Chemical Variation in Essential Oils from the Oleo-gum Resin of *Boswellia carteri*: A Preliminary Investigation

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Abstract
Frankincense, the oleo-gum resin of *Boswellia* species, has been an important element of traditional medicine for thousands of years. Frankincense is still used for oral hygiene, to treat wounds, and for its calming effects. Different *Boswellia* species show different chemical profiles, and *B. carteri*, in particular, has shown wide variation in essential oil composition. In order to provide insight into the chemical variability in authentic *B. carteri* oleoresin samples, a hierarchical cluster analysis of 42 chemical compositions of *B. carteri* oleo-gum resin essential oils has revealed at least three different chemotypes, (I) an α-pinene-rich chemotype, (II) an α-thujene-rich chemotype, and (III) a methoxydecane-rich chemotype.

Keywords: Frankincense, hierarchical cluster analysis, chemotypes, gas chromatography – mass spectrometry

Introduction

Frankincense has been used for millennia for its medicinal and aromatic properties, across the ancient and modern worlds. It appears in Ayurvedic and Chinese traditional medicine, in European, Middle Eastern, and indigenous medical traditions, and as a key component of religious ceremonies, making it one of the world's oldest globally-traded commodities.[1–10] Frankincense itself is a terpenoid oleo-gum-resin produced by trees in the genus *Boswellia*, which naturally serves as a deterrent to herbivory, insect attack, infection, and other potential damage.[11,12] *Boswellia* trees are characterized by papery, often exfoliating bark, compound leaves, and deciduousness, and are distributed across Africa, Arabia, and India.[13] While most species are tapped for local use, only a few species produce resins that are traded in significant volume: *Boswellia sacra, B. carteri, B. frereana, B. papyrifera*, and *B. serrata*. The resins are either used whole as medicine and incense, or are distilled into essential oil.

Boswellia carteri produces one of the highest quality frankincense resins. *B. carteri* and *B. sacra* are generally recognized as a single, highly variable species.[14] However, the two show differences in morphology, growth form, and resin chemistry, and therefore we here consider *B. carteri* as a separate species.[14,15] *B. carteri* is native to Somaliland and Somalia, growing on

limestone and volcanic rock between 5-1500 m in elevation.[3,14] The trees are tapped throughout their range by making incisions into the bark and collecting the exuded resin; traditional knowledge holds that resin yield and quality are affected by the trees' environment and management[16,17] (A. DeCarlo, personal communication with local elders). Traditionally tapping is done from April to September, but tapping intensity has increased over the past 5-10 years due to increased market interest[17] (A. DeCarlo, personal observations).

A perusal of the literature reveals very different chemical compositions of *Boswellia carteri* resin essential oils. Thus, for example, several commercial essential oils were rich in α-pinene,[18–20] others were rich in α-thujene,[19] octyl acetate,[21–23] β-caryophyllene,[24] or dihydrocitronellyl acetate.[25] However, analysis of commercial essential oil samples does not reveal any information about the botanical origin or quality of the resins, or if the oils had been adulterated.[26] In order to establish a baseline chemical profile from which to carry out more extensive analyses, we have analyzed 42 essential oils from *Boswellia carteri* by gas chromatography – mass spectrometry. The botanical identification of the source trees for all resin samples has been confirmed, each of the resin samples was hydrodistilled using the same (Clevenger) technique, and all essential oils were analyzed using GC-MS by the same operators under the same GC conditions.

Results and Discussion

Essential oils from the oleo-gum resins of *Boswellia carteri* (frankincense), collected from Somaliland or Puntland, Somalia, were obtained by hydrodistillation in yields ranging from 2.75% to 8.16% as pale yellow oils, which were analyzed by gas chromatography – mass spectrometry.

Hierarchical cluster analysis of the 42 essential oil compositions of *B. carteri* oleo-gum resins revealed five clusters based on dissimilarity (Fig. 1): (**Ia**) α-pinene/myrcene/ sabinene/limonene, (**Ib**) α-pinene/limonene, (**Ic**) limonene/α-pinene, (**II**) α-thujene, and (**III**) methoxydecane. Clusters **Ia-c** are all rich in α-pinene, and likely represent subgroups of the same chemotype. The essential oil compositions making up cluster **III**, however, are rich in methoxydecane, but poor in monoterpene hydrocarbons and represent a definitive chemotype. Group **II** is made up of essential oils dominated by α-thujene with lesser concentrations of *p*-cymene and also seems to be a distinct chemotype.

