

The Chemical Composition of Single-Tree *Boswellia frereana* Resin Samples

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Abstract

Frankincense is an aromatic terpenoid oleo-gum resin produced by trees in the genus *Boswellia*. It has been used for medicinal and religious purposes for millennia, and is today an important component in perfume and aromatherapy. The resin of *Boswellia frereana* is especially prized, and has been found to contain a high proportion of monoterpenes. However, previous studies have relied on commercial samples; in this study, we characterize the compositions of essential oil and DCM extract samples from 12 individual *B. frereana* trees. The triterpenoid fraction was largely consistent between samples, with lupeol (14.7%–32.5%), α -amyrin (13.0%–25.2%), 3-*epi*-lupeol (6.4%–14.2%), and β -amyrin (5.3%–8.0%) as the primary constituents. The essential oil showed more intersample diversity, but still represented a single, variable chemotype characterized by a moderate to high level of α -thujene (14.5%–43.9%) and a varying, often significant, level of α -pinene (3.0%–63.0%).

Keywords

essential oil, frankincense, olibanum, *Boswellia frereana*, maydi, Somaliland

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Introduction

Frankincense is an aromatic tree exudate, composed of 3 principal fractions: a volatile, typically monoterpene-rich essential oil, a complex polysaccharide gum, and a nonvolatile triterpene-based resin.¹ It has been used in traditional medicine, perfumery, and religious ceremonies for thousands of years. More recently, frankincense has become popular in aromatherapy and other types of alternative and complementary medicine.² Frankincense is produced by members of the genus *Boswellia* (Burseraceae), which is composed of small trees that specialize on arid conditions, often with exfoliating bark, compound leaves, and wind-dispersed seeds. There are ~24 species in the genus, although the exact number has been subject to recent changes.³

The oleo-gum resin essential oils of *Boswellia* vary significant from species to species. *Boswellia papyrifera* Hochst., found in Ethiopia, Eritrea, and Sudan, yields an essential oil dominated by octyl acetate and to a lesser degree octanol.^{4,7} *Boswellia sacra* Flueck. (syn. *Boswellia carteri* Birdw.) from Oman, Yemen, Somaliland, and Somalia, is variably dominated by α -pinene, α -thujene, or limonene, often with significant amounts of myrcene, sabinene, viridiflorol, β -caryophyllene, or *p*-cymene.^{6,9} Most other *Boswellia* species, such as *B. neglecta* S. Moore, *B. serrata* Roxb. ex. Colebr., *B. dalzielii* Hutch., *B. rivae* Engl., and the Socotran *Boswellia*, are similarly rich in these mono- and

sesquiterpenes.^{5,6,10–14} By contrast, *Boswellia occulta* Thulin, DeCarlo, & S.P. Johnson is dominated by methoxydecane and methoxyoctane, unusual components for a plant essential oil.^{15,16}

Boswellia frereana Birdw., endemic to Somaliland and Somalia, is one of the most commonly traded frankincense species. It is known for its large tears of resin, commonly used as chewing gum in the Arab world, and more recently has become popular for its essential oil.² Previous investigations on the essential oil of this species have all been on commercial samples, with mixed results: The different studies show varying levels of α -pinene, α -thujene, *p*-cymene, sabinene, and β -caryophyllene, possibly indicating multiple chemotypes.^{6,9,12} Some commercial *B. frereana* essential oils also contain methoxyalkanes, particularly methoxydecane (decyl methyl ether), and one study found resin

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that visually appeared to be *B. frereana* contained methoxyalkanes.¹⁷ In this study, we therefore aim to clarify the chemotypes of *B. frereana* by presenting a characterization of the oleogum resin essential oils from samples taken directly from individual trees in Somaliland.

Results

The *B. frereana* oleogum resin essential oils from 12 different individual trees were obtained by hydrodistillation in yields around 13% (w/w) as pale-yellow oils. Resin samples from 7 trees were also extracted using dichloromethane.

All essential oils were dominated by α -pinene (3.0%-63.0%) and α -thujene (14.5%-43.9%) (Table 1). Other major components included sabinene (3.3%-8.0%), *p*-cymene (1.7%-13.0%), and β -pinene (0.3%-4.1%). One sample also contained a significant quantity of α -phellandrene (6.0%) and its dimers (10.0%). Three groups were determined by the agglomerative hierarchical clustering (AHC) analysis (Figure 1): (1) one group represented by a single sample and defined by high levels of α -thujene; (2) a group comprising samples with high α -pinene (52.6%-63.0%) and moderate α -thujene (14.5%-17.4%), and (3) a group comprising samples with significant levels of both α -pinene (34.0%-53.5%) and α -thujene (21.4%-33.0%).

