Addis Ababa University

School of Graduate Studies

Phytochemical Investigation of the Resins of *Boswellia* Species Collected from Kebtele Area in Agew-Awi (Gojjam)

By: Asmare Melese

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July 2007

Phytochemical Investigation of the Resins of *Boswellia* Species Collected from Kebtele Area in Agew-Awi (Gojjam)

A Graduate Project Submitted to the School of Graduate Studies Addis Ababa University

In partial Fulfillment of the Requirements for the Degree of Masters of Science in Chemistry

By: Asmare Melese

July 2007

Dedicated to: My Mother Alefech Negussie

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ACKNOWLEDGEMENTS

I am extremely grateful to my research advisor Prof. Ermias Dagne for his continuous supervision, encouragement and guidance during the course of this project. I am also grateful to co-workers of the Natural Products Chemistry Project in particular Ato Tesfaye Yalew for GC, Ato Yoseph Atilaw and Ato Massreshaw Asnakew for running NMR spectra and technical assistance and W/ro Tsehay Dejene for motherly advice.

I am also indebted to Dr. Wendimagegn Mammo for his help particularly in running and interpreting 2D- NMR spectra. I am also very much grateful to Dr. Yonas Chebude and W/t Medhanit Mammo for running IR spectra, Dr. Kaj Vollesen for identification of the plant.

I would also like to acknowledge Ministry of Education for giving me the opportunity to participate in my postgraduate program and Natural Gum Processing and Marketing Enterprise (NGPME) for facilitating the fieldwork during specimen and sample collection.

My heartfelt thanks go to my parents for their unreserved support and encouragement. I am deeply indebted to my mother W/ro Alefech Negussie.

Abstract

Phytochemical Investigation of the Resins of *Boswellia* Species Collected from Kebtele Area in Agew-Awi (Gojjam)

By

Asmare Melese

Research Advisor: Prof. Ermias Dagne

The genus *Boswellia* is one of the 17 genera belonging to Bureseraceae family. In the course of this study leaves and barks of *Boswellia* species and its resins were collected from Kebtele area for botanical identification and chemical analysis. The species was identified by Dr. Kaj Vollesen (Kew Royal Botanical Garden, U.K.) as *Boswellia papyrifera*.

The essential oils of the resins from *Boswellia* sp. (Kebtele) and *B. papyrifera* (obtained from Markato) were isolated by hydrodistillation and some of the components identified by GC. The major components *Boswellia sp.* (Kebtele) were verticilla-4(20),7,11-triene (**BK1**) (65%), octylacetate (4%) and other four unknown components whose compositions of the oil near to 4 %. This analysis gave a different result compared to the report of Dekebo *et al.* [26], Hamm *et al.* [9] and *B. papyrifera* (obtained from Markato) with octyl acetate (≥56 %) and n-octanol (≥5 %) as their common major components and verticilla-4(20),7,11-triene was absent in any of the three. Basar *et al.* (2001)[34] isolated and identified verticilla-4(20),7,11-triene the first time in the essential oil of *B. carteri* (6% of the oil).

In this work it was also possible to isolate three compounds from the petrol extract of the resins: verticilla-4(20),7,11-triene, partially characterized diterpene (**BK3**) and uncharacterized white crystalline solid (**BK6**).

1. INTRODUCTION

1.1. The family Burseraceae

Burseraceae is a family represented by 17 genera and 500-600 species, wide spread in all tropical regions and extended to subtropics. They are trees or shrubs often spiny; often with latex, resins or oils which are strongly aromatic. It is often a dominant constituent of the vegetation in dry lowland areas. In Ethiopia 2 genera (*Boswellia* and *Commiphora*) and 58 species are present [1].

1.2. The genus *Boswellia*

The genus *Boswellia* has about 20 species occurring in the dry regions from west Africa to Arabia and south to north east Tanzania, also in India and one species in Madagascar. The genus is centered in northeast Africa where about 75 % of the species are endemic to the area. They are trees or shrubs; outer barks often peeling in parchmenty flakes, inner bark greenish, with watery aromatic resins, and wood with milky latex [1].

Frankincense or olibanum is the oleo-gum resin harvested from several different trees belonging to the genus *Boswellia*. The word frankincense is derived from the old French name "*frank encense*", meaning "*pure incense*". Frankincense is also known in Arabic as "*luban*", which means *''white''* or *''cream''*, in Greek "*libanos*" and in Ethiopia "*etan*" [2, 3. 4].

Frankincense has a wide use including incense in homes, formulation of a number of modern perfumes and as medicine [4]. Its volatile oils have

their own characteristic balsamic odours. Both resinoids (obtained by hydrocarbon extraction) and absolutes (obtained by alcoholic extraction) are used as fixatives and additives in perfumes [2, 4]. Olibanum is also used as components of adhesive plasters and fumigation powders, in chewing gums, ingredients for lotions, soaps, detergents and creams [2, 3, 4]. Frankincense is a complex mixture composed of about 5-9 % highly aromatic essential oil (mono- and sesquiterpenes), 65-85 % alcohol soluble resins (diterpenes, triterpenes), and the remaining water-soluble gums (polysaccharides) [4]. Mono- and sesquiterpenes are highly volatile compounds, diterpenes exhibit low volatility, triterpenes very low volatility and polysaccharides are not volatile [4, 9].

The major frankincense sources of the world today are Ethiopia, Somalia and northeast Kenya [5]. The principal frankincense producing species include *B. papyrifera* (Del.) Hochst, B. *pirottae* Chiov., *B. neglecta* S.Moore, *B. microphylla* Chiov., *B. rivae* Engl. and *B. ogadenesis* Vollesen occurring in Ethiopia [1], *B. sacra* Birdw. and *B. frereana* Birdw. occurring in Somalia, *B. serrata* Roxb.ex Coleber. occurring in India and *B. dalzielii* Hutch occurring in Nigeria.

The resin of *B. papyrifera* is a raw material of the Ethiopian frankincense commonly called "et*an"* or "*walya meke*r" in Amharic and widely collected in north Ethiopia. It is known in commerce as "Tigray or Eritrean Type". It is widely used in Ethiopia and other countries as incense at home and during religious ceremonies. It is also exported to different parts of the world where it is used for making adhesives, chewing gum and fragrance oil. The resin of *B. papyrifera* is considered of poorer quality than the product obtained from the Arabian and Somalian species. *B. pirottae* is a rare endemic species only known from north and central low land regions of Ethiopia. The resin of *B. rivae* is

known in commerce as "*Ogaden eta*n" because it is obtained from Ogaden area, while that of *B. neglecta* originating from Borena is traded as "*Borena etan*" [2].

In India the gum resin of *B. serrata* (called "*salai guggal*" in the vernacular) has been used in the traditional Ayurvedic medicine for treatment of inflammatory diseases. It is also used to cure rheumatoid arthritis. The ethanol extract of the resin of *B. serrata* is called "*sallaki*" is available in the Indian market [6].

Two species *B. sacra* (synonym *B. carteri*) and *B. frereana* occurring in Somalia are well known world wide as the source of high quality frankincense. Formerly *B. sacra* and *B. carteri* were regarded as two distinct species, the former is growing in Arabia and the latter is in Somalia. However, Thulin and Warfa indicated that the two should not be considered as two separate species since they differ only in the geographical location in which they are encountered [8, 9]. In contrast, Duperon presents an atomical argument for maintaining *B. sacra* and *B. carteri* as two distinct species [9]. More recently, in a paper on the analysis of triterpenes of various olibanum samples by HPLC, Mathe *et al.* found the same components in *B. sacra* and *B. carteri* olibanum [10].

B. sacra, which also occur in Yemen, is the source of the Arabian frankincense of classical times and is also known as "Bible incense" [2, 4]. Its resin, known in Somalia as "*beeyo"*, is exported to the European flavour and fragrance industries. The resin of *B. frereana* is known only from Somalia and prefers hot and humid climate and good supply of water to grow. The resin known locally as "*meydi*" is of superior quality due to its lemon sent, sweat taste and yellow color [2, 7].

B. dalzielii Hutch is a tree of the Savanna forest recognizable by its papery bark peeling off in a ragged manner. The bark yields a whitish gum resin, which dries readily and is friable [13].

It is customary to obtain resins from *Boswellia* species by making deliberate incision of the bark. The milky liquid that exudes gradually hardens on exposure to air into tears. The time of tapping, its duration and the interval between the individual tapping vary according to the species. Tapping involves removing small areas of the bark of tree using a scalpel-like tool, called *mengaff* in northern Ethiopia. After removing the resin, new tapping is made at the same place as in the previous ones [2, 4].

1.3. Ethnobotany of *Boswellia* Resins

Ethno- is a popular prefix these days, because it is a short way of saying 'that is the way other people look at the world' [11]. When used before the name of academic discipline such as botany, it implies that researchers are exploring local people perception of culturally used plants and their products. Ethnobotany is on how plants have been or are used, managed and perceived in human societies and includes plants for food, medicine, divination, dyeing, textiles, for buildings, tools, rituals, social life, music etc.

In Ethiopia, the resins and barks of different *Boswellia* species are used for different purposes. The resin of *B. papyrifera* is widely used as incense of choice by the Ethiopia Orthodox Church. It also enjoys a wide array of traditional uses as human medicine and insect repellant. The bark is chewed to treat stomach disturbances and to prevent or quench thirst. The bark of *B. neglecta* is used as varnish after boiling. The

frankincense is used as insect, rat and snake repellant and for the treatment of skin diseases. The resins of *B. microphylla* and *B. rivae* are used for tanning and to repel insects respectively [2].

In the traditional Indian Ayruvedic medicine many conditions are treated with the gummy resin of *B. serrata* (called "*salai gugga*l" in the vernacular). These include: diarrhea dysentery, ringworm, fever, skin and blood diseases, mouth sores, vaginal discharges, hair loss, hemorrhoids, syphilitic diseases and to stimulate the liver [13]. Ayruvedic medicine is a Hindu-based system of individualized healing that can be practiced in India for more than 2,000 years. It is a complex system that recognizes different human body types. Each of these types has different qualities that affect health and natural balance of the person.

The gum resin and different parts of *B. dalzielii* are widely employed in traditional medicine of Nigeria. The gum resin is used along with other medicines as a stomachic and for the treatment of veneral diseases. The bark is boiled in large quantities to make a wash for fever, rheumatism and also taken internally for gastrointestinal troubles. The root decoction boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The root decoction with *Daniellia oliveri* is used on wounds. In Nigeria (specifically in Adamawa State), the fresh bark is eaten to cause vomiting after a few hours to relieve symptoms of giddiness and palpitations [13].

In Somalia, the resin of *B. sacra* is used for embalming the dead and to treat cough and asthma [14]. Asthma is characterized by chronic bronchial airway inflammation resulting in increased mucus production and airway hyper-responsiveness. The underlying process appears to be an out-of-balance immune activity: overactive humoral immunity and

underactive cell-mediated immunity. The essential oil of frankincense is believed to be useful to treat asthma [14].

