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Analysis of major components of essential oils of *Boswellia* species by GC-MS

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Abstract
The aim of this study was to analyze the major components of essential oils of seven *Boswellia* species, *B. papyrifera, B. papyrifera* Kebtele*, B. frereana, B. neglecta, B. pirottae, B. sacra* and *B. rivae,* which were obtained by hydro-distillation of the finely powdered oleogum resin were analyzed using GC-MS method. The foremost components of each resin have been identified as n-octyl acetate, verticillia-4(20), 7, 11-trien, p-cymene, terpene-4-ol and 3-carene in *B. papyrifera, B. papyrifera* Kebtele, *B. frereana, B. neglecta* and *B. pirottae,* respectively. α-Pinene was detected as the major constituent in *B. sacra and B. rivae*.

Keywords: *Boswellia, papyrifera,* Kebtele, *frereana, neglecta, B. pirottae*

1. Introduction

The constituents of the essential oils of *Boswellia* species were first investigated by J. Stenhousein 1840 [1]. Stenhouse identified fourteen monoterpenoid constituents depending on the origin of the resins [2]. The yield of the volatile oil of frankincense obtained by hydro-distillation of the finely powdered oleogum resin derived from *Boswellia* species was reported in several articles [3-9]. Such studies revealed the presence of monoterpenes, sesquiterpenes and diterpenes [2, 4, 6, 10], *Fig.* 1. Monoterpenes found in *Boswellia sacra* Flűk (syn. *B. carteri* Birdw.), *Boswellia serrata* Roxb, *Boswellia papyrifera* Hochst., *Boswellia frereana* Birdw., *Boswellia neglecta* S. Moore, *Boswellia rivae* Engl. and *Boswellia pirottae* Chiov. have been reported in the literature [2, 4, 11-20] as shown in Table 1. Sesquiterpenes and diterpenes are also the main components of essential oil of *Boswellia* resins, Fig 2 and 3. The major sesquiterpenes and diterpenes are presented in Table 2. Compounds other than terpenes are *n*-octyl acetate, *n*-octanol, *n*-hexyl acetate, decanol, decyl acetate, geranyl acetate, dodecanol, hexyl hexanoate, octyl formate [8, 19].

Table 1: Monoterpenes reported from *Boswellia* species

Non-oxygenated monoterpenes
2-*β*-pinene (**1**), *α*-thujene (**2**), E-β-ocimene (**3**), 2,4(10)-thujadiene (**4**), camphene (**5**), sabinene (**6**), 1-*β*-pinene (**7**), myrcene (**8**), *α*-pinene (**9**), 2-carene (**10**) limonene (**11**), Z-*β*-ocimene(**12**), γ-terpinene (**13**), terpinolene (**14**), *p*-cymene (**15**), α-phellandrene (**33**), *β*-phellandrene (**34**), *δ*-3-carene (**42**), *o*-cymene (**43**), *m*-cymene (**44**), 2,5-dimethylstyrene (**59**)
Oxygenated monoterpenes
Perillene (**16**), isopentyl-2-methyl butanoate (**17**), isovalerate (**18**), *β*-thujone (**19**), *α*-campholene aldehyde (**20**), allo-ocimene (**21**), trans-pinocarveol (**22**), *p*-mentha-1,5-dien-8-ol (**23**), 4-terpineol (**24**), sabinyl acetate (**25**), myrtenal (**26**), *α*-terpineol (**27**), α-phellandrene epoxide (**28**), verbenone (**29**), *trans-(+)*-carveol (**30**), carvone (**31**), bornyl acetate(**32**), *cis*-verbenol (**35**), *trans*-verbenol (**36**), 1,8-cineole (**37**), α-pinene oxide (**38**), *p*-mentha-6,8-dien-2-one (**39**), neryl acetate (**40**), linalool (**41**), *cis*-1,2-limonen oxide (**45**), *trans*-1,2-limonene oxide (**46**), *trans*-sabinene hydrate (**47**), chrysanthenone (**48**), linalyl acetate (**49**), umbellulol (**50**), *trans*-pinocarveol (**51**), carvotanacetone (**52**), *cis*-1,2-epoxy-terpin-4-ol (**53**), *p*-mentha-1,5-diene-7-ol (**54**), *p*-cymen-8-ol (**55**), *m*-cymen-8-ol (**56**), thymol (**57**), carvacrol (**58**), pinocamphone (**60**), *trans*-dihydrocarvone (**61**), sabinaketone (**62**), *cis*-p-mentha-2,8-diene-1-ol (**63**), *trans-p*-mentha-1(7),8-diene-2-ol (**64**), *cis-p*-mentha-1(7),8-diene-2-ol (**65**), myrtenol (**66**), piperitenone (**67**), borneol (**68**), verbenone (**69**), *β*-myrcene (**70**), nerol (**71**), camphor (7**2**), *trans*-p-menth-2-en-1-ol (**73**), terpinyl acetate (**74**), *trans*-sabinol (**75**),*cis*-sabinol (**76**), pinocarvone (**77**), *trans*-carveol (**78**), *cis*-carveol (**79**), *cis*-p-menth-2-en-1-ol (**80**)

