1. Purpose

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*Boswellia* resins are described in numerous ancient texts and have been an important trade material for the civilizations located in the Arabian Peninsula and North Africa since at least the third millennium BCE. Frankincense (olibanum) is an exudate that seeps from injured bark of *Boswellia* species (Burseraceae). The oleogum resin obtained from *Boswellia serrata* is called Indian frankincense and is used in the Ayurvedic, Siddha, and Unani systems of traditional medicine. Additionally, its extracts and essential oils are used in soaps, cosmetics, foods, beverages, and incense products. This Laboratory Guidance Document aims to review the analytical methods used to authenticate natural oleogum resin from *B. serrata* and differentiate it from other *Boswellia* species, as well as other potential adulterants. This document can be used in conjunction with the *B. serrata* Botanical Adulterants Prevention Bulletin published by the ABC-AHP-NCNPR Botanical Adulterants Prevention Program in 2018.¹ From a historical perspective, a number of oleogum resins from *Boswellia* species have been used interchangeably for medicinal purposes around the world, and older “olibanum” pharmacopeial mono-
graphs consider more than one species as officially acceptable. Such interchangeable use is still observed today as several *Boswellia* species are offered as frankincense.\(^2,3\) However, Western botanical dietary supplements and the herbal medicine markets are dominated by products labeled to contain *B. serrata*, irrespective of whether a formal identification of the ingredient has been performed or not. Therefore, this laboratory guidance document has been written to help laboratory analysts to find appropriate analytical methods that allow the unambiguous identification of *B. serrata* oleogum resin and its extracts.

2. Scope

“*Boswellia serrata* oleogum resin” and “*Boswellia serrata* extract” monographs were issued in the *United States Pharmacopeia* (USP 33 NF 28) since 2010, and an official quality standards monograph has been published in the *European Pharmacopoeia* (Ph. Eur.) for Indian frankincense (*Olibanum indicum*) that will be used as an active ingredient of registered herbal medicinal products in the European Community.\(^4\) Additionally, a number of methods for *B. serrata* oleogum resin analysis are available in the peer-reviewed scientific literature. Thus, the review of analytical methods described here aims to identify the strengths and limitations of existing methods for differentiating *B. serrata* oleogum resin from other *Boswellia* species oleogum resins and its potential adulterants. Analysts can use this document to help guide the appropriate choice of techniques for their specific *B. serrata* products for qualitative purposes. Further, this document can be used by academic researchers and governmental regulatory laboratories for their specific purposes. The positive evaluation of a specific method for testing *B. serrata* material in the products’ particular matrix in this Laboratory Guidance Document does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratory using accepted protocols. Such protocols are outlined in the US Food and Drug Administration’s Final Rule for Current Good Manufacturing Practices for Dietary Supplements (21 CFR Part 111), by AOAC (Association of Official Analytical Chemists) International, International Organization for Standardization (ISO), World Health Organization (WHO), International Conference on Harmonisation (ICH), and national pharmacopeial bodies, as may be applicable, depending on the regulatory requirements of the country in which the *B. serrata* oleogum resin is being offered for sale, resale, and/or processing into finished consumer products.
3. Common and Scientific Names

3.1 Common names: Indian frankincense,\textsuperscript{5} boswellia, Indian olibanum-tree\textsuperscript{6}

3.2 Other common names:

\begin{itemize}
\item Arabic: kuurdur\textsuperscript{7}
\item Bengali: luban, salai,\textsuperscript{7} salgai\textsuperscript{8}
\item Chinese: chi ye ru xiang (齿叶乳香)
\item French: arbre à encens\textsuperscript{6}
\item German: Indischer Weihrauch\textsuperscript{9}
\item Hindi: madi, saler, salga, sali,\textsuperscript{7} labana,\textsuperscript{8} salia guggul,\textsuperscript{10} anduk, gugal, halar, kundur loban, lobhan, luban, salaga, salai, salar, salaran, salhe, sel-gond, vellakkun-turukkam\textsuperscript{11}
\item Italian: incenso indiano, olibano indiano\textsuperscript{12}
\item Marathi: salai cha dink,\textsuperscript{8} anduk, pahadidhup, saalayi, salai, salaphali, salphulia\textsuperscript{11}
\item Spanish: arbre à encens\textsuperscript{6}
\item Tamil: gugulu, parangisambirani, kundrikam, kungli, morada,\textsuperscript{7} kundurukam,\textsuperscript{8} parangisambirani, kungili\textsuperscript{10}
\item Telugu: anduga, kondagugi tamu\textsuperscript{6}
\item Tibetan: bo ga dkar po, bog dkar pa, po ga dkar po\textsuperscript{11}
\item Turkish: hint günülk ağacı\textsuperscript{14}
\end{itemize}

3.3 Latin binomial: Boswellia serrata Roxb.\textsuperscript{15,16}

3.4 Synonyms: Boswellia balsamifera Spreng., Boswellia glabra Roxb., Boswellia thurifera Roxb. ex Fleming, Boswellia serrata var. glabra (Roxb.) A.W.Benn., Chloroxylon dupada Buch.-Ham., Libanotus asiaticus Stackh., Liban us thuriferus Colebr.\textsuperscript{9,15,17}

3.5 Botanical family: Burseraceae

4. Botanical Description and Geographical Range

Many of the Boswellia species have been described multiple times with different names, which led to taxonomic confusion and nomenclatural controversies in the literature.\textsuperscript{18} Hence, the currently accepted scientific names are used, while the synonyms used in the original literature cited are noted in parenthesis throughout this document. In some publications, B. carteri is sometimes spelled B. carterii; the former spelling is correct and used throughout this document.

The genus Boswellia includes 28 species of trees and shrubs characterized by latex, oils, gum, and resin formation.\textsuperscript{15,18} In addition to B. serrata, the gum resin of B. freereana Birdw. (elemi frankincense), B. papyrifera (Caill. ex Delile) Hochst. (elephant tree), and B. sacra Flück., syn. B. carteri Birdw. (frankincense, olibanum) are also traded internationally under the common name frankincense.\textsuperscript{19-24} The oleogum resin is collected from wild trees by making incisions in the bark of the trunks and thick branches. Once the milky substance oozes out from the incision point, it is solidified by exposure to air.\textsuperscript{4,25} This air-dried gum resin exudate of B. serrata is called Indian frankincense, or Olibanum indicum, in the European Pharmacopoeia.\textsuperscript{26}

The American Herbal Products Association's Herbs of Commerce, 2nd edition, which specifies the common names for botanicals in the US botanical trade, assigns the common name Indian frankincense to B. serrata.\textsuperscript{5}

Boswellia serrata is a medium-to-large deciduous tree up to 18 m (59 ft.) in height and 2.4 m (7.87 ft.) in circumference. The bark is greyish green with outer flaking papery thin layers and a thick ashy to a reddish inner layer. Leaves are alternate, exstipulate, imparipinnate, 20-45 cm (7.87-17.7 in.) in length; leaflets are 2.5-8 cm x 0.8-1.5 cm (0.98-3.15 in. x 0.3-0.6 in.), sessile, lanceolate to ovate, crenate. Flowers are white, in stout racemes, 10-20 cm (3.9-7.87 in.) long, shorter than the leaves. The calyx is persistent, pubescent outside, 5-to-7-toothed; teeth are small, deltoid. Petals are 5-7 erect, free, and 0.5 cm (0.19 in.) long. Fruits are 1.3 cm (0.5 in.) long, trigonous, with three valves and three heart-shaped, 1-seeded pyrenes, winged, along with the margins. The specific name, serrata, means having toothlike projections on the edge and is referring to the toothed leaf margins.\textsuperscript{7,9}

Boswellia serrata habitats are marginal soils of limited fertility, often hot, dry, rocky ridges and slopes, as well as flat terrain in Bangladesh, India, Pakistan, and Sri Lanka, although it is critically endangered and possibly extinct in Sri Lanka.\textsuperscript{1,27,28} It occurs in 16 states of India and based on population surveys in India, forms relatively healthy forests, although projections suggest a slow decline in the tree population.\textsuperscript{27,29,30}

The geographical sourcing of Indian frankincense can inform marketers of the species they may have. Boswellia freereana is native to the Horn of Africa, in particular north-eastern Somalia. Boswellia papyrifera has a disjunct distribution between northeast tropical Africa (Chad, Eritrea, Ethiopia, Nigeria, Sudan, Uganda) and west tropical Africa (Cameroon, Central African Republic, Nigeria). Boswellia sacra (syn. B. carteri) is native to the Arabian Peninsula (Oman...
5. Adulterants and Confounding Materials of *Boswellia serrata* oleogum resin

For companies claiming to trade *B. serrata* oleogum resin, the inclusion of oleogum resin from other species, even other *Boswellia* species historically traded as frankincense, are considered adulterants. Therefore, these companies must ensure that the oleogum resin from other commercially important species such as *B. sacra* (syn. *B. carteri*), *B. frereana*, and *B. papyrifera* are not present. The oleogum resins from the latter species are imported from countries in the Gulf region and Africa and are sold in Indian markets under the same trade name used for *B. serrata* (*kundur*; in Ayurvedic texts *B. serrata* oleogum resin is also referred to as *shallaki*, *kunduru*, and *salai-guggul*). Some reports indicate that the oleogum resin of *Garuga pinnata* is sometimes sold as *B. serrata*. A survey on the quality of European and American botanical dietary supplements labeled to contain the oleogum resin of *B. serrata* (except one of the 17 products, which was labeled as “Boswellia extract”) showed the presence of *B. sacra* and *B. frereana* oleogum resins. Additionally, *B. papyrifera* and resins from tree species of the family Pinaceae are also known adulterants of *B. serrata* oleogum resin. Due to increasing scarcity of *Boswellia* trees in certain areas of India, instances of replacement of the tree oleogum with bark or with oil collected near the tree have been reported.

6. Identification and Distinction Using Macro-anatomical Characteristics

*Boswellia serrata* oleogum resin consists of translucent, small, roundish, or irregularly shaped, variable-sized pieces up to 3 cm (1.8 in). They sometimes form agglomerated masses up to 5 cm (1.96 in) long and 2 cm (0.8 in.) thick. The surface of the resin is covered with grey dust. The fracture is dull or slightly glossy, the fractured surface is waxy and translucent.