Boswellia carteri resin essential oils rich in α-pinene have been previously described in the literature.[18–20,24] Likewise, α-thujene-rich *B. carteri* essential oils have been described.[19] In this present study, we do not observe any *B. carteri* resin oils to be dominated by octyl acetate. While octyl acetate is often observed (19 of the 42 samples), the concentrations are relatively low (maximum concentration, 14.2%). In contrast, there have been several reports of octyl acetate-rich *B. carteri* resin oils. Abdel Wahab and co-workers described an essential oil from *B. carteri* resin from Somalia, but purchased from a drug market in Cairo, Egypt, to contain 60.0%

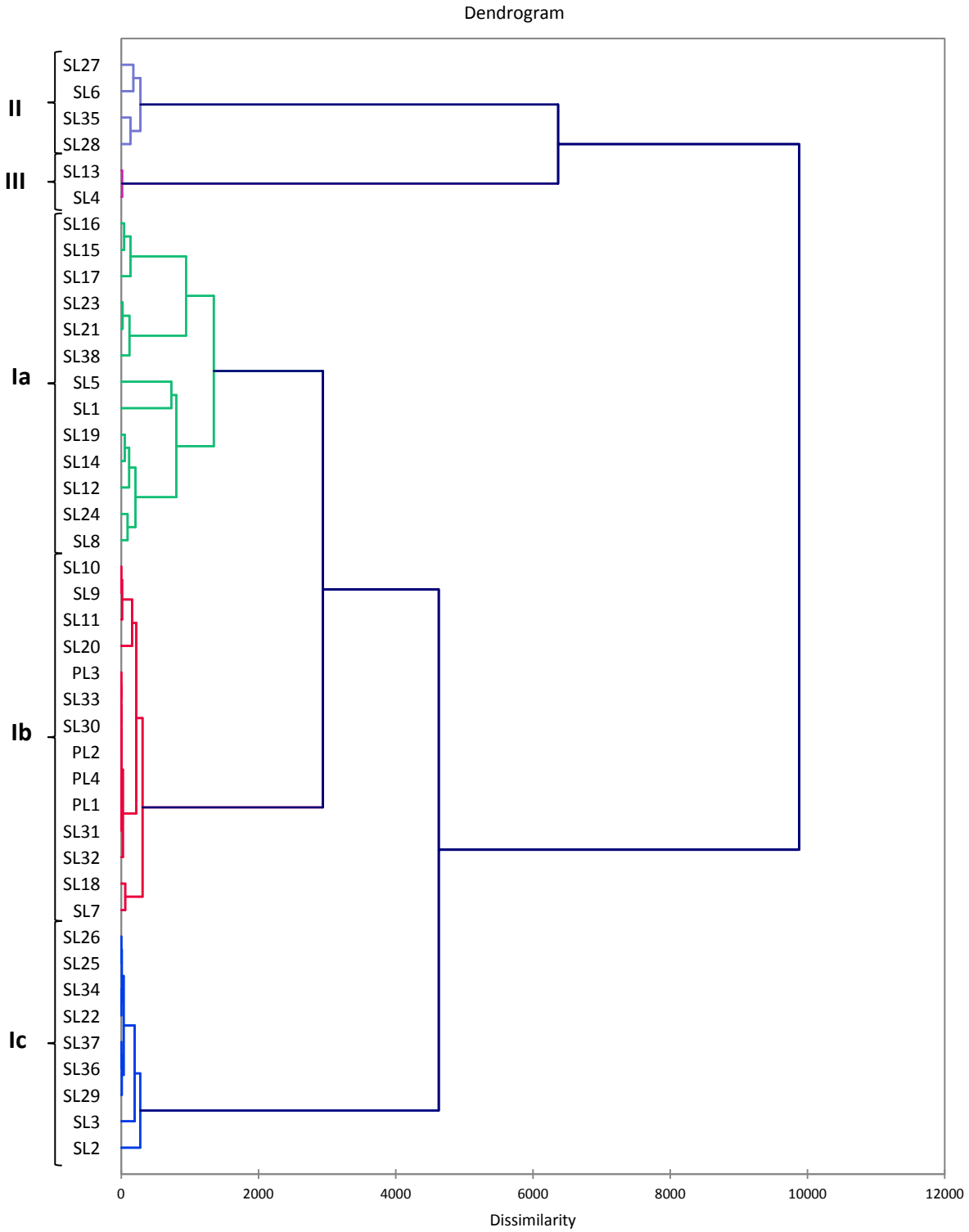

Figure 1. Dendrogram obtained from the agglomerative hierarchical cluster analysis of 42 *Boswellia carteri* oleo-gum resin essential oil compositions. "SL" refers to samples from Somaliland; "PL" refers to samples collected from Puntland, Somalia.

octyl acetate and 12.7% octanol.[21] Likewise, Simla Basar analyzed the essential oil from *B. carteri* resin from Egypt and found 39.3% octyl acetate and 11.9% octanol,[27] while a commercial resin sample (Minardi, Bagnacavallo-Ravenna, Italy) was composed of 45.2% octyl acetate and 3.1% octanol.[23] Interestingly, *Boswellia papyrifera* resin essential oils are dominated by octanol (3.4-17.8%) and octyl acetate (56.0-65.7%),[18,24,28–30] so it may be that the so-called *B. carteri* resins were mislabeled, the sources misidentified, or obtained from *B. carteri/B. papyrifera* hybrids.