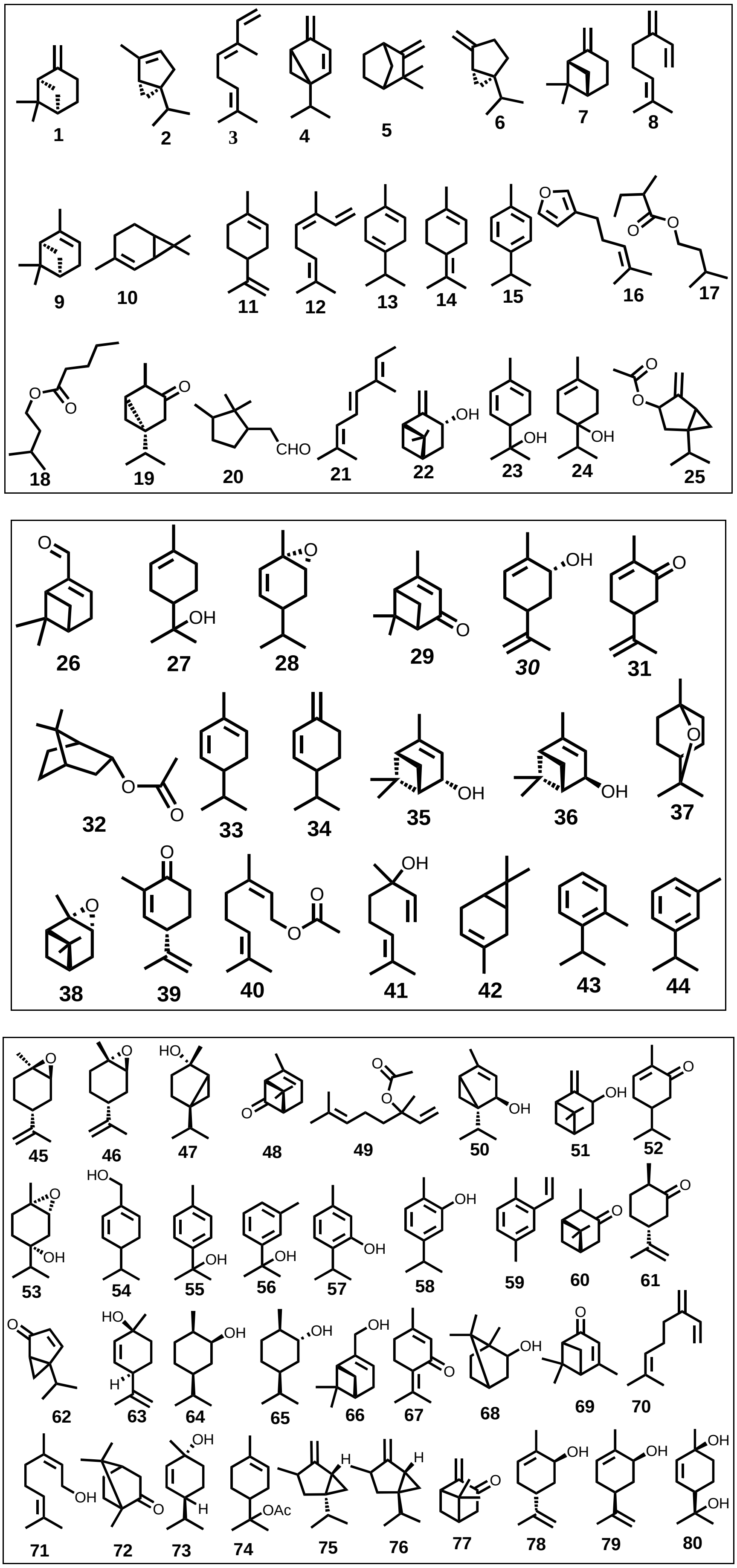


Fig 1: Structures of monoterpenes in the resin of Boswellia species

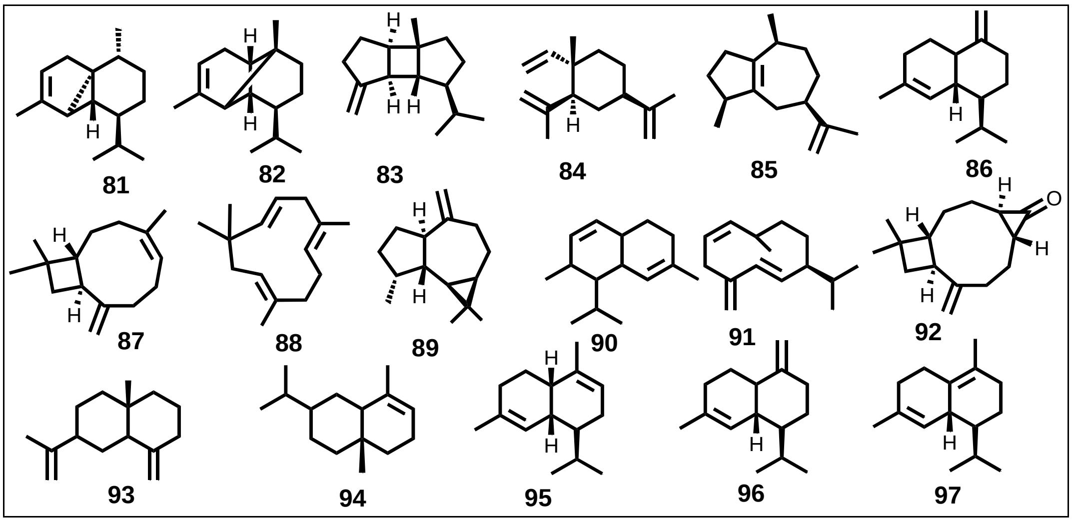


Fig 2: Structures of sesquiterpenes in the resin of *Boswellia* species

Table 2: Sesqui- and diterpenes reported from *Boswellia* species

Terpenes	Name of compounds
Sesquiterpenes [4]	α-cubebene (**81**), α-copaene (**82**), ß-bourbonene (**83**), β-elemene (**84**), α-gurjunene (**85**), γ-muurolene (**86**), E-aryophyllene (**87**), α-humulene (**88**), alloaromadendrene (**89**), α-amorphene (**90**), germacrene D (**91**), caryophyllene oxide (**92**), β-selinene (**93**), α-selinene (**94**), α-muurolene (**95**), γ-cadinene (**96**), δ-cadinene (**97**)
Diterpenes [2, 11, 21]	verticill-4(20),7,11-triene (**98**), cembrene (**99**), cembrene A (**100**), cembrene C (**101**), serratol (**102**), incensole (**103**), *iso*-incensole oxide (**104**), incensole acetate (**105**), incensole oxide (**106**), *iso*-incensole (**107**), *iso*-incensole acetate (**108**), *iso*-incensole acetate oxide (**109**), *m*-camphorene (**110**), *p*-camphorene (**111**), *α*-phellanderene dimer (**112**), cneorubenol (**113**), *iso*-serratol (**114**)