1.4. Biological activities of *Boswellia* species

Anti-cancer

Shanhan *et al.* [15] studied incensole and furanogermacene isolated from *B. sacra* for treatment of neoplastic lesions, particularly for the treatment of resistant neoplasia and immunodyseregulatory disorders. Incensole showed anti-tumor activity in various human carcinomas and melanomas.

Anti-inflammatory activity

Duweijua *et al.* studied the aqueous extract of two *Boswellia* species namely *B. dalzielii* and *B. sacra* for the anti-inflammatory activities. The aqueous extract of the resin of *B. dalzielii* has been found to significantly inhibit both the maximal edema response and the total edema response of carrageen induced rat edema [17].

In *in vivo* experiments, oral administration of an alcohol extract of *salai guggal* was shown to strongly inhibit antibody production and cellular response to sheep red blood cells, in mice and to inhibit the infiltration of polymorphonuclear leucocytes and reduce the volume of pleural exudates in carrageenan-induced pleurisy in rats. Later *in vitro* experiments elucidated the mechanism of these anti-inflammatory effects. In these experiments, boswellic acids were found to inhibit leukotriene synthesis via 5-lipoxygenase, but did not affect the 12-lipoxygenase or cyclooxygenase activities, nor did they prevent peroxidation of arachidonic acid by iron or ascorbate. Boswellic acids

were therefore shown to be specific, non-redox inhibitors of leukotriene synthesis, either interacting directly with 5-lipoxygenase or blocking its translocation [16].

Anti-microbial

Several species of *Boswellia* have exhibited anti-microbial activity: Methanol and aqueous extracts of *B. dalziellii* stem bark were found to exhibit broad spectrum inhibiting activity against bacteria, both Gram-positive and Gram-negative, and fungi. Methanol and aqueous extracts of *B. carteri* were found to significantly inhibit (>/90% inhibition at 100 microg/ml) hepatitis C virus (HCV) protease [18].

The volatile oil of *B. sacra* exhibited a more pronounced anti-microbial activity on the following tested organisms. These include *Staphylococcus aureus, Sarcinal lutea, Mycobacterium phlei, Bacillus subtilis, Esherichia coli and Neiseria catarrhalis* [14].

1.5. Review of the chemistry of *Boswellia* species

Previous phytochemical investigations on a few of the species have shown that resins contain appreciable amounts of volatile compounds in which monoterpenes predominate. Triterpenes are found to be the major components of the non-volatile components of these resins.

Diterpenes and triterpenes from *Boswellia* species

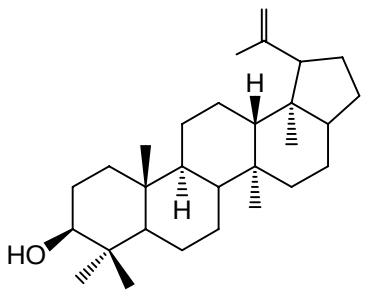
Chemical investigation by Proietti *et al*. [19] on the neutral extracts of frankincense produced by *B. frereana*, widely distributed in northern Somalia, led to the isolation of two triterpenes, lupeol (**1**) and epi-lupeol

(**2**). Later on chemical investigation by Fattoruso *et al.* [20] led to the isolation of triterpenes, 3β-acetoxy-16(S)-20(R)-dihydroxydammar-24-ene (**3**), 3β,20(S)-dihydroxydammar-24-ene (**4**), 3β-acetoxy-20(S)-dihydroxydammar-24-ene (**5**) and 20(S)-protopanxadiol (**6**).

Chemical investigation on the non-volatile neutral fractions of the resins of *B. carteri* results in the isolation and characterization of macro cyclic diterpenes such as incensole (**9**) [22], incensole oxide (**10**) and isoincensole oxide (**11**) [23].

Phytochemical investigation on the petrol extract of frankincense produced by *B. neglecta* led to the isolation of four known triterpenes, α-amyrin (**17**), canaric acid (**18**), α-amyron (**19**) and epi-α-amyrin (**20**) [24].

Pardhy and Bhattacharyya [21] isolated four pentacyclic triterpene acids from the chloroform extract of the resins of *Boswellia serrata* and characterized them as β-boswellic acid (**7**), actyl-β-boswellic acid (**8**), actyl-11-keto-β-boswellic acid (**12**), 11-keto-β-boswellic acid (**13**). In the same year Pardhy and Bhattacharyya [35] identified four tetracyclic triterpene acids from the hexane extract and characterized them as 3-ketotirucall-8, 24-dien-21-oic acid (**14**), 3-α-hydroxytirucall-8, 24-dien-21-oic acid (**15**), 3-β-hydroxytirucall-8, 24-dien-21-oic acid (16), 3-α-acetoxytirucall-8,24-dien-21-oic acid (**40**)



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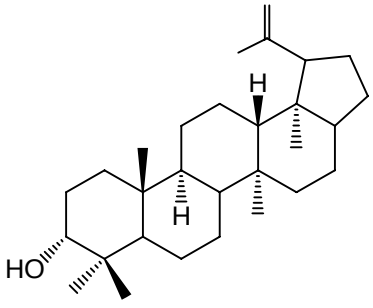

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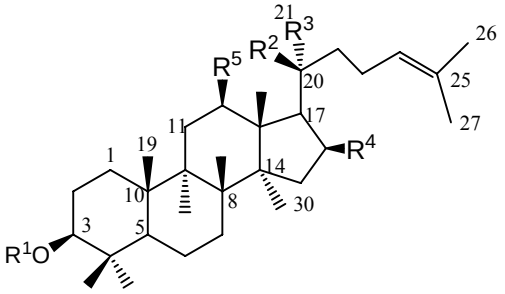
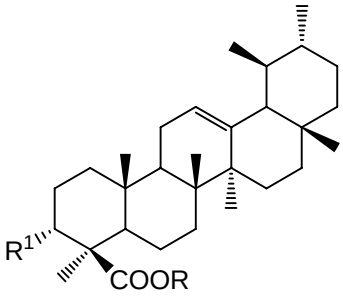


3: R^1=Ac; R^2=Me; R^3=OH; R^4=OH; R^5=H

4: R^1=H; R^2=OH; R^3=Me; R^4=H; R^5=H

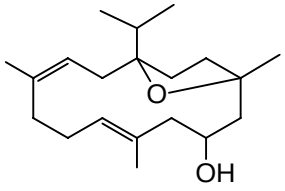
5: R^1=Ac; R^2=OH; R^3=Me; R^4=H; R^5=H

6: R^1=H; R^2=OH; R^3=Me; R^4=H; R^5=OH



7: R=H; R^1=H

8: R=H; R^1=OAc



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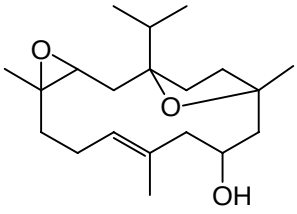

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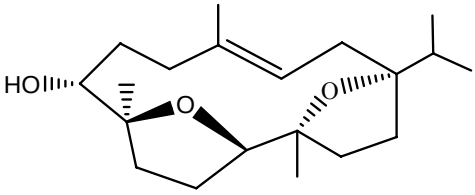

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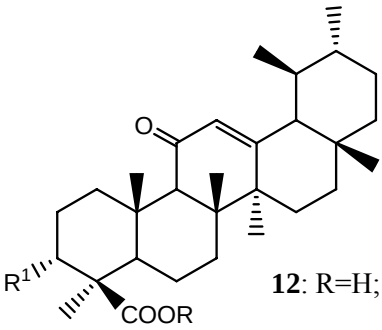
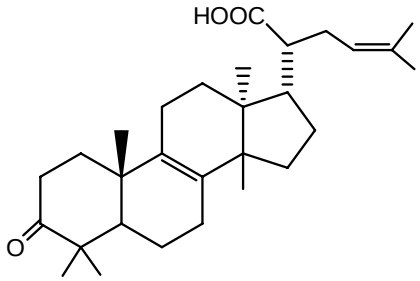


12: R=H; R^1=OAc

13: R=H; R^1=OH



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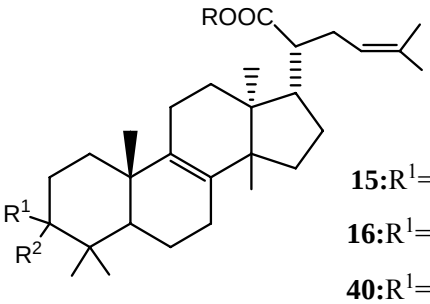
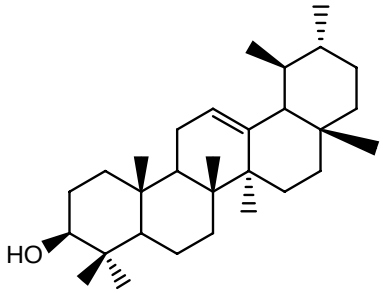


15: R^1=OH; R^2=H; R=H

16: R^1=H; R^2=OH; R=H

40: R^1=OAc; R^2=H; R=H



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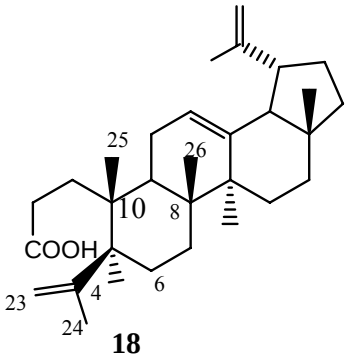

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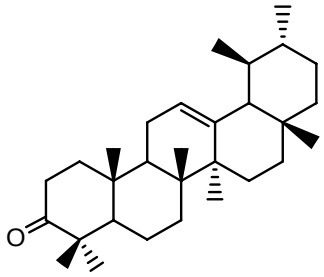

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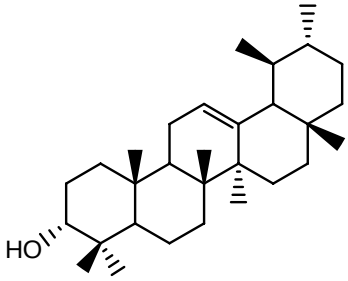

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Essential oils from *Boswellia* species

The chemical profile of the oil can be used as a chemotaxonomical marker to distinguish between the different commercial varieties of frankincense. The composition of the oil differs according to the climate, harvest conditions, and geographical source [32].

