.

The Burseraceae is a large family with 17-20 genera and 500-600 species, widespread in tropical and subtropical regions [12, 13]. 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 of North East Africa, where a large number of species are endemic to this area [15].

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 [16]. Holmes [17, 18] 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* [19]. The odor of myrrh is described as warm-balsamic, sweet, and somewhat spicy aromatic, sharp and pungent when fresh [20].

The gifts presented by the Maji to the infant Christ symbolized His life: "gold for royalty, rank incense for divinity, and myrrh for suffering" [20]. 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 [21]. 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 [19]. 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, respectively, are used on livestock against ticks [16]. The major use of myrrh is for burning as incense in religious ceremonies. The resin is distilled to yield volatile oils and these have their own characteristic balsamic odour which finds use in perfumery [13].

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. Thulin *et al.* [22] suggested that the myrrh of the Bible and the incense of the ancient Egyptians and of Classical times [19] was most likely the "perfumed myrrh" or "bissabol" from *C. guidottii* and not the medicinal myrrh from *C. myrrha.* "Bissabol" according to Holmes [23] 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 [22] 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 [24], 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 taken by Somali women after giving birth to a child. "Habak-hadi" is used in Somalia for the treatment of stomach complaints and diarrhoea [25]. It is also used topically for the treatment of wounds.

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 [13]. 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 [26]. The components of the essential oils obtained from only few *Commiphora* species have been investigated and these include: *C. terebinthina* and *C. cyclophylla* [27], *C. myrrha* [28, 29] and *C. rostrata* [30]. The oils from C. *rostrata* are distinguished by the presence of the homologous ketones starting from 2-octanone, 2-nonanone, 2-decanone etc [30]. 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 for their distinctive aroma and flavor, even though they are often minor constituents of the oil [31]. 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. In this study resin was collected from *Commiphora kataf* tree and its essential oil constituents were analyzed. The results are discussed hereunder.

Materials and Methods

General

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 μmfilm 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 refracto meter, 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). Spot detection on TLC was performed by using UV (254 nm, 365 nm) and spray reagent 1% vanillin in H_2SO_4.
UV-Vis spectrum was measured with GENESY's spectrometer (200-600nm) in methanol at room temperature.
NMR data was generated with 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR, for the compound isolated from *Withania somnifera*, TMS as internal standard and $CDCl_3$.
The FT-IR samples were prepared using spectral grade KBr pellets and made into pellets and spectral analysis was made using FT-IR, Perkin Elmer, 1600 series, in the range between 4000 cm^{-1} and 400 cm^{-1}.

Plant materials
The root of *Withania somnifera* was collected in May 25, 2010 from Limunabilbilo Woreda, Arsi Zone, Oromia Region, Ethiopia. The plant was identified by botanist Mr.Retta Regasa Department of Biology, Hawassa College of Teachers Education. Plant specimen was deposited at the herbarium of Hawassa College of Teacher Education with voucher number ws /31.
Plant materials of C. *kataf*were collected on two occasions in January, and April, 2008 from Gamo Gofa (Konso). Konso is located 587 km South of Addis Ababa. Naturally exuded resins were collected for the phytochemical investigation. 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.) as C. *kataf* (*C. erythreae)* (Ehrenb.) Engl. (1883) and voucher specimen has been deposited at the National Herbarium, Addis Ababa University with numbers given Tegene 1/072769.

Extraction
The collected root of *Withania somnifera* was chopped into small pieces and air dried under shade for 30 days and milled to suitable size by using mortar and pestle. About 300 g of the powdered root of *Withania somnifera* was soaked in 1.5L n-hexane in a round bottom flask, at room temperature. The round bottom flask was put on an orbital shaker and left for 48 hours at a speed of 120 revolutions per minute. After 48 hrs the solution was filtered using Whatman filter paper. The filtered solution was concentrated using rotary evaporator at reduced pressure and a temperature of about 36-38 ^oC. The marc left was further extracted with chloroform and methanol consecutively, likewise.
Hydro-distillation of *C. kataf* resin was performed 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.