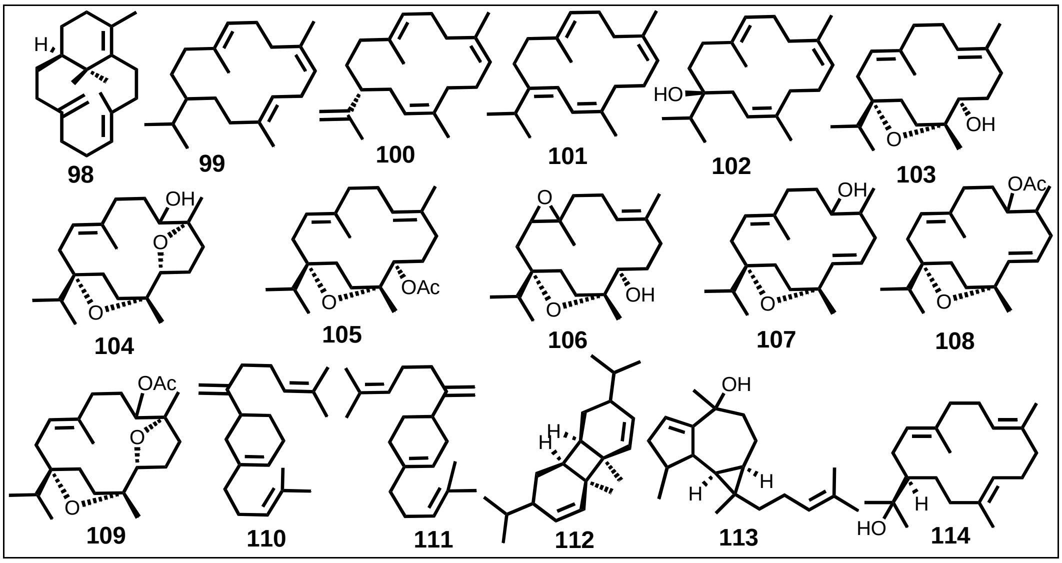


Fig 3*:* Structures of diterpenesin the resin of *Boswellia* species

The main constituents of *B. papyrifera, B. papyrifera Kebtele, B. frereana, B. neglecta, B. pirottae, B. sacra and B. rivae* resins in the same GC-MS condition have not been reported yet, so, the primary objective of this study was to analyze the major constituents of these resins using GC-MS method.

2. Materials and Methods

Most of the resin materials used for the investigations reported here were obtained from the natural product research lab of AAU department of chemistry. The material was already correctly assigned as authentic frankincense resin material in the stock of professor Ermias's research lab collection and also fresh samples were properly identified by National Herbarium of Ethiopia, Addis Ababa University. Authentication were done by Prof. Sebsebe Demissew and A to Melaku Wondafrash of National Herbarium experts. The resin used in this work for analysis were collected from different matured tree of the same location.