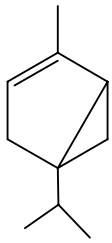
Table 1. The major components of the essential oils from different *Boswellia* species

Name of the species	Major components	Ref.
B. neglecta	α-thujene (**21**) (19.2%), α-pinene (**22**) (16.7%) and terpinen-4-ol (**26**) (12.5%)	[25]
B. rivae	limonene (**25**) (14.8%)	[25]
B. pirottae	*trans*-verbenol (**30**) (15.5%) and terpinen-4-ol (**26**) (14.6%)	[25]
*B. papyrifera***	n-octylacetate (**27**) (56 %), n-octanol (**28**) (8%) and limonene (6 %)	[26]
*B. papyrifera***	n-octylacetate (**27**) (64.6 %), incensole acetate (10.8 %) and n-octanol (**28**) (13.9%)	[9]
B. serrata	α-thujene (**21**) (61.4%), α-pinene(**22**) (7.7%) and sabinene (**29**) (5.1%)	[27]
B. serrata	α-thujene (**21**) (50%), p-cymene (**24**) (14.0%) and β-pinene (**23**) (6.2%)	[28]
*B. serrata**	α-pinene (**22**) (73.3%)	[29]
B. dalzielli	α-pinene (**22**) (45.7%), α-terpinene (11.5%), trans-sabinene hydrate (4.6%), cis-p-menth-2-en-1-ol (2.9%), α-campholenal (2.7%), caryophyllene oxide and α-phellandrene (**38**) (2.3%).	[33]
B. *frereana*	α-thujene (**21**) (10.1%), p-cymene (**24**) (4.3%) and an unknown compound (25.8%)	[30]
B. carteri	α-thujene (**21**) (19.2%), sabinene (**29**) (9.4%), limonene (**25**) (7.8%) and α-pinene (**22**) (7.2%)	[30]
B. carteri	α-pinene (**22**) (41.0%) and limonene (**25**) (12.8%)	[14]
*B. carteri***	n-octylacetate (**27**) (60.0%), n-octanol (**28**) (12.7%) and p-cymene (**25**) (8.7%).	[31]
*B. carteri***	n-octylacetate (39.3 %), n-octanol (11.9 %), α-pinene (10.9 %) and verticilla-4(20),7,11-triene (6 %)	[34]

* Shows the essential oil component of the bark, the rest are obtained from the resins

** The essential oils compositions of *B. carteri* (analyzed by Wang *et al.*, 1993 and Basar *et al.*, 2001) showed octyl acetate and octanol as the main components. However, these reported chemical composition correspond to the pattern which was observed in *B. papyrifera* with certified botanical origin (analyzed by Dekebo *et al.*, 1999 and Hamm *et al.*, 2001). Basar *et al.* isolated and identified the first time verticilla-4(20),7,11-triene in *B. carteri* keeping the above discrepancy as it is.

Recently, Botros *et al.* reported the essential oil constituents of the resin of *B. careteri.* It was found to contain monoterpenes (13.1%), sesquiterpenes (1%), and diterpenes (42.5%). The major components of the oil were duva-3,9,13-trien-1,5α-diol-1-acetate (**31**) (21.4%), octyl acetate (**27**) (13.4%), *o*-methyl anisole (**32**) (7.6%), naphthalene decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2-pentenyl) (**33**) (5.7%), thunbergol (**34**) (4.1%), phenanthrene-7-ethenyl-1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl (**35**) (4.1%), α-pinene (3.1%), sclarene (**36**) (2.9%), 9-*cis*-retinal (2.8%), octyl formate (1.4%), verticiol (**37**) (1.2%) decyl acetate (1.2%), *n*-octanol (1.1%) [32].



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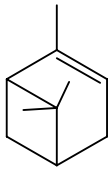

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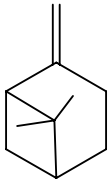

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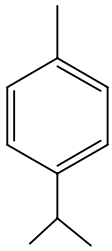

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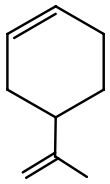

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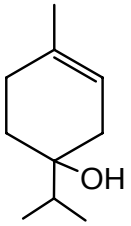

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$CH_3(CH_2)_7OAc$

27

$CH_3(CH_2)_7OH$

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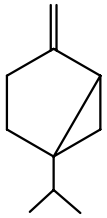

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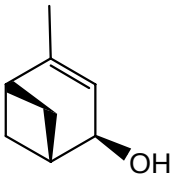

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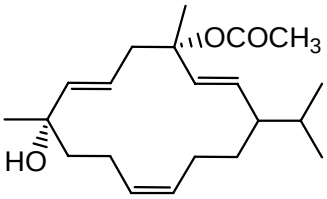

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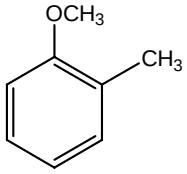

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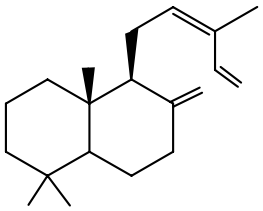

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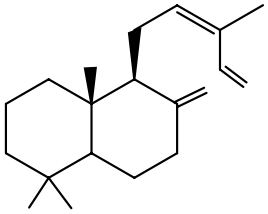

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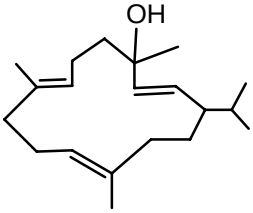

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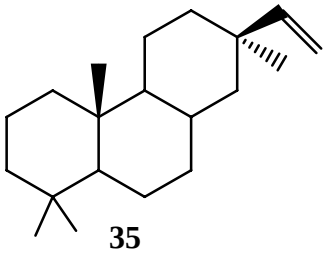

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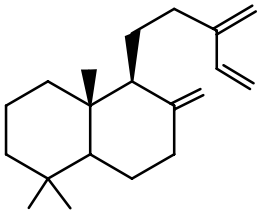

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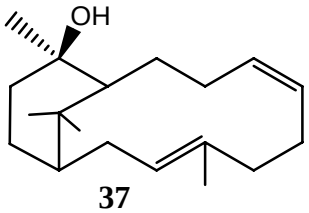

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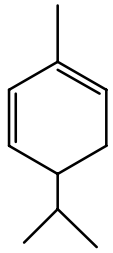

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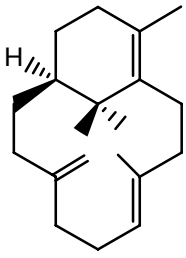

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1.6. Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products. Fortunately, despite their structural diversity, they have a simple unifying feature by which they are defined and may be easily classified. Their carbon skeleton is built up from the union of two or more of the isopentyl (isoprene) units which are usually linked in a head-to-tail manner, with more notable exceptions. Terpenes are classified by the number of carbon units they contain: hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpens (C_{15}), diterpenes (C_{20}), sesterpenes (C_{25}), triterpenes (C_{30}) and tetratrepenes (C_{40}) [37, 38].

Terpenes have a common biosynthetic origin based on the mevalonic acid derivative isopentyl pyrophosphate. This is formed from acetyl CoA via mevalonic acid [37, 38].

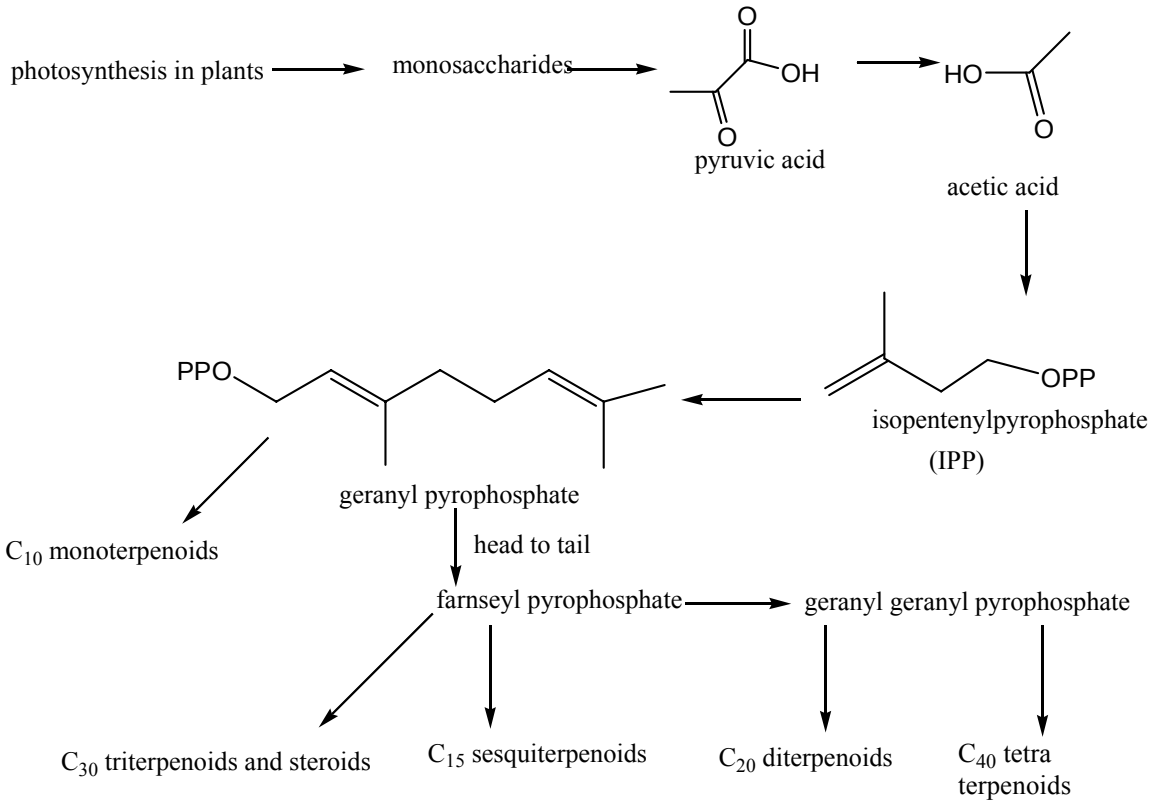
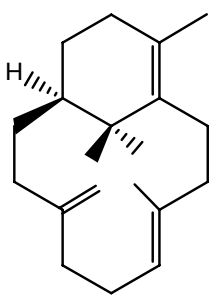


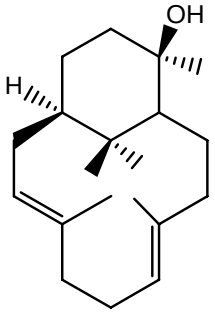
Figure 1: Biosynthesis and biogenic relation ships of terpenes

1.7. Biogenetic formation of verticillane type diterpenes

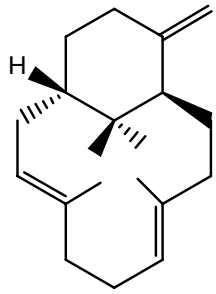
Only a few members of verticillane type diterpenes are known. The isolation of verticillol, a corresponding tertiary alcohol to verticilla-4(20),7,11-triene (**39**), was first reported from *Sciadopitys verticillata.* Moreover, a hydrocarbon, *ent*-verticillene, along with some hydroxylated derivatives was identified as a constituent of the Japanese liverwort *Jackiella javanica.*



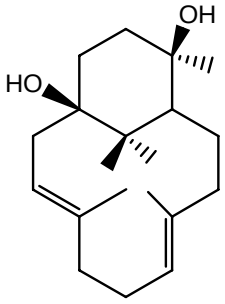
verticilla,4(20),7,11-triene



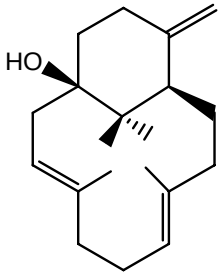
vericillol



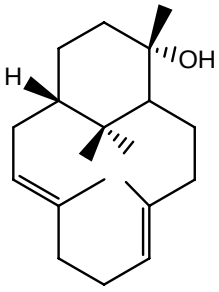
ent-verticillene



ent-13-*epi*-verticillanediol



ent-isoveticillenol



ent-verticillol

Fig. 2. Known verticillane type diterpenoids

It is assumed that verticillanes origin from geranylgeranyl pyrophosphate and are derived from cembrene derivatives by an 11,15-cyclisation (Fig. 3).

