



Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples

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Abstract

Trees from the genus *Boswellia* (Burseraceae) are traditionally used as a medicine, a fumigant, in various cosmetic formulations and in aromatherapy in several countries around the world. This plant produces a commercial oil known as frankincense which has a woody, spicy and haunting smell. Frankincense oil has several pharmacological properties, of which many elude to the anti-infective potential. Variation in the chemical composition of this oil has been reported in literature. These factors prompted an investigation to study the commercial frankincense oils from various international suppliers. Twenty essential oils were analyzed by gas chromatography coupled to mass spectrometry. Considering the major constituents, the oils were found to be qualitatively similar. However, there was immense quantitative variation for certain oil constituents. The components identified and their range in the oils include α -pinene (2.0–64.7%); α -thujene (0.3–52.4%); β -pinene (0.3–13.1%); myrcene (1.1–22.4%); sabinene (0.5–7.0%); limonene (1.3–20.4%); p-cymene (2.7–16.9%) and β -caryophyllene.

FEEDBACK 



► This is the first scientific comparison of the antimicrobial and essential oil composition of a number of commercial frankincense oils. ► The publication highlights differences in constituents and antimicrobial efficacies between samples, thus providing a standard with which to compare quality.

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Keywords

Antimicrobial; *Boswellia* spp.; Essential oil; Gas chromatography coupled to mass spectrometry (GC-MS)

1. Introduction

Frankincense is the common name given to the aromatic resin produced by a group of trees belonging to the genus *Boswellia* (Burseraceae). The three main frankincense-producing species are *Boswellia carteri* Birdw., *B. frereana* Birdw. (Somalia), and *B. serrata* Roxb. from north-western India (Frank et al., 2009). The trees have a pale papery brown bark with a thick brown inner resiniferous layer (Thulin and Warfa, 1987). Incisions are made in the trunks of the aged trees where the resinous exudates are tapped. This gum hardens into an orange-brown gum resin known as olibanum or more commonly known as frankincense. The oil which has a woody, spicy and haunting smell is usually obtained through steam distillation of the frankincense gum resin and remains one of the most important commercial essential oils available on the international market.

The name frankincense is derived from the ancient French term “franc enens” meaning “pure incense” and is possibly best known through the biblical story of the “Three Wise Men” who delivered gold, frankincense and myrrh as gifts for the baby Jesus (Dharmananda, 2003). Frequent references in the Old Testament and ancient texts have subsequently led to the extensive use of frankincense in religious rituals and currently the oils are used as a major ingredient in incense formulations which are burned in Jewish, Roman Catholic



The oil is said to exhibit antiseptic, astringent, cicatrisant and sedative properties and alleviates the pain caused by rheumatism (Shealy, 1998, Stevensen, 1998, Wootton, 2005). There have been a number of publications supporting these various pharmacological claims (Banno et al., 2006, Borrelli et al., 2006, Michie & Cooper, 1991, Singh et al., 2008) and even its use in combination therapy with other medicinal plants has been documented (Dharmananda, 2003, Moussaieff et al., 2005, Scarborough, 1983, Shen & Lou, 2008). A number of randomized clinical trials have been undertaken on *Boswellia* spp. and include studies on asthma, arthritis, collagenous colitis and Crohn's disease (Ernst, 2008).

The chemical composition of various frankincense oils (Al-Harrasi & Al-Saidi, 2008, Başer et al., 2003, Dekebo et al., 1999, Hamm et al., 2005, Kasali et al., 2002, Strappaghetti et al., 1982) and their constituents differ according to the climate, harvest conditions and geographical distribution (Mikhaeil et al., 2003). Frankincense oils on the international market are obtained from several sources and distributed by various companies. Oil obtained from several species are sold under the same name as “frankincense oil” therefore a study was undertaken to record the chemical variation for twenty commercial frankincense oils. Furthermore, as chemical composition inevitably may have an impact on the pharmacological activities, the antimicrobial activities for all twenty samples were comparatively assessed.

