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Original Research Article

Identification and characterization of diterpenes from ethyl acetate fraction of stem barks of *Boswellia papyrifera* (del) hochst, sudanese medicinal plant

Ibrahim Abdurrahman[a,b]*, Hu Yu-Lai[b], Yang Cai- Xia[b], Tuhami E. Hagr[c]

[a] *Department of Basic Science, University of Zalingei, Zalingei, Sudan*

[b] *College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou, P.R. China*

[c] *College of Applied and Industrial Sciences, University of Bahri, Khartoum, Sudan*

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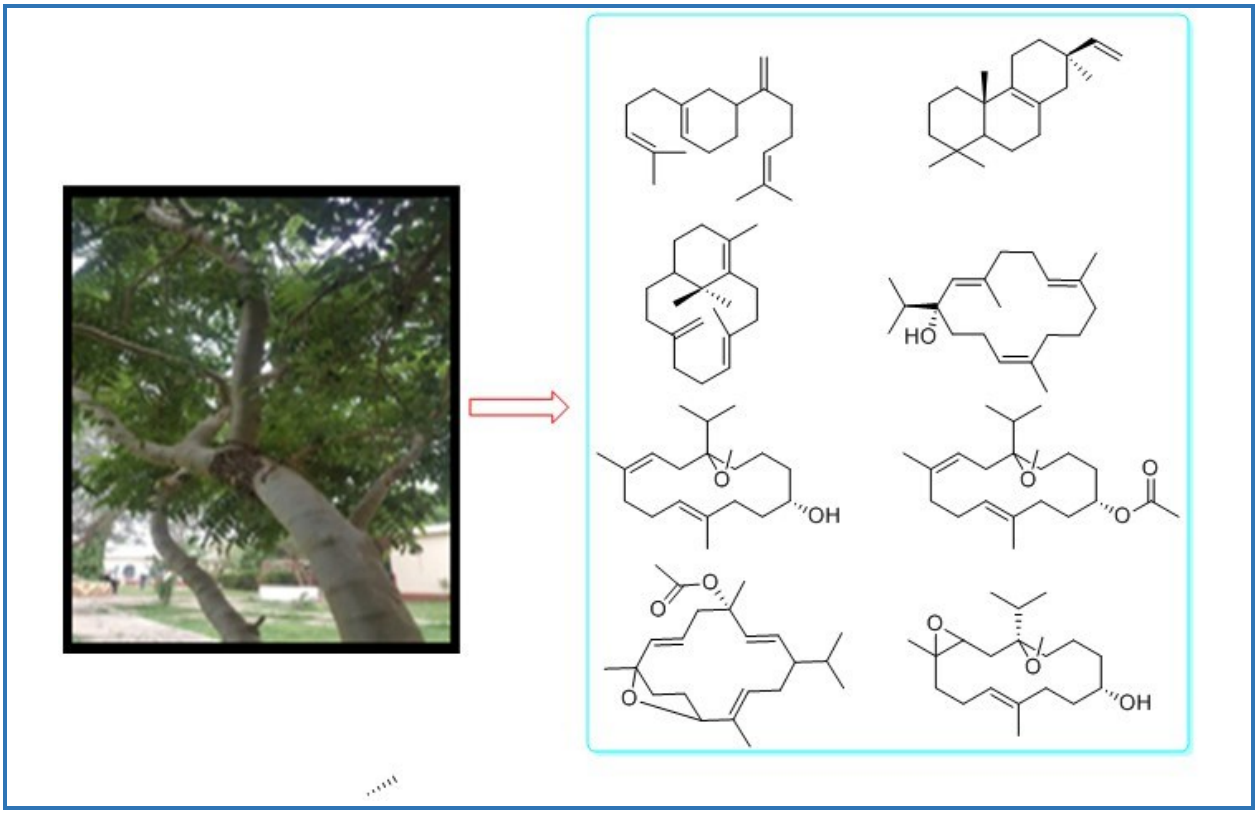
ABSTRACT

In this study, the diterpen compounds present in the ethyl acetate fraction of stem bark of *Boswellia papyrifera* and grown in Sudan were investigated using gas chromatography-mass spectrometry (GC-MS), based on the interpretation of the mass spectra fragmentation data, matching their mass spectra with NIST databases, and by the comparison of the mass spectra with those of the reference compounds published in literature. A total of nine diterpenes were identified as m-camphorene **1**, pimaradiene **2,** verticilla-4(20),7,11-triene **3,** serratol **4**, incensole **5,** incensyl acetate **6,** duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate **7,** incensole oxide **8**, incensole oxide acetate **9**, respectively. Among them compound **1**, **2**, **4** and **7** were reported here for the first time from this plant.

Corresponding author, email: Moniter123@outlook.com (I. Abdurrahman).
Fax: +024 996 858 8527.

Graphical Abstract



Introduction

The genus *Boswellia* belongs to the family [1] as represented by 17 genera and 500-600 species, widespread in tropical and subtropical regions. It grows in dry land region from Northeastern Africa (Somalia, Sudan, Ethiopia, and Eritrea) to South Asia (Oman and Yemen and India) [2–5]. *Boswellia papyrifera* in Sudan is a common savanna tree found in rocks or hill slopes, occurring in Bahr El-ghazal, Blue Nile, Nuba mountains and in Darfur, *Boswellia papyrifera* occurs around Zalingei, Rodom and Jabel Marra [6]. Properties of the *Boswellia papyrifera* have been exploited for years in traditional Sudanese medicine to alleviate pain and inflammation. It is used in medicinal preparations for the treatment of amenorrhoea, diarrhea, cough, asthma, and bronchitis as an ingredient of embalming fluid, a diuretic stimulant and an emmenagogue. But, its essential oil and absolute oil are used as fixatives in perfumes, soaps, creams, lotions and detergents [7, 8]. The *Boswellia papyrifera* is known to contain several acidic triterpenes, some of them showed anti-inflammatory, analgesic, antileukemic, immunosuppressant, and hepatoprotective activities. In recent years the interest in preparations, based on *boswellia* resins as a treatment for several diseases, ascended again. Previous studies of *Boswellia* species chemistry was found to be a

complex mixture composed of about 65-85% alcohol-soluble resins (Triterpenes, diterpenes), 5-9% highly aromatic essential oil (Mono and sesquiterpenes) and the remaining water-soluble gums (Polysaccharides).