### Table 1. Scientific names, family, and common names of confounding species and adulterants of *Boswellia serrata* oleogum resin

<table>
<thead>
<tr>
<th>Species a</th>
<th>Synonym(s) a</th>
<th>Family</th>
<th>Common name b,c,d</th>
<th>Other common names b,c,d</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Boswellia frereana</em> Birdw.</td>
<td></td>
<td>Burseraceae</td>
<td>Elemi frankincense</td>
<td>African elemi, elemi, olibanum tree</td>
</tr>
</tbody>
</table>

a According to Plants of the World Online, Kew Science
b According to Medicinal Plants Names Services, Kew Science
c According to Flora of Peninsular India
d According to the American Herbal Products Association's *Herbs of Commerce*, 2nd edition
* Since there is no detailed information about the Pinaceae resins, they are not included in the table.
which the tree was grown (see section 8). Hence, studies on macro-anatomical differences among oleogum resins of *Boswellia* spp. are limited; macroscopic identification is inadequate to authenticate *B. serrata* oleogum resin or detect adulteration.

### 7. Identification and Distinction using Micro-anatomical Characteristics

Microscopically, rectangular cork cells, very few yellowish oil globules, and numerous small or large, oval to round, or rhomboidal crystalline resin fragments (Figure 1a) are present in *B. serrata* oleogum resin powder.9 Although high quality resin samples contain hardly any bark tissue fragments, dissolving resin samples in ethanol helps find any such plant tissues, but this ethanol-dissolved resin is rather sticky and difficult to study under the microscope. After this cleaning process, the most abundant tissues are cork cells (Figure 1d); prismatic calcium oxalate crystals up to 40 μm in diameter and mainly lined up along the sclerenchyma fibers (Figure 1e); or parenchymal cells. Debris from fibers, resin canals (Figure 1b and 1c), xylem consisting of lignified vessels occurring in small groups, isolated, ovoid, or rectangular sclereids with channeled walls (Figure 1f), and stone cells with thickened walls are occasionally visible.

Despite a comprehensive literature search, no information was found on distinctive micro-anatomical characteristics of the resins of other Boswellia species. A microscopic investigation of several oleogum resin samples, including *B. dalzielii*, *B. frereana*, *B. neglecta*, *B. papyrifera*, *B. rivae*, *B. sacra*, *B. serrata*, and *Commiphora myrrha* did not provide sufficiently clear distinction criteria, although the two myrrh samples contained a large amount of stone cells (Figures 2a and 2b) that might be helpful to differentiate between the species. However, more data is needed to confirm this. Based on the currently available information, it is not possible to distinguish *B. serrata* oleogum resin from others and detect adulterations by microscopic test methods.

### 8. Organoleptic Identification

*Boswellia serrata* oleogum resin is greenish-yellow, reddish-brown, or dull yellow to orange in color, and the color changes according to the quality. Generally, the oleogum resin is graded into four groups according to its flavor, color, shape, and size. The superfine grade is translucent, very light, yellow, free from bark and other impurities; quality I is brownish yellow, less translucent, and free from bark and impurities; quality II is brownish, semitranslucent, and may have some impurities; and quality III is dark brown, opaque and with impurities.8,9,26,39,40 The odor of *B. serrata* oleogum resin is slightly aromatic, characteristically balsamiferous, and sweet, and it tastes somewhat bitter and pungent. It burns readily and emanates an agreeable characteristic balsamic resinous aroma. The resin of *B. sacra* exhibits a yellow-brown color after drying while the resin of *B. frereana* is pale yellowish. The resin of *B. frereana* is not bitter like that of *B. sacra* and is much appreciated for chewing particularly among Arabs. When burnt as incense the fragrance of *B. frereana* is less heavy than that of *B. sacra*.9,39,41,42 The fragrance of *B. papyrifera* resin is somewhat sweet and comparable to an orange due to its high octyl acetate content, a fragrance quite specific to *B. papyrifera*.43

It is known that exposure to moisture can change the color and shape of the *B. serrata* oleogum resin, and significant variations exist between different species, provenances, climates, resin flow-inducing treatments, age classes, and population structures.18 Additionally, autoxidation, polymerization, and enzy-
matic reactions cause the darkening of the color of the resin. The identification at species-level of the major raw Boswellia products in trade (i.e., gums, resins, extracts) is reportedly possible by experienced traders, based on color and taste. However, it remains unclear if species identification at the global level by non-experts would be feasible, and whether the available tests are sufficiently reliable to identify lesser-known species or highly processed and deliberately adulterated products.27

**Genetic Identification and Distinction**

Genetic studies on Boswellia species have mainly focused on two aims—revealing the phylogenetic relationships among Boswellia species and evaluating the inter- and intra-population genetic diversity for the conservation of threatened species. However, those studies were carried out using Boswellia leaves.18,44-46 There are only a few studies on the genetic identification and distinction of B. serrata oleogum resin. Two methods described in the literature were evaluated in this review: Shanmughanandhan et al.47 and Tripathi et al.48

Shanmughanandhan et al. used the DNA sequences of the genomic regions rbcL and ITS2 as barcodes, and a DNA reference library for 187 species of Indian herbs to assess the botanical authenticity and potential adulteration of 93 retail herbal products purchased in India. The authors tested only one B. serrata product, in which DNA from a Lamiaceae species (rbcL) and fenugreek (Trigonella foenum-graecum, Fabaceae) (ITS2) were detected.47 As these species have never been recorded as adulterants before, the authors suggested that these results might have been due to incidental pieces of woody plant materials that adhered to the sticky gums, proving the sensitivity of the DNA molecular diagnostic tool in detecting the foreign matter.

In India, Commiphora wightii gum (guggul) is sometimes adulterated with “salai guggul gum” that is obtained from B. serrata. The aroma of both gums is similar, and it is difficult to notice the adulteration by morphological investigations. A DNA-based method was generated for the detection of B. serrata gum as an adulterant in oleogum resin of C. wightii. A single set of primers was designed for the intron region of ribosomal protein Sl 6 (rpsl6). The primer set amplified with extracted DNA of B. serrata yielded single amplicons of 150 bp with leaf and oleogum resin samples, while in C. wightii the same primer set gave two different amplicons of 150 bp and 350 bp with leaf and oleogum resin samples. The amplicon of
150 bp was common for both species but the 350 bp amplicon can be used to discriminate *C. wightii* and *B. serrata*. The disadvantage of this method might be that using a relatively long DNA sequence of 350 bp* can cause some false negative results even when including control samples.*

**Comments:**

For molecular studies, a paramount requirement is the isolation of DNA of sufficient quantity, length, and purity for selected identification methods. However, extraction of intact and high-quality DNA may be challenging, especially when working with processed ingredients such as extracts. Based on data on genetic testing of cannabis (*Cannabis sativa*, Cannabaceae) resins, certain compounds in the resin can interfere with DNA extraction and amplification. Therefore, the DNA extraction had to be optimized to recover amplifiable DNA from the resin. A particularity of plant exudates such as mucilages, gums, and resins, which occur in the form of viscoelastic gels, is that they have adhesive properties. Thus, many microorganisms, insects, and different plant particles from airborne contamination can stick to these plant products. This may create an obstacle in genetic identification and distinction since DNA from accidental contaminants is likely to be present and may provide false positive results, especially when using amplification based methods and Sanger sequencing. Second-generation sequencing (also known as next-generation sequencing), which can sequence DNA from hundreds or thousands of species in parallel, can potentially alleviate parts of this problem, through issues like amplification bias can remain if an amplicon approach (amplification of a section of the genome, e.g., DNA barcoding) is taken. Nevertheless, DNA methods are often inappropriate or not conclusive for identity testing of gum extracts, essential oils, or certain other processed materials; therefore, the authentication and detection of adulteration is best done by chemical means in these cases.

10. Chemical Identification and Distinction

10.1 Chemistry of *Boswellia serrata* and potential adulterants and confounding materials

*Boswellia serrata*

*Boswellia serrata* oleogum resin contains about 30-65% resin, 5-10% essential oil, and 20-23% polysaccharides composed of arabinose, galactose, and xylose. Different classes of terpenoid compounds, such as monoterpenes, sesquiterpenes, diterpenes, and pentacyclic and tetracyclic triterpenes, are found in *Boswellia* resins.

Several authors have investigated the composition of *B. serrata* essential oil. The composition appears to be quite variable depending on the origin of the samples (Table 2). Major monoterpenes in the essential oil of *B. serrata* are α-thujene, along with myrcene, camphene, 3-carene, p-cymene, hashishene, limonene, linalool, methyl chavicol, α-phellandrene, β-phellandrene, α-pinene, sabinene, γ-terpinene, κ-terpineol, and tetrahydro-linalool. Classen et al. list methyl

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Gupta et al.53</th>
<th>Hamm et al.55</th>
<th>Niebler and Buettner a,36</th>
<th>Schmiech et al.54</th>
<th>Singh et al.56</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Carene</td>
<td>0.5-4.2</td>
<td>0.2</td>
<td>1.6</td>
<td>0.5</td>
<td>3.6-9.6</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>2.2-3.5</td>
<td>4.7</td>
<td>4.3</td>
<td>1.1</td>
<td>1.6-2.8</td>
</tr>
<tr>
<td>Limonene</td>
<td>1.3-3.7</td>
<td>2.6</td>
<td>2.4</td>
<td>4.6</td>
<td>0.7-8.5</td>
</tr>
<tr>
<td>Methyl chavicol</td>
<td>n.d.</td>
<td>8.9</td>
<td>12.3</td>
<td>6.0</td>
<td>0.0-4.6</td>
</tr>
<tr>
<td>Myrcene</td>
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<td>7.0</td>
<td>4.1</td>
<td>41.4</td>
<td>n.d.</td>
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<tr>
<td>α-Pinene</td>
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<td>3.4</td>
<td>4.8</td>
<td>14.7</td>
<td>0-11.2</td>
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<tr>
<td>Sabinene</td>
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<td>3.4</td>
<td>2.3</td>
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<tr>
<td>α-Thujene</td>
<td>22.5-69.8</td>
<td>11.7</td>
<td>15.2</td>
<td>14.5</td>
<td>22.7-47.4</td>
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</tbody>
</table>

*a Average of 11 samples
n.d.: not determined

* DNA barcoding methods amplify segments of genomic regions. Amplification success depends – in large parts – on the presence of DNA fragments that can be amplified using the designated primers. However, the longer the sequence to be amplified, the bigger the risk that it may not be present in its entirety (since certain processing steps, e.g., heat or UV irradiation can degrade DNA) and hence cannot be detected in a material.
chavicol (estragol) as a typical *B. serrata* essential oil constituent that is absent in oils of other *Boswellia* species.