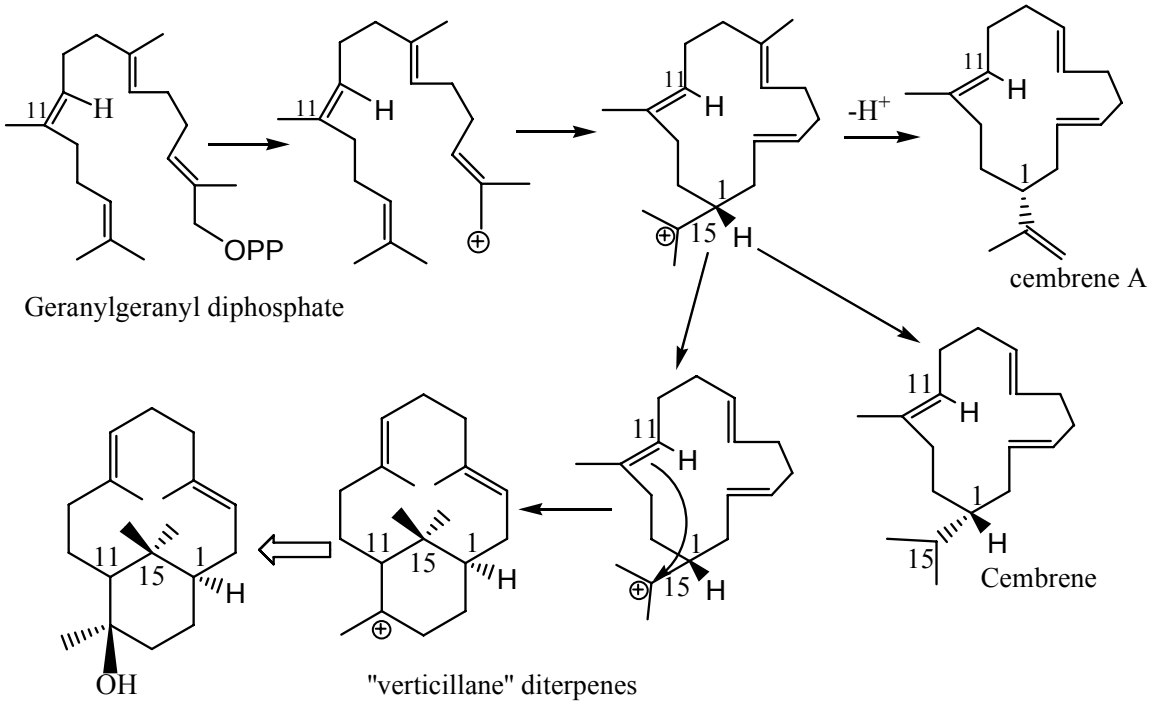


Fig. 3. The biogenetic formation of verticillane type diterpenes via the cembrene skeleten. Note that numbering of the skeletons is different in acyclic precursor and bicyclic verticillol.

1.7. Objective of the study

The main objectives of this project are:

- to check the preliminary information about the plant by collecting specimen for the botanical identification
- to undertake phytochemical study on the resins of this species.

2. RESULTS AND DISCUSSIONS

2.1. Some Information about Place of Specimen and Resins Collection (Kebtele)

Amhara Region has 10 Zones. Agew- Awi is one of the 10 Zones in the Amhara Region of Ethiopia. Agew -Awi is bordered on the south by the Oromiya Region, on the west by Benishangule Gumuz Region, on the north west by North Gondar Zone and on the north and east by West Gojjam Zone.

Figure 4: Map showing the different Regions of Ethiopia

Figure 5: Map showing the different Zones of the Amhara Region

The administrative center of Agew-Awi is Ingibara (448 km from Addis Ababa): other towns include Chagni (505 km from Addis Ababa) and Dangila (485 km from Addis Ababa). Jawe Woreda is a newly established Woreda separated from Dangila. It is 78 km west of Dangila in the partially completed road.

Kebtele is found in Jawe Woreda. It is 78 km north of Jawe. The road from Jawe to Kebtele is not properly constructed; therefore transport is possible by four-wheel drive cars only from the end of October to the beginning of June with some difficulty. Alefa Woreda in North Gondar borders Kebtele in the north. Transport from Addis Ababa to Jawe Woreda has two alternatives one is through Chagni and the other is through Dangila. After reaching Chagni one should travel through Mandura and Pawe Woredas in Metekel Zone Benishangul Gumuz Region, which are 7 km and 60 km away from Chagni respectively. The distance from Pawe Woreda to Jawe Woreda is 37 km. For the time being transport from Dangila Woreda to Jawe Woreda is impossible. This is due to two reasons:

1. The road from Dangila to Jawe is incomplete.
2. The Natural Gum Processing and Marketing Enterprise (NGPME) branch office is found in Chagni. This office gives the necessary information about the place (Kebtele) and resins collection.

In Kebtele there are some harvesting sites namely Barbas, Denbih, Kokolag, Kubaba, Zerzer, Zenzen, Mariamweha and Ayshal.

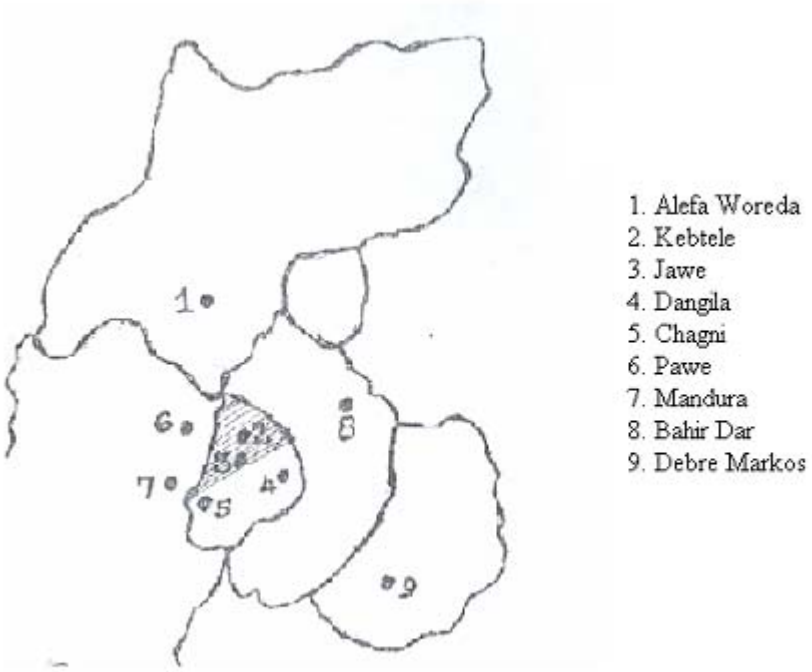


Figure 6: Map showing the different areas mentioned in the report

2.2. Plant Material

The preliminary information obtained from Natural Gum Processing and Marketing Enterprise (NGPME) indicated that the *Boswellia* species (obtained from Kebtele) was different from *B. papyrifera* (obtained from Tigray, Guba and other areas) by taking moisture content, color and taste of the resins and the color of the bark as the basic criteria for differentiating the species. The resin of *Boswellia* species (obtained from Kebtele) has high moisture content and white color and its bark is grey. The resin of *B. papyrifera* has low moisture content and light brown and its bark is pale yellow. Compared to the resin of *B. papyrifera*, the resin of the *Boswellia* species from Kebtele is very soft and best as natural chewing gum. This information makes us eager to go the area and collect specimen and resins for identification and chemical analysis respectively. We are also interested to gather ethnobotanical and other necessary informations of the plant and the place.

Fig. 7: The resin of *Boswellia* sp. (Kebtele) (Picture by A. Melese)

Based on the fieldwork that took seventeen days the following informations were obtained. This species is the predominant constituent of the vegetation in the area. Local name of the plant is called "*walya*" in Amharic. The tree is approximately 7-10 m, with outer bark white to greyish that peels into flakes and occurs on the rocky slopes between altitudes of 850 and 1100 m. When the bark is incised milky exudates flows out and solidifies within 5-6 days.

Figure 8: *Boswellia* sp. (Kebtele) tree (Picture by A. Melese).

The harvester and local people used the resin to quench thirst and the tree for fire respectively. According to the foresters working in the area, the plant has leaves from May to November, in the rest of the months the tree becomes leafless if present very small and on the branch. Flowers are available from December to February. Fruits are also available from January to April. The first and the last taping were done on December first and June first respectively.

2.3. Specimen collection and identification

The plant material used in this study was collected in April, 2007 from each of the harvesting areas in Kebtele. Plant specimen (immature leaves, barks and fruits) and resins were collected for botanical identification and chemical analysis respectively. The specimen and the resins were sent to England for identification. Based on the available specimen, the plant was identified as *B. papyrifera* by Dr. Kaj Vollesen (Kew Royal Botanical Garden, U.K.). According to the message he sent

us, he needed flowering, fruiting and mature leaves from the same population to see it again if there are any morphological differences between the well known *B. papyrifera* and ours. We call our plant material *Boswellia* sp. (Kebtele). Here Kebtele is the place of specimen and resins collection.

2.4. TLC analysis of some *Boswellia* species

The chloroform extracts of the resins of *B. papririfera, Boswellia* sp. (Kebtele), *B. carteri* and *B. frereana* were analyzed on analytical TLC. The spots are visible after spraying with vanillin in sulfuric acid and heating with hot air gun. As shown in the Fig.7 and Fig. 8 below, incensole acetate (BP4), which is the biomarker of *B. papririfera* [9, 36], is absent in any of the other three species. From the analytical TLC one can easily understand the differences among *Boswellia* sp. (Kebtele) and the other three.