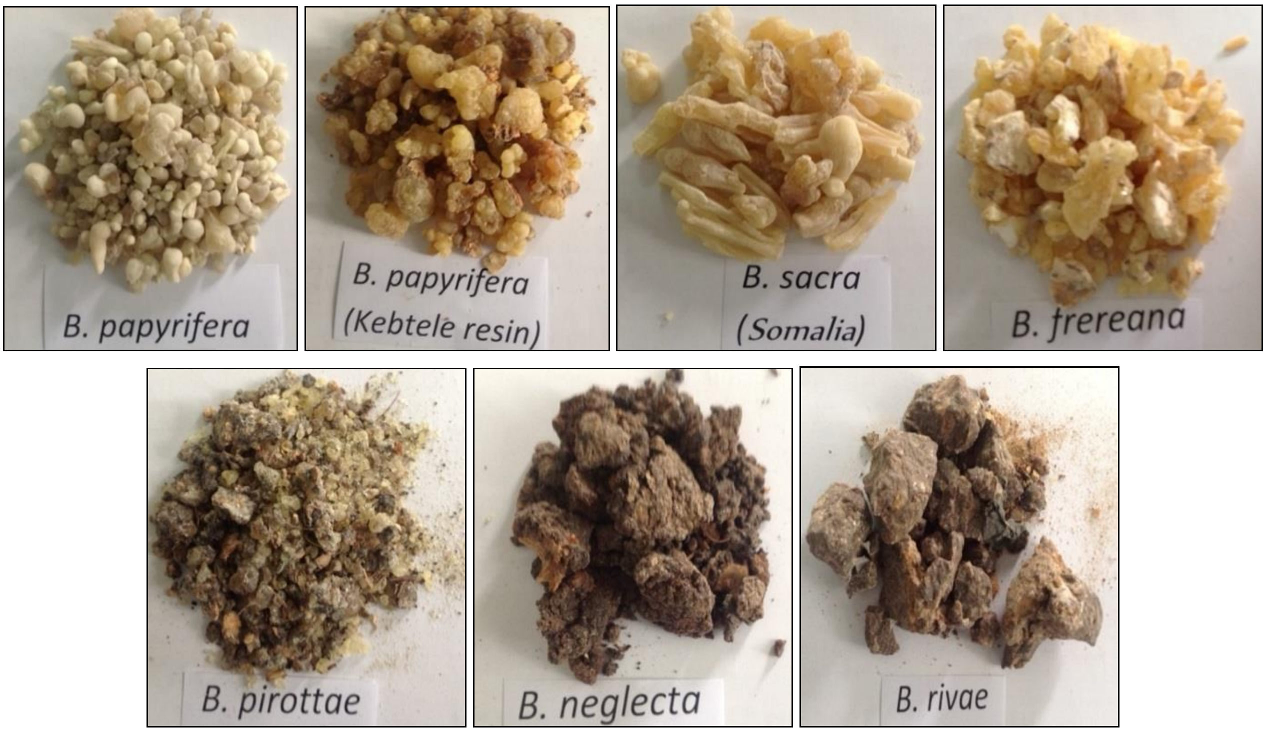


Fig 1: Photos of resins (By Dr. Sisay A.)

Finely ground Boswellia resins (50 g of each) were subjected to hydro-distillation using Clevenger's apparatus until complete exhaustion. Extractions were done for three hours.
The oils were collected, dried over anhydrous sodium sulfate and kept in the refrigerator until analysis. The data was recorded using GC-MS Agilent Technologies. GC-system-7820A, MS-5977E MSD detector with column size 30m x 0.250mm, 0.25 micron- 60 to 350 ^{o}C.

Table 3: Lists of Resins Analysis

Scientific name	Local name	Collection Year	Herbarium number	Collection site
B. papyrifera	*Nech etan* (Amh.)	Feb, 2017	072817	Sekota
B. papyrifera (Kebtele)	*Walya meker* (Amh)	Jan, 2017	E. Dagne	Agew-Awi
B. sacra (Syn. B. carteri)	*Beyo*	Feb, 2017	E. Dagne	Somalia
B. frereana	*Maydi*	Feb, 2017	E. Dagne	Somalia
B. pirottae	*Yetan-Zaf* (Amh)	Mar, 2017	072815	Chis-Abay
B. neglecta	*Borena Etan* (Amh.)	Jan, 2017	072813	Borena
B. rivae	*Ogaden Etan* (Amh)	Jan, 2017	072808	Ogaden

3. Results and discussion

The essential oils of seven *Boswellia* species were obtained by steam distillation of the finely powdered oleogum resin. The yields of the volatile oils are presented in Table4. The strongly aromatic oils were separated from the water layer by adding chloroform and dried with anhydrous Na_2SO_4. The oil obtained from *B. papyrifera*Kebtele resin was small when compared with the rest resins investigated here.

