Original Research Paper

Natural Product Communications
Volume 17(7): 1–6

DOI: 10.1177/1934578X221117411
journals.sagepub.com/home/npx
SAGE

Inter-Tree Variation in the Chemical Composition of *Boswellia papyrifera* Oleo-Gum-Resin

Anjanette DeCarlo[1], Salah Agieb[2], Stephen Johnson[3], Prabodh Satyal[1] and William N. Setzer[1,4]

Abstract

Frankincense is a fragrant resin produced by *Boswellia* species, and has been used for centuries as a perfume, medicine, and incense, and is an important cosmetic and therapeutic product today. A number of studies have been conducted on the resin essential oils, but many have used commercial sources outside of the country of origin, leading to potential taxonomic confusion or misidentification. Individual *Boswellia papyrifera* resin samples were each obtained directly from 11 individual trees in Sudan, hydrodistilled, the volatile phytochemicals determined by gas chromatographic methods, and the chemical compositions subjected to cluster analysis. All samples were very similar, with high levels of octyl acetate (49.5%-81.0%) and octanol (6.5%-13.7%), and varying levels of diterpenoids (6.6%-32.7%). The cluster analysis indicated 3 highly similar groups, defined by (1) relatively higher levels of octyl acetate (58.9%-81.0%), but with low levels of diterpenoids (6.6%-18.6%); (2) relatively lower levels of octyl acetate (49.5%-61.3%), but with a higher proportion of diterpenoids (19.0%-22.8%); and (3) with octyl acetate (51.6%) and diterpenoids (32.7%).

Keywords

frankincense, olibanum, *Boswellia papyrifera*, essential oils, chemical composition, chemical ecology

Received: June 29th, 2022; Accepted: July 15th, 2022.

Introduction

Frankincense is a natural plant resin widely valued for its aromatic and therapeutic uses.[1] It is produced by species in the genus *Boswellia* (Burseraceae: Sapindales), a group of approximately 24 species of small trees often featuring compound leaves, exfoliating bark, and a deep red resiniferous bark layer.[2] The resin is produced and stored in resin canals in the bark, and is exuded when the bark is broken, either by an animal (such as a boring beetle) or intentionally by humans to extract the resin.[2]

Frankincense has been used and traded internationally for thousands of years for its use in traditional medicine, perfumery, cosmetics, and religious ceremonies. It is still used for these purposes today.[1] In addition, essential oils and extracts derived from frankincense resins have become ingredients in supplements, aromatherapy, and complementary/alternative medicine. The essential oils and heavy terpenes in the resins have shown notable biological activity.[3]

A number of studies have been conducted on frankincense essential oils, but many of these used resins obtained from commercial sources outside of the country of origin, potentially leading to taxonomic confusion. Most *Boswellia* species produce essential oils dominated by mono- and sesquiterpenes, particularly α-pinene, α-thujene, sabinene, limonene, myrcene, *p*-cymene, and β-caryophyllene.[3] *Boswellia occulta* is a unique exception, with an essential oil dominated by methoxyalkanes.[4]

Boswellia papyrifera is found across Sahelian east and central Africa, with major populations in Ethiopia, Eritrea, and Sudan, and is perhaps the most-traded frankincense species in terms of volume.[2, 5] The essential oil has been reported to contain high levels of octyl acetate and octanol; however, many of these studies have been conducted on commercial samples obtained outside of the species' range states.[6–10] Significant concerns about the conservation status and sustainability of trade of *B papyrifera* have been raised, making proper identification of these resins a key priority.[5] In this study, we present a characterization of the resin essential

[1]The Aromatic Plant Research Center, Lehi, UT, USA
[2]Independent Consultant, Muscat, Sultanate of Oman
[3]FairSource Botanicals, LLC, Fox Island, WA, USA
[4]University of Alabama in Huntsville, Huntsville, AL, USA

Corresponding Authors:
Anjanette DeCarlo, The Aromatic Plant Research Center, 230 N 1200 E, Suite 100, Lehi, UT 84043, USA.
Email: anjanettedecarlo@gmail.com
William N. Setzer, Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.
Email: wsetzer@chemistry.uah.edu

Table 1. Chemical Components Identified in *Boswellia Papyrifera* Individual Tree Resin Samples (**A-K**) From South Kordofan, Sudan.