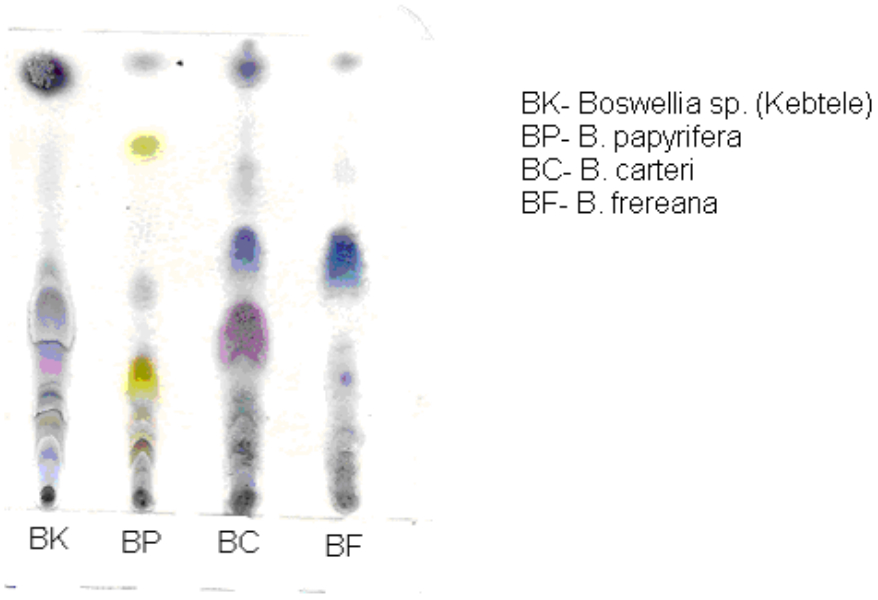


Figure 9: Analytical thin layer chromatogram for $CHCl_3$ extracts (Petrol/$CHCl_3$ 1.6:2.4)

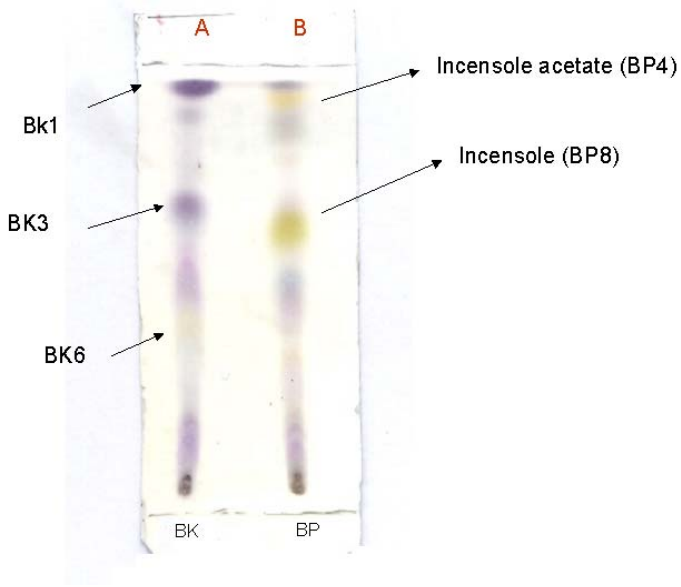


Fig. 10: Analytical thin layer chromatogram of the $CHCl_3$ extracts (Petrol/CHCl3/EtOAc 2:1.5:0.5)

TLC has been done for both the hydrocarbon fraction of the essential oils of *Boswellia* sp. (Kebtele) and its crude chloroform and petrol extracts. As mentioned in the experimental part, the petrol and chloroform extracts show the same TLC spots and the spot of the hydrocarbon fraction of the oil was also one of spots in the TLC of petrol and the chloroform extracts of the resins.

2.5. Essential oils analysis of *Boswellia* sp. (Kebtele) and *B. papyrifera*

The essential oils of the resins of *Boswellia* sp. (Kebtele) (79-111C) and *B. papyrifera* (from Markato) (86-95A) were obtained by hydrodistillation. GC analysis of the oils was undertaken and the result is presented below.

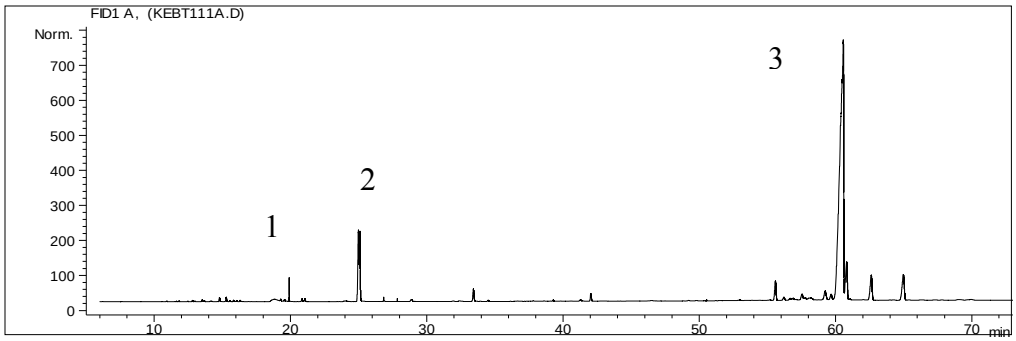


Fig.11. Typical gas chromatogram of the hydrodistillate of *Boswellia* sp. (Kebtele)
1: unknown (4.8%) 2: n-octyl acetate (4.2%) 3: **BK1** (65.1 %)

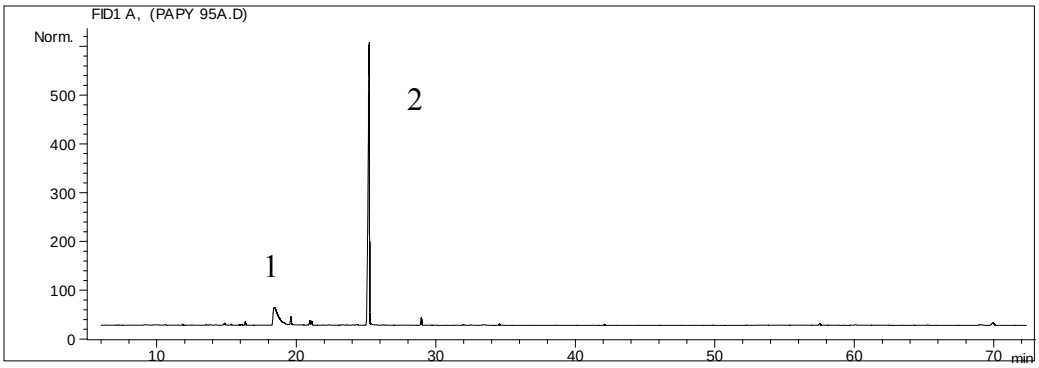


Fig.12. Typical gas chromatogram of the hydrodistillate of *B. papyrifera*
1: n-octanol (5 %) 2: n-octyl acetate (71 %)

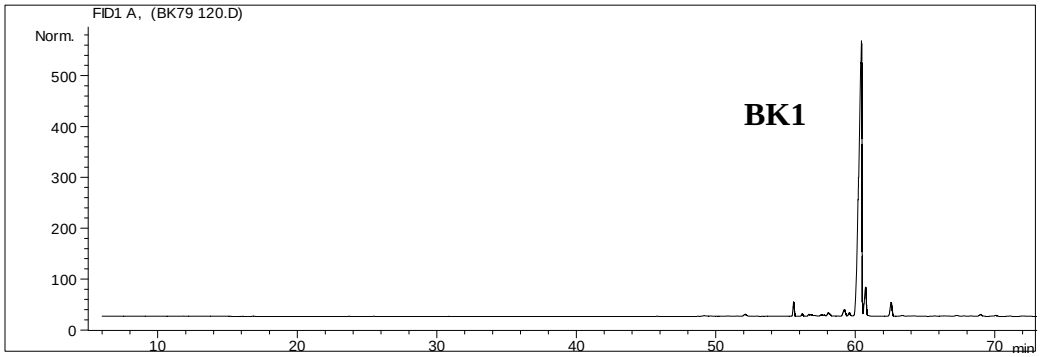


Fig.13. Typical gas chromatogram **BK1** (80%)

2.6. Isolation of compounds from the extracts of resins of *Boswellia* sp. (Kebtele)

Resins of *Boswellia* sp. (Kebtele) were extracted in petrol to give yellowish extract (75 %). The marc was extracted further with chloroform to give a yellowish extract (12.5%). Similarly, the marc of chloroform extract was extracted with EtOAc to give yellowish extract (2.5 %). Direct extraction with chloroform yielded 70 %.

The direct chloroform extract of the resins was applied to flash column chromatography (FCC) and eluted with petrol and EtOAc to give hydrocarbon and oxygenated fractions respectively. The hydrocarbon fraction was subjected to FCC and eluted with 100 % petroleum ether resulting in the isolation of one compound, coded as **BK1**. As shown in Fig.10, GC has been done for **BK1**. The result showed that **BK1** was the major component of the essential oil of the resins of *Boswellia* sp. (Kebtele).

The petrol extract of the resins of *Boswellia* sp. (Kebtele) was subjected to FCC and eluted with EtOAc in petrol with increasing polarities resulting in the isolation of three compounds. The three compounds isolated were coded as: **BK1**, **BK3** and **BK6**.

2.3.1. Characterization of BK1

This compound is colorless oil and is also found in the hydrodistillate (65%). The IR spectrum (KBr) shows a strong absorption band at 2923 cm^{-1} is due to aliphatic CH_3 and CH_2 groups. A weak absorption bands at 3029 cm^{-1} indicate the presence of olefinic CH. Absorption band at 1641.3 cm^{-1} is indicative of C=C stretching.

^{1}H-NMR spectrum (Appendix 1, Table 2) of **BK1** in $CDCl_3$ showed four singlets (integrating for three protons each) at δ 0.96, 1.0, 1.6 and 1.7 for two geminal and for two allylic methyl groups, respectively. Three olefinic protons were observed at δ 5.15, 4.70 and 4.62. Two multiplets were also detected at δ 2.46 and 2.80 each corresponding to one proton.

^{13}C- NMR (Appendix 2, Table 2) and DEPT-135 spectra (Appendix 3) of **BK1** in $CDCl_3$ indicate that it has 20 carbon atoms: four methyl groups, nine methylene groups, two methine groups, five quaternary carbons of which six resonated in the region corresponding to olefinic carbons at δ 108.2, 127.6, 129.6,133.5, 136.5 and 153.7.

BK1 is quite similar with the previously reported data of verticilla-4(20),7,11-triene[34] isolated and identified the first time in *B. carteri*. The chemical shifts of the carbon and proton atoms of **BK1** are compared with literature data of verticilla-4(20),7,11-triene in Table 2.

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **BK1** compared with lit. data for verticilla-4(20),7,11-triene by Basar *et al.* (2001).