2. Materials and methods

2.1. Essential oil analysis

Twenty frankincense oil samples were purchased at various herbal shops or pharmacies. The taxonomic identity of the species is based on labeling information on each of the 20 purchased products and include *Boswellia carteri* ($n = 9$; samples BC1 to BC9), *B. neglecta* ($n = 1$; BN10), *B. sacra* ($n = 2$; BS11 and BS12), *B. thurifera* ($n = 1$; BT13), *B. frereana* ($n = 3$; BF14 to BF16) and *Boswellia* species ($n = 4$; Bsp17 to Bsp20) were sourced from various international suppliers. It should be noted that *B. sacra* and *B. carteri* are today considered synonyms and the former name should have precedence (Thulin and Warfa, 1987). The oils were analyzed using gas chromatography coupled to a mass spectrometer (Agilent 6890 N GC system coupled directly to a 5973 MS) equipped with a HP-Innowax polyethylene glycol column (60 m × 250 μ m i.d. × 0.25 μ m film thickness). A volume of 1 μ l was injected (using a split ratio of 200:1) with an autosampler at 24.79 psi and an inlet temperature of 250 °C. The GC oven temperature was



identifications were made by comparing mass spectra from the total ion chromatogram and retention indices using NIST®, Mass Finder® and Flavour® GC-MS libraries.

The relationship between percentage composition of the oil samples was analysed by cluster analysis using the NTSYS software (Rohlf, 1992). Correlation was selected as a measure of similarity and the unweighted pair-group method with arithmetic average was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) where a very high correlation ranged between 0.90 and 1.00, high between 0.70 and 0.89 and moderate correlation between 0.40 and 0.69.

2.2. Antimicrobial activity

The antimicrobial activity was evaluated using the minimum inhibitory concentration (MIC) microdilution method (Eloff, 1998) taking into account modifications for assaying essential oils (Carson et al., 1995). The following micro-organisms were included; *Staphylococcus aureus* ATCC 12600 and *Bacillus cereus* ATCC 11778 (Gram-positive), *Escherichia coli* ATCC 25922 and *Proteus vulgaris* ATCC 33420 (Gram-negative) and the yeast *Candida albicans* ATCC 10231. Culture selection was based on their related pathogenesis and traditional use. Sterile distilled water (100 µl) was introduced into all wells of a sterile 96 well microtitre plate. The *Boswellia* oils were diluted in acetone at starting stock concentrations of 128 mg/ml and 100 µl was transferred into the first rows. Serial doubling dilutions were performed and the cultures yielding an approximate inoculum size of 1×10^8 colony forming units (CFU)/ml were introduced. Optimal incubation conditions (37 °C for 24 h for bacteria and 48 h for the yeast) followed. Commercial antimicrobials (ciprofloxacin for bacteria and amphotericin B for yeasts) at starting concentrations of 0.01 mg/ml and 0.10 mg/ml respectively were included as positive controls in all MIC repetitions to validate microbial sensitivity. Negative controls were included to confirm that the diluent (acetone) had no effect on antimicrobial activity, the cultures remained pure and that the media was sterile. A 0.4 mg/ml *p*-iodonitrotetrazolium violet solution (INT) was prepared and 40 µl transferred into all the inoculated wells. The microtitre plates inoculated with bacteria were examined after 6 h to determine a colour change in relation to the concentration of microbial growth. The yeast *C. albicans* was examined after 24 h. Assays were undertaken in triplicate and the mean documented in Table 2.



that *B. sacra* (1867) and *B. carteri* (1870) are today considered synonyms and the former name should have precedence (Thulin and Warfa, 1987).

GC-MS results mostly indicate quantitative variation (Table 1). The variable constituents of the oils comprise of: α -pinene (2.0–64.7%); myrcene (1.1–22.4%); sabinene (0.5–7.0%); β -caryophyllene (0.1–10.5%); limonene (1.3–20.4%); α -thujene (0.3–52.4%); *p*-cymene (2.7–16.9%); β -pinene (0.3–13.1%). β -caryophyllene-oxide is present in concentrations \leq 6%. α -Copaene, α -humulene and δ -cadinene are present in concentrations \leq 4.5% (Table 1). The constituent having the most quantitative variability was α -pinene (2.0–64.7%) and α -thujene (0.3–52.4%) (Table 1).

Table 1. Major constituents (% area > 1) of frankincense oil (*Boswellia* spp.) samples.