Table 4: Percentage of essential oils from *Boswellia* species

***Boswellia* species**	**Yield g)**	**Oil content (%)**
B. papyrifera resin	2.3	4.5
B. papyrifera Kebtele resin	1.0	2.0
B. sacra (Syn, B. carteri) resin	2.5	5.0
B. frereana resin	2.0	4.0
B. rivae resin	1.5	3.0
B. neglecta resin	2.0	4.0
B. pirottae resin	1.5	3.0

The essential oils were analyzed by GC-MS and components were identified through comparison of the fragmentation patterns in the resulting mass spectra with those published in the literature and by using National Institute of Standards and Technology (NIST) mass spectral database. Additionally, the data generated for the essential oils obtained from the resin of *B. papyrifera*, *B. neglecta* and *B. rivae* were compared with those reported by Aman *et al.* [19], whereas, the data obtained from *B. sacra* and *B. frereana*data were compared with those reported by Hamma *et al.* [6] and Mertens *et al.* [8]. There have been no reports on the GC-MS analysis of the essential oils of *B. papyrifera*Kebtele resin. However, GC analysis of this resin was reported by Asmare with verticillantriene as major and n-octyl acetate as minor components [11]. As shown in Table 5 and Fig. 2, the major components of the essential oils of the *Boswellia* species analyzed in this work are presented. Some variations were observed with the data reported in the literature [6, 8, 19] and these may be due to seasonal and geographical conditions.

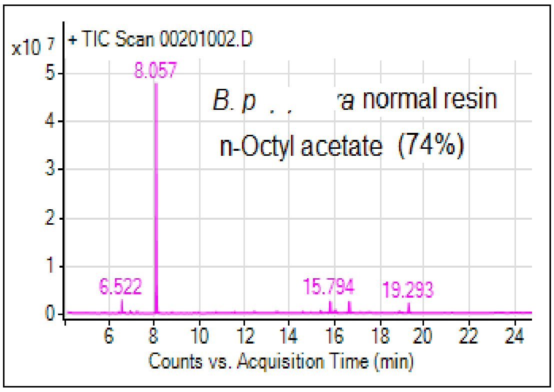
Table 5: Main components of essential oils from resins of *Boswellia* species

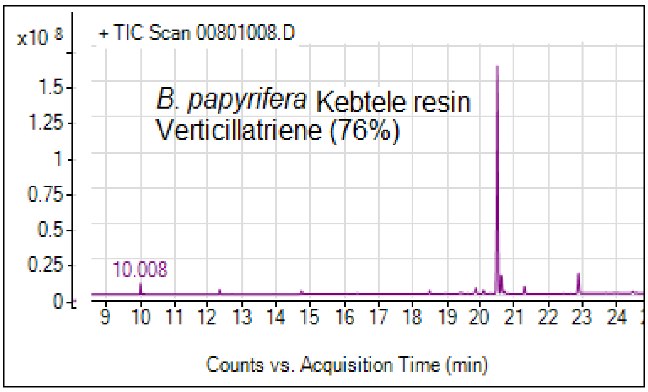
RI[a]	Major oils	A	B	C	D	E	F	G	RI[b]
921	β-Thujene	-	-	-	12.0	6.5	2.6	3.6	916