Phytochemical screening
Phytochemical screening tests were carried out on the crude extracts (n-hexane, chloroform and methanol) following the standard procedures of Visweswari *et al*. [32] and Kokate [33] in order to investigate the types of secondary metabolites present in the plant species under investigation.

Compound isolation
TLC profile analyses were carried out in the chloroform extract using varied proportions of n-hexane:ethyl acetate solvent systems to identify the appropriate solvent for column chromatography. The chloroform extract (12 g) was adsorbed on 20 g of silica gel and was subjected to column chromatography. The column was packed by n-hexane to achieve least polarity to the mobile phase during the beginning of elution. Elution was conducted with increasing gradient of n-hexane/ethyl acetate. A total of 150 fractions (25ml each) were collected and analyzed by TLC. Fractions 23-36showed three spots and were combined and further fractionation was conducted in small column in n-hexane/ethyl acetate solvent system and a total of 10 fractions (20 mL each) were collected. TLC analysis of fraction 7 showed single spot with R_f value (0.67) in (4:1) n-hexane/ethyl acetate solvent system. Finally (90mg) white solid was obtained and coded as SYA. Spectroscopic data of SYA was generated using UV, IR, ^1H and ^{13}C NMR techniques, for structure elucidation.

Results and Discussion
Ethnobotany and chemical analysis of *Commiphora kataf* Ethnobotany
The *Commiphora* plant is widely grown in Ethiopia (Arbaminch and Konso) because it is suitable for hedge and fence. In Arbaminch area the tree is known as "Tsedaki" (Amharic), the Konso people call it "Qahatita". However, the name "Kokomarritta" is used specifically for C. *kataf.* The name "Qahatita" means 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 or as remedy for any disease.

Isolation and analysis of the essential oil
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. The essential oil of the resin of *Commiphora kataf* was obtained by hydro-distillation. GC and GC/MS analysis of the oil was undertaken and the result is presented below (Table 1).

Table 1: Physical data on the isolated essential oil of *C. kataf* resin.

Species	Resin (in g)	Oil (in mg)	% yield	$[\alpha]_D$	Ref. Ind.
C. kataf	15	35	0.2	-10.0	1.472

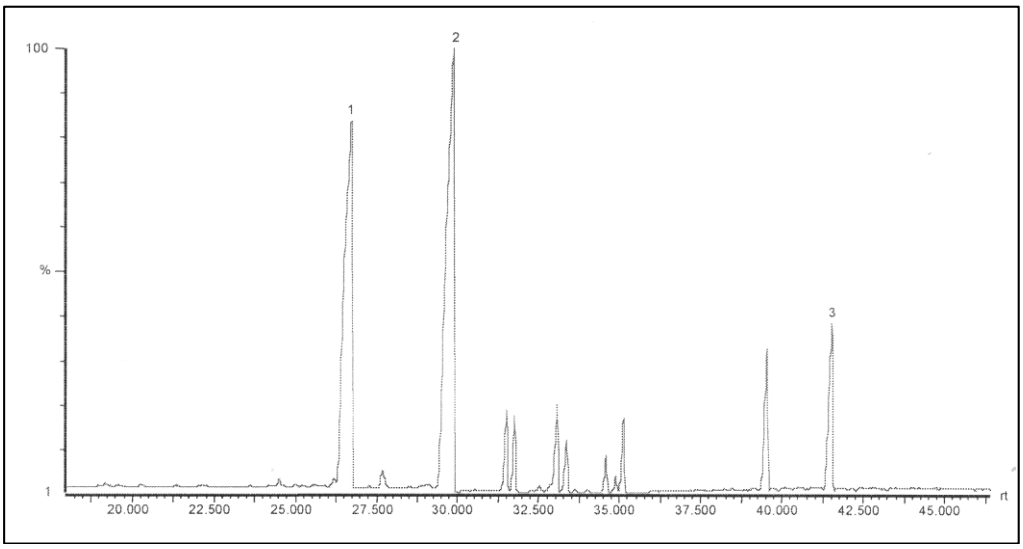

Fig 1: Gas chromatogram of hydro-distillate of C. *kataf* 1: *α*-copaene (28.5%) 2: *trans-caryophyllene* (33.4%) 3: *β*-cadinene (6.7%)