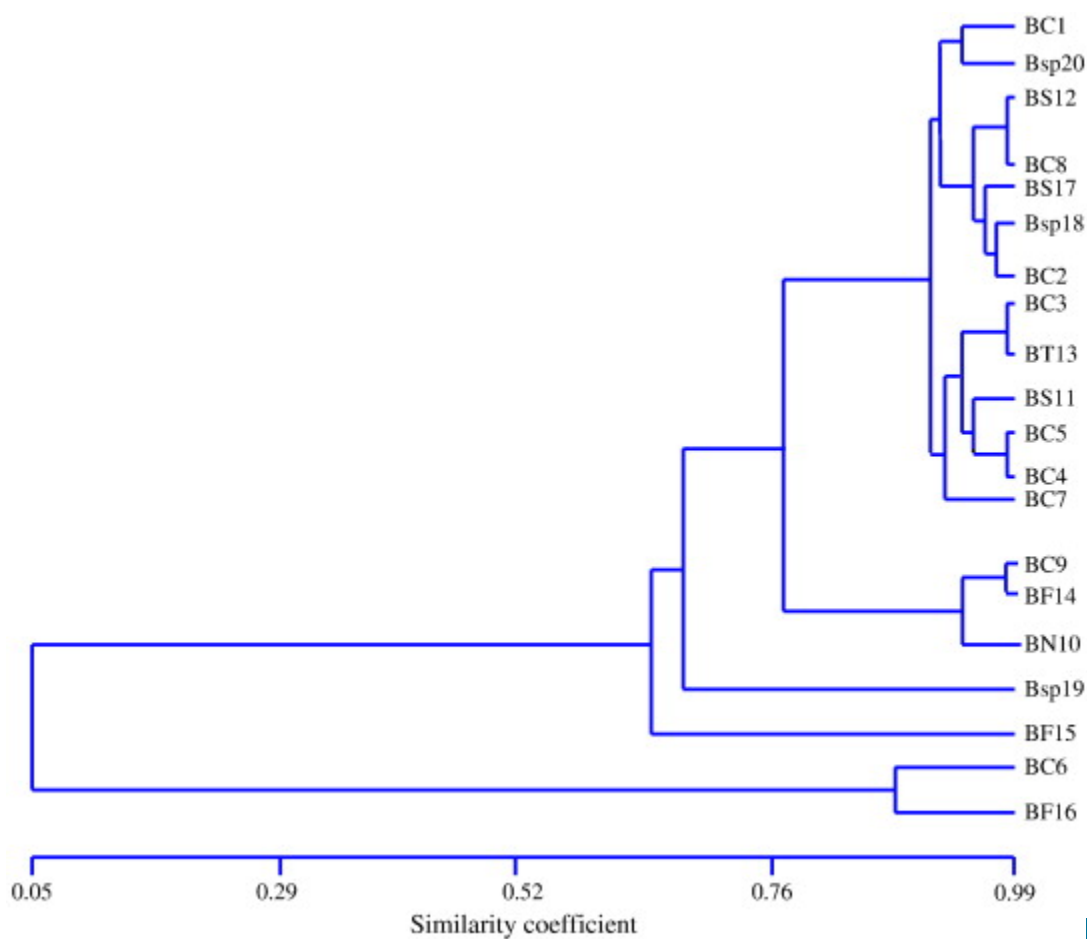
RRI	Compounds	<i>Boswellia</i> spp. oil samples													
		BC1	BC2	BC3	BC4	BC5	BC6	BC7	BC8	BC9	BN10	BS11	BS12	BT13	B
1016	α -Pinene	17.0	22.8	30.1	27.6	40.4	4.8	22.3	23.6	12.0	43.4	22.5	18.3	28.0	6
1019	α -Thujene	4.6	8.3	1.6	7.9	7.7	52.4	2.1	12.0	5.3	4.7	3.9	11.2	0.7	–
1104	β -Pinene	1.1	1.6	1.1	1.4	1.5	0.3	1.1	0.8	1.0	13.1	1.1	0.9	2.0	2
1117	Sabinene	4.4	4.1	3.5	3.9	4.4	5.6	4.3	3.8	0.5	2.3	6.9	4.1	3.8	7
1159	Myrcene	6.8	5.5	6.0	6.8	8.8	4.0	–	2.5	9.9	–	5.5	3.5	5.6	2
1193	Limonene	14.9	14.9	20.4	14.6	15.8	2.6	11.9	18.3	12.7	1.9	11.2	13.1	14.6	3
1270	<i>p</i> -Cymene	6.2	4.7	3.6	4.6	5.3	3.4	3.7	4.5	4.4	8.6	5.9	4.7	2.7	5
1448	α -Copaene	1.6	1.2	1.2	1.0	0.6	0.3	1.6	1.4	1.2	–	1.5	1.3	1.0	–
1596	β -Caryo- phyllene	7.0	8.0	6.9	5.6	2.7	0.3	7.8	6.3	10.5	0.1	7.6	7.2	5.8	–
1674	α Humulene	3.2	2.8	2.8	2.3	1.2	0.2	3.1	2.3	4.4	1.1	2.5	2.7	3.6	–
1763	δ -Cadinene	2.1	2.1	1.5	1.4	–	–	3.0	1.5	3.2	–	1.9			



Total	71.7	78.2	80.3	77.1	88.4	73.9	60.9	79.3	67.8	75.2	70.5	72.1	71.1	8
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BC: *Boswellia carteri*; BN: *Boswellia neglecta*; BS: *Boswellia sacra*; BT: *Boswellia thurifera*; BF: *Boswellia frereana*; Bsp: *Boswellia* species.