Sesquiterpenes and monoterpene are highly volatile compounds; polysaccharides which are not volatile diterpenes exhibit low volatility, and, also, triterpenes exhibit very low volatility [9–12]. The major constituents isolated from the non-volatile fractions of bark and resin of *Boswellia paparifera* were incensole, incensyl acetate, verticilla-4(20),7,11-triene, incenosole oxide, and incenosole oxide acetate and more especially penta or tetracyclic triterpenoids belonging to oleanane, ursane, lupane, dammarane and tirucallane groups [13–15] however, diterpenes like incensole and incensole acetate are considered as specific biomarkers for this plant. In the present work, we identified and confirmed the structures of the different diterpenes by gas chromatography-mass spectrometry (GC-MS) from the ethyl acetate fraction of stem bark of *Boswellia papyrifera* for the first time.

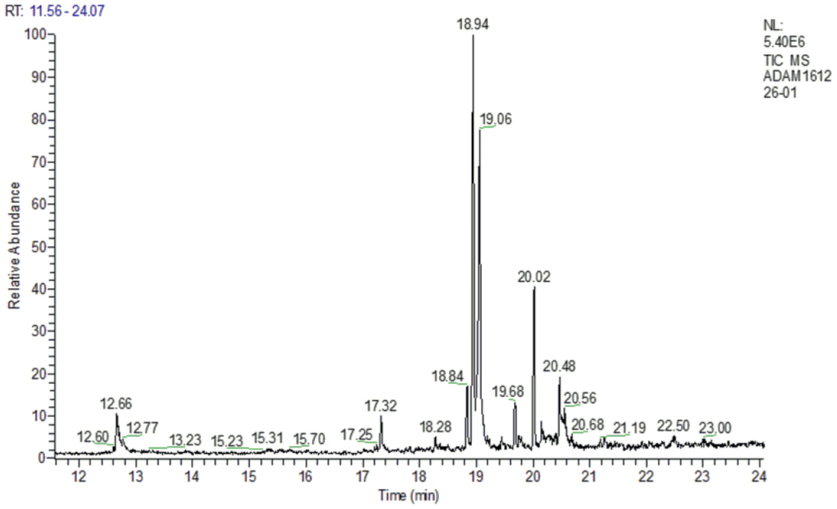


Figure 1. Chromatogram of ethyl acetate fraction of *Boswellia papyrifera*

Experimental

Materials and methods

The barks of *Boswellia papyrifera* were collected in May 2016 from the Zalingei area, central Darfur state, west of Sudan, and the plant was authenticated by prof. G.A. Yagoub, department of botany, faculty of agriculture, University of Zalingei, where a voucher specimen (No. 20161016) has been deposited in the herbarium of author's laboratory. All the chemicals and solvents were commercial grade and used after further purification. Petroleum ether (PE), ethyl acetate (EtOAc),

methanol (MeOH), chloroform ($CHCl_3$), were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfate was purchased from Fluka (Buchs, Switzerland). Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mesh, Qingdao Haiyang Chemical Group Company, China), TLC were carried out on GF_{254} silica gel plates (Merck, Qingdao Haiyang Chemical Group Company, China). Gas chromatography-mass spectrometry (PerkinElmer, USA), and syringes (Hamilton Bonaduz AG, Switzerland), Polyethylene plastic bags, ceramic mortar and pestle (Haldenwanger, Germany), rotary evaporator and heating mantle (Buchi, Switzerland), a digital analytical balance (Mettler Toledo, Model AG 204, Switzerland) are among the equipment and instruments that were used in the study.

Table 1. The chemical constituents of the ethyl acetate fraction of stem barks of *Boswellia papyrifera* by GC-MS

Identity	(t_R) min	M.F	M.W	Components
1	12.66	$C_{20}H_{32}$	272	m-Camphorene
2	17.32	$C_{20}H_{32}$	272	Pimaradien
3	18.84	$C_{20}H_{32}$	272	verticilla-4(20), 7,11-triene
4	18.94	$C_{20}H_{34}O$	290	serratol
5	19.00	$C_{20}H_{34}O_2$	306	incensole
6	19.06	$C_{22}H_{36}O_3$	348	incensyl acetate
7	19.68	$C_{22}H_{34}O_3$	346	duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate
8	20.02	$C_{20}H_{34}O_3$	322	incensole oxide
9	20.48	$C_{22}H_{36}O_4$	364	incensole oxide acetate

Extraction and fractionation method

The stem barks of the *Boswellia papyrifera* were air-dried at room temperature for four weeks, grinded, and homogenized to uniform powder by ceramic mortar and pestle and saved. The stem barks powder (2 kg) was extracted three times with 95% EtOH at room temperature (Each 7 days × 4 L). Then, the filtrates were combined, concentrated in vacuum (Rotary evaporation) in order to remove the organic solvent and dried. Then, a total of 400 g of ethanolic extracts was subjected to column chromatography (15×150 cm column) over silica 2000 g, the column was eluted with chloroform until no elute came out yield 10 g of dried extracts fraction **I**, and then the column was eluted with EtOAc yield 18 g, fraction **II**, which were chromatographed on 300 g of silica gel (Merck kiesel gel 300-400 mech) used cholorform/MeOH gradient (50:1, 1:1). Then, all the fractions were checked by Thin-layer chromatography (TLC) using the cholorform/MeOH 20:1, 15:1, 10:1, 5:1 and