The natural resinous triterpenoids can be grouped into the following 2 types: pentacyclic triterpenes (ursanes, oleananes, lupanes) and tetracyclic triterpenes (tirucallane-type, dammarane-type, and euphane-type). Main triterpene constituents of *B. serrata* oleogum resin of biological interest are members of the boswellic acids (BAs) that are pentacyclic triterpenes of the ursane- and oleanane-types. They are 9,11-dehydro-α-boswellic acid, 9,11-dehydro-β-boswellic acid, 3-O-acetyl-9,11-dehydro-α-boswellic acid, 3-O-acetyl-9,11-dehydro-β-boswellic acid, 11-keto-α-boswellic acid (KβBA), 11-keto-β-boswellic acid (KαBA), 3-O-acetyl-11-keto-α-boswellic acid (AKαBA), 3-O-acetyl-11-keto-β-boswellic acid (AKβBA), 3-α-O-acetyl-α-boswellic acid (AαBA), and 3-α-O-acetyl-β-boswellic acid (AβBA). Crude oleogum resin typically contains up to 30% BAs in total, while ethanol extracts of frankincense contain higher levels of BAs. Thus far, BAs can be regarded as specific chemical markers for the *Boswellia* genus.8,9,18,43,54,58-61

Oleogum resins of *B. serrata* from India are characterized by a high content of the deacetylated pentacyclic triterpene acids (PTAs), αBA (1.53%), βBA (4.44%), KβBA (0.81%), and lupeolic acid (LA) (0.52%). The ratio of the signal intensities of the non-acetylated BAs to the acetylated ones (αBA/AαBA and βBA/AβBA) is < 1 in the case of frankincense extracts from *B. sacra*, but is > 1 in the case of frankincense extracts from *B. serrata*.63 Based on the analysis of 14 samples, the peak height ratio of KβBA to AKβBA was found to be 1:1 in *B. serrata* using high performance liquid chromatography (HPLC) at 250 nm.43 Additionally, the mean of the KβBA, KαBA, AKβBA, and AKαBA contents of *B. serrata* oleogum samples were determined as 0.79, 0.02, 1.14, and 0.04%, respectively. The total 11-keto-boswellic acid (KBA) contents of *B. serrata* samples (n=7) differed from those of *B. sacra* (n=11) with higher values of 0.3–2.29%.54

Other terpenoid acids such as tirucallic acids (elemonic acid, α- and β-elemolic acid, and 3-α-O-acetyl-8,24-dien-tirucallic acid), 3-α-O-acetyl-lupeolic acid (ALA), α-amyrin, and ursolic acid, besides sesquiterpenes (e.g., elemol), diterpene alcohols (cembrene A, incensole, isoserratol, and serratol), and diterpene hydrocarbons (m- and p-camphorene) have also been identified.43,56,59,64,65 Lupeolic acids, tirucallic acids, roburic acids, and some neutral terpenic compounds found in the *Boswellia* genus cannot be regarded as specific chemical markers for a certain species in general. However, in combination with other compounds, they may be helpful for species classification in some cases.43

Headspace solid-phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS) analysis of *B. serrata* oleogum resins revealed the presence of a diterpenic compound, isoincensole acetate, which is absent in *B. frereana* and *B. papy-
Boswellia serrata oleogum resins. Additionally, p-anisaldehyde, elemicin, isocaryophyllene, methyl chavicol, and methyl eugenol have been determined as chemical markers that can be used to distinguish B. serrata oleogum resin from B. sacra, B. frereana, and B. papyrifera. In contrast to B. sacra, the ratio of α-thujene to α-pinene is usually higher than one (average 3.1) and the monoterpene β-myrcene is found in higher quantity in B. serrata samples. Furthermore, the three sesquiterpenes Z-α-trans-bergamotene, β-bourbonene, and kessane are mentioned as chemical markers for B. serrata.36

Boswellia serrata oleogum resin must contain not less than 1.0% of the keto derivatives of βBA in dried resin, calculated as the sum of KβBA and AKβBA according to the United States Pharmacopeia (USP) monograph.66 The European Pharmacopoeia (Ph. Eur.) monograph mentions the minimum limits of KβBA and AKβBA as 1.0% each in the dried drug.26 In American and European markets, B. serrata is mainly sold in the form of resin extracts. Extracts may be standardized based on the content of total acids, organic acids, BAs (60–70%), AKβBA (30%), or AβBA (20%).35

Boswellia frereana

The oleogum resin of B. frereana has a monoterpene composition very similar to that of B. sacra or B. serrata. It contains low amounts of sesquiterpenes and is devoid of diterpenes of the incensole type. Although there is substantial variability based on the origin of the material, B. frereana oleogum resin contains high concentrations of α-phellandrene dimers, which make 12.9-20.2% of the relative peak area of volatiles measured by SPME-GC-MS, or 0-10% of the essential oil.67 The major triterpenic compound in the methanol extract of B. frereana oleogum resin is lupeol. Lupenone, 3-epi-lupeol, α- and β-amyrone, 3-epi-α-amyrin, 3-epi-β-amyrin, and β-amyrin are the other triterpenes detected, while lupeolic acid, boswellic acids, and their respective O-acetyl derivatives are absent in B. frereana resin samples.57,62,63,68

Investigations of the B. frereana essential oil indicate that it is mainly composed of bornyl acetate, camphene, p-cymene, eucalyptol, limonene, α-pinene, sabinene, 4-terpineol, α-thujene, and trans-verbenol.18,36,64,69 Trans-sabinene hydrate acetate was detected in B. frereana, yet in only three of four samples, and it was absent in B. papyrifera, B. sacra, and B. serrata. However, truly characteristic is the presence of large amounts of dimers of α-phellandrene, with 2,7-diisopropyl-4a,4b-dimethyl-1,2,4a,4b,7,8a,8b-octahydrodibiphenylene being the most abundant one.55 Additionally, high amounts of α-thujene are a feature shared with B. serrata.36

Boswellia papyrifera

The chemical composition of B. papyrifera oleogum resin is markedly different from that of other Boswellia species. Boswellia papyrifera contains small amounts of monoterpenes (bornyl acetate, camphene, eucalyptol, limonene, linalool, linalool oxide, β-myrcene, α-pinene, β-pinene, α-terpineol, and thujone as the main constituents), sesquiterpenes (e.g., calamenene), diterpenes (e.g., cembrene A, incensole, incensole acetate, incensole oxide, incensole oxide acetate, and verticillia-4(20),7,11-triene), and triterpene acids (αBA, βBA, βKBA, βKBA, elemonic acid, α- and β-elemic acid, 3-α-O-acetyl-8,24-dien-tirucallic acid, 3-β-O-acetyl-8,24-dien-tirucallic acid, 3-α-O-7,24-dien-tirucallic acid, and 3-α-hydroxy-lup-20(29)-en-24-oic acid). The presence of a large amount of n-octyl acetate (56-64.6% of essential oil) with a lesser amount of n-octanol (8-13.9% of essential oil) is a unique feature that makes B. papyrifera clearly distinguishable from other Boswellia species.18,36,43,54,55,70-73 Addi-
tionally, incensole, its oxide and acetate, and verticillia-4(20),7,11-triene are the main diterpene constituents in *B. papyrifera* oleogum resin.\textsuperscript{42,55} Incensole acetate and verticillia-4(20),7,11-triene can be used as specific chemical markers for this species.\textsuperscript{43,74,75} Linear carboxylic acids from pentanoic acid to lauric acid were also identified in *B. papyrifera* oleogum resin by headspace SPME-GC-MS.\textsuperscript{55}

*Boswellia papyrifera* oleogum resin samples from three different origins (Ethiopia, Eritrea, and Sudan) were investigated, and their KβBA, KαBA, AKβBA, AKαBA contents were determined as 0.35, 0.02, 2.81, and 0.15% respectively. They contained more total KBAs (0.31–0.48%) and a higher proportion of AKαBA (3.4–6.4% of total acetyl-11-keto-boswellic acid content) than *B. sacra*.\textsuperscript{54}

**Boswellia sacra**

The predominant monoterpenes in *B. sacra* were found to be α-pinene, β-myrcene, limonene, and E-β-ocimene in company with bornyl acetate, camphene, α-campholenal, 3-carene, carveol, carvone, cuminal, p-cymenol, myrtanal, myrtenol, α-phellandrene, β-pinene, pinocarveol, sabinene, 4-terpineol, α-thujene, trans-verbenol, and verbenone. β-Caryophyllene is the major sesquiterpene along with allo-aromadendrene, γ-cadinene, τ-cadinol, caryophyllene oxide (syn. caryophyllene epoxide), cedrol, α-copaene, β-elemene, β-eudesmene, eudesmol, germacrene, α-humulene, γ-muurolene, and α-selinene. Characteristic oleogum resin compounds such as incensole, isoincensole, isoincensole acetate, isoserratol, serratol, 3-β-OH-tirucallol, and cembrane A are the main diterpenes.\textsuperscript{36,55,59,69,76} Among them, serratol and incensole are known as *Boswellia*-specific diterpene alcohols. β-Caryophyllene oxide, isoincensole, and its acetate, γ-cadinene, δ-cadinene, τ-cadinol, and cedrol seem to be typical chemical markers for this species.\textsuperscript{36,43}

Triterpenes in *B. sacra* oleogum resin are lupeolic acid (LA), 3-O-acetyl-lupeolic acid (ALα), αBA, βBA, KβBA, AαBA, AβBA, AKβBA, α- and β-amyrenone, 3-epi-α- and 3-epi-β-amyrin.\textsuperscript{63,68} Oleogum resin of *B. sacra* from Oman (*n*=11) are characterized by a high proportion of the acetylated PTAs, AαBA (2.36%), AβBA (4.09%), AKβBA (3.13%), and ALA (2.18%). *Boswellia sacra* exhibits a unique combination of low total content KBAs (0.10-0.28%) and a low proportion of KαBA (1.1–6.6% of KαBA versus 94.6–98.9% KβBA).\textsuperscript{54,62}