			Concentration, %										
RI_{calc}	RI_{db}	Compound[a]	A	B	C	D	E	F	G	H	I	J	K
920	919	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	tr	tr	tr	---	tr	tr	tr	tr	tr	tr	tr
926	927	α-Thujene	0.1	0.1	tr	tr	tr	0.1	tr	tr	tr	0.1	tr
933	933	α-Pinene	1.5	1.4	0.9	0.5	1.0	1.6	0.5	0.5	0.5	0.8	0.4
949	950	Camphene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
972	972	Sabinene	0.1	0.1	0.1	tr	0.1	0.1	tr	tr	tr	0.1	tr
977	978	β-Pinene	0.1	0.1	0.1	tr	0.1	0.1	tr	tr	tr	0.1	tr
989	989	Myrcene	0.5	0.4	0.3	0.1	0.3	0.6	0.2	0.2	0.2	0.5	0.3
993	992	6-Methylhept-5-en-2-ol	tr	---	---	tr	tr	tr	tr	---	tr	tr	tr
994	994	Mesitylene	---	---	---	---	tr	---	---	tr	---	---	---
1004	1004	Octanal	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1007	1007	α-Phellandrene	tr	tr	tr	---	tr	tr	tr	tr	tr	tr	tr
1009	1009	2-Methylanisole	---	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
1009	1009	δ-3-Carene	tr	---	---	---	---	---	---	---	---	---	---
1012	1012	Hexyl acetate	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr
1017	1017	α-Terpinene	tr	tr	tr	---	tr	tr	tr	tr	tr	tr	tr
1024	1024	*p*-Cymene	0.1	tr	tr	---	tr	0.1	tr	tr	tr	tr	tr
1029	1030	Limonene	5.6	3.6	4.8	1.4	2.4	5.4	2.0	1.2	1.9	2.6	2.4
1030	1031	β-Phellandrene	tr	tr	tr	---	tr	0.1	tr	tr	tr	0.1	tr
1032	1032	1,8-Cineole	0.1	0.1	tr	tr	0.1	0.1	tr	tr	tr	0.1	tr
1035	1034	(*Z*)-β-Ocimene	0.4	0.2	0.1	0.1	0.2	0.4	0.1	0.1	0.2	0.3	0.2
1046	1045	(*E*)-β-Ocimene	2.2	2.5	1.4	0.8	2.0	3.4	1.1	0.9	1.6	3.0	1.9
1058	1057	γ-Terpinene	tr	tr	tr	---	tr	0.1	tr	tr	tr	tr	tr
1072	1069	1-Octanol	12.5	8.5	9.6	6.5	10.5	13.7	8.5	8.1	13.0	12.1	10.8
1085	1086	Terpinolene	tr	tr	---	---	tr	tr	tr	---	tr	tr	tr
1099	1099	Linalool	0.3	0.3	0.1	0.1	0.4	0.5	0.2	0.1	0.3	0.4	0.2
1105	1104	Nonanal	tr	---	---	---	tr	tr	tr	---	---	---	---
1119	1120	3-Octyl acetate	---	---	---	---	---	---	tr	---	---	---	---
1127	1135	6-Methyl-5-hepten-2-yl tiglate	0.7	0.3	0.2	0.2	0.4	0.3	0.4	0.2	0.2	0.5	0.4
1127	1127	*allo*-Ocimene	---	---	---	---	tr	---	---	---	---	---	---
1129	1129	Octyl formate	0.1	tr	tr	tr	0.1	0.1	tr	0.1	tr	tr	0.1
1145	1145	*trans*-Verbenol	tr	---	---	---	---	---	---	---	---	---	---
1180	1180	Terpinen-4-ol	tr	---	---	---	tr	tr	---	---	tr	tr	tr
1193	1192	Methyl salicylate	---	---	---	---	---	---	---	---	tr	---	tr
1195	1195	α-Terpineol	0.1	0.1	tr	tr	0.1	0.1	tr	---	tr	0.1	tr
1214	1214	Octyl acetate	49.5	60.9	65.5	81.0	62.8	58.9	61.2	51.6	59.7	71.6	61.3
1220	1218	*trans*-Carveol	tr	tr	tr	---	tr	---	tr	---	---	---	---
1224	1226	Nerol	tr	---	---	---	---	---	---	---	---	---	---
1226	1227	Citronellol	tr	---	---	---	---	---	---	---	---	---	---
1232	1232	*cis*-Carveol	tr	---	---	---	---	---	---	---	---	---	---
1244	1242	Carvone	tr	---	---	---	---	---	---	---	---	---	---
1250	1249	Geraniol	tr	---	---	---	---	---	---	---	---	---	---
1272	1271	1-Decanol	tr	---	---	---	---	---	tr	---	tr	---	tr
1284	1285	Bornyl acetate	tr	tr	tr	---	tr	tr	tr	tr	tr	tr	tr
1310	1309	Nonyl acetate	---	---	---	---	---	---	---	tr	---	---	---
1322	1322	Myrtenyl acetate	---	---	---	---	---	---	---	tr	---	---	---
1346	1346	α-Terpinyl acetate	---	---	---	---	---	---	---	---	---	---	tr
1349	1349	Citronellyl acetate	0.2	0.2	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.2
1357	1355	*iso*-Terpinyl acetate	tr	tr	tr	---	tr	---	tr	tr	tr	tr	tr
1358	1361	Neryl acetate	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1364	1367	Decanoic acid	---	---	---	---	tr	---	---	---	---	---	---
1374	1375	α-Copaene	0.1	0.1	tr	---	0.1	tr	tr	tr	tr	tr	0.1
1378	1378	Geranyl acetate	0.1	0.3	0.1	tr	0.2	0.1	0.1	0.1	0.1	0.1	0.1
1385	1384	Hexyl hexanoate	0.1	---	---	---	---	---	---	tr	tr	---	---
1408	1408	Decyl acetate	0.4	0.4	0.3	0.1	0.5	0.3	0.4	0.4	0.3	0.3	0.4
1452	1451	(*E*)-β-Farnesene	---	---	---	---	---	---	---	tr	---	---	---
1499	1500	11-Undecenyl acetate	tr	tr	tr	---	tr	---	---	tr	tr	tr	tr