1:1 as mobile phases. Fractions showing similar results on TLC were combined together to provid three fractions (**F_{II-I}, F_{II-II},** and **F_{II-III}**), and, then, the fraction **F_{II-I}** was analyzed by GC-MS in order to identify compounds **1** to **9**.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) were performed utilizing a 500 series Perkin Elmer Clarus (GC) coupled with PerkinElmer Clarus (MS) electron ionization analyzer mass spectrometer at 70 eV. Coloumn was fused, silica capillary type was DB-17 (30 m × 0.25 mm i.d.) and temperature of the oven was programmed at 80-280 °C at a rate of 10 °C/min with helium as mobile phase; moreover, the detector (FID) and injector temperatures were both maintained at 250 °C.

Results and discussion

The chemical constituents of the ethyl acetate fraction of stem barks of *Boswellia papyrifera* were analyzed by GC-MS, the identified components were confirmed by the interpretation of their mass spectra fragmentation data, matching their mass spectra with NIST databases, and by comparison of the obtained mass spectra with those of the published literature data [16–24], which have already been reported from similar compounds of other *Boswellia* species and as well as in other plants. The corresponding result (Chromatogram) was presented in (Figure 1 and Table 1).

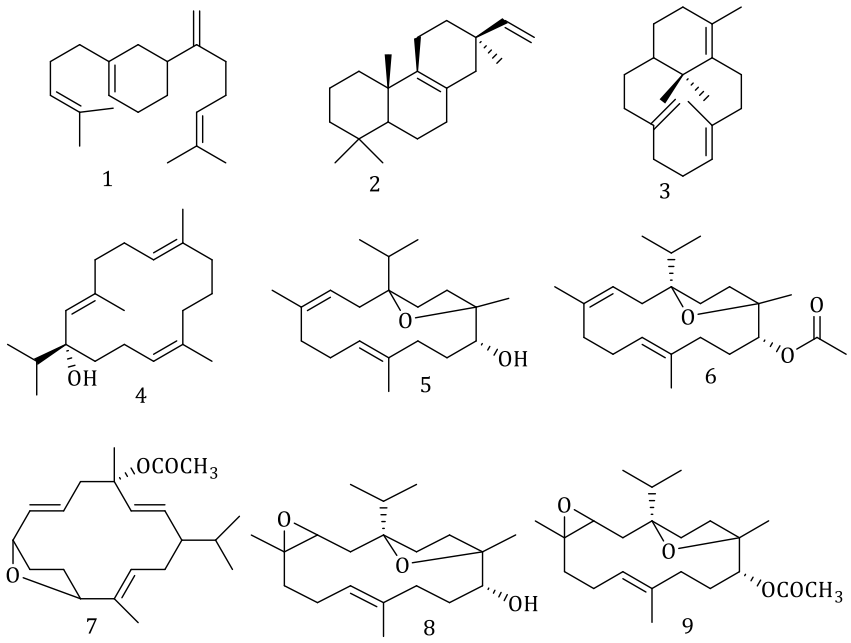
The first peak which appeared at 12.66 min was identified as m-camphorene 1. which produced molecular ion signal at (M^+) m/z=272 in its mass spectrum (Scheme 1), corresponding to an elemental composition of $C_{20}H_{32}$. The fragmentation pattern of m-camphorene primarily showed cleavages, 2-methylbut-2-ene from molecular ion at m/z=204 with the formula of $C_{15}H_{124}$, which produced fragment with an elemental composition of $C_{10}H_{16}$, gave rise to peak at m/z=136 and was followed by RDA reaction explaining the base peak at m/z=69. The possible fragmentation patterns are illustrated in Scheme 2.

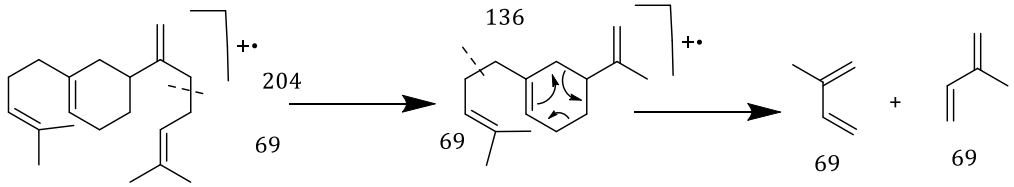
Another compound appeared at 17.32 min was produced molecular ion peak signal at (M^+) m/z=272 in its mass spectrum (Figure 2), and corresponded to an elemental composition of $C_{20}H_{32}$. A fragment ion peak signal at m/z=257 could be produced by loss of an CH_3 group from m/z=272. Cleavage of the ethylene group from molecular ion at m/z=257 produced fragment with an elemental composition of $C_{17}H_{25}$ which gave rise to peak at m/z=229. This diterpene is found to be pimaradien 2. The possible fragmentation patterns are shown in Scheme 3.

Another diterpene was also appeared at 18.84 min which produced molecular ion (M^+) m/z=272 in its mass spectrum that corresponded to formula $C_{20}H_{32}$. The fragmentation mechanism shows the

initial cleavage of allyl methyl group from the molecular ion and then undergoes RDA reaction in the cyclohexene ring to produce peak signal at m/z=257 representing the base peak in its mass spectrum (Figure 4). Accordingly, compound **3** was identified as verticilla-4(20),7,11-triene. The possible fragmentation patterns is depicted in (Scheme 4).