Although *B. sacra* and *B. carteri* are generally recognized as single species, chemotypes in the literature vary widely, with α-pinene-, α-Thujeone-, limonen-, 1-methoxydecane- and E-β-ocimene-dominant chemotypes being reported. Much of this may be due to differential geography, environment, and tree management, although some variation is likely due to wrong identification of the source trees and a lack of testing resins directly collected from properly identified plants.\textsuperscript{59,64,76}

**Garuga pinnata**

*Garuga pinnata* leaves and stem bark contain sitosterol, stigmasterol, and campesterol, fatty acids, a mixture of long-chain esters, and aliphatic compounds, as well as tannins and waxes. From the leaves also garugarin and amentoflavone were isolated. However, the resin of this plant has not been studied in detail so far. Only the occurrence of α-amyrin, its 3α-epimer, butyrosperrmol, and dammaradienol was reported.\textsuperscript{77,78}

**Pinaceae**

In Niebler and Buettner’s study, two commercial *B. serrata* gum resin samples were found to contain unexpected constituents that do not commonly occur in *B. serrata* oleogum resin.\textsuperscript{36} Longifolene (34.3% and 19.0% of the total peak area) was the highest peak in both chromatograms and α-longipinene (1.7% and 2.1%), longicyclene (2.9% and 2.8%), longicamphylene-none (0.44% and not detected), longiborneol (0.46% and 0.26%), and longiborneol acetate (3.0% and 2.3%) were also identified. These sesquiterpenes are characteristic for Pinaceae resins, and it is reported that a sample of Norway spruce (*Picea abies*, Pinaceae)
resin has a similar but not identical composition. The authors hypothesize these two products may have been adulterated with a Pinaceae resin.\textsuperscript{36} This family contains the most important genera of resin producers of which the genus \textit{Pinus} clearly ranks first with regards to commercial relevance. On the other hand, species in the genera \textit{Picea}, \textit{Abies}, and \textit{Larix} are also used as resin sources.\textsuperscript{79,80} Since the botanical origin of the adulterant Pinaceae resin is not clearly identified in the study of Niebler and Buettner, no detailed data on the composition of pine resins are given in this section.

Comments: The scientific literature on the chemical composition of commercial oleogum resin from \textit{Boswellia} samples contains many conflicting reports, some of which may be due to the use of misidentified species as the research material. This confusion is likely due to the use of commercial samples that were purchased from local markets without proper taxonomic identification.\textsuperscript{18} \textit{Boswellia carteri} has long been confused with \textit{B. papyrifera}, and \textit{B. carteri} is also often treated as a different species from \textit{B. sacra}. As an example, Başar analyzed a botanically certified sample from Ethiopia labeled as \textit{B. carteri}, but the chemical profile presented resembled that of \textit{B. papyrifera}.\textsuperscript{43,64} In some publications, Latin names of the analyzed commercial samples are not mentioned, and the oleogum resins were simply named as Indian frankincense, African frankincense, Aden type frankincense, or Eritrean type frankincense. While \textit{B. serrata} is called Indian frankincense, Aden type is usually sourced from \textit{B. sacra} and Eritrean type is from \textit{B. papyrifera}. Using vernacular names alone can cause confusion and may result in inconsistent chemical results.\textsuperscript{43,60,72}

Frankincense species display major differences in their chemical composition. They can be identified without the need for exact quantification of compounds and based on primarily qualitative and semiquantitative features. Generally, monoterpenes are not particularly well suited for differentiation purposes, since results of essential oil studies show a large variability in this compound class across all species. The monoterpene profile can serve only as an indicator for a certain species and must be combined with further characteristics to identify to the species level. Better suited are sesqui- and diterpenes, as well as other compound classes. However, many of the commercial extracts are devoid of the essential oil, and therefore the measurement of volatile terpenes may not be an option to authenticate these materials.

On the other hand, BAs can be used for chemotaxonomic purposes since their contents and ratios are changing distinctly between \textit{B. serrata} oleogum resin and its adulterants (Table 4).

As mentioned before, the ratio of the non-acetylated BAs to the acetylated ones (\(\text{\(\alpha\)}\text{BA}/\text{\(\alpha\)}\text{BA} and \(\beta\text{BA}/\text{\(\alpha\)}\text{BA}\)) is <1 in \textit{B. sacra} but is >1 in \textit{B. serrata} samples. The results of the studies given in Table 4 are compatible with these observations. The \textit{B. sacra} sample examined in Mannino et al.\textsuperscript{81} and the \textit{B. serrata} sample used in Ganzera and Khan’s study\textsuperscript{82} are not included in the table since these were commercial samples without proper authentication, and the species label claims are not in agreement with the BA ratios described above. According to Paul,\textsuperscript{43} the sample

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Compound</th>
<th>\textit{B. frereana}</th>
<th>\textit{B. papyrifera}</th>
<th>\textit{B. sacra}</th>
<th>\textit{B. serrata}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diterpenes</td>
<td>Incensole</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Incensole acetate</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Serratol</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>(\alpha)-Humulene</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(\beta)-Caryophyllene</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Boswellic Acids</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Tirucallic Acids</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Lupeolic Acids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ large quantity, ++ average quantity, + detectable, - not detectable

\(a\) The lack of reporting a specific constituent in a given \textit{Boswellia} species may not necessarily mean that it is not present, as not all these species have been thoroughly investigated.

\(b\) This table is a summary of the HPLC results of the dissertation of Paul.\textsuperscript{43} \textit{Boswellia frereana} results are added to the table according to the references mentioned in section 10.1, and all the results are confirmed by the relevant references in the same section.
named as *B. serrata* in the publication by Ganzera and Khan\(^82\) may be *B. papyrifera* oleogum resin, since the chemical composition fits best with this species (quite remarkable is the AK\(\beta\)BA concentration of 5.4%, unlike the amounts found by Paul in *B. serrata*). Similarly, the *B. sacra* sample analyzed by Mannino et al.\(^81\) has an \(\alpha\)BA and \(\beta\)BA content of 11.0 and 11.2%, respectively, that is not compatible with the results of other studies on *B. sacra* samples. Thus, this sample may belong to another species.

Adding to the authentication challenges is the use of proprietary manufacturing processes selectively enriching certain BAs (particularly AK\(\beta\)BA), or BA fractions in extracts. Such processes will lead to marked changes in the BA fingerprint, rendering species identification using BAs as marker compounds in these extracts impossible.

In some of the studies on *Boswellia* species, information about the origin and identification of the correct species of commercial frankincense is insufficient. Thus, Schmiech et al.\(^62\) created a rather complex formula that enables the classification of frankincense samples. The concentrations of three PTAs (AK\(\beta\)BA, \(\beta\)BA, and A\(\alpha\)BA) are required to define a characteristic index, which is called the *Boswellia* index (Bos\(_i\)).

\[
Bos_i = (\text{[AK}_{\beta}\text{BA]} - 1) \times \left(\frac{[A\beta\text{BA}] + 1}{[\beta\text{BA}] + 1}\right) \times (\text{[AK}_{\beta}\text{BA]} + [A\beta\text{BA}] + [\beta\text{BA}] + 1) \times ([A\beta\text{BA}] - [\beta\text{BA}])
\]

Table 4. Mean boswellic acid contents (%) and boswellic acid index (Bos\(_i\)) of oleogum resin samples of *B. serrata* and other *Boswellia* species\(^a\)

<table>
<thead>
<tr>
<th>Species</th>
<th>Origin</th>
<th>Reference</th>
<th>K(\beta)BA</th>
<th>AK(\beta)BA</th>
<th>(\alpha)BA</th>
<th>(\beta)BA</th>
<th>A(\alpha)BA</th>
<th>A(\beta)BA</th>
<th>Total</th>
<th>Bos(_i)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. dalzielli</td>
<td>Burkino Faso</td>
<td>Schmiech(^62)</td>
<td>1.17</td>
<td>5.34</td>
<td>1.33</td>
<td>1.74</td>
<td>2.73</td>
<td>3.14</td>
<td>15.45</td>
<td>134,344</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
<td>Schmiech(^62)</td>
<td>0.99</td>
<td>7.22</td>
<td>1.62</td>
<td>2.03</td>
<td>3.92</td>
<td>3.80</td>
<td>19.58</td>
<td>303,423</td>
</tr>
<tr>
<td></td>
<td>Senegal</td>
<td>Schmiech(^62)</td>
<td>1.39</td>
<td>6.83</td>
<td>1.61</td>
<td>1.68</td>
<td>2.97</td>
<td>2.81</td>
<td>17.29</td>
<td>142,486</td>
</tr>
<tr>
<td>B. frereana</td>
<td>Somalia</td>
<td>Schmiech(^62)</td>
<td>&lt;LOQ(^c)</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>B. neglecta</td>
<td>Somalia</td>
<td>Schmiech(^62)</td>
<td>0.11</td>
<td>0.01</td>
<td>2.31</td>
<td>6.74</td>
<td>0.13</td>
<td>0.62</td>
<td>9.91</td>
<td>71 to 888</td>
</tr>
<tr>
<td></td>
<td>Kenya</td>
<td>Schmiech(^62)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.16</td>
<td>0.00</td>
<td>0.04</td>
<td>0.30</td>
<td>0 to 5</td>
</tr>
<tr>
<td>B. papyrifera</td>
<td>Eritrea</td>
<td>Paul(^43)</td>
<td>0.36</td>
<td>4.51</td>
<td>1.13</td>
<td>2.07</td>
<td>2.09</td>
<td>3.57</td>
<td>13.73</td>
<td>114,673</td>
</tr>
<tr>
<td></td>
<td>Ethiopia</td>
<td>Schmiech(^62)</td>
<td>0.44</td>
<td>4.39</td>
<td>1.09</td>
<td>2.36</td>
<td>2.14</td>
<td>3.00</td>
<td>13.43</td>
<td>34,172</td>
</tr>
<tr>
<td></td>
<td>Eritrea</td>
<td>Schmiech(^62)</td>
<td>0.34</td>
<td>2.78</td>
<td>0.55</td>
<td>1.64</td>
<td>1.43</td>
<td>1.99</td>
<td>8.74</td>
<td>7,196</td>
</tr>
<tr>
<td></td>
<td>Sudan</td>
<td>Schmiech(^62)</td>
<td>0.32</td>
<td>1.68</td>
<td>1.03</td>
<td>3.18</td>
<td>2.02</td>
<td>4.65</td>
<td>12.88</td>
<td>32,469</td>
</tr>
<tr>
<td>B. rivae</td>
<td>Ethiopia</td>
<td>Schmiech(^62)</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>B. sacra</td>
<td>Oman</td>
<td>Paul(^43)</td>
<td>0.14</td>
<td>1.68</td>
<td>1.76</td>
<td>3.51</td>
<td>2.61</td>
<td>4.66</td>
<td>14.35</td>
<td>23,681</td>
</tr>
<tr>
<td></td>
<td>Oman</td>
<td>Schmiech(^62)</td>
<td>0.18</td>
<td>3.13</td>
<td>0.71</td>
<td>2.07</td>
<td>2.36</td>
<td>4.09</td>
<td>12.55</td>
<td>13,137 to 307,249</td>
</tr>
<tr>
<td>B. serrata</td>
<td>India</td>
<td>Mannino(^81)</td>
<td>1.90</td>
<td>0.40</td>
<td>6.93</td>
<td>6.22</td>
<td>0.16</td>
<td>0.62</td>
<td>16.24</td>
<td>-1,431</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>Paul(^43)</td>
<td>0.92</td>
<td>0.95</td>
<td>2.41</td>
<td>5.05</td>
<td>1.22</td>
<td>4.01</td>
<td>14.56</td>
<td>-7,132</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>Büchele(^60)</td>
<td>2.02</td>
<td>1.43</td>
<td>2.45</td>
<td>4.60</td>
<td>0.47</td>
<td>2.54</td>
<td>13.51</td>
<td>-1,343</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>Schmiech(^62)</td>
<td>0.81</td>
<td>1.18</td>
<td>1.53</td>
<td>4.44</td>
<td>1.02</td>
<td>2.93</td>
<td>11.91</td>
<td>-21,112 to -2,909</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>Indena(^4)</td>
<td>1.06</td>
<td>1.16</td>
<td>2.70</td>
<td>6.55</td>
<td>1.64</td>
<td>3.24</td>
<td>16.34</td>
<td>-19,393</td>
</tr>
<tr>
<td></td>
<td>Africa</td>
<td>Indena(^4)</td>
<td>0.79</td>
<td>5.25</td>
<td>6.23</td>
<td>5.24</td>
<td>5.90</td>
<td>4.84</td>
<td>28.25</td>
<td>-29,199</td>
</tr>
</tbody>
</table>

