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Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients?

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ABSTRACT

Ethnopharmacological relevance: Essential oils obtained from resins of *Boswellia carteri* Birdw. and *Commiphora myrrha* (Nees) Engl., commonly known as frankincense and true myrrh respectively, have been used extensively since 2800 BCE for the treatment of skin sores, wounds, teeth, inflammation, and urinary tract diseases in traditional medicine; for preparation of mummification balms and unguents; and also as incense and perfumes. Since ancient times, burning of frankincense and myrrh in places of worship for spiritual purposes and contemplation (a ubiquitous practice across various religions) had hygienic functions, to refine the smell and reduce contagion by purifying the indoor air.

Aim of the study: The general purpose of the study was to assess the *in vitro* antimicrobial potential of the liquid and vapour phases of *B. carteri* and *C. myrrha* essential oils and burn incense, as well as to test the effectiveness of their *in situ* application to cleanse microbially-contaminated air within the ambient of an investigated 17th-century church.

Materials and methods: The chemical composition of *B. carteri* and *C. myrrha* essential oils, obtained by hydrodistillation of frankincense and true myrrh oleo gum resins was determined using GC/MS, and antimicrobial properties of their liquid and vapour phases were assessed by the broth microdilution and microatmosphere diffusion methods. Chemical analysis of burn incense fume obtained using bottle gas washing with dichloromethane as a solvent was performed by GC/MS, while its antimicrobial activity was evaluated using a modified microatmosphere diffusion method to evaluate germination inhibition for fungi and CFU count reduction for bacteria. The *in situ* antimicrobial activity of *B. carteri* burn incense and essential oil vapour phase was assessed in the sealed nave and diaconicon of the church, respectively.

Results: The dominant compounds of *B. carteri* EO were *α*-pinene (38.41%) and myrcene (15.21%), while *C. myrrha* EO was characterized by high content of furanoeudesma-1,3-diene (17.65%), followed by curzerene (12.97%), *β*-elemene (12.70%), and germacrene B (12.15%). Burn incense fume and soot had *α*-pinene (68.6%) and incensole (28.6%) as the most dominant compounds, respectively. *In vitro* antimicrobial assays demonstrated high bacterial and fungal sensitivity to the liquid and vapour phases of EOs, and burn incense fume. *In situ* application of *B. carteri* EO vapour and incense fume resulted in reduction of air-borne viable microbial counts by up to 45.39 ± 2.83% for fungi and 67.56 ± 3.12% for bacteria (EO); and by up to 80.43 ± 2.07% for fungi and 91.43 ± 1.26% for bacteria (incense fume).

Conclusions: The antimicrobial properties of essential oil derived from frankincense, a compound with well-known traditional use, showed that it possesses a clear potential as a natural antimicrobial agent. Moreover, the results suggest possible application of *B. carteri* EO vapour and incense fume as occasional air purifiers in sacral ambients, apart from daily church rituals.

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1. Introduction

Microorganisms, including archaea, bacteria and fungi, in addition to lichens and insect pests, are constantly causing a multitude of problems for the conservation of heritage monuments, as well as all types of historical artefacts stored and exhibited in museums and private art collections, due to their pronounced biodeteriorative potential (Sterflinger and Piñar, 2013). To repair recent or progressive microbiological damage, a limited range of physical and chemical methods are available nowadays (Allsopp et al., 2004). Chemical treatments, conducted using liquid biocides and/or fumigation, imply utilization of a small number of synthetic biocides approved by the European Union's Biocidal Products Directive (BPD) (EU No. 528/2012). In the selection of an appropriate agent to be used for the control of microbial contamination of heritage premises, several characteristics, such as pronounced antimicrobial potential, minimal or lack of toxicity for the personnel present in the immediate vicinity, low risks of environmental pollution, and no interference with structural components of art works, must be taken into consideration. Natural products (specialized metabolites) obtained from plants used in traditional medicine throughout the world, products such as essential oils (EOs), are known to possess antimicrobial capacity, and, regardless of the lack of sufficient scientific evidence, are considered more or less harmless to humans. This has prompted worldwide application of EOs in medicine, aromatherapy, and various forms of consumer products (Lahlou, 2004; Camarda et al., 2007). Since EOs are predominantly composed of various types of chemical compounds, ones such as terpenes, terpenoids, aldehydes, alcohols *etc.,* many of which are volatile (Laird and Phillips, 2011), they represent natural alternatives to commercial synthetic agents, since microorganisms are less likely to develop resistance.

Plants from the genera *Boswellia* and *Commiphora* (Burseraceae), which occur solely in dry and arid regions of the southern part of the Arabian Peninsula (Yemen and Oman), India, Madagascar, north-east Africa, Somalia, Kenya, Ethiopia and the Sudan, produce the culturally and commercially important oleo gum resins frankincense or olibanum and myrrh, respectively (Baser et al., 2003; Hamm et al., 2005). Hardened aromatic resinous exudates are for the most part obtained naturally or from incisions made in the bark of *Boswellia carteri* Birdw. and *Commiphora myrrha* (Nees) Engl., the most important sources of olibanum and true myrrh. The EOs obtained by hydrodistillation of these gum resins are usually very dense and have a warm, sweet, and spicy scent (Mikhaeil et al., 2003).