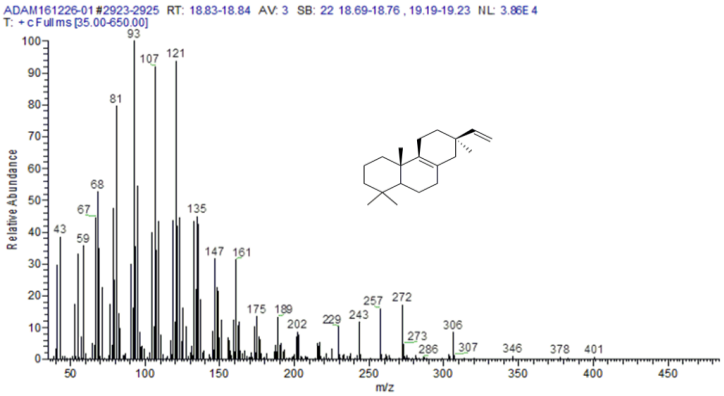
Scheme 1. Structure of diterpenes identified in ethyl acetate fraction from stem barks of *Boswellia papyrifera.* Numbers correspond to compounds listed in Table 1

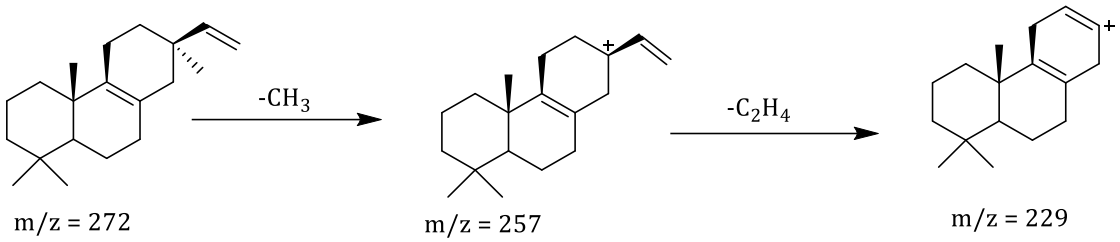




Scheme 2. Possible fragmentation patterns of m-camphorene **1**

Figure 2. Mass spectrum showing presence of pimaradien **2**





Scheme 3. Possible fragmentation patterns of pimaradien 2

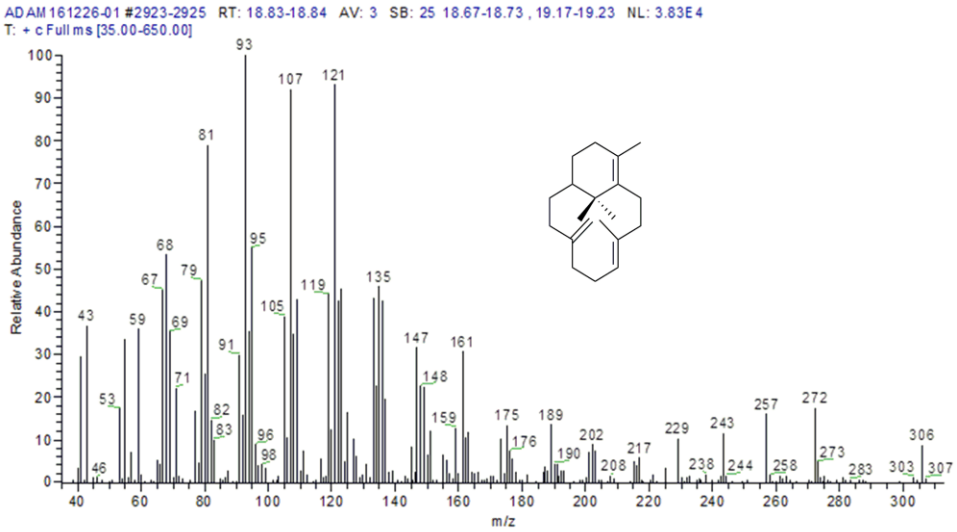
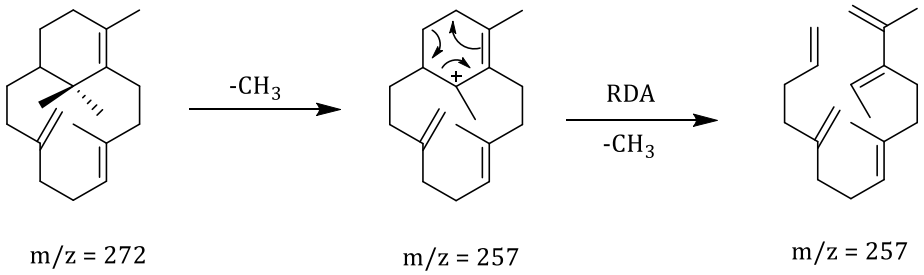


Figure 4. Mass spectrum showing presence of verticilla-4(20),7,11-triene **3**



Scheme 4. Possible fragmentation patterns of verticilla-4(20),7,11-triene **3**

The peak at 18.94 min on GC chromatogram, produced molecular ion peaks signal at (M^+) m/z=290 in its mass spectrum (Figure 5), corresponded to an elemental composition of $C_{20}H_{34}O$. There are peaks that appeared in the mass spectra at 229, 247, and 272. Elimination of the isopropyl group from molecular ion at m/z=290 produced fragments with an elemental composition of $C_{17}H_{27}O$ which gave rise to peak at m/z=272, whereas the fragment ion signal m/z=229 is produced by cleavage of H_2O group from molecular ion 272. Fragment ion peak signal

at m/z=247 could be produced by loss of an isopropyl group from m/z=290. This compound was identified as serratol **4** (Figure 5), showing the possible fragmentation patterns.