These results are supported by the cluster analysis. Several clusters can be observed with samples BC9, BF14 and BN10 being strongly correlated ($r > 0.94$). Similarly, sample BC6 and BF16 also showed a strong correlation due to their high content of α -pinene. A very high correlation was observed between the remaining samples with the exception of sample Bsp19 and BF15 which showed moderate correlation ($r < 0.70$) (Fig. 1). The high correlations obtained between the samples are due to the presence of monoterpenes such as α -pinene, β -pinene, α -thujene, sabinene, myrcene limonene and *p*-cymene which are present in higher quantities (Fig. 1).





The GC-MS data from a variety of *B. carteri* resins studied from the north-east region of Somalia also indicated quantitative variability where α -pinene (10.3–37.7%), α -phellandrene (12.2–41.8%) and limonene (6.4–19.6%) were present as major constituents. The comprehensive review of [Mertens et al. \(2009\)](#) elegantly summarizes the published compositional data of frankincense volatiles and reiterates the chemotypic variation and vast quantitative variance for certain constituents.

When investigating samples obtained from the north western region of Somalia, the only compound present in all six samples was α -pinene ranging from 1.0–62.9% ([Hall, 2000](#)). [Hamm et al. \(2005\)](#) reported the headspace volatiles of various *Boswellia* samples. Major compounds identified in two sources were α -pinene (23.2% and 6.3%), β -myrcene (4.4% and 4.5%), limonene (22.4% and 10.2%), α -copaene (1.6% and 5.5%), β -caryophyllene (6.9% and 16.9%), α -humulene (1.1% and 5.2%); caryophyllene oxide (2.0% and 13.1%). Although our results are mostly congruent with published data, isoincensole and its derivatives, present as biomarkers in previous studies were not detected in any of the 20 samples studied here. An earlier study also documented the presence of α -pinene, (-)-limonene, (+)- α -thujene, *p*-cymene, β -pinene, myrcene and isoincensolein from a *B. carteri* resin sample from Ethiopia. A number of other compounds were also noted; however whether they existed as major or minor constituents is not clear as relative percentages were not noted ([Basar et al., 2001](#)).

3.2. Antimicrobial evaluation

The twenty frankincense oil samples were investigated for their antimicrobial efficacy against five test micro-organisms ([Table 2](#)). Antimicrobial activity against *S. aureus* varied between 4–16 mg/ml, with a mean average of 8.1 ± 2.5 mg/ml depending on the oil sample studied. Essential oils having MIC values of 2 mg/ml or lower are considered to be noteworthy ([Van Vuuren, 2008](#)). In light of this, studies undertaken with the pathogen *B. cereus* exhibited the most noteworthy antimicrobial activity with six samples having MIC values ≤ 2 mg/ml. The greatest variation was noted between samples BC2, BF15 and Bsp20 (MIC values of 1.5 mg/ml) and sample BC8 (8.3 mg/ml). Antimicrobial activity against *E. coli* varied between 4.0–12.8 mg/ml, with a mean average of 6.2 ± 1.8 mg/ml depending on the oil sample investigated. Antimicrobial activity against *P. vulgaris* displayed the greatest variability between samples where MIC values ranged between 2.0 and 12.0 mg/ml with a standard

Table 2. The mean MIC (mg/ml) for frankincense oil samples where $n \geq 3$.