\(a\) The results of the articles which have determined the content of six major boswellic acids in the *Boswellia* oleogum resin samples are demonstrated in this table. Studies on processed materials or pharmaceutical products are not included.

\(b\) Mean value (except for Schmiech\(^62\)).

\(c\) Limit of quantification

\(d\) Data on BA contents of two different batches of authentic *B. serrata* oleogum resins were provided by Indena, SpA (Milan, Italy)
and to locate their geographical origin (The results of Schmiech et al. are summarized in Table 4). Additionally, the Bos values of oleogum resin samples examined in other studies are shown in Table 4. The values are compatible with the results of Schmiech et al.,62 except that of a B. serrata sample investigated by Ganzera and Khan,82 and the B. sacra sample examined by Mannino et al.81 Hence, Bos might be a rather convenient tool to classify Boswellia samples based on just three boswellic acids. However, for more accurate classification, additional samples of the main Boswellia species in trade and the additional constituents of the PTA fraction of the oleogum resin must be investigated.

10.2 Laboratory methods
Various analytical methods are used to identify B. serrata oleogum resin, assess its quality, and/or determine evidence of adulteration. Unless otherwise noted, all methods summarized below are based on chemical analysis of the oleogum resin. Table 7 in section 11 provides a summary of different methods of analysis of B. serrata.

10.2.1 Titration
The methods published by Gupta et al.83 and Rajpal84 were evaluated in this review.

Comments: Although the non-aqueous titrimetric methods for the quantification of total BAs used by Gupta et al. and Rajpal are cited in Kamal et al.,85 Mehta et al.,86 and Schmiech et al.,87 the full texts of these studies are not accessible. According to Schmiech et al.,87 many suppliers promote their frankincense products with BA contents over 80% determined by non-aqueous titration. Such high concentrations often cannot be confirmed in quality control laboratories or by academic researchers using chromatographic methods.84 Titrimetric methods estimate unselectively all acidic compounds, not only BAs. Additionally, Boswellia gum resin extracts are standardized by different acids, such as the organic acid, total acid, or the BA content. As BAs represent only a minor portion of the total acid content, a product claiming to contain 65% total acids or organic acids is not equivalent to a product claiming 65% BAs. Moreover, different analytical methods may result in different BA content. Hence, the BA content of a product was determined to be 70% when quantified by titration, but did not exceed 35-45% when quantified with HPLC.35 Thus, titration is not a highly selective and accurate quantification method for the analysis of BAs, and is not specific enough and therefore not appropriate for authentication of Boswellia oleogum resins and respective extracts.

10.2.2 Thin-layer chromatography (TLC)/High-performance thin-layer chromatography (HPTLC)
Methods from the following sources were evaluated in this review: USP 2022,66,88 Ph. Eur. 10.8,26 The Ayurvedic Pharmacopoeia of India,8 HPTLC Association,89 Hairfield et al.,90 Krohn et al.,91 Mehta et al.,86 Meier and Spriano,92 Paul et al.,23 and Pozharitzkaya et al.93

Comments: TLC and HPTLC are simple and common methods used for differentiation of Boswellia species (Figure 4) as well as the qualitative analysis of boswellic acid derivatives in frankincense. Methanol is a commonly used solvent to extract frankincense for TLC and HPTLC analysis; such extracts are usually developed on silica gel plates with various mobile phases, and anisaldehyde, sulfuric acid, and antimony pentachloride reagents are used for derivatization. Densitometric analysis of the developed plates can be carried out to quantify the BAs before and after derivatization.

According to the TLC method of USP, the methanol extract obtained from B. serrata oleogum resin exhibits two main zones due to KβBA and AKβBA. After dipping the plate in 10% sulfuric acid in methanol and heating, βBA and AβBA zones are also exhibited.66,88 Ph. Eur. mentions the presence of zones due to KβBA and AKβBA along with other weak quenching zones in UV light at 254 nm.26 The Ayurvedic Pharmacopoeia of India uses a TLC method for analyzing B. serrata oleogum resin, its hydro-alcoholic extract, and its water extract and uses βBA as a reference substance.8 Hairfield et al.90 compared TLC of methyl esters of acids obtained after exposing diethylether extracts of B. serrata, B. sacra, B. papyrifera, and B. frereana, and those of other resins (myrrh [Commiphora myrrha, Burseraceae], mastic [Pistacia lentiscus, Anacardiaceae], elemi [Canarium luzonicum, Burseraceae], and dammar [gums of select genera from Dipterocarpaceae trees]) to diazomethane. While the TLC easily distinguished frankincense from the other resins that contain triterpene acids, the sample preparation is cumbersome and the use of diazomethane represents a safety hazard.90 Chemical constituents of Boswellia spp. are not in the focus of the study by Hairfield et al., and there is no discussion about the chemical markers of these species.90

Paul et al. developed a TLC method to compare certified and commercial resin samples belonging to B. serrata, B. sacra, and B. papyrifera. Plates were analyzed by UV detection (254 nm) and dyeing with anisaldehyde reagent. All species have the classic BAs in common, as KβBA, AKβBA, αβA+βBA, and
AαBA+KBBA were present in all three species. Incenseole and incenseole acetate were noted as specific chemical markers for *B. papyrifera*, although the former has been reported from other *Boswellia* species (see 10.1). β-Caryophyllene oxide was suggested as a marker compound for *B. sacra*. *Boswellia serrata* neither exhibited bands for incenseole acetate nor β-caryophyllene oxide, but could be identified by marked serratol (green, Rf = 0.46) and 3-oxo-8,24-dien-tirucallic acid (blue, Rf = 0.22) spots.23

The HPTLC Association gives details of an HPTLC method, adopted in the *USP Dietary Supplements Compendium*, for the analysis of *B. serrata* and *B. sacra* oleogum resins. HPTLC fingerprints of *Boswellia serrata*, *B. sacra*, dammar, *Commiphora mukul*, *C. myrrha*, and *Styrax tonkinensis* oleogum resins are included in this document.89 There is no discussion about the chemical markers of *Boswellia* species or composition of relevant species in this study. Meier et al.92 used the TLC method proposed by Ph. Eur. on HPTLC plates to detect BAs in the oleogum resin samples of three *Boswellia* spp. In Indian frankincense (*B. serrata*), AKβBA and KβBA were present in a balanced ratio (AKβBA/KβBA=0.8-1.3), frankincense from the Arabian peninsula (*B. sacra*) contained AKβBA at a level 5- to 10-fold higher than KβBA (AKβBA/KβBA=5.5-10), and the frankincense from Somalia (*B. frereana*) contained almost no BAs. In some of the HPTLC studies, densitometry was used for the determination of the BA content of *B. serrata* oleogum resin.86,91,93 Krohn et al.91 developed a rapid, simple, and partially validated HPTLC method for the quantitative determination of KβBA and AKβBA in the oleogum resin of *B. serrata* with spot visualization under UV light and scanning at 250 nm. HPLC combined with a diode array detector (HPLC-DAD) and HPTLC densitometry were used to determine KβBA and AKβBA contents of *B. serrata* oleogum resin by Mehta et al. The quantitative results of both analytical methods did not show any major differences, although a trend to lower values was found for the KβBA and AKβBA content measured by HPLC.86 Lastly, Pozharitskaya et al.93 quantified KβBA, AKβBA, AβBA, and βBA in authenticated and two commercial *B. serrata* extracts. The proposed HPTLC method for the simultaneous quantification of the four major BAs was found to be simple, precise, specific, sensitive, and accurate and can be used for routine qual-