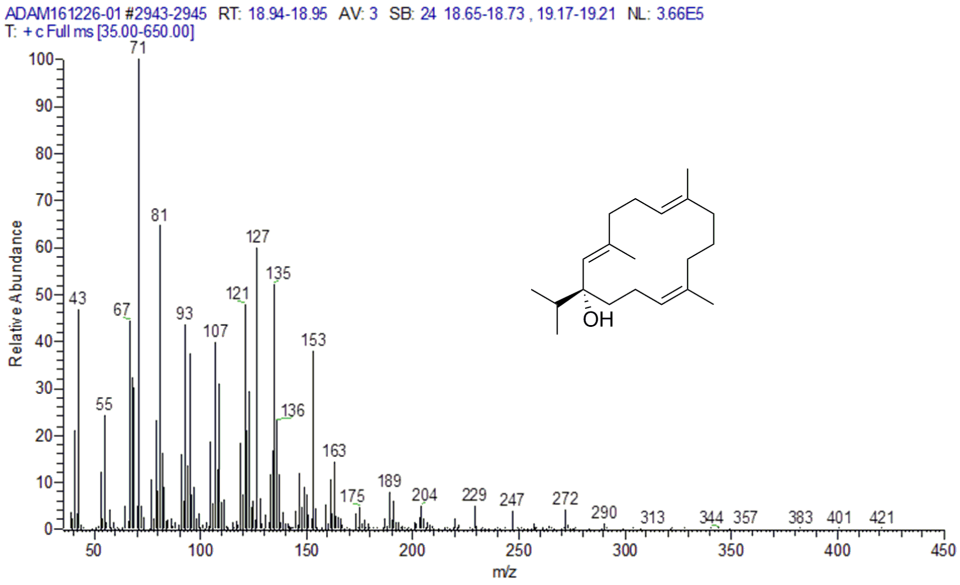
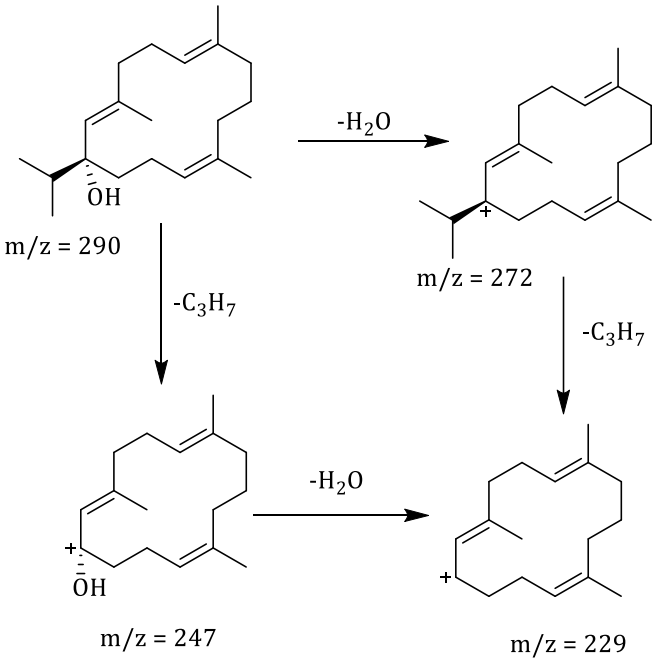


Figure 5. Mass spectrum showing presence of serratol **4**

Scheme 5. Possible fragmentation patterns of serratol **4**



The compound appeared at 19 min on GC chromatogram which produced molecular ion peak signal at (M^+) m/z=306 in its mass spectrum (Figure 6) corresponded to an elemental composition of $C_{20}H_{34}O_2$. Cleavage of the isopropyl group from molecular ion at m/z=306 produced fragment

with an elemental composition of $C_{17}H_{27}O_2$ which gave rise to peak at m/z=263. This compound was found to be incensole **5**. The possible fragmentation patterns is shown in (Scheme 6).