The total number of components is 13 with *α*-copaene (28.5%), *trans*-caryophyllene (33.4%), accounting for 61.9% of the oil (98.6%), making this resin an excellent source of these industrially important chemicals. Other constituents found in the essential oil include: *β*-pinene (7.4%), *β*-cadinene (6.7%), *α*-pinene (6.2%), caryophylleneoxide (4.7%), *β*-selinene (2.4%), *α*-humulene (2.3%), alloaromadendrene (1.9%), *δ*-cadinene (1.8%), *α*-selinene (1.6%), *γ*-cadinene (0.9%), and germacrene (0.7%).*α*-Pinene is the main constituent of turpentine, widely distributed in conifers and other plants. It is important intermediate in the manufacture of synthetic fragrant compounds and also used as a flavoring ingredient. It undergoes cationic polymerization to give terpene resins.

Chemical analysis of the root of *Withania somnifera* Extraction

The yields of the crude extracts of *Withania somnifera* are presented in Table 2 below.

Table 2: The percent yield of crude extracts.

Solvent	Mass of plant root (g)	Extract (g)	Yield (%)
n-Hexane	300*	1.9	0.63
Chloroform		22	7.33
Methanol		10	3.33

*the plant root extraction was done sequentially from n-hexane to chloroform to methanol.

As can be seen in Table 2 above, the crude extract yield increased up on increasing the solvent polarity from n-hexane to chloroform it however, decreased when the solvent polarity increased further to methanol. This indicates that most of the chemical constituents in the plant stem bark are moderately polar. The small yield of n-hexane and methanol extracts only allowed performing phytochemical screening.

Phytochemical screening

A variety of herbs and herbal extracts contain different secondary metabolites with biological activity that can be of valuable therapeutic index. These secondary metabolites are natural products or plant constituent are responsible for medicinal properties of plants to which they belong. Phytochemical screening tests of different solvent extract of stem bark of *Withania somnifera* revealed the presence of medicinally active secondary metabolites. Phytochemical screening of the n-hexane extract revealed the presence of terpenoids, alkaloids, phenols and tannins and the absence of saponins, glycoside and steroids. In the chloroform extract terpenoids, alkaloids, phenols, tannins, glycosides, and steroids were present while saponins were absent. The methanolic extract showed the absence of onlyglycosides and the presence of alkaloids, terpenoids, tannins, glycosides, phenols, and saponins.

Alkaloids isolated from *W. somnifera* are used for the treatment of tumors, nocturnal leg pain caused by vascular contraction and diarrhea. These compounds possess anti-microbial activity and sedative effects. Many alkaloids are used as anesthetics and have encouraging effects on psychotic or hypertensive patients without inducing sleep [34]. Flavonoids from this plant showed properties like anti-oxidant, strengthening of capillary walls, reducing osteoporosis, get better blood cholesterol levels, and lower risk of cancer and coronary heart diseases [35]. Steroids have much medicinal values including: reduction of cholesterol levels, affecting immune system, and helping cell growth [36-38]. Terpenoids are used for anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, anti-cancer and inhibit cholesterol synthesis [39]. In Ethiopia traditionally *Withania somnifera* is used for the treatment of fever however, pharmacologically it is used for antimicrobial and for treatment of diarrhea.

Structure elucidation of compound SYA

The compound SYA: 90 g obtained as white powder.

UV-Vis absorption at λ_{max}230nm indicates that SYA contains non conjugated alkene functionality.

