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Chemical analysis of incense smokes used in Shaxi, Southwest China: A novel methodological approach in ethnobotany

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ABSTRACT

Aim of the study: Characterization and comparative analysis of the main VOCs (volatile organic compounds) present in the smoke of 11 experimentally combusted plant species used as incense in Shaxi, Southwest China. Substances which may be responsible for the pleasant smell of the smokes as well as substances with a potential pharmacological activity are discussed.
Materials and methods: We adopt the dynamic headspace sorption method for the collection of smoke samples as a novel methodological approach in ethnobotany. The VOCs were identified using gas chromatography–mass spectrometry (GC–MS). Principal component analysis and canonical discriminant analysis were performed using PASW statistics (Version 18.0.2).
Results: Among the identified compounds were 10 monoterpenoids, 7 sesquiterpenoids, 6 linear hydrocarbons, 6 methoxy phenolics, 2 benzenoids, 2 polycyclic aromatic hydrocarbons, and 2 fatty acids. Based on their volatile profiles, the species are well clustered intraspecifically and separated interspecifically. The most abundant among the compounds potentially responsible for the pleasant smells of the smokes are methyl salicylate (12.28 ± 3.90%) for *Gaultheria fragrantissima* leaves, δ-cadinene (15.58 ± 2.29%) for *Juniperus squamata* wood, and α-Pinene for *Cupressus funebris* branches (9.16 ± 7.73%) and *Pistacia weinmanniifolia* branches (19.52 ± 8.66%). A couple of substances found are known for pharmacological activity, such as methylsalycilate, beta-caryophyllene and cedrol.
Conclusions: The species used by the local people in Shaxi for incense differ clearly with respect to the chemical compounds of their smoke. Further, incense contains substances, which are of pharmacological interest and might support medicinal uses of smoke. Cedrol with its pleasant smell and sedative properties may be an important factor why specific plants are chosen as incense. Our findings support the idea that the effects of the use of incense as well as medicinal smoke depend on both, the cultural as well as the pharmacological context.

1. Introduction

Plant-derived smokes are used in medicinal as well as ritual contexts worldwide. As a vector for drug administration, plant derived-smokes are used for a wide range of indications in different medicinal systems around the globe (Mohagheghzadeh et al., 2006; Braithwaite et al., 2008). Also, the use of incense in rituals is documented for many cultures (e.g. Case et al., 2003; Bhattarai et al., 2006; Weckerle et al., 2006). Medicinal applications are reported for approximately 740 plant species while ritual and religious uses are documented for around 400 species (Pennacchio et al., 2010:4). Plant-derived smokes can be inhaled actively or passively as ambient smoke. The volatile combustion products present in smoke are formed through processes like hydrolysis, oxidation, dehydration and pyrolysis (Simoneit, 2002). While some of these substances, such as benzopyrenes, are known to be toxic others may be of pharmacological significance. These two aspects of smoke, the pharmacological and the cultural, can blend or intertwine, such as with the ancient use of tobacco as incense, medicine, and for religious purposes (Robicsek, 2004). In medicine, the intertwining of cultural and pharmacological aspects of healing has been put forward with the concepts of the placebo effect, and later, the meaning response (Moerman and Jonas, 2002). The meaning response conceptualizes how both the administered drug and the cultural meaning of the treatment context influence the efficacy of a medicinal treatment. Therefore, in analogy to medicine, it might be argued that the effects that rituals including incense have on human beings are also dependent on both the cultural and the pharmacological context.

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Chemical analyses of the composition of incenses have mainly been focusing on derived essential oils and extracts (e.g. Roveri et al., 1998; Basar et al., 2001; Case et al., 2003; Basar, 2005). Studies on the volatile combustion products of incense species and their derivatives are relatively rare (Basar, 2005; Hamm et al., 2005; Feist et al., 2007; Yamada and Yatagai, 2007). The latter study identified large numbers of preponderantly cyclic or polycyclic hydrocarbons including monoterpenes, sesquiterpenes, as well as diterpenes in the smoke of Sugi, a leafy incense made from the Japanese Cedar *Cryptomeria japonica* (Thunb. ex L. f.) D. Don, Cupressaceae.

Here we aim at the characterization and comparative analysis of the main VOCs (volatile organic compounds) present in the smoke of experimentally combusted plant species used as incense in Shaxi, Southwest China. Substances which may be responsible for the pleasant smell of the smokes as well as substances with a potential pharmacological activity are discussed. We adopt the dynamic headspace sorption method for the collection of smoke samples as a novel methodological approach in ethnobotany. This VOC-collection technique is widely applied for chemical investigation of VOCs in areas such as floral scent ecology, perfume industry, or food chemistry (e.g. Fabre et al., 2002; Kaiser, 2006; Schiestl et al., 2010). In contrast to alternative methods it allows not only for smoke collection in the laboratory but also in the field and is thus very suitable for ethnobotanical studies. Compared to the indirect approach of analysing incense distillates or extracts this sampling technique allows for a direct collection and analysis of incense in its locally used form.