(Continued)

Table 1. Continued.

RI_{calc}	RI_{db}	Compound[a]	Concentration, %										
			A	B	C	D	E	F	G	H	I	J	K
1507	1508	β-Bisabolene	tr	---	---	---	tr	---	---	tr	---	---	tr
1515	1518	δ-Cadinene	---	---	---	---	tr	---	---	---	---	---	---
1524	1527	Methyl dodecanoate	---	---	---	---	tr	---	---	---	---	---	---
1561	1559	Dodecanoic acid	0.2	0.4	0.1	---	0.4	tr	0.1	0.1	0.1	---	0.1
1581	1581	Hexyl octanoate	0.1	---	---	---	---	tr	tr	tr	tr	---	tr
1582	1582	Octyl hexanoate	0.1	---	---	---	---	tr	tr	tr	tr	---	tr
1697	1691	Tridec-(4*E*)-en-1-yl acetate	0.1	tr	tr	---	0.1	tr	0.1	0.1	tr	---	tr
1764	1769	Benzyl benzoate	tr	---	---	---	---	---	---	---	---	---	---
1777	1779	Octyl octanoate	tr	---	---	---	---	---	---	tr	tr	---	---
1908	1907	Isopimara-9(11),15-diene	---	---	---	---	---	---	---	tr	---	---	---
1934	1934	(3*Z*)-Cembrene A	0.2	tr	0.2	0.1	tr	tr	0.1	0.1	tr	tr	0.2
1950	1951	(3*E*)-Cembrene A	2.9	1.7	2.6	0.6	2.0	1.3	2.5	2.0	1.3	0.9	3.0
1965	1960	Neocembrene	tr	0.1	tr	---	0.2	tr	tr	0.1	---	---	---
1971	1979	Biflora-4,10(19),15-triene	0.1	0.1	tr	---	0.1	tr	0.1	0.1	0.1	tr	tr
1995	1992	α-Pinacene	1.0	1.0	0.5	0.2	0.9	0.5	0.6	0.8	0.6	0.3	0.6
2009	2012	Verticilla-4(20),7,11-triene	11.2	9.0	7.1	6.8	7.3	7.2	14.8	24.7	13.6	3.7	10.9
2038	2038	Thunbergol A	0.4	tr	0.3	---	tr	tr	0.2	0.2	0.1	tr	0.4
2133	2138	*iso*-Cembrol	0.2	0.2	0.1	---	0.2	0.1	0.2	0.2	0.1	tr	0.2
2143	2143	Incensole	1.5	1.7	0.8	0.3	1.6	1.0	1.1	0.1	1.0	0.5	1.0
2147	2144	Linoleic acid	---	---	---	---	---	---	---	---	---	---	0.1
2162	2164	Incensyl acetate	4.9	4.8	3.0	0.9	4.4	2.6	3.2	4.3	2.9	1.2	2.9
2266	2264	*iso*-Incensyl acetate	tr	tr	---	---	tr	---	tr	tr	---	---	---
		Monoterpene hydrocarbons	10.6	8.5	7.7	2.9	6.1	11.9	3.8	2.8	4.5	7.4	5.2
		Oxygenated monoterpenoids	0.8	1.0	0.3	0.1	1.0	1.1	0.4	0.4	0.5	1.0	0.6
		Sesquiterpene hydrocarbons	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
		Diterpenoids	22.4	18.6	14.7	8.7	16.9	12.8	22.8	32.7	19.7	6.6	19.0
		Benzenoid aromatics	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
		Fatty acids and derivatives	63.7	70.4	75.6	87.7	74.7	73.2	70.7	60.5	73.3	84.6	73.0
		Total identified	97.5	98.6	98.4	99.4	98.7	98.9	97.7	96.3	98.0	99.6	97.9