Although β-caryophyllene is observed in most *B. carteri* resin oil samples (37 of 42) in this study, the concentrations are relatively low (maximum concentration, 5.3%). There are commercial *B. carteri* essential oils that are rich in β-caryophyllene, however, including a sample from Scents of the Earth (Sun City, CA, USA) with 66.9% β-caryophyllene[24] and a sample from Améo Essential Oils (Zija International, Lehi, UT, USA) with 22.2% β-caryophyllene (W.N. Setzer, unpublished). Therefore, although not identified in the present study, there may also be a β-caryophyllene-rich chemotype of *B. carteri*.

The methoxydecane chemotype of *B. carteri* has been previously described.[31] These workers found oleo-gum resin oils rich in both methoxyoctane (5.5-11.7%) and methoxydecane (30.7-54.9%), consistent with the five essential oil compositions found in cluster **III**. The methoxydecane-rich chemotype, therefore, represents either a very distinct chemotype of *B. carteri* or a new taxon of *Boswellia*.

Conclusions

There are five different chemical clusters based on the chemical compositions of *Boswellia carteri* oleo-gum resin essential oils, with at least three distinct chemotypes based on the hierarchical cluster analysis in this study, a chemotype rich in α-pinene, a chemotype rich in α-thujene, and a chemotype dominated by methoxydecane. It is not apparent what factors may be involved in the chemical variations observed, but may include abiotic factors such as sunlight, precipitation, elevation, soil composition; or may involve human disturbance such as overgrazing or frankincense overharvesting.[32] Current research is underway in the field and in our laboratories to find correlations between environmental and human factors with *Boswellia* essential oil chemistry.

Experimental Section

Plant Resin Material

Boswellia carteri resin samples were either collected (January-February, 2017) by our field team (A. DeCarlo and S. Johnson) directly from trees growing in Somaliland and identified by S. Johnson, or obtained from suppliers in Somaliland or Puntland, which were collected by local indigenous tree tappers. Samples directly from trees were taken opportunistically, as local security permitted; commercial samples were taken from regional warehouses, with locations, landowners, and origins indicated by markings on the bags. These warehouses are located a

few hours from the harvesting areas and are the first stop where resins are stored before sorting. We have a high degree of certainty of the botanical provenance of the commercial samples, as they were taken during spontaneous visits to the warehouses, preventing preparation of deception. Additionally, we sampled only from bags from locations the field team previously visited and confirmed to be producing *B. carteri*. *B. carteri* resins were packed in plastic bags and shipped to dōTERRA International (Pleasant Grove, UT) for hydrodistillation and analysis.

Hydrodistillation

Hydrodistillations of *Boswellia carteri* oleoresin samples were carried out in an all-glass Clevenger-type apparatus. The resin and water were mixed in a ratio of 1:6 and the mixture was stirred constantly during hydrodistillation. Hydrodistillation times varied (180-300 min) but were continued until no more oil was apparent in the distillate.

Gas Chromatographic – Mass Spectral Analysis

Each of the essential oils of *Boswellia carteri* oleoresins were analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. Injector temperature was 250 °C and the ion source temperature was 200 °C. The GC oven temperature program was programmed for 50 °C initial temperature, temperature increased at a rate of 2 °C/min to 260 °C. A 5% *w*/*v* solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature,[33] and stored in our in-house library.[34]

Hierarchical Cluster Analysis

The chemical compositions of the *B. carteri* oleo-gum resin essential oils, obtained from dōTERRA International (Pleasant Grove, UT), were used in the cluster analysis. The 42 essential oil compositions were treated as operational taxonomic units (OTUs), and the concentrations (percentages) of 57 components were used to determine the chemical associations between these frankincense essential oils using agglomerative hierarchical cluster (AHC) analysis using XLSTAT Premium, version 19.5.47159 (Addinsoft, Paris, France). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method.

Supplementary Material

Boswellia carteri oleoresin essential oil compositions used in the cluster analysis.

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Author Contribution Statement

A.D. and S.J. collected the oleoresin samples; A.P. and P.S. collected the GC-MS data; L.B. carried out hydrodistillation of the resins; W.N.S. analyzed the GC-MS data. All authors contributed to the writing and editing of the manuscript.

Conflicts of Interest Statement

The authors declare no conflicts of interest. The funding sponsor, dōTERRA International, played no role in the design of the study; in the collection, analysis, or interpretation of the data; conclusions of the study; or in the decision to publish the results.

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