946	α-pinene	-	-	48.0	6.1	20	9.0	37	942
951	Sabinyl propipnoate	-	-	-	2.1	-	-	-	-
972	β-Phellandrene	-	-	14.0	3.2	7.0	0.8	-	969
980	β-pinene	-	-	-	-	-	-	6.0	973
1001	β-myrcene	-	-	10.5	-	-	-	-	994
1006	α-Phellandrene	-	-	-	-	-	-	4.0	1003
973	Sabinene	-	-	-	-	7.3	-	-	963
1009	3-Carene	-	-	9.0	-	22	-	-	1006
1019	p-Cymene	-	-	-	27.0	-	6.7	23	1014
1021	E.β-Ociemene	-	-	10.6	-	-	-	-	1016
1023	Limonene	-	-	-	-	1.1	6.1	18	1020
1025	o-cymene	-	-	-	-	9.0	-	-	1022
6.46	γ-Tepinene	-	-	-	-	2.0	1.0	-	1054
1065	n- Octanol	-	8.0	-	-	-	-	-	1063
1081	Linalool	-	-	-	3.0	-	-	-	1077
1054	α-Thujen-ol	-	-	-	-	-	-	3.0	1049
1136	Trans- Verbenol	-	-	-	-	8.0	3.7	5.9	1131
1185	Terpene-4-ol	-	-	-	8.0	1.5	51	-	1182
1196	α-Terpineol	-	-	-	2.0	-	16.0	-	1193
1114	α-Phellandren-8-ol	-	-	-	-	1.2	-	-	-
1224	n-Octyl acetate	2.7	74.2	-	2.5	-	-	-	1220
1226	Cis-verbenone	-	-	-	-	-	2.0	-	1221
1390	β-Bourbonene	-	-		3.0	-	-	-	1388
1402	Carophyllene	-	-	7.6	-	-	-	-	1399
1672	Cembrene	-	4.0	-	-	-	-	-	1661
1816	Dimer of α-phellandrene	-	-	-	12.2	-	-	-	1810
2188	Incensole acetate	-	6.5	-	-	-	-	-	2182
1964	Cembrene C	2.4	-	-	-	-	-	-	1959
1987	Isoverticillene	1.1	-	-	-	-	-	-	-
2010	Verticillatriene	76.3	7.2	-	-	-	-	-	2004
2015	Verticillene	5.3	-	-	-	-	-	-	2006
2023	Cneorben-10-ol	7.2	-	-	-	-	-	-	-
	Total	95.0	99.9	99.7	81.0	84.3	99.0	99.6	

A = *B. papyrifera* Kebtele, B = *B. papyrifera*, C = *B. sacra*, D = *B. frereana*, E = *B. pirottae*, F = *B. neglecta*, G = *B. rivae*, RI[a] = RI of present work, RI[b] = RI of literature

As shown in the table the major component reported for B. sacra by Hamm *et al.* was *E-β*-Ocimene (32%) however in this work its major component was detected as *α*-Pinene (48%) [6]. Same author mentioned *α*-Pinene as the major component for *B. frereana* (38%), *B. neglecta* (21%) and *B. rivae* (16%), whereas, in the preset work p-Cymene (48%), Terpene-4-ol (51%) and *α*-Pinene (37%), respectively. Moreover, Aman *et al.* reported that limonene (15%) and Terpen-4-ol (13%) as a major component of *B. rivae* and *B. neglecta*, respectively [22].The major components of *B. pirotae*were analysed as Trans-Verbenol and Terpen-4-ol by Ahmed Al-Harrasi *et al* [8]. And *Aman et al*., where as, 3-Carene (22%) was found as the major oil in this work. The major component of *B. papyrifera* reported in this work was inconsistence with other authors except the percent yield. However, B. papyrifera Kebtele resin showed verticillaster as a major component.