While the chemotypic groups of the essential oils were defined by monoterpenes, the extract groups were defined by the triterpenes (Figure 2). While 3 groups were identified, all samples were highly similar, with major components including lupeol (14.7%-32.5%), α -amyrin (13.0%-25.2%), 3-*epi*-lupeol (6.4%-14.2%), α -pinene (0.4%-13.9%), α -thujene (3.6%-12.9%), and β -amyrin (5.3%-8.0%) (Table 2).

Discussion

In this study, we aimed to clarify the chemical composition of the oleogum resin essential oils produced by *Boswellia frereana* (frankincense) trees in Somaliland (northern Somalia). Consistent with previous studies, the oleo-gum resin essential oils were found to contain large amounts of α -pinene and α -thujene, with smaller amounts of sabinene, *p*-cymene, and α -phellandrene dimers.^{6,9,12} However, while the samples showed a range of variation, they were not dissimilar enough to be considered separate chemotypes; rather than 2 distinct chemotypes, one dominated by α -pinene and one dominated by α -thujene, as we expected, most samples contained appreciable levels of both monoterpenes. We conclude that the samples in this study show only a single essential oil chemotype, one characterized by a moderate to high level of α -thujene and a varying, often significant, level of α -pinene.

Methoxyalkanes were not detected, even as trace components, in any of the samples tested. This is particularly interesting given that we sampled *B. frereana* trees sympatric to the *B. occulta* trees known to produce methoxyalkanes.¹⁸ Methoxyalkanes are extremely rare as natural products, having been reported botanically only from *B. occulta* and one cultivar of Chinese tree peony.^{18,19} However, *B. occulta* resin is commonly mixed with other frankincense resins from *B. sacra* and *B. frereana* in Somaliland, leading to commercial essential oils sold as *B. sacra*/*B. carteri* which are in fact a mixture of *B. sacra* and *B. occulta*.¹⁸ Our results do not preclude the possibility that *B. frereana* may produce methoxyalkanes, but we conclude that it is more likely that commercial *B. frereana* essential oils containing methoxydecane or methoxyalkanes are in fact mixtures of *B. frereana* and *B. occulta*.

We found very little variation in the triterpenoid components from tree to tree, even between trees that have relatively

Table 1. Major Constituents (% Area >1) of Essential Oils Distilled From *Boswellia frereana* Individual Tree Oleo-gum Resin Samples.

Compound	A	B	C	D	E	F	G	H	I	J	K	L
α -Thujene	43.9	25.5	16.2	33.1	31.5	32.9	25.9	17.4	14.5	27.3	21.4	16.2
α -Pinene	3.0	37.3	63.0	44.8	34.0	34.5	42.7	60.9	58.4	53.5	43.5	52.6
Thujadiene	1.3	0.4	0.4	0.8	2.2	0.9	1.0	0.1	0.3	0.7	0.4	0.7
Camphene	0.1	0.8	0.2	0.7	0.9	0.8	1.1	1.0	1.8	0.9	1.2	1.5
Sabinene	8.0	5.9	3.3	5.3	6.6	6.9	6.1	3.9	3.3	4.8	5.1	4.2
β -Pinene	0.3	2.8	3.3	2.2	2.4	2.7	3.5	3.4	4.1	2.9	3.4	3.9
α -Phellandrene	6.0	4.6	0.3	0.1	0.2	2.9	1.7	2.5	0.0	0.3	2.6	0.2
<i>p</i> -Cymene	13.0	5.7	3.3	1.9	6.5	5.7	5.1	2.2	3.2	1.7	5.5	5.2
Limonene	2.7	1.8	1.1	0.5	1.0	1.1	1.3	1.1	1.1	0.6	2.1	1.6
β -Phellandrene	2.5	1.3	0.3	0.2	0.3	1.1	1.0	0.7	0.1	0.3	1.0	0.2
1,8-Cineole	0.3	0.8	0.5	1.1	0.5	0.4	0.2	0.7	0.1	0.4	0.3	0.9
β -Thujone	0.5	0.3	0.1	0.5	1.2	0.6	0.3	0.0	0.3	0.2	0.2	0.4
Terpinen-4-ol	1.5	0.6	0.4	1.8	1.1	1.2	0.7	0.4	1.0	0.4	0.5	0.7
Bornyl acetate	0.2	1.3	1.2	0.7	1.3	1.0	1.5	1.0	1.7	1.0	1.5	1.9
β -Bourbonene	0.0	0.0	0.7	0.0	0.0	0.1	0.0	0.0	2.2	1.2	0.0	0.0
α -Phellandrene dimer A (RI 1792)	7.5	3.6	0.6	0.0	0.3	0.8	1.9	1.1	0.1	0.1	4.6	0.7
α -Phellandrene dimer B (RI 1798)	1.3	0.6	0.1	0.0	0.0	0.1	0.3	0.2	0.0	0.0	0.9	0.0
α -Phellandrene dimer C (RI 1826)	1.2	0.5	0.1	0.0	0.0	0.1	0.3	0.1	0.0	0.0	0.7	0.0