Historically, the use of frankincense and myrrh dates back to 2800 BCE, when as mentioned in ancient Egyptian medical records, they were used as burn incense and in perfumes, as well as for the preparation of unguents and balm for mummification. Their combined use is well documented in the 'Papyrus Ebers', a collection of prescriptions dating from approximately 1500 BCE wherein they were prescribed for the treatment of skin sores and wounds (Michie and Cooper, 1991). Thus, frankincense and myrrh were well known at the time of writing of the Bible, in which they were extensively cited and are considered the most often mentioned aromatic resins (De Rapper et al., 2012). In Christianity, gold, frankincense, and myrrh are key parts of the *Christmas story*, as they were brought as gifts for the baby Jesus by the Three Wise Men (Balthasar, Melchior and Gaspar) (Gospel of Matthew 2:11, New Testament). In the past, burning of frankincense and myrrh in temples and other places of worship produced fumes used to reduce the smell and contagion by purifying the air (Michie and Cooper, 1991). The antimicrobial properties of frankincense and myrrh vapours and oils have been known since the 11th century BCE, when the Sumerians used myrrh to treat teeth and intestinal parasitic worms, while the antimicrobial use of frankincense can be traced back to the 11th century CE, when the Persian philosopher and physician Avicenna applied frankincense oil to treat inflammation and infections of the urinary tract (Michie and Cooper, 1991). Nowadays, numerous studies have been undertaken in support of the traditional use of frankincense and myrrh as immune-enhancing, anaesthetic, antiallergenic, antiinflammatory, antirheumatic, antianxiety, antidepressive, anticancer, antioxidant, and antimicrobial agents (Michie and Cooper, 1991; De Rapper et al., 2012; Shen et al., 2012).

The principal goal of this study was to evaluate the *in vitro* and *in situ* antimicrobial potential of burn incense, as well as the liquid and vapour phases of *B. carteri* and *C. myrrha* EOs obtained by hydrodistillation of olibanum and true myrrh resins against bacteria and fungi isolated from the air of an investigated church.

"*Bearing in mind the wisdom of the ancients, in this article we demonstrate the value of incense burning above and beyond just for religious ceremony by presenting evidence indicating powerful antimicrobial activity of Boswellia carteri essential oil and burn incense fume. This evidence suggests their possible use as air purifiers in sacral ambients.*"

Authors

2. Material and methods

2.1. Experimental heritage site

The old Church of the Holy Ascension is located on the southwestern slopes of the Suva Planina Mountains, in the village of Veliki Krčimir (Gornje Zaplanje, Gadžin Han, Serbia) (43° 05′ 28″ N, 22° 12′ 40″ E). Built in the 17th century of large blocks of dressed stone (siga) in lime mortar, it represents an elongated hemisphaerical vaulted structure (6.5 × 12 m) with a semicircular apse covered with a gabled roof. With a pair of pilasters along the side walls the interior is divided into two aisles. Fragments of the church's wall paintings with scenes from the Old and New Testaments are still preserved mostly in the nave, in the altar area and on the western façade (Deljanin, 1995). At the present time, the church is categorized as a cultural monument of great importance by a directive of the Institute for Protection of Cultural Monuments of Niš (SK305, "Official Gazette of RS" No. 28/83).

2.2. Tested microbial isolates

2.2.1. Fungal isolates and culture conditions

The following fungal isolates were used for *in vitro* determination of the antifungal efficiency of EOs and burnt incense fume: *Aspergillus flavus* (BEOFB 313m), *Aspergillus niger* (BEOFB 343m), *Aspergillus europaeus* (BEOFB 381m), *Cladosporium cladosporioides* (BEOFB 1821m), *Cladosporium uredinicola* (BEOFB 1841m), *Curvularia australiensis* (BEOFB 713 m), *Penicillium bilaiae* (BEOFB 1131m), *Penicillium lanosum* (BEOFB 1161m), and *Penicillium atrosanguineum* (BEOFB 1171m). The fungi used in this study are saprotrophic or pathogenic/toxigenic species with cosmopolitan distribution, predominantly airborne and small-spored (Florian, 2004), obtained from air of the investigated church (Unković et al., 2017) and determined *via* ITS I and β-tubulin gene sequencing. Isolates were maintained in cryovials with 1.5 mL of 30% glycerol at − 75 °C (Vivar et al., 2013) deposited in the fungal culture collection of the University of Belgrade - Faculty of Biology (BEOFB). Conidia suspensions (1.0×10^5 CFU mL^{-1}) were prepared according to the protocol given in Unković et al. (2018) and stored at − 20 °C. Prior to experiments, dilutions of the inocula were cultured on solid MEA to check their validity and verify the absence of contamination.

2.2.2. Bacterial isolates and culture conditions

Bacterial isolates were obtained from air of the investigated church using an air sampler (MAS-100 Eco, Merck) with airflow set to 100 L min^{-1}. The BHI (brain heart infusion, Lab M) and TSA (tryptic soy agar, Merck) growth media were used for cultivation of bacteria. Isolates were incubated at 30 °C for 24 h, aerobically. On the basis of morphological analysis and the Gram reaction, 23 bacterial isolates were considered for further analysis. Suspensions were adjusted to