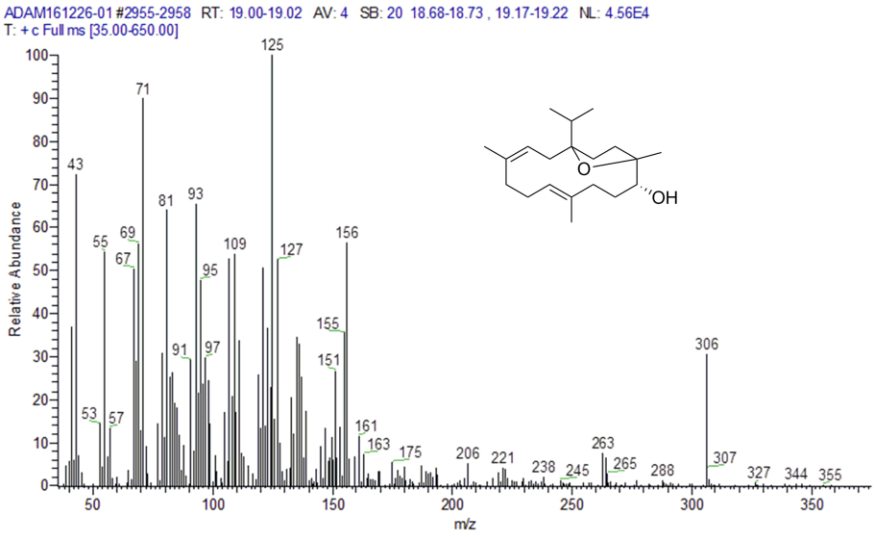
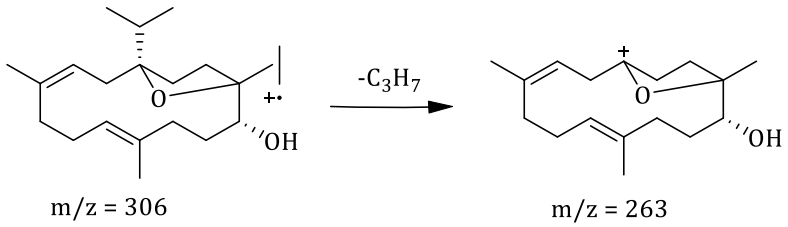
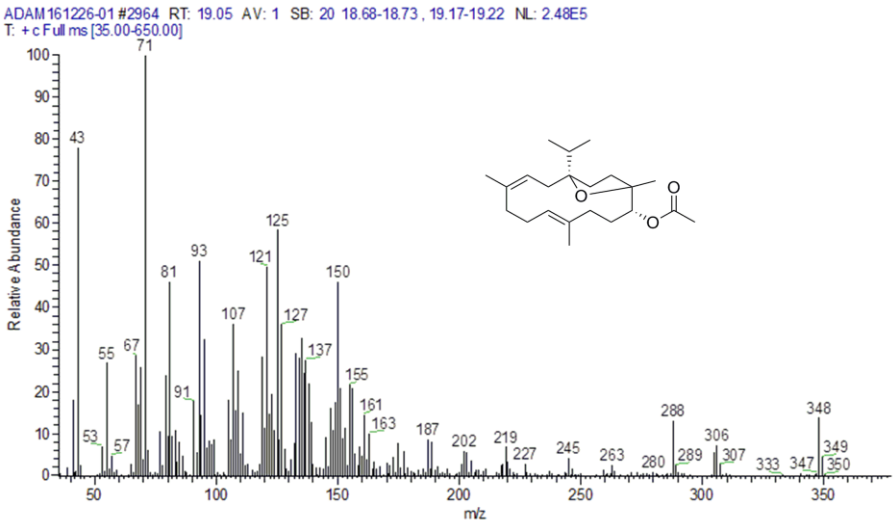


Figure 6. Mass spectrum showing presence of incensole **5**



Scheme 6. Possible fragmentation patterns of incensole **5**

Figure 7. Mass spectrum showing presence of incensyl acetate **6**

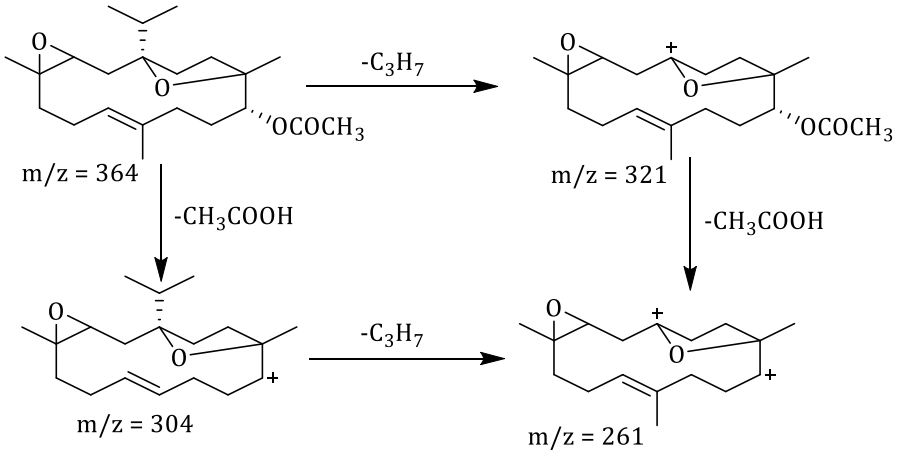


Another compound identified in this research study appeared at 19.06 min on GC chromatogram and gave a molecular ion peak signal at (M^+) m/z=348 in its mass spectrum (Figure 7), which corresponded to an elemental composition of $C_{22}H_{36}O_3$. The peaks appeared in the mass spectra at 245, 288, and 305. Elimination of the isopropyl group from molecular ion at m/z=348 produced fragment with an elemental composition of $C_{19}H_{29}O_3$ which gave rise to peak at m/z=305, whereas the fragment ion signal m/z=288 is produced by cleavage of acetic acid group from molecular ion (M^+). Fragment ion peak signal at m/z=245 could be produced by loss of an isopropyl group from m/z=288. This compound was identified as incensyl acetate 6. The possible fragmentation patterns are illustrated in Scheme 7.

The compound appeared at 19.68 min on GC chromatogram, identified as duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate **7** by molecular ion peaks signal at (M^+) m/z=346 in its mass spectrum (Figure 8), corresponded to an elemental composition of $C_{22}H_{34}O_3$. There are three peaks appeared in the mass spectra at 243, 286, and 303 which can be explained by elimination of the isopropyl group from molecular ion at m/z=346 as they produced fragment with an elemental composition of $C_{19}H_{27}O_3$, and gave rise to peak at m/z=303, whereas the fragment ion signal m/z=289 is produced by cleavage of acetic acid group from molecular ion (M^+). Fragment ion peak signal at m/z=243 could be produced by loss of an isopropyl group from m/z=289. The possible fragmentation patterns are illustrated in Scheme 8.