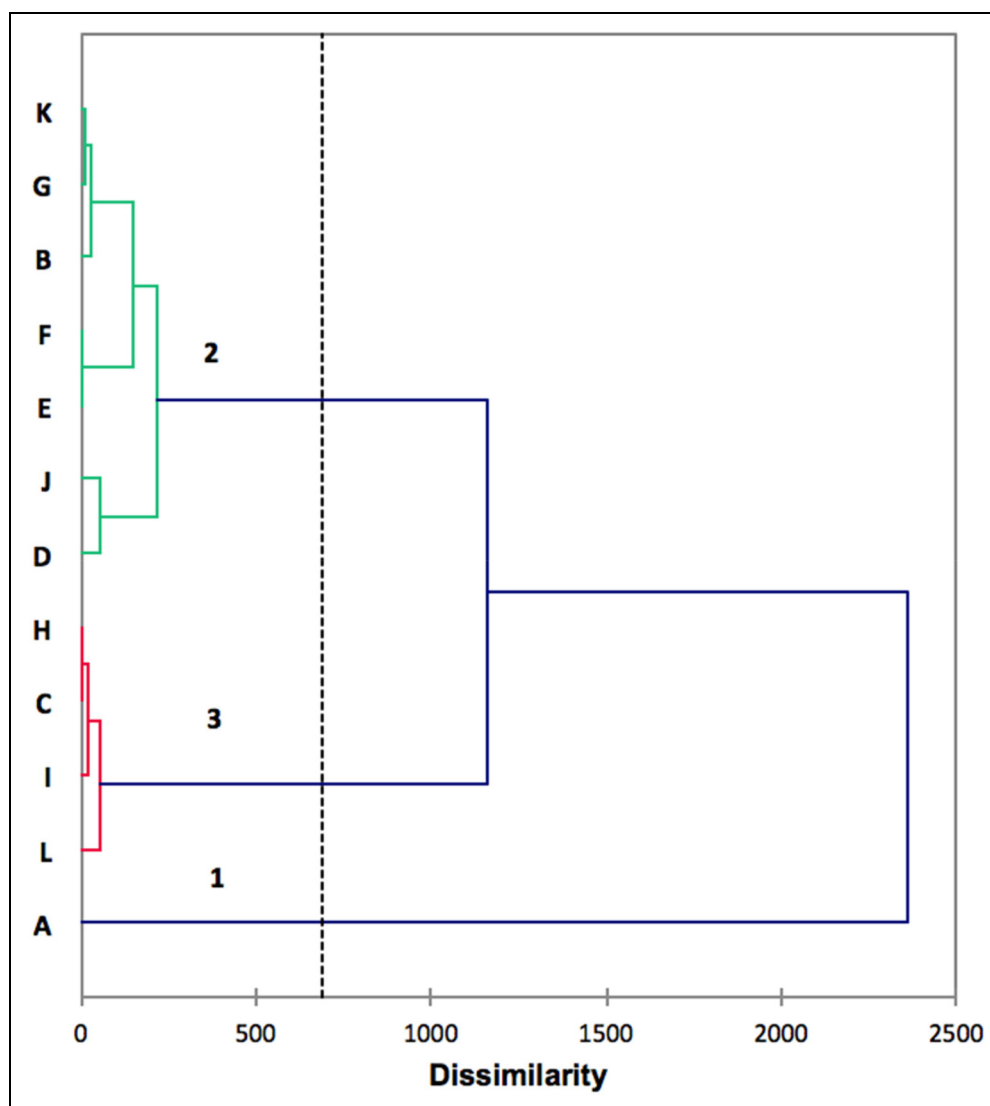


Figure 1. Dendrogram obtained from agglomerative hierarchical cluster analysis of the essential oils distilled from 12 *Boswellia frereana* individual tree oleo-gum resin samples from Somaliland.