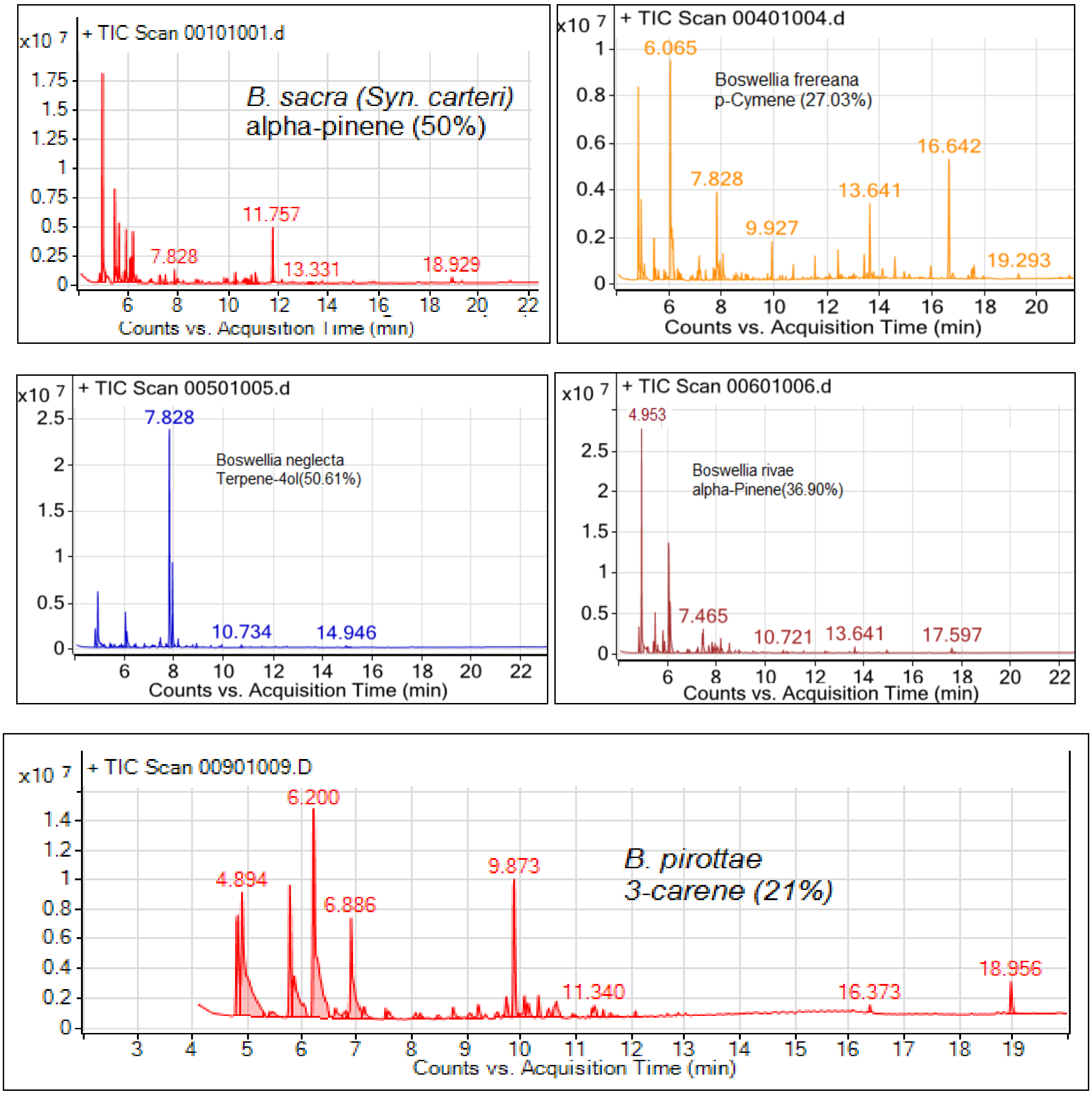


Fig 2: GC-MS Chromatograms of the oils of *Boswellia* resins

4. Conclusion

The major components of the essential oils of the *Boswellia* species analyzed in this work are n-octyl acetate (74%), verticillia-4(20),7,11-triene (76%), p-cymene (27%), terpene-4-ol (50%) and 3-carene (27%) in *B. papyrifera*, *B. papyrifera* Kebtele, *B. frereana*, *B. neglecta*and *B. pirottae*, respectively. α-Pinene (48% and 36%) was the major constituent in *B. sacra* and *B. rivae*. Other essential oils obtained from *B. papyrifera* Kebtele resin were cembrene A, cembrene C, verticillene, and is verticillin.

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