Figure 4. HPTLC analysis of *Boswellia* oleogum resin samples. Figure 4a: Underivatized, UV 254 nm (showing 11-keto-β-boswellic acid at Rf ~ 0.33 and 3-O-acetyl-11-keto-β-boswellic acid at Rf ~ 0.42), Figure 4b: Anisaldehyde reagent, white light, Figure 4c: Anisaldehyde reagent, UV 366 nm. Lane 1: Anethole, Thymol, with increasing Rf. Thymol is an orange zone at Rf ~ 0.56 in Figure 4b. Anethole is a dark zone at Rf ~ 0.62 in Figure 4a. Lanes 2-6, 8: B. serrata, samples from India, lane 7: B. serrata, sample from Iran, lanes 9-10: B. freerana, lanes 11-12: B. neglecta, lanes 13-15: B. sacra (samples in lanes 13 and 14 were labeled B. carteri), lanes 16-17: B. papyrifera, lane 18: B. occulta, lane 19: B. riva, lane 20: B. dalzielli, lane 21: Commiphora myrrha

Conditions as described by the HPTLC Association89 with slight modifications. Developing solvent: Cyclohexane, diethyl ether, glacial acetic acid 7:4:1 (v/v/v); Concentration: 50 mg/mL; Application volume in Figure 4a: 0.5 µL, in Figures 4b and 4c: 0.5 µL for reference compounds and 0.2 µL for *Boswellia* spp. Samples provided by the American Herbal Pharmacopoeia (Scotts Valley, CA), Verdure Sciences (Noblesville, IN), and the Aromatic Plant Research Center (Lehi, UT).
ity control and the quantification of these compounds in plant materials.\textsuperscript{93}

Based on the detailed examination of specific chemical marker compounds for three different \textit{Boswellia} spp. (\textit{B. serrata}, \textit{B. sacra}, and \textit{B. papyrifera}) the TLC method published by Paul et al.\textsuperscript{23} is a suitable option to differentiate oleogum resins from these species. However, many dietary supplements contain \textit{Boswellia} oleogum resin extracts with BAs in modified proportions (e.g., some companies use oxidation and acetylation steps after extraction of the oleogum resin to selectively increase AKβBA contents).\textsuperscript{94} Also, the essential oils of \textit{Boswellia} spp. are used in perfume or cosmetic industry. Some companies utilize the leftover resin after removing the essential oil by steam distillation to obtain the BAs and offer the resulting extract as a dietary supplement ingredient. Thus, these commercial samples will not contain volatile compounds that can be used as chemical markers. In such cases, the HPTLC method described by Krohn, may be a suitable alternative.\textsuperscript{91} On the other hand, the method offered by the HPTLC Association is useful for the fingerprint analysis of unprocessed oleogum resin samples (i.e., samples in which the volatile constituents are still present), although no data on the origin of the analytes are provided.\textsuperscript{89}

\subsection*{10.2.3 Infrared spectroscopy}

The following papers were included for this review: Archier et al.\textsuperscript{95} Al Shidhani et al.\textsuperscript{96} and Rehman et al.\textsuperscript{97}

\textbf{Comments:} Archier et al. used Fourier transform infrared absorption spectrometry (FTIR) to compare 11 frankincense samples originating from Yemen (three), Somalia (four), Djibouti (two), Sudan (one), and India (one) with potassium bromide as micropellets. There were noteworthy differences in the incenses based on geographical origin. Indian frankincense (\textit{B. serrata}) is distinguished from the other samples by the fingerprint in the 450-900 cm\textsuperscript{-1} range, in particular by the intense bands at 779 cm\textsuperscript{-1}, 692 cm\textsuperscript{-1} and, most importantly, at 459 cm\textsuperscript{-1}.\textsuperscript{95}

Two studies used near-infrared spectroscopy (NIR) coupled with partial least squares (PLS) regression analysis to quantify incensole in \textit{B. papyrifera}, \textit{B. sacra}, and \textit{B. serrata} oleogum resin samples,\textsuperscript{96} or to determine the AKβBA content in \textit{B. sacra} plant parts and
various fractions of its resin exudates. For incensole, the NIR spectrometer was used in absorption mode in the wavelength range between 700 and 2500 nm. The PLS model obtained has an $R^2$ value of 98% with a correlation of 0.99 and a good prediction capability with root mean square error for prediction (RMSEP) value of 3.2%. The methanolic extract of B. papyrifera resin had the highest concentration of incensole (18.36%) while only trace amounts (0.16-0.39%) were found in the fractions of B. sacra and no incensole in B. serrata. Similarly, the PLS model for AKβBA in the range from 0.1 ppm to 100 ppm showed a good correlation with an $R^2$ value of 99% and a root mean square error of cross-validation value of 0.39%. In both publications, the findings were in agreement with data from HPLC analyses, suggesting that NIR spectroscopy in combination with PLS is an alternative method for the quantification of incensole and AKβBA in Boswellia oleogum resins. However, neither of the studies analyzed a large number of samples or included all relevant adulterant species. While NIR holds much promise as a screening method, more data are needed to assess its usefulness to detect adulteration.

NIR and FTIR spectroscopy methods are fast and non-destructive methods for frankincense analysis. The FTIR spectra of B. serrata oleogum resins show significant differences from its adulterant species and frankincense samples from different origins, and this technique can be used as a screening method for authentication. A large number of authenticated samples are needed for statistics. No data on the ability of IR spectroscopy to determine the identity of B. serrata extracts have been retrieved; therefore, it is not known how reliable it can be in authenticating more highly processed products.

10.2.4 Hyperspectral imaging

The publication by Zhang et al. was evaluated in this section.

Comments: Hyperspectral imaging is a non-destructive and time-saving technique that is widely applied to agricultural product inspection along with origin identification and quality control of plant materials. It collects spectral information for each pixel of an image, reflecting external characteristics, internal physical structure, and chemical composition of the samples. Hyperspectral imaging was used to collect spectral data in the 410–2500 nm range of frankincense oleogum resin of various quality categories from India, Ethiopia, and Somalia. (Latin names of the Boswellia species used in this study were not mentioned.) The algorithm framework discriminative marginalized least squares regression (DMLSR) was used for feature extraction of respective data. Then, the discriminative collaborative representation with Tikhonov (DCRT) regularization was applied for classifying the geographical origin and quality level of frankincense in samples from three different origins. The final average origin classification accuracy obtained with the best model was higher than 90%, and the standard deviation was below 7%. The quality classification results of samples from the three geographical regions achieved an accuracy of above 80%. This showed that hyperspectral imaging has some promise as a screening method for origin classification, although achieving 90% accuracy is not sufficient for a robust quality control method. In addition, more research is needed to determine its suitability for species discrimination. Another disadvantage is the relatively large number of samples needed to create the model.

10.2.5 High-performance liquid chromatography (HPLC) and ultrahigh-performance liquid chromatography (UPLC)

Methods described in the following literature were evaluated in this review: USP 2022, Ph. Eur. 10.8, The Ayurvedic Pharmacopoeia of India, Börner et al., Büchele et al., Frank and Unger, Ganzera and Khan, Kamal et al., Katragunta et al., Mannino et al., Mehta et al., Meins et al., Paul, Schmiech et al., Shah et al., Sharma et al., Subbaraju et al., and Zhang et al.

Comments: Since HPLC methods do not require a derivatization of the sample and allow the rapid and efficient qualitative as well as quantitative analysis of frankincense samples, HPLC combined with DAD or other detectors are used in many studies. An assessment of these studies can be found in Table 5. The methods by Mannino et al., and Mehta et al. were not listed in the table since an extraction time of 10 days and 36 hours, respectively, make these methods impractical for a quality control laboratory. In Table 5, run times up to 25 min. are acknowledged as short, 25-50 min. as moderate, and run times more than 50 min. are categorized as long.

One of the challenges in authenticating Boswellia serrata extracts is the presence of highly enriched or chemically modified ingredients on the market. The extracts are mainly enriched in AKβBA, which is known as an inhibitor of 5-lipoxygenase, one of the key enzymes in the regulation of inflammatory processes, and thus has been promoted as a marker for the biological activity of B. serrata oleogum resin. However, these modified extracts do not exhibit the same chemical composition as genuine B. serrata.
oleogum resin, and therefore species assignment is almost impossible. Accordingly, the methods listed below, which determine the species based on chemical composition, are appropriate only for materials that contain BAs in the proportions found in natural frankincense oleogum resin.

Since USP\(^{66}\) and Ph. Eur.\(^{26}\) specify the quality of \(B.\) serrata oleogum resin according to its AK\(\beta\)BA and K\(\beta\)BA contents, most of the published HPLC studies are focused on the quantification of these two compounds, even if many commercial products are standardized to their total BA contents. Boswellic acids and other triterpenoids are also detected at different wavelengths based on their absorption maxima, e.g., 205-210 nm for \(\alpha\)BA, \(\beta\)BA, as well as lupeolic acid, and 280 nm for 9,11-dehydro-\(\alpha\)- and -\(\beta\)-boswellic acids (250-254 nm is used for K\(\beta\)BA).\(^{54,60,62,81,87,102}\)

Since acidic compounds tend to show peak tailing on reversed-phase material, using an acidic mobile phase significantly improves the peak shape and symmetry.\(^{82}\) Thus, either acetic acid,\(^{60,66,101}\) formic acid,\(^{100,104}\) trifluoroacetic acid,\(^{43}\) or phosphoric acid\(^{26,82}\) are usually added to the mobile phases for BA analysis. Although volatile acids such as acetic acid, formic acid, and trifluoroacetic acid have the advantage of being compatible with a mass spectrometric detector,\(^{60}\) the addition of phosphoric acid is advantageous for UV detection at low wavelengths since other acids will result in a considerable baseline drift below 215 nm (the UV cutoff of some acids is even higher).\(^{82}\)