The compound which appeared at 20.02 min and produced molecular ion peak signal at (M^+) m/z=322 in its mass spectrum (Figure 9), which corresponded to an elemental composition of $C_{20}H_{34}O_3$. Fragment ion peak signal at m/z=303 could be produced by loss of an H_2O group from m/z=322. Cleavage of the isopropyl group from molecular ion at m/z=303 produced fragment with an elemental composition of $C_{17}H_{25}O_2$ which gave rise to peak at m/z=261. This diterpene is found to be incensole oxide 8. The possible fragmentation patterns are shown in Scheme 9.

Scheme 7. Possible fragmentation patterns of incensyl acetate **6**



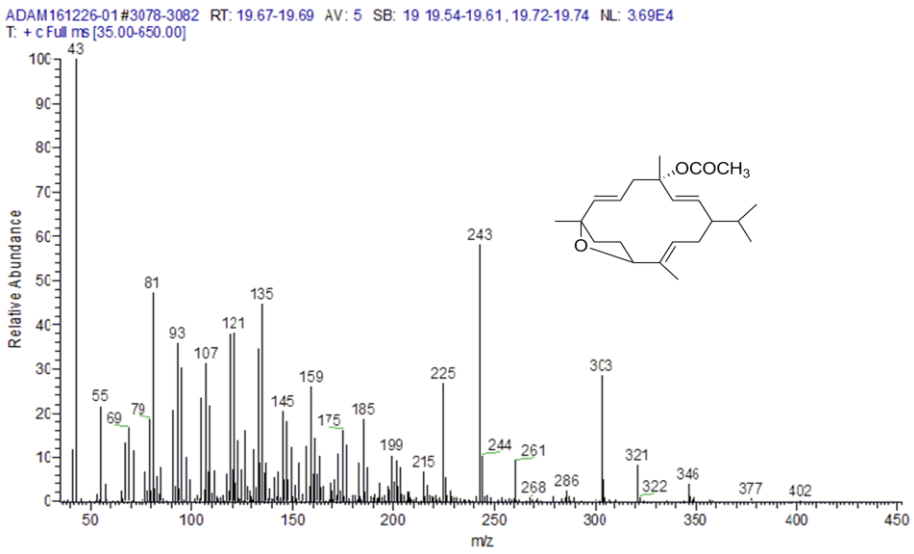
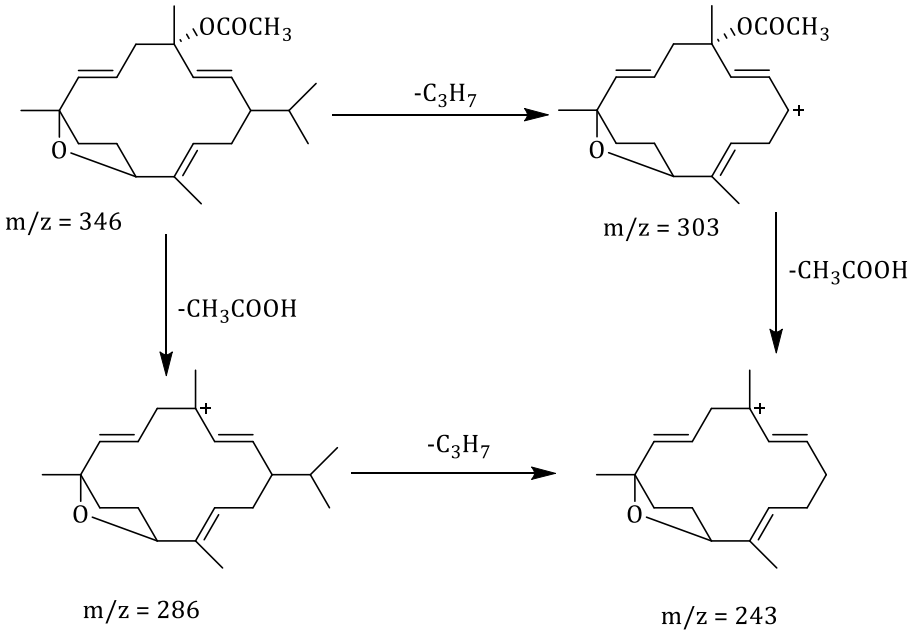


Figure 8. Mass spectrum showing presence of duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate **7**



Scheme 8. Possible fragmentation patterns of duva-3,9,13-trien-1α-ol-5,8-oxide-1-acetate **7**

The peak appeared at 20.48 min which is a produced molecular ion peaks signal at (M+) m/z=364 in its mass spectrum (Figure 10), corresponds to an elemental composition of $C_{22}H_{36}O_4$. Three peaks appeared in the mass spectra at 261, 304, and 321 could be explained by elimination of the isopropyl group from the molecular ion at m/z=364. They produced fragment with an elemental composition of $C_{19}H_{29}O_4$ and gave rise to peak at m/z=321, whereas the fragment ion signal m/z=304 is produced by cleavage of acetic acid group from the molecular ion (M^+). Fragment

ion peak signal at m/z=261 could be produced by loss of an isopropyl group from m/z=304. This compound was identified as the incensole oxide acetate 9. The possible fragmentation patterns are shown in Scheme 10.