No.	**BK1**		verticilla-4(20),7,11-triene	
	δ ^{13}C -NMR	δ ^{1}H -NMR	δ ^{13}C -NMR	δ ^{1}H -NMR
1	43.7	1.46-1.53 (m)	44.1	1.40-1.44
2	31.7	1.65-1.69, 2.0-2.1	32.0	1.68-1.73, 2.04-2.12
3	32.5	2.80(td), 2.0-2.1	33.0	2.04-2.12, 2.81
4	153.7	-	153.3	-
5	36.2	2.0-2.1, 2.24-2.30	36.6	2.04-2.12, 2.22-2.29
6	29.6	2.0-2.1, 2.0-2.15	29.9	2.04-2.12, 2.15-2.18
7	129.6	5.15(dd)	130.0	5.18
8	133.5	-	133.5	-
9	39.3	2.45(tm), 1.96-2.03	39.6	1.96-2.03, 2.45
10	25.7	1.40-1.47	26.1	1.40-1.44,2.18-2.23
11	136.5	-	136.8	-
12	127.6	-	128.0	-
13	30.4	2.04-2.12, 1.80-1.90	30.7	2.04-2.12, 1.74-1.80
14	25.4	2.12-2.16, 2.20-2.30	25.8	2.12-2.15, 2.22-2.29
15	37.3	-	37.6	-
16	33.2	1.02(s)	33.4	1.02
17	26.6	0.96(s)	26.9	0.95
18	20.9	1.7(s)	21.0	1.6
19	16.7	1.6(s)	16.8	1.5
20	108.2	4.70, 4.62	108.9	4.80, 4.81

Both the **HMBC** (Appendix 6, Table 3) and **HSQC** (Appendix 5) spectral data of **BK1** are in good agreement with the proposed structure. **H**etronuclear **S**ingle **Q**uantum **C**orrelation (**HSQC**) experiment correlates

the chemical shift of proton/s with the chemical shift of directly bonded carbon atom. The **HSQC** spectra of **BK1** (Appendix-5) showed protons at δ 4.70 and 4.62 attached to carbon at δ 108.2 (C-20) this indicates there is an exocyclic double bond in **BK1**, a proton at δ 5.15 connected to carbon at δ 129.6 (C-7). Methyl group signals at δ 0.96, 1.02, 1.60 and 1.70 directly bonded to carbons at δ 26.5(C-17), 33.2(C-16), 16.7(C-19) and 20.9(C-18), in the ^{13}C-NMR spectrum, respectively. The protons at δ 2.45 and 2.81 were found to correlate the carbons at δ 39.3 (C-9) and 32.5(C-3). Their low field was related to their proximity with the double bond system. In addition the methine carbon at δ 43.7(C-1) was correlated to the multiplet at δ 1.46-1.53.

Hetronuclear **M**ultiple **B**ond **C**orrelation (**HMBC**) experiment gives information about coupling of hydrogen and carbons that are two or three bonds away. The **HMBC** (Appendix 6) of **BK1** between protons and carbons is presented in Table 3.

Table 3: **HMBC** (^{1}H→^{13}C) NMR correlation data for **BK1** (verticilla-4(20)7,11-triene)

C. No.	δ 13C(ppm)	HMBC (^{1}H→^{13}C)
1	43.7	H ^{1}→C ^{11}, C ^{12}, C ^{14}, C ^{15}
3	32.5	H ^{3}→C ^{1}, C ^{4}, C ^{20}
7	127.6	H ^{7}→C^{5}, C^{6}
9	39.3	H ^{9}→C^{10}, C^{11}
16	33.2	H ^{16}→C ^{1}, C ^{11}, C ^{15}, C ^{17}
17	26.6	H ^{17}→C ^{1}, C ^{11}, C ^{15}, C ^{16}
18	20.9	H ^{17}→C ^{11}, C ^{12}, C ^{13}
19	16.7	H ^{19}→C ^{7}, C ^{8}, C ^{9}
20	108.2	H ^{20}→C ^{5}

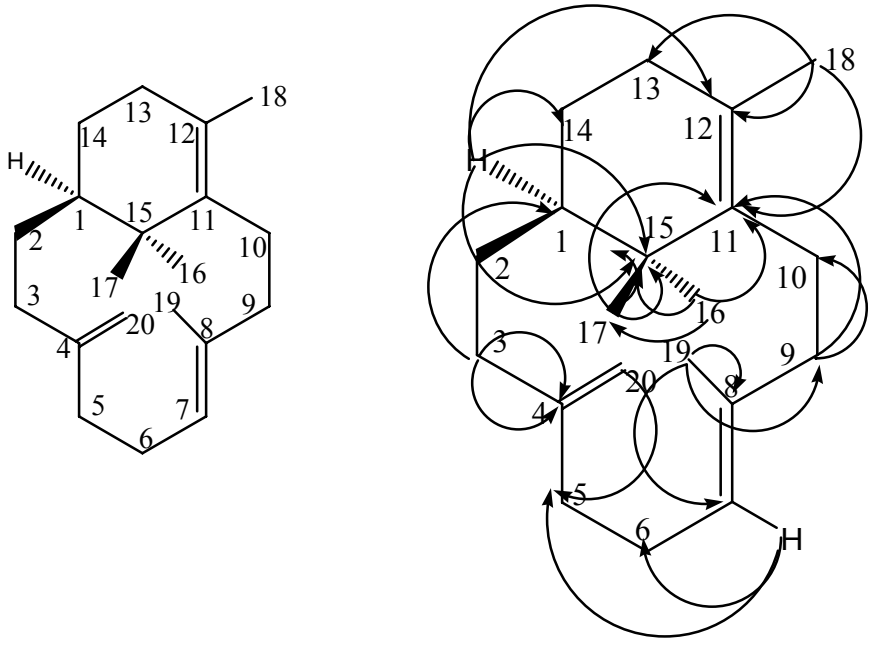


Figure 14: Verticilla-4(20),7,11-triene (**BK1**) and its selected **HMBC** ($^{1}H \rightarrow ^{13}C$) correlation

2.6.2. Partial characterization of BK3

The ^{1}H-NMR spectrum (Appendix 7) of **BK3** showed olefinic proton at δ 4.95 (1H, tm), methyl protons at δ 0.806 (3H, d), 0.858 (3H, s), 1.01 (3H, s), 1.46 (3H, s) and 1.53 (3H, s). One triplet at δ 0.00 and multiplet (ddd) at δ 0.482 were also detected, each corresponding to one proton.

The ^{13}C-NMR (Appendix 8, Table 4) and DEPT-135 spectra (Appendix 9, Table 4) confirmed the presence of 20 carbons: 5 methyl groups, 6 methylene groups, 6 methine groups and three quaternary carbons, of which one resonated in the region corresponding to oxygenated carbon at δ 74.7, two resonated in the olefinic region at δ 125.0 (CH) and δ 130.8 (C). This data revealed the presence of one trisubstituted double bond.

Table 4: ^{1}H NMR, ^{13}C NMR, DEPT-135 and $^{1}H \leftrightarrow {}^{1}H$ **COSY** data of **BK3** in $CDCl_3$.

Carbon number	^{1}H NMR (δ in ppm)	^{13}C NMR (δ in ppm)	DEPT-135 (δ in ppm)	$^{1}H \leftrightarrow {}^{1}H$ COSY
1	1.30-1.40,1.64-1.71	25.8	25.8	$H^{1} \leftrightarrow H^{11}$, $H^{1} \leftrightarrow H^{2}$
2	1.08-1.15, 1.50-1.60	29.2	29.2	$H^{2} \leftrightarrow H^{1}$, $H^{2} \leftrightarrow H^{3}$
3	1.78-1.91 (m)	38.8	38.8	$H^{3} \leftrightarrow H^{2}$, $H^{3} \leftrightarrow H^{4}$, $H^{3} \leftrightarrow H^{20}$
4	1.68-1.76 (ddd)	39.2	39.2	$H^{4} \leftrightarrow H^{3}$, $H^{4} \leftrightarrow H^{5}$, $H^{4} \leftrightarrow H^{11}$
5	0.00 (t)	22.2	22.2	$H^{4} \leftrightarrow H^{7}$, $H^{4} \leftrightarrow H^{3}$
6	-	22.4	-	-
7	0.458 (ddd)	27.3	27.3	$H^{7} \leftrightarrow H^{5}$, $H^{7} \leftrightarrow H^{8}$
8	1.20-1.30, 1.45-1.50	18.8	18.8	$H^{8} \leftrightarrow H^{7}$, $H^{8} \leftrightarrow H^{9}$
9	1.38-1.42, 1.52-1.60	37.6	37.6	$H^{9} \leftrightarrow H^{8}$
10	-	74.7	74.7	-
11	1.60-1.70	58.3	58.3	$H^{11} \leftrightarrow H^{1}$, $H^{11} \leftrightarrow H^{3}$
12	0.71-0.83, 1.30-1.40	43.4	43.4	$H^{12} \leftrightarrow H^{13}$
13	1.95-2.00	25.3	25.3	$H^{13} \leftrightarrow H^{12}$, $H^{13} \leftrightarrow H^{14}$
14	4.95 (tm)	125.0	125.0	$H^{14} \leftrightarrow H^{13}$, $H^{14} \leftrightarrow H^{16}$, $H^{14} \leftrightarrow H^{17}$
15	-	130.8	130.8	-
16	1.46 (s)	17.6	17.6	-
17	1.53 (s)	25.7	27.7	-
18	0.858 (s)	13.4	13.4	-
19	1.01 (s)	32.2	32.2	-
20	0.806 (d)	16.6	16.6	$H^{20} \leftrightarrow H^{3}$

The position of hydrogen on carbon was deduced from the **HSQC** correlation spectrum (Appendix 12). Methyl group signals at δ 0.806 (3H, d), 0.858 (3H, s), 1.01 (3H, s), 1.46 (3H, s), 1.53 (3H, s) were found to correlate to carbons at δ 16.6, 13.4, 32.2, 17.6, 25.7, in the ^{13}C-NMR spectrum, respectively. The protons at δ 0.00 (t) and 0.482 (ddd) were found to correlate to the carbons at δ 22.2 and 27.3. Their high field shift was related to their attachment in cyclo propane system. The olefinic proton δ 4.95 (tm) was found to be connected to the carbon at δ 125.0.

Connectivity of the carbon atoms was deduced from the ^{1}H↔^{1}H **COSY** (Appendix 11, Table 4) and **HMBC** spectrum (Appendix 13, Table 5). In the **HMBC** spectrum pertinent correlation were observed between the olefinic quaternary carbon at δ 130.8 (C 15) with the methyl protons H-17 and H-16 and vinylic proton H-14, the vinylic carbon at δ 125.0 (C-14) with H-12, H-13, H-16 and H-17, the methyl carbon at δ17.6 (C-16) with H-14 and H-17, methyl carbon at δ 25.7(C-17) with H-14 and H-16. The above correlations led to partial skeleton **I** Fig. 15.

Partial skeleton **II** (Fig.15) is based on the methyl protons of H-18 and the methine protons at H-5 and H-7. From the HMBC H-18 showed a strong correlation with the two methine carbons at δ 22.2 (C-5) and δ 27.3 (C-7), the methylene carbon at δ 43.4 (C-12), the aliphatic quaternary carbon at δ 22.4 (C-6). The methine proton H-7 showed a correlation with the methyl carbon at δ 13.4 (C-18), the aliphatic quaternary carbon at δ 22.4 (C-6), the methine carbons at δ 39.2 (C-4) and δ 22.2 (C-5) and a methylene carbon at δ 18.2 (C-8). The methine proton H-6 showed a correlation with the methyl carbon at δ 13.4 (C-18), the aliphatic quaternary carbon at δ 22.4 (C-6), the methine carbons at δ 39.2 (C-4) and δ 22.2 (C-7). The two partial skeletons, **I** and **II,** has a common carbon (C-12).