different essential oil compositions. Our results were largely consistent with other studies performed on commercial resin samples. As with previous studies, we found no boswellic acids or lupeolic acids, which are common in other *Boswellia* species.^{20,21} This is not surprising, however, considering that these components may not be volatile enough to be detected by GC-MS. Previous work has identified lupeol and 3-*epi*-lupeol as the major triterpenoid components of *B. frereana* resin²¹; we likewise found significant levels of lupeol and 3-*epi*-lupeol. However, we also found α -amyrin and to a lesser degree β -amyrin to be present in significant quantities, components that have been previously reported from *B. frereana* but not in significant quantities.²²

We thus conclude that the samples in this study show a single, somewhat variable chemotype rich in α -thujene, α -pinene, lupeol, and α -amyrin. However, further sampling

across the species' range may reveal additional chemotypes, especially with regard to the triterpenoids.

Methods and Materials

Collection of Resins

Resins from individual *B. frereana* trees were collected by Ahmed Mohamed Dhunkaal from the Sanaag region of Somaliland (northern Somalia), near the town of Ceel Dibir, during October 2019 (Table 3, Figure 3). The resins were sealed in plastic bags and shipped to the Aromatic Plant Research Center (Lehi, UT, USA) for analysis. The trees were photographed and a voucher specimen was collected and deposited in the University of Hargeisa Herbarium (Voucher #HARG000497). The voucher specimen was identified by Faisal Jama.

