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 (0.1–10.5%). The antimicrobial activity (minimum inhibition concentration assay) of the oils was investigated against five reference test organisms and the activity ranged from 4–16 mg/ml (Staphylococcus aureus); 1.5–8.3 mg/ml (Bacillus cereus); 4.0–12.0 mg/ml (Escherichia coli); 2.0–12.8 mg/ml (Proteus vulgaris) and 5.3–12.0 mg/ml (Candida albicans).

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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 and Greek Orthodox ceremonies (Wootton, 2005). The ancient Egyptians used frankincense as a fumigant which also was an important ingredient in the embalming process (Shealy, 1998). The myriad of pharmacological properties attributed to frankincense includes its use in the treatment of inflammation, wound healing, skin diseases, urinary tract infections, gynaecological disorders, as an immunostimulant and for the treatment of respiratory infections.

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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 and Cooper, 1991; Singh et al., 2008) and even its use in combination therapy with other medicinal plants has been documented (Dharmananda, 2003; Moussiaeff et al., 2005; Scarborough, 1983; Shen and Lou, 2008). A number of randomized clinical trials have been undertaken on Boswellia spp. and include studies on asthma, arthritis, collageneous colitis and Crohn’s disease (Ernst, 2008).

The chemical composition of various frankincense oils (Al-Harrasi and Al-Saidi, 2008; Bašer 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 (Mikhaiel et al., 1982). 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 μm i.d. × 0.25 μm film thickness). A volume of 1 μl 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 60 °C for 10 min, then 220 °C at a rate of 4 °C/ min for 10 min and followed by a temperature of 240 °C at a rate of 1 °C/min. Helium was used as carrier gas at a constant flow of 1.2 ml/min. Spectra were obtained on electron impact at 70 eV, scanning from 35 to 550 m/z. The percentage composition of the individual components were quantified by integration measurements using flame ionization detection (FID, 250 °C) and n-alkanes were used as reference points in the calculation of relative retention indices (RRI). Component 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 1×10^6 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.

3. Results and discussion

3.1. Chemical variation

The twenty commercial frankincense oils samples were sourced from various international outlets. Although the oil samples are labeled differently by the suppliers, it should be noted 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%); sabine (0.5–7.0%); β-caryophyllene (0.1–10.5%); limonene (1.3–20.4%);
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, isoicenone 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 isoicenonein 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 deviation of ±3.0. Sample BT13 exhibited the highest

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] | *S. aureus* ATCC 12600 | *B. cereus* ATCC 11778 | *E. coli* ATCC 5922 | *P. vulgaris* ATCC 33420 | *C. albicans* ATCC 10231 |
|--------------------------|---------------------------------------------|------------------------|-----------------------|-------------------------|--------------------------|--------------------------|
| BC1 Boswellia carteri    | 5.0                                         | 4.0                    | 8.0                   | 3.0                     | 12.0                     |
| BC2 Boswellia carteri    | 8.0                                         | 1.5                    | 8.0                   | 12.8                    | 8.0                      |
| BC3 Boswellia carteri    | 8.0                                         | 4.0                    | 8.0                   | 3.0                     | 6.6                      |
| BC4 Boswellia carteri    | 6.0                                         | 2.0                    | 12.0                  | 3.0                     | 8.0                      |
| BC5 Boswellia carteri    | 6.0                                         | 3.0                    | 6.0                   | 3.0                     | 8.0                      |
| BC6 Boswellia carteri    | 16.0                                        | 4.0                    | 6.0                   | 3.0                     | 8.0                      |
| BC7 Boswellia carteri    | 10.4                                        | 4.0                    | 6.0                   | 3.0                     | 5.3                      |
| BC8 Boswellia carteri    | 8.0                                         | 8.3                    | 4.0                   | 3.0                     | 8.0                      |
| BC9 Boswellia carteri    | 10.4                                        | 4.0                    | 6.0                   | 3.0                     | 5.3                      |
| BN10 Boswellia neglecta | 6.0                                         | 2.0                    | 6.0                   | 3.0                     | 6.6                      |
| BS11 Boswellia sacra     | 4.0                                         | 3.0                    | 4.0                   | 3.0                     | 8.0                      |
| BS12 Boswellia sacra     | 8.0                                         | 2.0                    | 6.0                   | 3.0                     | 8.0                      |
| BT13 Boswellia thurifera | 10.0                                        | 4.0                    | 6.0                   | 3.0                     | 6.0                      |
| BF14 Boswellia freeneana | 8.0                                         | 3.0                    | 6.0                   | 3.0                     | 6.0                      |
| BF15 Boswellia freeneana | 4.0                                         | 1.5                    | 4.0                   | 3.0                     | 6.0                      |
| BF16 Boswellia freeneana | 12.0                                        | 6.6                    | 4.0                   | 12.8                    | 12.0                     |
| Bsp17 Boswellia spp.     | 9.3                                         | 3.0                    | 6.0                   | 5.0                     | 6.0                      |
| Bsp18 Boswellia spp.     | 8.0                                         | 3.0                    | 6.0                   | 3.0                     | 6.0                      |
| Bsp19 Boswellia spp.     | 6.0                                         | 4.0                    | 6.0                   | 3.0                     | 8.0                      |
| Bsp20 Boswellia spp.     | 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               |
sensitivity (2.0 mg/ml) towards the Gram-negative pathogens studied. Antimicrobial activity against C. albicans demonstrated moderate to poor activity with MIC values ranging between 5.3–12.0 mg/ml, with a mean average of 7.4±1.9 mg/ml.

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 and Lindequeist, 2005; Mothana et al., 2009; Schillaci et al., 2008; Weckesser et al., 2007), few studies have recorded the antimicrobial activity of B. carteri, B. freerea, 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 and Kunicka, 2003; Pauli and Kubeczka, 1997; Rios and 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 immunomodulatory effect act as a combined anti-infective.

The frankincense oils distributed by various international companies are similar in their composition with mostly quantitative variation noted. The oils exhibited good to poor in vitro antimicrobial activity (1.5–16 mg/ml) depending on the pathogen studied. Seemingly no industry standard in terms of oil composition exists which defines a good frankincense oil. Quality assessment is mostly of a chemosensory nature and it still remains unclear what the contribution of the individual compounds are to the specific organoleptic properties which defines frankincense. In view of the commercial interest in frankincense it remains an import challenge to develop a quality control protocol to achieve uniformity in the market for this important fragrance material.

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