The partial skeleton **III** (Fig. 15) is based on the methyl proton H-20 and methine protons H-3 and H-4. From the **HMBC** H-20 showed strong correlation with methine carbons at δ 38.8 (C-3) and δ 39.2 (C-4) and a methylene carbon at δ 29.2 (C-2). This partial skeleton has a common carbon (C-4) with partial skeleton **II**.

The partial skeleton **IV** (Fig.15) is based on methyl protons H-19, methine proton H-11 and methylene proton H-9. Methyl protons H-19 showed correlation with the oxygenated quaternary carbon at δ 74.7 (C-10), the methine carbon at δ 58.3 (C-11) and methylene carbon at δ 37.6 (C-9). The methylene proton H-9 showed correlation with the quaternary oxygenated carbon at δ 74.7 (C-10) and methylene carbon at δ 18.2 (C-8). This partial skeleton has a common carbon C-8 with the partial skeleton **II.** The ^{1}H-^{1}H **COSY** also confirmed the protons of C-8 coupled with the protons of C-7 and C-9. Hence the two fragments can be connected.

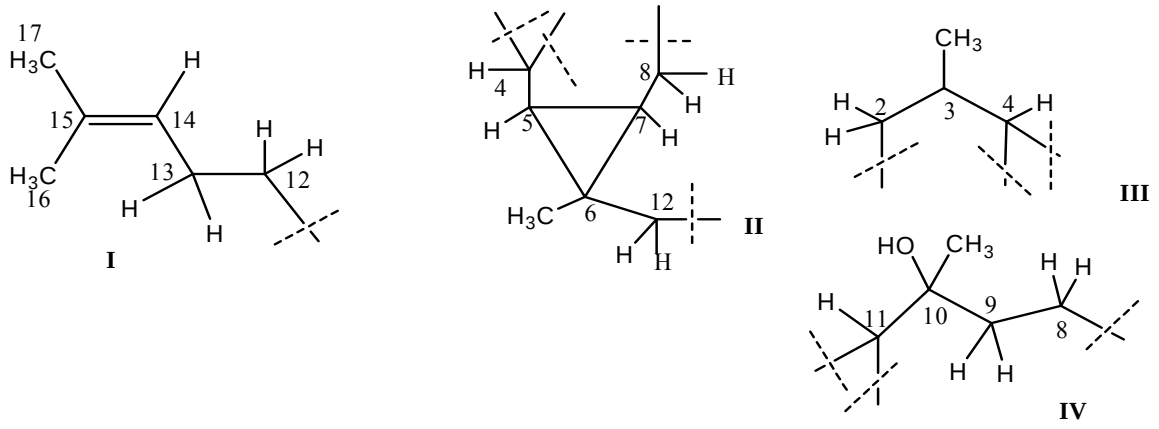


Figure 15: Suggested partial skeletons of BK3

The proximity of C-1, C-2 and C-11 (δ 25.7, 29.2 and 58.3) was deduced from the correlations of in the ^{1}H-^{1}H **COSY** (**Co**rrelation **Sp**ectroscopy) spectrum (Appendix 11). The above findings and the application of the isoprene rule lead to the proposed structure of **BK3** as shown in the Fig. 16.

Table 5: **HMBC** ($^{1}H \rightarrow ^{13}C$) NMR correlation data for **BK3**

C. No.	δ ^{13}C(ppm)	**HMBC** ($^{1}H \rightarrow ^{13}C$)
3	38.8	$H^{3} \rightarrow C^{2}, C^{20}, C^{4}, C^{5}$
4	39.2	$H^{4} \rightarrow C^{11}, C^{5}, C^{7}, C^{3}, C^{20}$
5	22.2	$H^{5} \rightarrow C^{3}, C^{4}, C^{6}, C^{7}, C^{8}, C^{12}, C^{18}$
7	27.3	$H^{7} \rightarrow C^{3}, C^{4}, C^{6}, C^{7}, C^{12}, C^{18}$
9	37.6	$H^{9} \rightarrow C^{8}, C^{10}$
16	17.6	$H^{16} \rightarrow C^{13}, C^{14}, C^{15}$
17	25.7	$H^{17} \rightarrow C^{13}, C^{14}, C^{15}$
18	13.4	$H^{18} \rightarrow C^{5}, C^{6}, C^{7}, C^{12}$
19	32.2	$H^{19} \rightarrow C^{9}, C^{10}, C^{11}$
20	16.6	$H^{20} \rightarrow C^{2}, C^{3}, C^{4}$

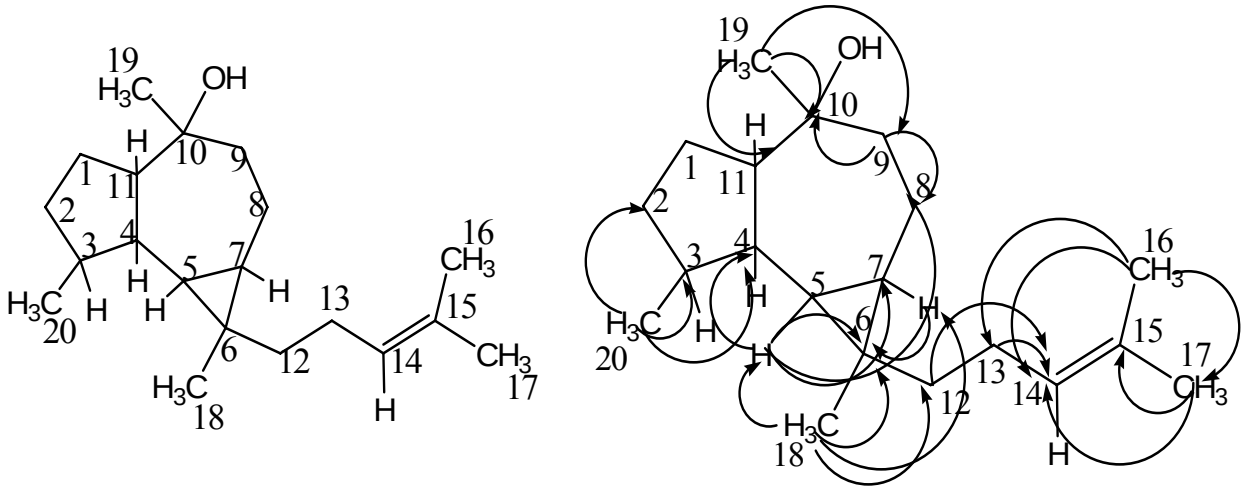


Figure 16: Proposed structure for **BK3** with its selected **HMBC** ($^{1}H \rightarrow ^{13}C$) correlations.

2. EXPERIMENTAL

3.1. General

The essential oils from the plant materials were obtained by hydrodistillation at atmospheric pressure using round bottom flask fitted with a Clevenger type apparatus and a glass condenser. The oils were separated from the distillate, washed with diethyl ether and dried over anhydrous sodium sulphate.

GC was run using Hewlett-Packard HP 6890 GC series equipped with FID and HP-5 capillary column (cross linked 5 % diph, 95 % dimethyl polysiloxan, 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 using N_2 as carrier gas. The injector and detector temperature were 220 ^{0}C and 270 ^{0}C, respectively.

Melting point was determined in capillary tube with a digital electro thermal melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF_{254} (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) and spraying with vanillin in H_2SO_4 and by heating with hot air gun. Flash column chromatography was performed using silica gel 60 (230-400 mesh) Merck. Samples were applied on the top of the column by adsorbing on silica gel. The speed of the mobile phase was increased by applying pressure from the top.

^{1}H and ^{13}C NMR spectra in $CDCl_3$ were recorded using the solvent peak as reference (chloroform: δ H 7.26 and δ C 77.10). ^{1}H, ^{13}C NMR and 2D NMR were obtained on Brucker Advance instrument at 400 MZ and 100

MZ, with TMS and solvent as internal standards and δ values are given in ppm relative to TMS internal standard. IR spectra were recorded with a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr.

3.2. Plant Material

The plant material used in this study was collected in April, 2007 from Kebtele area 78 km N of Jawe, a newly established Woreda 78 km west of Dangila (485 km from Addis Ababa). Local name of the plant is called "*walya*" in Amharic. The tree is approximately 7-10 m, with outer bark white to grayish that peel into flakes. When the bark is incised milky exudates flows out and solidifies within 5-6 days. Leaves and bark collected to aid the botanical identification of the species. The species was identified by Dr. Kaj Vollesen (Kew Royal Botanical Garden, U.K.)

3.3. Hydodisillation and isolation of the oil

The dry resin *Boswellia* sp. (Kebtele) (100 g) was ground and placed in a round bottom flask fitted with Clevenger type apparatus and hydrodistilled for 2 h at atmospheric pressure to yield 300 mg of oil. The strongly aromatic oil was separated from the water layer by adding diethyl ether and dried by anhydrous Na_2SO_4.

Similarly the resin of *B. papyrifer*a (100 g) was ground and hydrodistilled for 2 h to yield the essential oil (500 mg, 0.5 %)

The oil of *Boswellia* sp. (Kebtele) (170 mg) was then applied to CC over flash silica gel (10 g) and eluted with petroleum ether (150 ml) and EtOAc (150 ml) to yield hydrocarbon (100 mg, 60 %) and oxygenated (5o mg, 30 %).

3.4. Coding system

B stands for the genus name *Boswellia*, **K** stands for the place of specimen and resin collection and the numbers behind **P** and **K** indicate the location of the compounds starting from the highest R_f value to the lowest. TLC examination of the crude extracts revealed the presence of at least 9 spots when sprayed with vanillin in sulfuric acid. Thus, **BK1**, **BK3** and **BK6** indicate the first, third and sixth compounds respectively.

3.5. Extraction and compound isolation from the resins *Boswellia* sp. (Kebtele)

The pulverized resin of *B. kebtele* (20 g) was soaked in petrol for one day using shaker at room temperature and concentrated to give a yellowish extract (15 g, 75 %). The marc was extracted further with chloroform three times to give a yellowish extract (2.5 g, 12.5 %). Similarly, the marc after chloroform was extracted with EtOAc to give 0.5 g (2.5 %). This shows a total of 90 % organic solvent soluble constituents are present in the resin. Direct extraction with chloroform yielded 70 %. TLC has been done to both the hydrocarbon fraction of the oil, the petrol extract and the direct chloroform extract of the resin; the result shows the spots in the hydrocarbon fraction of the oil are also present in the chloroform and petrol extracts.