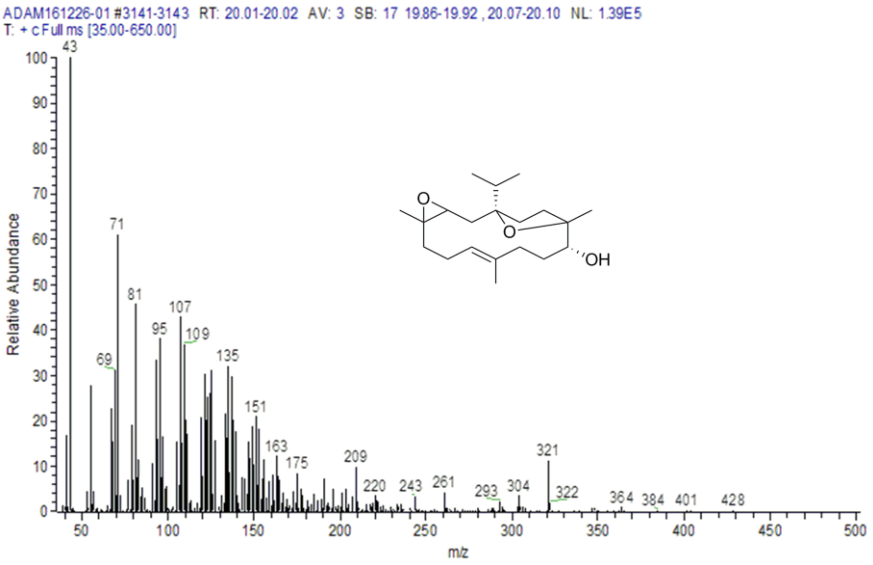
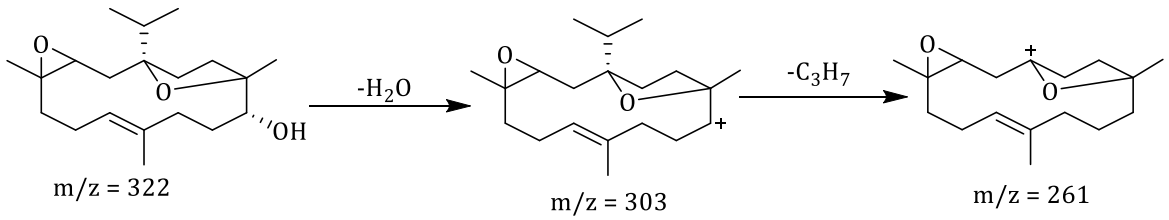
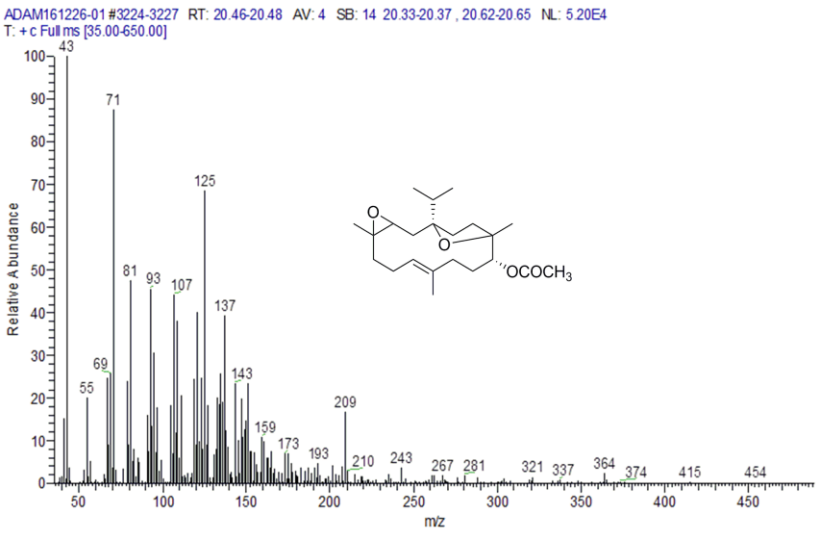


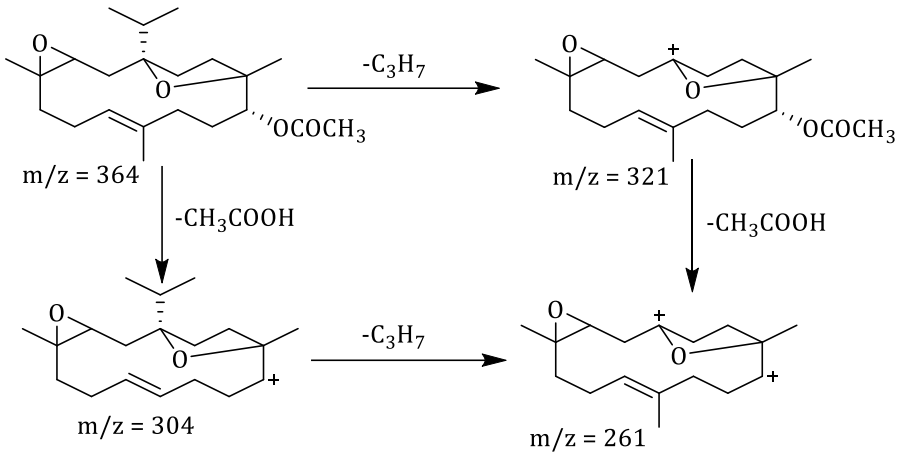
Figure 9. Mass spectrum showing presence of incensole oxide **8**



Scheme 9. Possible fragmentation patterns of incensole oxide **8**

Figure 10. Mass spectrum showing presence of incensole oxide acetate **9**





Scheme 10. Possible fragmentation patterns of incensole oxide acetate **9**

Conclusion

In the present study, nine diterpens in the ethyl acetate fraction of stem bark of *Boswellia papyrifer* have been identified using GC-MS analysis. The existence of these bioactive chemical compounds proved the use of this plant for various ailment by traditional medical practitioners. Among the identified compounds, m-camphorene, pimaradiene**,** serratol, and 9,13-trien-1α-ol-5,8-oxide-1-acetate were reported for the first time.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the authors.

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