[a]The compounds were determined based on the comparison of RI and MS fragmentation patterns.
Abbreviations: RI_{calc}, Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5 column; RI_{db}, Retention index from the databases; tr, trace (< 0.05%); ---, not detected.

oils of vouchered single-tree samples taken in South Kordofan, Sudan.

Results

The *B papyrifera* resin essential oils of 11 individual trees (samples **A-K**) were obtained by hydrodistillation in yields of 1.32% to 2.72% (w/w) as colorless to pale yellow oils. The chemical composition of each sample is reported in Table 1.

All of the samples were dominated by fatty acids and derivates, particularly octyl acetate (49.5%-81.0%) and octanol (6.5%-13.7%). They were also rich in diterpenoids, particularly verticilla-4(20),7,11-triene (6.8%-24.7%), incensyl acetate (0.9%-4.9%), (3*E*)-cembrene A (0.6%-3.0%), and incensole (0.3%-1.7%). Monoterpenes and oxygenated monoterpenoids were present in modest amounts, primarily limonene (1.2%-5.6%) and (*E*)-β-ocimene (0.8%-3.4%). Sesquiterpenes and benzenoids were present only in very small or trace amounts.

A hierarchical cluster analysis carried out on samples indicates that they are highly similar (Figure 1). The cluster analysis indicates sample **H** has 94% similarity to the others and all of the other samples have a 99% similarity. Nevertheless, 3 groups could be delineated: (Group **1**, **B-F**, **J**)) defined by relatively higher levels of octyl acetate (58.9%-81.0%), but with low levels of diterpenoids (6.6%-18.6%); (Group **2**, **A**, **G**, **I**, **K**) defined by relatively lower levels of octyl acetate (49.5%-61.3%), but with a heightened proportion of diterpenoids (19.0%-22.8%); and (Sample **H**) with octyl acetate (51.6%) and diterpenoids (32.7%).

Discussion

In this study, we aimed to clarify the composition of *B. papyrifera* resin essential oil, using confirmed vouchered single-tree samples. This revealed a composition unlike any other type of frankincense; while most frankincense species have resin essential oils composed primarily of mono- or sesquiterpenes,[2] the samples in this study were dominated by fatty acids and their