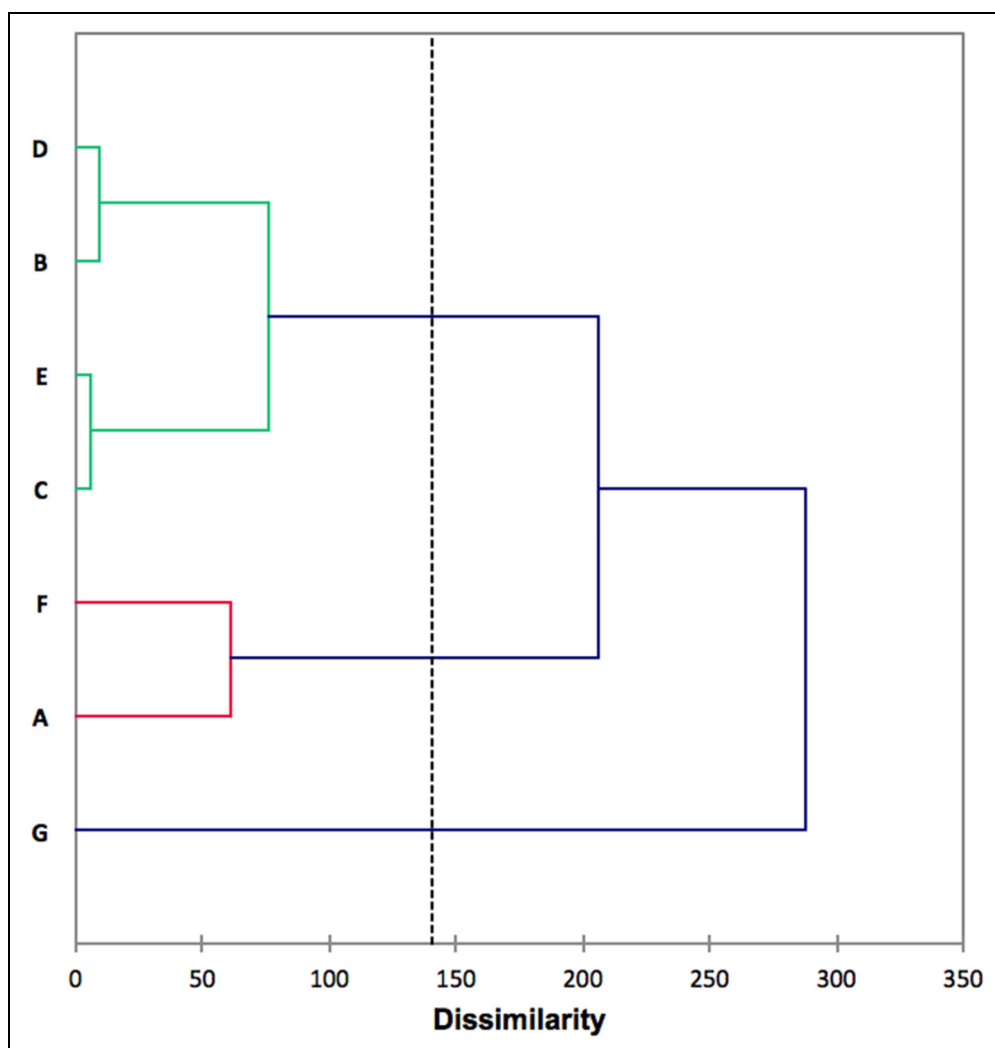


Figure 2. Dendrogram obtained from agglomerative hierarchical cluster analysis of the dichloromethane extracts obtained from 7 *Boswellia frereana* individual tree oleo-gum resin samples from Somaliland.

Table 2. Major Constituents (% Area >1) of Dichloromethane Extracts Obtained From 7 *Boswellia frereana* Individual Tree Oleo-gum Resin Samples.

Compound	RI	A	B	C	D	E	F	G
α -Thujene	926	9.6	6.3	4.6	5.8	3.6	12.9	7.4
α -Pinene	934	0.4	9.9	13.0	9.0	13.9	2.0	12.8
Sabinene	972	1.2	1.0	0.8	1.0	0.7	1.6	1.1
α -Phellandrene	1007	1.8	0.8	0.2	0.0	0.0	0.1	0.3
<i>p</i> -Cymene	1024	1.5	0.7	0.5	0.2	0.3	0.9	0.6
α -Phellandrene dimer A	1798	3.1	2.1	0.4	0.0	0.1	0.3	0.8
β -Amyrin ^a	3345	8.0	5.4	6.4	5.8	6.1	5.5	5.3
Unidentified lanostane	3373	1.7	1.6	1.7	1.3	1.6	1.6	1.7
α -Amyrin ^a	3395	15.2	13.0	17.4	13.6	16.2	13.1	25.2
Lupeol ^a	3402	32.5	30.0	24.0	32.4	26.6	27.9	14.7
Unidentified ursane	3404	0.4	1.2	2.2	1.3	2.3	1.8	2.0
3- <i>epi</i> -Lupeol ^a	3413	6.4	11.8	12.5	10.3	13.1	14.2	12.1
α -Amyrin acetate ^a	3499	1.3	1.4	2.0	1.1	1.9	2.4	1.6
Unidentified triterpenoid	3649	3.7	3.3	2.7	4.6	2.0	3.4	3.3

^aIdentification of triterpenoids is tentative only and largely based on mass spectral fragmentation; there are few retention data reported in the literature for comparison.

Table 3. *Boswellia frereana* Sample Collection Sites Near the Town of Ceel Dibir in the Sanaag Region of Somaliland.

Sample	GPS coordinates	Elevation (m)
A	10.51675N, 46.25948E	551
B	10.51680N, 46.26018E	547
C	10.51790N, 46.26057E	538
D	10.51820N, 46.26072E	535
E	10.47090N, 46.39307E	389
F	10.47030N, 46.39248E	399
G	10.40785N, 46.34010E	429
H	10.41140N, 46.32222E	559
I	10.42993N, 46.31598E	598
J	10.43165N, 46.31735E	595
K	10.40020N, 46.34263E	439
L	10.51617N, 46.25983E	556
M	10.58250N, 47.18528E	518

Hydrodistillation of Resins

Hydrodistillations of the resin samples of *B. frereana* were carried out using an all-glass Clevenger apparatus as previously described.⁸

Dichloromethane Extraction of Resins

Resin samples from 7 individual trees were extracted with dichloromethane at room temperature, filtered, and evaporated to give the *B. frereana* extracts.

Gas Chromatography-Mass Spectrometry

The *B. frereana* resins were analyzed by GC-MS with a Shimadzu GCMS-QP2010 Ultra with ZB-5 capillary column as previously described.⁸ Identification of the chemical components was carried out by comparison of the retention indices determined with respect to a homologous series of normal alkanes and our comparison of their mass spectra with those reported in the literature²³ and our own in-house library.²⁴

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**Figure 3.** Map showing area where *B. frereana* samples were collected (circle).

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: S. Johnson declares a business interest in a company producing products from *B. frereana*; it is not expected that this paper will affect that company. The rest of the authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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