Several authors have aimed to discover specific patterns in the PTA composition of oleogum resins of different \(Boswellia\) species,\(^{35,43,60,62,63,81}\) and used HPLC-UV for determining the chemical composition of oleogum resin samples or commercial \(Boswellia\) products.\(^{35,60,63,82,87,99-101}\) Most of the developed methods are validated, but system suitability parameters (e.g., column efficiency, tailing factor, resolution) have been published only for the USP 2022 and Ph. Eur. methods. In some of the studies, high performance liquid chromatography electrospray ionization mass spectrometry (HPLC/ESI-MS),\(^{63,81}\) ultrahigh-performance liquid chromatography-diode array detection (UHPLC-DAD) coupled to an electrospray ionization time of flight mass spectrometer (ESI-Q-ToF-MS)\(^{100}\) and HPLC with tandem mass spectrometry detection (HPLC-MS/MS)\(^{87}\) were used for comparative analysis. A multivariate statistical analysis of the data revealed differences in the triterpenic acid composition that could be assigned to distinct \(Boswellia\) species.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample Set</th>
<th>Method</th>
<th>Analyte(s)</th>
<th>Isocratic (I)/Gradient (G)</th>
<th>Pro(s)</th>
<th>Con(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP 2022</td>
<td>Oleogum, dry extract</td>
<td>LC-UV</td>
<td>AKβBA and KβBA</td>
<td>I</td>
<td>System suitability parameters are given, relatively inexpensive, simple sample prep, moderate run time (45 min)</td>
<td>Only 2 BAs are measured</td>
</tr>
<tr>
<td>Ph. Eur. 10.8</td>
<td>Oleogum resin</td>
<td>LC-UV</td>
<td>AKβBA and KβBA</td>
<td>G</td>
<td>System suitability parameters are given, relatively inexpensive, simple sample prep, moderate run time (28 min)</td>
<td>Only 2 BAs are measured</td>
</tr>
<tr>
<td>Ayurvedic Pharmacopoeia of India</td>
<td>Oleogum resin, dry water, and hydro-alcoholic extract</td>
<td>LC-UV</td>
<td>βBA</td>
<td>I</td>
<td>Relatively inexpensive</td>
<td>βBA is not sufficient for species authentication, relatively long sample prep time (45 min) and long run time (more than 50 min)</td>
</tr>
<tr>
<td>Börner99</td>
<td>Tablets, oily capsules, hard gelatin capsules</td>
<td>LC-MS</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives</td>
<td>G</td>
<td>Moderate run time (35 min)</td>
<td>Long sample prep time (100 min), MS equipment is expensive</td>
</tr>
<tr>
<td>Büchele et al.60</td>
<td>Oleogum resins of 2 Boswellia spp., tablets (5 batches), capsules (2 batches)</td>
<td>HPLC-DAD</td>
<td>βBA, LA, KβBA, 9,11-dehydro-α-BA, 9,11-dehydro-β-BA, and their acetylated derivatives</td>
<td>G</td>
<td>Validated, specific, and sensitive method</td>
<td>Long extraction time (3 h), long run time (66 min), Indian and African frankincense terms are used, Latin names are not mentioned</td>
</tr>
<tr>
<td>Frank and Unger63</td>
<td>Oleogum resins of 4 Boswellia spp., tablet (1)</td>
<td>LC/LC/ESI-MS</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives</td>
<td>G</td>
<td>Online extraction process allows the injection of high sample volumes and removal of unwanted extract components, good resolution</td>
<td>Long sample prep time (more than 70 min), long run time (65 min), MS equipment is expensive</td>
</tr>
<tr>
<td>Ganzera and Khan82</td>
<td>Oleogum resins, commercial products (4)</td>
<td>HPLC-DAD</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives</td>
<td>G</td>
<td>Validated, moderate run time (35 min), simple and quick sample prep, good resolution</td>
<td>Poor peak shape of AβBA, no information on injection volume</td>
</tr>
<tr>
<td>Kamal et al.85</td>
<td>Oleogum resin</td>
<td>HPLC-DAD</td>
<td>AβBA, βBA, KβBA, and KβBA</td>
<td>I</td>
<td>Validated, moderate run time (40 min)</td>
<td>Long extraction time (135 min), MS equipment is expensive</td>
</tr>
<tr>
<td>Katragunta et al.100</td>
<td>Oleogum resin extracts (4), capsules (6), tablets (3), powders (2), gel formulations (2), tea fusion material (1)</td>
<td>UPLC-DAD-MS</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives, tirucallic acids</td>
<td>G</td>
<td>Validated, short run time (17 min), good resolution</td>
<td>Long extraction time (30 min), baseline separated, good resolution</td>
</tr>
<tr>
<td>Meins et al.35</td>
<td>Tablets (7), capsules (6), soft capsule (1), sachets (3)</td>
<td>HPLC-MS</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives</td>
<td>G</td>
<td>Validated, moderate run time (35 min), compounds are baseline separated, good resolution, matrix composition does not affect the results</td>
<td>Long sample prep time (100 min), MS equipment is expensive</td>
</tr>
<tr>
<td>Paul43</td>
<td>Oleogum resins of 3 Boswellia spp.</td>
<td>HPLC-DAD</td>
<td>Boswellic, lupeolic, tirucallic acids, and other terpenes</td>
<td>G</td>
<td>Validated, offline 2D chromatography method was generated to separate coeluting compounds</td>
<td>Long analysis time</td>
</tr>
<tr>
<td>Schmiech et al.62</td>
<td>Oleogum resins of 9 Boswellia spp. (41)</td>
<td>HPLC-MS/MS</td>
<td>8 Boswellic and lupeolic acids</td>
<td>G</td>
<td>Validated, moderate run time (30 min), baseline separated, good resolution</td>
<td>Long sample prep time (100 min), MS equipment is expensive</td>
</tr>
<tr>
<td>Shah et al.101</td>
<td>Oleogum resin extract, tablets (2), capsule (1)</td>
<td>HPLC-UV</td>
<td>KβBA and AβKBA</td>
<td>I</td>
<td>Validated, short run time (17 min), moderate sample prep time (30 min)</td>
<td>Only two BAs are measured</td>
</tr>
<tr>
<td>Sharma et al.102</td>
<td>Oleogum resin</td>
<td>HPLC-DAD</td>
<td>8 Boswellic and tirucallic acids</td>
<td>I</td>
<td>Validated</td>
<td>Long extraction time (9 h), long run time (50 min), no information on injection volume</td>
</tr>
<tr>
<td>Subbaraju103</td>
<td>Oleogum resins (2), extracts (5)</td>
<td>HPLC-DAD</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives</td>
<td>G</td>
<td>Partially validated, moderate run time (35 min), compounds are baseline separated, good resolution</td>
<td>Long extraction time (120 min)</td>
</tr>
<tr>
<td>Zhang104</td>
<td>Oleogum resins of 3 Boswellia spp. (55)</td>
<td>UPLC-DAD-MS</td>
<td>βBA, βBA, KβBA, and their acetylated derivatives, serratol, tirucallic acids</td>
<td>G</td>
<td>Validated, short run time (22 min)</td>
<td>Relatively long extraction time (60 min), no information on injection volume</td>
</tr>
</tbody>
</table>
The method developed by Katragunta et al.\textsuperscript{100} can be a good option for analyzing \textit{Boswellia} oleogum resins by HPLC, with a short run time (17 min), good resolution of BAs, and comprehensive characterization of secondary metabolites and adulterants in different types of herbal formulations that are labeled to contain \textit{Boswellia} oleogum resin extracts. Although sample preparation time is long (135 min), the method is validated, and it can be used for both qualitative and quantitative analysis. Another validated method has been published by Ganzera and Khan;\textsuperscript{82} it has a short sample preparation time (10 min) with a moderate run time (35 min). It allows the accurate determination of six individual BAs in methanol extracts of oleogum resin samples as well as in multi-component preparations with a gradient mobile phase composed of 0.05% phosphoric acid and an acetonitrile/water mixture.

### 10.2.6 Capillary electrochromatography

**Comments:** Capillary electrochromatography (CEC) is a little-known hybrid technique, combining the selectivity of the HPLC with the efficiency of capillary electrophoresis (CE). The driving force in CEC is the electroosmotic flow, generating a plug-like velocity profile, enabling high separation efficiencies and the use of small particles as stationary phase. Most published methods have used modified capillary electrophoresis instruments to perform the analysis.\textsuperscript{106} Ganzera et al. used CEC for the analysis of six major BAs in the resin of \textit{B. serrata}. The influence of relevant parameters (stationary phase, composition of mobile phase, voltage, temperature) was investigated and optimized. Baseline separation of selected compounds was possible in 20 min and the quantitative determination of six BAs in plant material was achieved. Due to their different absorption maxima, the standard compounds were detected either at 210 or 254 nm. The resulting data were precise (δmax = 3.3% based on peak area) and in good agreement with results obtained by HPLC analysis of the same sample (δmax = 4.1% between CE and HPLC results). The method can be used to determine BA contents of resin samples but, in this case, peak resolution was poorer than what can be achieved using HPLC. Additionally, the capillary column was lab-made and, to our knowledge, is not commercially available. Based on a number of shortcomings, e.g., time investment to manufacture the column, lack of commercial equipment, and poor separation results, there are no obvious reasons to use capillary electrochromatography to authenticate \textit{Boswellia} oleogum resin.

### 10.2.7 Supercritical Fluid Chromatography

The method described in the following literature was evaluated in this review: Zwerger and Ganzera\textsuperscript{107}.

**Comments:** Supercritical Fluid Chromatography (SFC), also known as convergence chromatography, is an increasingly popular separation technique that uses a supercritical fluid (CO\textsubscript{2}) as a mobile phase. An SFC system coupled to a DAD detector and a mass spectrometer was used by Zwerger and Ganzera.\textsuperscript{107} Under optimized conditions, six BAs (αBA, βBA, KβBA, and their acetylated derivatives) could be separated in under 6 min. This validated method can easily be hyphenated to mass spectrometry, which is helpful to tentatively assign further compounds (mainly derivatives of tirucallic acid) and to increase the assay’s sensitivity. Its practical applicability was confirmed by analyzing six commercial products (containing \textit{B. serrata} resin and/or extract), which mainly contained βBA as dominant triterpene, yet in extremely variable amounts ranging from 0.9% to 16.9%. SFC-DAD showed to be slightly less sensitive than UPLC-DAD, and peak overlapping in some of the extracts was observed; however, in five out of six dietary supplements containing \textit{B. serrata} oleogum resin all six marker compounds could be assigned. Advantages are the fast separation and the low amounts of organic solvents needed. The method can be used to determine BA contents of resin samples and can be used to detect adulteration.