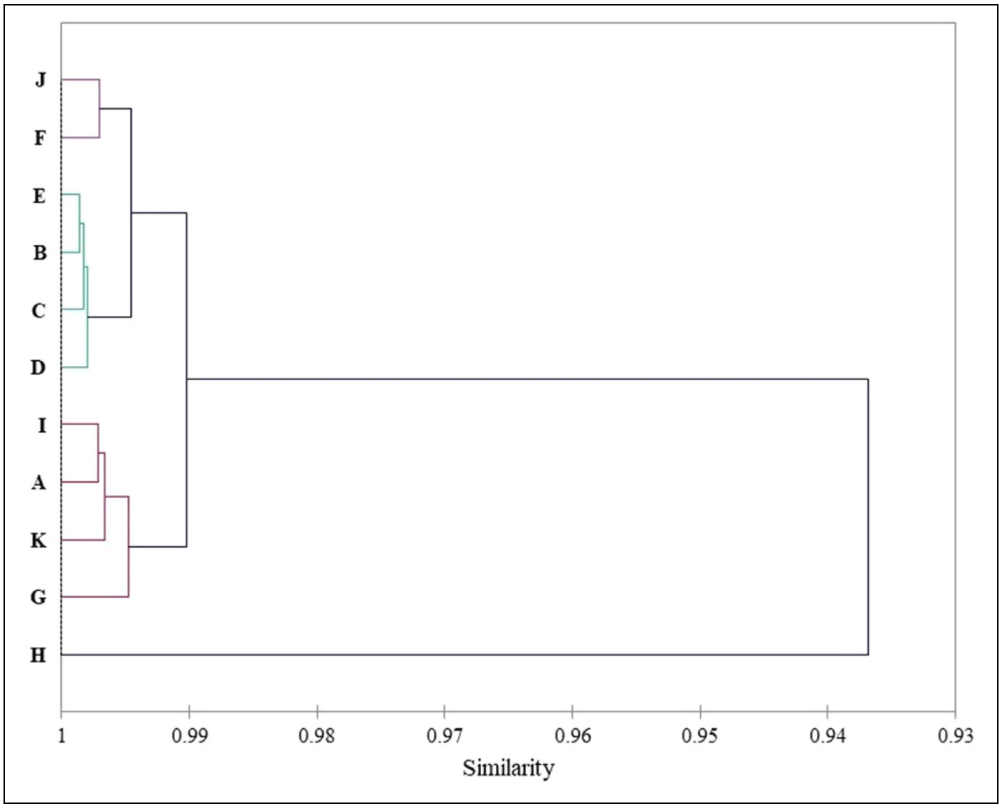

Figure 1. Agglomerative hierarchical cluster (AHC) analysis based on the concentrations of chemical constituents.

derivatives, primarily octyl acetate and octanol. This fits with previous work that reported similar compositions in commercial resins sold as *B papyrifera*.[6–10] Other studies have reported samples of *Boswellia carteri* rich in octyl acetate and octanol; however, these were obtained from commercial sources outside of the species' range state.[11–14] Based on our results and previous work,[15] these were likely misidentified samples of *B papyrifera*.

Also of note is the presence of mesitylene, a benzene derivative, in trace amounts in 2 of the samples. Mesitylene is a common urban VOC resulting from combustion, and its presence in essential oils has been suggested to be potentially indicative of contamination with combustion fumes (pers. comm. with testing labs). As the resin samples in this study were not exposed to combustion fumes, we conclude that the component is naturally occurring; it has been found in other essential oils as well.[16–19]

Other frankincense resin essential oils are markedly different. *Boswellia frereana* produces a variable profile rich in α-thujene and α-pinene, with modest levels of *p*-cymene and sabinene.[9, 11, 20] *Boswellia dalzielii* is high in α-pinene, sometimes with large amounts of myrcene and limonene,[21, 22] while *Boswellia serrata* is typically high in α-thujene with other terpenes such as myrcene, kessane, sabinene, and α-pinene.[23]

Boswellia sacra has a highly variable essential oil composition. The Arabian populations are typically dominated by α-pinene.[24–26] The populations in Somaliland and Somalia (often described under the synonym *B carteri*) are more variable. There is a chemotype rich in α-thujene and *p*-cymene, as well as a variable chemotype rich in α-pinene. The α-pinene chemotype is so far known to break into 3 subgroups: (1) with varying levels of α-pinene, myrcene, sabinene, limonene, and other monoterpenes, (2) dominated by α-pinene with lower levels of limonene, and (3) dominated by limonene with lower levels of α-pinene.[15]