Analysis of IR spectrum (ν_{max} in cm^{-1}): weak broad band at 3294 showed presences of O-H stretching, very weak peak at 1630 indicates presence of non-conjugated C=C bond. The strong band at 2923 and 2854 represents C-H stretch (-CH_3 and -CH_2) of saturated compounds, the strong sharp band at 1461indicates C-H bending of –CH_3 and -$CH_2$and the band at 1377indicates C-O bond.

^{1}H-NMR spectrum of compound SYA (δ in ppm): 3.22(td,1H,H-3), 4.71(d,1H,H-29),4.58(d,1H, H-29), 2.43(td,1H,H-19), 1.7(s,3H,H-30),1.10(s, 3H,H-23),0.98(s, 3H,H-26),0.95-0.71(s, 9H,H-24,H-25& H-27),0.68(s, 3H,H-28).

^{13}C-NMRspectrum of compound SYA (δ in ppm): showed a total of thirty different carbons. The signalsat38.8, 40.8,37.17,

43.0, and 42.8 are quaternary carbons C-4,C-8,C-10,C-14,andC-17carbons respectively (Figure 2).The signal at 78.9 is due to C-3 oxygen bonded carbon and the signals at, 150.9,and109.3 are the olefinic carbons C-20 and C-29 respectively.

DEPT-135spectra (δ in ppm): displayed eleven peaks that correspond to methylene (CH_2) groups: 109.3, 40.0, 38.7, 35.5, 34.3, 29.0, 28.0, 27.4, 25.1, 20.9, and 18.0 for C-29, C-22, C-1, C-16, C-7, C-21, C-2, C-15, C-12, C-11, and C-6, respectively. Seven upward peaks correspond to methyl (CH_3) groups: 27.4, 19.3, 18.0, 16.1, 15.9, 15.3, and 14.5 for C-23, C-30, C-28, C-25, C-26, C-24, and C-27, respectively. The five peaks at 55.3, 50.4, 48.3, 47.9 and 38.0 correspond to methyne (CH) carbons; C-5, C-9, C-18, C-19 and C-13, respectively.

Comparison of ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of compound **SYA** with the reported data of lupeol [40] is shown below (Table 3). The IR, UV-Vis, and NMR data discussed above is consistent with the reported compound (1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a, 5a, 5b, 8, 8, 11a-hexamethy l-1-prop-1-en-2-yl-1, 2, 3, 4, 5, 6, 7, 7a, 9, 10, 11, 11b, 12, 13, 13a, 13b-hexa decahydro cyclopenta [a] chrysen-9-ol commonly known as lupeol data [40].

Lupeol has a complex pharmacology, displaying antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemo-preventive properties [41]. Animal models suggest lupeol may act as an anti-inflammatory agent. A 1998 study found lupeol to decrease paw swelling in rats by 39%, compared to 35% for the standardized control compound indomethacin [42]. One study has also found some activity as a Dipeptidyl peptidase-4 inhibitor and prolyl oligopeptidase inhibitor at high concentrations (in the millimolar range) [43]. It is an effective inhibitor in laboratory models of prostate and skin cancers [44-46].

Table 3: Comparison of ^{1}H- & ^{13}C-NM Rand DEPT-135 data of compound SYA with reported lupeol data [40]