### 10.2.8 Gas chromatography (GC)

Methods described in the following review and summarized in Table 6 are: Başar,\textsuperscript{64} Hairfield et al.,\textsuperscript{90} Hamm et al.,\textsuperscript{55} Niebler et al.,\textsuperscript{36} Paul,\textsuperscript{43} Schmiech et al.,\textsuperscript{54} and Singh et al.\textsuperscript{56}

**Comments:** Various gas chromatography (GC) methods have been developed for the characterization and differentiation of \textit{Boswellia} species based on the chemical composition of the oleogum resins. A flame ionization detector (FID) and/or mass spectrometry are used for the detection. With the exception of Hairfield et al.\textsuperscript{90} and Başar,\textsuperscript{64} the publications have focused on essential oil analysis of various \textit{Boswellia} species. Essential oils of resin samples are obtained by hydrodistillation, then diluted with dichloromethane or pentane before analysis, although other appropriate solvents may also be used. In some of the studies, the headspace solid phase microextraction (SPME) technique was applied to analyze the oleogum resin directly in order to avoid the distillation step. Headspace SPME is a non-destructive method, and it concentrates volatile and semi-volatile compounds allowing their detection even at trace levels without the
need to dissolve the sample. Additionally, non-volatile compounds such as fats, waxes, and polysaccharides, often dominant in gums, are not extracted by such a method, thus avoiding the fastidious sample pretreatment required for classical GC-MS analysis.\textsuperscript{55} Due to the low volatility of diterpenes, their determination is highly dependent on SPME method parameters and on the volatilization process parameters (duration, temperature) in the case of the essential oils. However, the enrichment by SPME from raw gum resin material appears to yield higher proportions of the diterpenoids than in essential oils obtained by hydrodistillation, for both \textit{B. sacra} and \textit{B. serrata}.\textsuperscript{36}

None of the methods described in this section are validated. Those proposed by Hamm et al.\textsuperscript{55} and Niebler et al.\textsuperscript{36} may enable the unambiguous determination of adulteration of \textit{B. serrata} oleogum resin with other \textit{Boswellia} species, i.e., \textit{B. frereana}, \textit{B. papyrifera}, and \textit{B. sacra}, by means of their specific chemotaxonomic markers. Given the fact that many commercial \textit{Boswellia} extracts are obtained from gums after the essential oil has been removed and sold to the fragrance industry, the usefulness of gas chromatography for species identification based on the composition of volatile constituents is restricted to crude oleogum resins. Compared to HPLC-UV, the need for derivatization of the samples makes GC a much less attractive method for the analysis of boswellic and other triterpene acids.

## Conclusion

Although macroscopic and organoleptic characteristics of frankincense are well known, the identification of major raw \textit{Boswellia} products in trade is possible only by experts, based on color and taste. Macroscopic and organoleptic assays are not sufficiently reliable to identify processed materials, such as extracts enriched in BAs. Isolating high quality DNA from oleogum resin samples is challenging, and there is a relatively high risk of contamination by foreign matter due to the sticky nature of the gums; therefore, genetic studies on \textit{Boswellia} species aiming to detect adulteration are limited and more data are needed to assess the usefulness of DNA-based methods for oleogum resin authentication. Again, the usefulness of DNA-based assays to authenticate \textit{Boswellia} extracts is

<table>
<thead>
<tr>
<th>Table 6: Comments on the published GC methods for \textit{B. serrata} oleogum resin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basar</strong>\textsuperscript{54}</td>
</tr>
<tr>
<td><strong>Hairfield</strong>\textsuperscript{90}</td>
</tr>
<tr>
<td><strong>Hamm</strong>\textsuperscript{55}</td>
</tr>
<tr>
<td><strong>Niebler</strong>\textsuperscript{36}</td>
</tr>
<tr>
<td><strong>Paul</strong>\textsuperscript{41}</td>
</tr>
<tr>
<td><strong>Schmiech</strong>\textsuperscript{54}</td>
</tr>
<tr>
<td><strong>Singh</strong>\textsuperscript{56}</td>
</tr>
</tbody>
</table>
very limited. Titration is one of the easiest and lowest-cost methods to measure “BA” contents of whole or powdered oleogum resins and their extracts, but the method suffers from a lack of specificity and accuracy due to interference from other acidic compounds and is also inappropriate to detect adulteration.

For materials containing essential oils, such as the crude frankincense oleogum resins, GC-MS/FID is a useful technique. Of interest are the methods published by Hamm et al.,55 and Niebler et al.,36 which may detect adulteration of B. serrata oleogum resin with other Boswellia species. Other methods, such as capillary electrochromatography and supercritical fluid chromatography are not widely used in the herbal and dietary supplement industry, and there are not enough scientific data to determine whether they are adequate to detect adulteration of whole or powdered oleogum resins, their extracts, or commercial products.

As explained in section 10.2.5, many commercial “Boswellia serrata” extracts have been chemically modified to contain high concentrations of AKβBA. In most of these cases, the determination of the species of origin is not feasible anymore by the tests described in this laboratory guidance document. Methods that confirm the species in extracts based on the chemical composition are appropriate for native, natural B. serrata oleogum resins.

If a method based on the metabolite composition is chosen for authentication, the TLC method offered by Paul et al.23 might be a suitable option to differentiate Boswellia oleogum resins from each other; additionally, the HPTLC method reported by Krohn51 can be used for routine quality control and the quantification of the four major BAs in frankincense materials. The HPLC and UHPLC methods summarized in Table 5 can be used for qualitative and quantitative purposes. The validated methods described by the USP, Ph. Eur., Katragunta et al.,100 and Ganzera and Khan82 are considered suitable choices, with the last method being of interest due to the simple sample preparation, medium run time, and good peak resolution. However, none of these methods has been assessed for their ability to determine the correct species in mixed samples, i.e., samples where several Boswellia species have been mixed together. In such cases, the determination of some of the specific chemical marker compounds outlined in section 10.1 by HPLC-UV-MS is likely to provide the best results.

References

### Table 7. Comparison of the different techniques to characterize/authenticate *B. serrata* oleogum resin

<table>
<thead>
<tr>
<th>Method</th>
<th>Applicable to</th>
<th>Pro</th>
<th>Con</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic</td>
<td>Whole oleogum resin</td>
<td>No solvents required</td>
<td>No systematic anatomy studies exist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quick</td>
<td>No automation/statistics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inexpensive</td>
<td>Need for botanically authenticated reference material</td>
</tr>
<tr>
<td>Microscopic</td>
<td>Powdered oleogum resin</td>
<td>Inexpensive Few solvents required</td>
<td>No automatic/statistics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No distinctive characteristic elements currently known</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Obtaining tissue fragments from the oleogum resin is challenging</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reference material needed</td>
</tr>
<tr>
<td>Organoletic</td>
<td>Whole oleogum resin</td>
<td>Quick Inexpensive Color and odor can give an idea about adulteration</td>
<td>Difficult or impossible for highly processed products</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not appropriate for certain processed materials (i.e., oleogum resins devoid of essential oil)</td>
</tr>
<tr>
<td>Genetic</td>
<td>Whole or powdered oleogum resin</td>
<td>Able to detect small amounts of non-target species Only method for botanical blend No reference material needed when database established</td>
<td>Isolation of high-quality DNA is difficult from resins and gums Oligum resins are highly adhesive and they are prone to contamination with incidental amounts of foreign matter Cannot distinguish among plant parts Labor-intensive sample preparation and analysis Expensive equipment DNA in certain processed materials cannot be detected No info about the ability of this method to detect substitution by other <em>Boswellia</em> species</td>
</tr>
<tr>
<td>Titration</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Quick Inexpensive</td>
<td>Lack of specificity and accuracy Results are not comparable to results from HPLC Presence of other <em>Boswellia</em> species cannot be detected Method only suitable for quantitation of total “BAs”</td>
</tr>
<tr>
<td>TLC/HPTLC</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Quick and efficient Basic equipment affordable for smaller labs Able to detect small amounts of adulterants</td>
<td>High-end equipment somewhat expensive Need for authenticated botanical reference materials</td>
</tr>
<tr>
<td>IR, NIR, FTIR</td>
<td>Whole or powdered oleogum resin,b extracts</td>
<td>No sample preparation needed Short analysis time State-of-the-art statistical evaluation Environmentally friendly approach</td>
<td>Accuracy and precision for low-concentration compounds insufficient Limited quantitative information Large number of authenticated samples needed for statistics</td>
</tr>
<tr>
<td>Hyperspectral imaging</td>
<td>Whole oleogum resin</td>
<td>Non-destructive technique Environmentally friendly approach</td>
<td>Low accuracy Large number of samples needed to establish a model</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Standard equipment in laboratories Able to detect small amounts of adulterants Both quantitative for chemical profiling and quantitative for marker compounds Suitable for routine analysis</td>
<td>Equipment somewhat expensive</td>
</tr>
<tr>
<td>HPLC/UPLC-MS</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Able to detect small amounts of adulterants Qualitative and quantitative</td>
<td>Equipment expensive</td>
</tr>
<tr>
<td>Capillary electro chromatography</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Qualitative and quantitative</td>
<td>No info about the ability of this method to detect presence of other <em>Boswellia</em> species Lower sensitivity than HPLC-UV No commercial equipment available</td>
</tr>
<tr>
<td>Supercritical fluid chromatography</td>
<td>Whole or powdered oleogum resin,a extracts</td>
<td>Effective and fast Qualitative and quantitative Environmentally friendly approach</td>
<td>No info about the ability of this method to detect other <em>Boswellia</em> species, but results are expected to be similar to HPLC-UV</td>
</tr>
<tr>
<td>GC-MS/FID</td>
<td>Whole or powdered oleogum resin,a extracts, essential oil</td>
<td>Able to detect small amounts of adulterants Qualitative and quantitative</td>
<td>Equipment expensive Mainly for volatile constituents, other compounds need derivatization prior to analysis</td>
</tr>
</tbody>
</table>

*a* Whole, cut, and powdered material only suitable for analysis after extraction  
*b* With NIR and FTIR whole or powdered material can be analyzed without prior extraction; for IR, prior extraction is necessary.
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48. Tripathi N, Thakur V, Pooniya S, et al, inventors; India; assignee. A DNA based method for verification of *Boswellia serrata* gum as adulterant in gum oleo-resin of *Commiphora wightii* by extraction of DNA of high quality from them and development of a primer set for its amplification. India: Indian Pat Appl; 2019:IN201921005333.