Frankincense oil sample	Species [as noted on the commercial product]	Test pathogen				
		<i>S. aureus</i> ATCC 12600	<i>B. cereus</i> ATCC 11778	<i>E. coli</i> ATCC 5922	<i>P. vulgaris</i> ATCC 33420	<i>C. albicans</i> ATCC 10231
BC1	<i>Boswellia carteri</i>	5.0	4.0	8.0	3.0	12.0
BC2	<i>Boswellia carteri</i>	8.0	1.5	8.0	12.8	8.0
BC3	<i>Boswellia carteri</i>	8.0	4.0	8.0	3.0	6.6
BC4	<i>Boswellia carteri</i>	6.0	2.0	12.0	3.0	8.0
BC5	<i>Boswellia carteri</i>	6.0	3.0	6.0	3.0	8.0
BC6	<i>Boswellia carteri</i>	16.0	4.0	6.0	3.0	8.0
BC7	<i>Boswellia carteri</i>	10.4	4.0	6.0	3.0	5.3
BC8	<i>Boswellia carteri</i>	8.0	8.3	4.0	3.0	8.0
BC9	<i>Boswellia carteri</i>	10.4	4.0	6.0	3.0	5.3
BN10	<i>Boswellia neglecta</i>	6.0	2.0	6.0	3.0	6.6
BS11	<i>Boswellia sacra</i>	4.0	3.0	4.0	3.0	8.0
BS12	<i>Boswellia sacra</i>	8.0	2.0	6.0	3.0	8.0
BT13	<i>Boswellia thurifera</i>	10.0	4.0	6.0	2.0	6.0
BF14	<i>Boswellia frereana</i>	8.0	3.0	6.0	3.0	6.0
BF15	<i>Boswellia frereana</i>	4.0	1.5	4.0	3.0	6.0
BF16	<i>Boswellia frereana</i>	12.0	6.6	4.0	12.8	12.0
Bsp17	<i>Boswellia</i> spp.	9.3	3.0	6.0	5.0	6.0
Bsp18	<i>Boswellia</i> spp.	8.0	3.0	6.0	3.0	6.0



Bsp20	<i>Boswellia spp.</i>	8.0	1.5	6.0	3.0	7.0
Mean with std dev		8.1 ± 2.5	3.4 ± 1.7	6.2 ± 1.8	4.0 ± 3.0	7.4 ± 1.9
Ciprofloxacin control		0.3 × 10 ⁻³	0.2 × 10 ⁻³	0.6 × 10 ⁻³	0.6 × 10 ⁻³	–
Amphotericin control		–	–	–	–	0.6 × 10 ⁻³

Although the antimicrobial efficacies have been studied on various *Boswellia* spp. (Adamu et al., 2005, Adelakun et al., 2001, Baratta et al., 1998, Kudi et al., 1999, Mothana & Lindequist, 2005, Mothana et al., 2009, Schillaci et al., 2008, Weckesser et al., 2007), few studies have recorded the antimicrobial activity of *B. carteri*, *B. frereana*, *B. neglecta* and *B. thurifera*. For *B. carteri*, only two recent studies could be found. Camarda et al. (2007) investigated the antimicrobial efficacy of *B. carteri* against *E. coli*, *P. aeruginosa* and three strains of *S. aureus*. Noteworthy inhibitory activity were found against all pathogens and the highest sensitivity was noted for *P. aeruginosa* with inhibitory activities as low as 6.6 µg/ml. Conversely in another study, albeit using disc diffusion assays, the essential oil of *B. carteri* was investigated for inhibitory activity against a Methicillin resistant *Staphylococcus aureus* (MRSA) strain where it was found to have no inhibitory activity (Chao et al., 2008). In our study, moderate (4.0 mg/ml for samples BS12 and BF15) to poor activity (16.0 mg/ml for sample BC6) was noted against a reference *S. aureus* strain (ATCC 12600). Disc diffusion assays on essential oils are known to be problematic and often yield false negative results due to the volatility of the oil. A proportion of the oil is inclined to be lost due to evaporation (Cos et al., 2006, Janssen et al., 1987, Kalemba & Kunicka, 2003, Pauli and Kubeczka, 1997, Rios & Recio, 2005). This together with sample variation and difference in bacterial strain could explain the variance between our study and that noted previously. Using multivariate techniques no correlation could be found between the antimicrobial activity and the oil composition of various samples.

There is evidence that *Boswellia* spp. exhibits immunomodulatory activity (Badria et al., 2003, Chevrier et al., 2005, Khajuria et al., 2008, Mikhaeil et al., 2003, Pungle et al., 2003). One could therefore postulate that even though the antimicrobial efficacies found in the twenty samples of this study were not all remarkable, they may have a synergistic mode of action, whereby the antimicrobial activity together with immunomodular effect act as a combined anti-infective.

The frankincense oils distributed by various international companies are similar



challenge to develop a quality control protocol to achieve uniformity in the market for this important fragrance material.

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