Carbon numbering	^{13}C-NMR data of compound SYA(ppm)	Reported ^{13}C-NMR data of lupeol[40]	DEPT-135 data of compound SYA(ppm)	^{1}H-NMR data of compound SYA(ppm)	Reported ^{1}H-NMR data of lupeol[40]	Type of carbon
C-1	38.7	37.257	38.7			CH_2
C-2	28.0	29.933	28.0			CH_2
C-3	78.9	78.512	78.9	3.22(dd, 1H,H-3)	3.20(dd, 1H,H-3)	CH
C-4	38.8	38.790	-----			C
C-5	55.3	55.380	55.3			CH
C-6	18.3	18.089	18.3			CH_2
C-7	34.2	34.363	34.2			CH_2
C-8	40.8	40.089	-----			C
C-9	50.4	50.520	50.4			CH
C-10	37.1	42.917	-----			C
C-11	20.9	21.015	20.9			CH_2
C-12	25.1	25.223	25.1			CH_2
C-13	38.0	38.948	38.0			CH
C-14	43.0	43.087	-----			C
C-15	27.4	27.513	27.4			CH_2
C-16	35.5	35.670	35.5			CH_2
C-17	42.8	40.918	-----			C
C-18	48.3	48.388	48.3	2.43(td,1H,H-19)		CH
C-19	47.9	48.076	47.9			CH
C-20	150.9	150.98	-----			C
C-21	29.8	28.076	29.8			CH_2
C-22	40.0	38.139	40.0			CH_2
C-23	27.4	29.787	27.4	1.10(s, 3H,H-23)		CH_3
C-24	15.3	14.634	15.3			CH_3
C-25	16.1	16.208	16.1			CH_3
C-26	15.9	16.062	15.9	0.98(s, 3H,H-26)		CH_3
C-27	14.5	15.455	14.5			CH_3
C-28	18.0	18.408	18.0	0.68(s, 3H, H-28)		CH_3
C-29	109.3	109.56	109.3	4.70, 4.58(s)	4.57,4.69(brs)	C=C
C-30	19.3	19.316	19.3	1.7(s,3H,H-30)		CH_3

As an anti-inflammatory agent, lupeol functions primarily on the interleukin system. Lupeol decreases IL-4 (interleukin 4) production by T-helper type 2 cells [41, 47]. Lupeol has been found to have a contraceptive effect due to its inhibiting effect on the calcium channel of sperm (CatSper) [48]. It is reported to exhibit a spectrum of pharmacological activities against various diseases such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer [49].

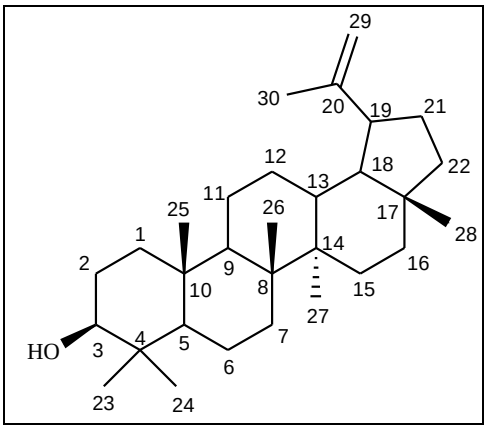

Fig 2: The structure of lupeol (carbon numbering is only for structure elucidation).

Conclusion

Although the Phytochemical investigation of *Withania somnifera* was done in different countries worldwide, this is the first study performed in Ethiopia. The phytochemical screening of the root of *Withania somnifera* revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and glycosides. Chromatographic separation of the chloroform extract yielded the compound (1R, 3aR, 5aR, 5bR, 7aR, 9S, 11aR, 11bR, 13aR, 13bR)-3a, 5a, 5b, 8, 8, 11a-hexamethy l-1-prop-1-en-2-yl-1, 2, 3, 4, 5, 6, 7, 7a, 9, 10, 11, 11b, 12, 13, 13a, 13b-hexadecahydrocyclopenta [a]chrysen-9-ol commonly known as lupeol that has a wide spectrum of pharmacological activities against various diseases such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer. This therefore, makes *Withania somnifera* one of the medicinally important plants.

The essential oil extracted through hydro-distillation from the resin of *Commiphora kataf* contained 13 components of which *α*-copaene (28.5%) and *trans*-caryophyllene (33.4%) accounting for 61.9% of the oil. The industrially important components *β*-pinene (7.4%) and *α*-pinene (6.2%) are the other constituents of the essential oil. It however, is highly recommended to isolate more compounds from the root of *Withania somnifera* and from the resin of *Commiphora kataf.*

Conflict of Interest

The authors have not declared conflict of interest.

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