Isolation: 5.8 g of the direct chloroform extract of the resin of *Boswellia* sp. (Kebtele) was applied to CC over flash silica gel (60 g) and eluted with petroleum ether (300 ml) and EtOAc (300 ml) to yield hydrocarbon (1 g, 17.2 %) and oxygenated (4.5 g, 77.6 %). The hydrocarbon fraction (200 mg) was applied to CC over flash silica gel (15 g) and eluted with 100 % petroleum ether. Out of the seven fractions collected, fraction one (90

mg, 45%) gave nearly pure verticilla-4(20),7,11-triene which was coded as **BK1**.

The petrol extract (7.5 g) of the resin of *Boswellia* sp. (Kebtele) was applied on CC over flash silica gel (70 g) and eluted with petrol/ EtOAc with increasing polarities. Out of the 23 fractions (25 ml each) collected, fraction number 1 (**BK1**), 8 (**BK3**) and 22 (**BK6**) gave pure spots.

Verticilla-4(20),7,11-triene(**BK1**): Colorless oily liquid ; The IR v_{max}(KBr) cm^{-1} 2923, 1444.8 cm^{-1}, 1382.8 cm^{-1}, 1641.3; ^{1}H NMR (400 MHz, $CDCl_3$): υ 0.95 (3H, s, H-16/17), 1.01 (3H, s, H-16/17), 1.60 (3H, s, H-19), 1.70 (3H, s, H-18), 5.18 (1H, dd, H-7), 4.83 (1H, s, H-20), 4.80 (1H, s, H-20), 2.81 (1H, ddd, H-3), 2.45 (1H, dt, H-9); ^{13}C NMR and spectrum are shown in Table 2 and Appendix 2 respectively.

BK3: colorless resin; ^{1}H NMR (400 MHz, $CDCl_3$): υ 1.46 (3H, s, H-16/17), 1.53 (3H, s, H-16/17), 1.01 (3H, s, H-20), 0.805 (3H, d, H-18), 0.856(3H, s, H-18), 4.95 (1H, tq, H-14), 0.482 (1H, ddd, H-7), 0.00 (1H, t, H-9); ^{13}C-NMR and spectrum are shown in Table 4 and Appendix 9 respectively.

BK6: A white crystalline (mp 217-220 ^{o}C)

4. CONCLUSIONS AND RECOMMENDATIONS

In this work the major component of the essential oil *Boswellia* sp. (Kebtele) and the solvent extract of the resin has been analyzed. The major component of the essential oil of *Boswellia* sp. (Kebtele), verticilla-4(20),7,11-triene, was reported only in *B. carteri* (6 % of the oil) by Basar

et al.. The petrol extract of the resins gave three compounds, **BK1**, **BK3** and **BK6**.

In our analysis of the essential oil *Boswellia* sp. (Kebtele) and *B. papyrifera* (from Markato), verticilla-4(20),7,11-triene (65 %) and octyl acetate (71 %) are the predominant major compounds respectively. Hence further work is recommended to

- Identify the cause/s for the remarkable variation in the composition of the oils of the same species.
- Collect specimen of the species, which contain matured leaves, barks, flowers and fruits for further identification of the species.
- Isolate additional compounds from the petrol extract of the resins which were left because of time constraint.

5. REFERENCES

[1] Vollesen, K. (1989). Burseraceae. In: Hedberg, I. and Edwards, S. (Eds.), Flora of Ethiopia. National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia, **3**: pp. 442-447.

[2] Dekebo, A. (2001). Phytochemical Studies on the Resin of some *Commiphora* and *Boswellia* Spp., PhD Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

[3] Kindeya, G.H. (2003). Ecology and Management of *Boswellia Papyrifera* (Del.) Hochst. Dry Forest in Tigray, Northern Ethiopia. PhD Thesis, Culvillier Verlag, Gottingen, Germany.

[4] Tucker, A.O. (1986). Frankincense and Myrrh, *Economic Botany*, **40**: 425-433.

[5] Provan, G.J., Alexander, I. and Waterman, P.G. (1987). Monoterpene-rich resins from some Kenyan Burseraceae. *Flavour Frag. J.*, **2**:115-119.

[6] Ammon, H.P., Mack, J., Sinngh, G.B. and Safayhi, H. (1991). Inhibition of Leukotriene B4 formation in rat peritoneal neutrophils by an ethanolic extracts of the gum resin exudates of *B. serrata. Planta Med.*, **57**: 203-207.

[7] Svoboda, K.P., Hampson, J.B. and Hall, L *Boswellia* from Somalia, a source of high quality frankincense. Medicinal Plant Consevation, Vol. **7**, IUCB, Bann, pp. 16-19.

[8] Thulin, M. and Warfa, A.M. (1987). The Frankincense Trees (*Boswellia* Spp., Burseraceae) of Northern Somalia and Southern Arabia. *Kew Bulletin*, **42**: 487-500.

[9] Hamm, A., Bleton, J., Connan, J. and Tchapla, A. (2005). A Chemical investigation by headspace SPME and GC-MS of volatile and semi-volatile terpenes in various olibanum samples. *Phytochemistry*, **66**: 1499-1514.

[10] Mathe, C., Culiolic, G., Archier, P. and Viellcazes, C. (2004). High performance liquid chromatographic analysis of triterpenoids in commercial frankincense. *Chromatographia,* **60**: 493-499.

[11] Martin, J.G. (1995). Ethnobotany. A "People and Plants" Conservation Manual (1st ed.), Great Britain at the University press, Cambridge. pp. XX-XXI.

[12] Singh, G.B. and Atal, C.K. (1998). Pharmacology of Extract of Sallia Guggal ex-*Boswellia serrata*, a Non-Steroidal Anti-Inflammatory Agent. Agents and Actions, **18**: 407-412.

[13] Nwinyi, F.C., Binda, L., Ajoku, G.A., Aniagu, S.O., Enworom, N.M., Orisandipe, A., Kubmaraw D. and Gamandie, K.S. (2004). Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for the anti-microbial activities and gastrointestinal effects. *African Journal of Biotechnology*, **3**: 284-288.

[14] Wahab, A.S.M., Abuutab, E.A., El-Zulham, S.M. and El-Fallah, N. (1987). The essential oils of olibanum. *Planta Med.*, 382-384.

[15] Shanhan, p. and Elisabeta, T. (2002). Incesole and Furanogermacrene Compounds in The Treatment of Neoplastic lesion and Microorganisms, pct.Int. PPI. W00253, **138**: 1155-1161. (Chem. Abst. 137 (2002)8844b).

[16] Ammon, H.P., Safayhi, H., Sabieraj, J.. (1993). Mechanism of anti-inflammatory actions of curcumine and boswellic acids. J. Ethnopharmacology, **38**: 113-119.

[17] Duweiejula, M., Zoitlin, I.J., Waterman,P.G., Chapman, J., Mhango, G.J. and Provan, G.J. (1993). Anti-Inflammatory Activities of Resins from Some Species of the Plant Family Burseraceae. *Planta Med.*, **59**: 12-16.

[18] Adelkun, E.A., Finbar, E.A., Agha, S.E. and Makinda, A.O. (2002). Anti- microbial Activity of *Boswellia dalzielii* Stem Bark. *Filotherapia*, **72**: 822-824.

[19] Proietti, G., Strappaghetti, G. and Corsano, S. (1981). Triterpenes of *Boswellia frereana. Planta med.*, **41**: 417-418.

[20] Fattorusso, E., Santacroce, C. and Xaason, F. (1985). Dammarane triterpenes from the resin of *Boswellia frereana. Phytochemistry*, **24**: 1035-1036.

[21] Pardhy, R.S. and Bhattacharyya, S.C. (1978). Tetracyclic Triterpene Acids from the Resin of *B. serrata* Roxb. *Indian J. Chem.*, **16B**: 176-178.

[22] Corsano, S. and Nicoletti, R. (1967). The Structure of Incensole. *Tetrahedron*, **23**: 197-1984.

[23] Forcellese, M.L., Nicoletti, R. and Petrossi, U. (1972). The structure of isoincensole oxide. *Tetrahedron*, **28**: 325-331.

[24] Dekebo, A., Dagne, E., Odd, R.G. and Aren, J.A. (2002). Triterpenes from the Resin of *Boswellia neglecta. Bull. Chem. Soc. Ethiop.*, **16**: 87-90.

[25] Dekebo, A., Baser, K.H.C., Demirici, B. and Dagne, E. (2003). Essential Oils of some *Boswelia* Spp., Myrrh and Opapanax. *Flavour Frag. J.*, **18**: 153-158.

[26] Dekebo, A., Zewedu, M. and Dagne, E. (1999). Volatile Oils of Frankincese from the *Boswelia papyrifera. Bull. Chem. Soc. Ethiop.*, **13**: 93-96.

[27] Verghese, O., Joy, M.T., Retamare, J.A., Malinskas, G.G., Catalon, C. and Gros, E.G. (1987). A fresh look at the constituents of Indian olbanum oil. *Flav. Fragr. J.*, **2**: 99-102.

[28] Maiupetit, P., (1985). New constituents in olibanum resinoid and essential oil. *Perfum. Flavor.*, **9**: 19-37.

[29] Kasali, A.K., Adio, A.M., Oyedesi, A.O., Ishilakun, A.O. and ` Adefenwa, M. (2002). Volatile constituents of *Boswellia Serrata* Roxb. (Burseraceae) bark. *Flav. Fragr. J.*, **17**; 462-464.

[30] Chivari, G., Galletti, G.C., Piccaglia, R. and Mohamud, M.A. (1991). Differenial between resins of *B. carteri* and *B. frereana* (Frankincense) of Somalia origin. *J. Essent. Oil Res.*, **3**: 185-186.

[31] Wang, W., Zhu, Y.X., Liu, L.Z., Link, D.K., Qin, X.L. and Tian, J.G. (1993). Analysis of the chemical constituents of essential oil of *Boswellia carteri* birdwood from Somalia. *Yaowu Fendi Zazhi*, **13**: 170-173.

[32] Botros, R.M., Galali, T.M., Farid, A.B., and Mohamed, M.A. (2003). Chemistry and Immunomodulatory Activity of Frankincese Oil. *Z. Natutforsh,* **58**: 230-238.

[33] http://findarticles.com/p/articles/mi-qa 4091/is-200603/ai-n17176762/pg 1-2.

[34] Basar, S., Koch, A. and Konig, W.A. (2001). A Verticillane-type diterpenes from *Boswellia carteri* essential oil. *Flavour Fragr. J.*, **16**: 315-318.

[35] Pardhy, R.S. and Bhattacharyya, S.C. (1978). Tetracyclic Triterpene Acids from the Resin of *B. serrata* Roxb. *Indian J. Chem.*, **16B**: 171-173.

[36] Endale, M. (2004). Phytochemical Investigation on the Resin of *B. papyrifera* (Del.) Hochest. MSc Thesis. Addis Ababa, Ethiopia.

[37] Kaufman, P.B., Cseke, L.J., Duke, W.S. and James, A. (1999). Natural product chemistry from plants, CRC press LLC; USA.

[38] Kurt, B., Torssell, G. (1997). Natural product chemistry; a mechanistic, biosynthetic and ecological approach, 2nd ed., Swedish pharmacological press: Stockholm, Sweden.