Other *Boswellia* species such as *Boswellia rivae* and *Boswellia neglecta* have been found to contain similar mono- and sesquiterpenes.[6, 7, 11] *B occulta* produces an extremely different resin essential oil, dominated by methoxyalkanes such as methoxydecane and methoxyoctane.[4] Thus, *B papyrifera* and *B occulta* are the only 2 known *Boswellia* resin essential oils that are not dominated by mono- and sesquiterpenes. This is of particular interest currently, as sustainability concerns have led to the current consideration of *B papyrifera* for listing under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Conclusions

In this work, the resin essential oils from individual *B papyrifera* trees from South Kordofan, Sudan have been obtained and analyzed. The authenticity of each resin is established and there is no possibility of contamination or adulteration. The information provided can be used to verify and validate *B papyrifera* essential oils considered for commercialization. A key concern is whether the resin of *B papyrifera* could be distinguished from that of other *Boswellia* species; these results suggest that it is easily distinguished by its chemical composition and scent.

Materials and Methods

Resin Collection

A resin sample from each *B papyrifera* tree was collected by Salah Agieb from the South Kordofan region of Sudan, during June 2020. Samples were collected from trees at least 50 m apart in the Taroabah Natural Forest (11.74° N, 31.06° E) and the Al Faidh Um-Abdalla Natural Forest (11.74° N, 30.88° E). Each resin sample was sealed in a plastic bag and the samples were then dispatched to the Aromatic Plant Research Center (Lehi, UT, USA) for gas chromatographic analysis. A voucher specimen of the tree has been deposited in the Soba Forestry Research Center Herbarium in Khartoum, Sudan (Note: The Soba Forestry Center, where the voucher specimen is deposited does not assign voucher numbers, rather they list by name of species and APRC as the research institution). The voucher specimen was identified by Abdelgadir Ahamed Abdalla.

Resin Hydrodistillation

Each of the *B papyrifera* resin samples was subjected to hydrodistillation using an all-glass Clevenger apparatus as previously described.[15] Hydrodistillation times varied between 3 and 6 h but were continued until no more oil was apparent in the distillate.

Gas Chromatography–Mass Spectrometry

The *B papyrifera* resin essential oils were subjected to GC-MS analysis as previously described[15]: Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments) with a ZB-5 ms capillary column (5% phenyl polydimethylsiloxane, 60 m × 0.25 mm × 0.25 μm film thickness) (Phenomenex); electron impact (EI) mode (electron energy = 70 eV), scan range = 40 to 400 atomic mass units, scan rate = 3.0 scans/s. The GC oven temperature program: start at 50 °C, ramp up to 260 °C (2 °C/min). For each essential oil sample, 1.0 μL of a 5% (w/v) solution in dichloromethane was injected with a splitting mode of 24.5:1. Retention index (RI) values were calculated using a homologous series of *n*-alkanes. The chemical compositions of the essential oils were determined based on comparison of the RI and the mass spectral fragmentation pattern for each component, which were available in the Adams database[27] and our own in-house library.[28]

Gas Chromatography–Flame Ionization Detection

Quantitation of the *B papyrifera* resin essential oil components was accomplished by GC-FID analysis using a Shimadzu GC 2010 (Shimadzu Scientific Instruments) equipped with a flame ionization detector, a split/splitless injector, and Shimadzu AOC-20i autosampler, with a ZB-5 capillary column (60 m × 0.25 mm × 0.25 μm film thickness) (Phenomenex) as previously described.[15]

Hierarchical Cluster Analysis

The *B papyrifera* resin essential oil compositions were used for an agglomerative hierarchical cluster (AHC) analysis. The 11 resin essential oil compositions were treated as operational taxonomic units (OTUs), and the percentages of 8 major components (limonene, (*E*)-β-ocimene, 1-octanol, octyl acetate, (3*E*)-cembrene A, verticilla-4(20),7,11-triene, incensole, and incensyl acetate) were used to determine the compositional similarities between the *B papyrifera* essential oils using XLSTAT Premium, version 2018.1.1.62926 (Addinsoft). Similarity was determined using Pearson correlation, and clustering was defined using the unweighted pair-group method with arithmetic mean (UPGMA).

Acknowledgements

This work was carried out as part of the activities of the Aromatic Plant Research Center (APRC, https://aromaticplant.org/).

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Salah Agieb and Stephen Johnson own shares in companies trading in frankincense products; this paper is not expected to affect those shares. The other author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

Ethical approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

ORCID iD

William N. Setzer https://orcid.org/0000-0002-3639-0528

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