1.1. Incense plants in Shaxi, Southwest China

Among the rural population of Southwest China the use of incense plants either burned freshly, dried, or in the form of joss sticks is very common. They are used to communicate with deities and spirits of the surroundings but also for the treatment of diseases such as respiratory ailments (Weckerle et al., 2006; and unpublished data). This study builds on our ethnobotanical research in Shaxi, northwest Yunnan, China, which started in 2005 and is continuing since (Weckerle et al., 2009; Huber et al., 2010). The area belongs to the eastern Himalayan foothills and is mainly inhabited by the Tibeto-Burman Bai people (for details see Staub and Weckerle, submitted for publication). In Shaxi, powdered incense plants are used and either burned in a censer or as joss sticks. Incense is burned for both the communication with spiritual entities and for personal well-being. Incense powders always comprise several plant species, foremost among these are *Cupressus funebris*, *Gaultheria fragrantissima*, and *Ligustrum sempervirens* (for details see Staub and Weckerle, submitted for publication). Here, we analyse the composition of smoke of the main eleven species used for the production of incense powders in Shaxi.

2. Methodology

2.1. Plant material

Table 1 gives an overview of the analysed incense plants and the respective voucher specimens. Plant material for smoke analysis was air dried in plaited baskets in the shadow. Specimens are deposited at the herbarium of the Kunming Institute of Botany (KUN) and the herbarium of the University of Zurich (Z/ZT). Research was conducted according to the Convention on Biological Diversity (CBD).

2.2. Smoke collection

Smoke was collected using the dynamic headspace sorption method (Huber et al., 2005), using a glass filter filled with 4 mg of Porapak Q (Mesh size 80/100; Alltech Associates Inc., Deerfield, IL, USA) enclosed by a layer of quartz wool and glass beads (0.3 mm, Merck KGaA, Darmstadt, Germany) on both sides. One adsorbent trap was placed inside a polyethylene terephthalate (PET) cooking bag that was closed at one end. One gram of dried plant material was burned in a fuming hood using a butane gas-fuelled micro torch (Prince, Micro Torch GT-3000, Japan). The same plant organs were burned as used by the local people for production of incense powders (see Table 2). Once the flame died out and the plant material smoldered, the bag was placed over the smoke plume, and 1.5 l of air was drawn through the filter which was connected to a battery-operated vacuum pump (Personal Air Sampler; SKC Inc., Eighty-Four, PA, USA) using PTFE tubing (Maagtechnic, Dübendorf, Switzerland). Before use, all the filters were cleaned with 100 μl acetone and 300 μl hexane and left to dry. Surrounding air samples were taken as control samples for ambient contaminants. After sampling, the trapped volatiles were eluted with 50 μl hexane. All samples were stored in sealed glass vials at −20 °C for subsequent gas chromatographic (GC) analysis.

Table 1
Analysed incense plants.

Scientific name	Family	Specimen numbers
Artemisia sp.	Asteraceae	100523–4/1 100530–1/1
Chamaecyparis cf. *obtusa* (Siebold & Zuccarini) Endlicher	Cupressaceae	100530–1/4
Cornus oblonga Wallich	Cornaceae	100529–3/1 100608–1/1
Cupressus funebris Endlicher	Cupressaceae	100523–3/1 100608–1/3 100606–1/1
Gaultheria fragrantissima Wallich	Ericaceae	100530–1/6 100619–1/1
Juniperus squamata Buchanan-Hamilton ex D. Don	Cupressaceae	100530–1/5
Ligustrum sempervirens (Franchet) Lingelsheim	Oleaceae	100529–2/1 100602–1/1 100608–1/4 100622–1/1 100609–3/1
Lyonia ovalifolia (Wallich) Drude	Ericaceae	100609–4/2
Pistacia weinmanniifolia J. Poisson ex Franchet	Anacardiaceae	100609–2/1
Ternstroemia gymnanthera (Wight & Arnott) Beddome	Theaceae	100609–4/1

2.3. Chemical analysis

Quantitative analysis of the smoke samples was conducted using gas chromatography with flame ionization detection (GC-FID; Agilent 6890N; Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with an HP5 column (5%-Phenyl-methylpolysiloxane, 30 m × 0.32 mm i.d. × 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). Hydrogen served as carrier gas at a flow of 1.8 ml min^{-1}. To all samples, 100 ng of n-octadecane (purity 99.8%; Fluka, Buchs, Switzerland) was added as internal standard. One microlitre of each sample was injected pulsed splitless (inlet temperature 300 °C). The oven was programmed to heat from 50 °C to a maximum temperature of 300 °C at a rate of 10 °C min^{-1}; the oven was then kept at 300 °C for a 3 min post run. Chromatogram outputs were recorded by the Chemstation program (Agilent Technologies, Version E. 02.00.493) for qualitative and quantitative analysis.

The volatile compounds were identified using gas chromatography–mass spectrometry (GC–MS). The GC–MS runs were performed on an Agilent 6890N gas chromatograph coupled with an Agilent 5975 mass selective detector (GC; Agilent Technologies, Palo Alto, CA, USA) equipped with an HP5-MS column