

Boswellia Serrata Essential Oils: Comparison of the Chemical Composition, Antimicrobial and Antioxidant Activity of the Commercially Available One and Hydrodistilled from Commercial Resin

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Abstract This paper was written with the aim to give comparison of the commercially available *B. serrata* essential oil and hydrodistilled from resin that is also commercially available on the Serbian market. In that order, GC/MS analysis was performed, chemical components identified and quantified and obtained results served as the basis for interpreting the findings from the antimicrobial and antioxidant tests. The essential oil from frankincense resin was isolated by Clevenger's distillation. In the essential oil hydrodistilled from commercial resin a total of 28 components were identified with octanol acetate (40.6%) as dominant one, while in the commercial one 18 components were identified and the most represented was α -thujene (75.9%). The antimicrobial activity of essential oils was determined by the disc-diffusion method against nine microorganisms. Both isolated and commercial oil exhibited similar inhibitory strengths (mm of inhibition zone) against: *S. aureus* ATCC 25923 (10.66 and 12.66), *B. cereus* house strain (16.33 and 11.33), *E. coli* ATCC 25922 (11.33 and 10.66) with that difference that only commercial one inhibited *C. albicans* ATCC 2091 (12.66). The results for antioxidant activity determined spectrophotometrically by DPPH test favoured commercial essential oil under the same conditions after 40 minutes of incubation.

Keywords: essential oil, *Boswellia serrata*, GC/MS analysis, antioxidant activity, antimicrobial activity

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1. Introduction

Boswellia serrata also known as Indian frankincense tree, belongs to the family *Burseraceae* and is widespread in dry regions of India, Yemen, Nigeria and Pakistan, as well in Arabian Peninsula [1,2]. The *Boswellia* tree produces a resin that is also known by the names: frankincense, sallaki, idigenous olibanum, luban [3]. This frankincense has a trading history between Europe and China for the past 5,000 years [4]. Since ancient times, this natural resin has been used for embalming and in cultural ceremonies due to its incense. Members of Hindu, Babylonian, Persian, Roman, Chinese, Greek, and ancient American civilizations practiced burning this resin to soothe their souls and please their gods with the smoke and fragrance it produces [5]. It is a coloured, exuded like "tear" shaped solid with odour described as dry, slightly green, fresh-balsamic and tenacious. When pyrolysed, it gives well known incense characteristic of the churches [6]

(both Orthodox and Catholic [4]).

B. serrata is considered the 'true frankincense' producing tree. Interest in the scientific study of this plant species is on the rise, and modern science has revealed that its oleo-gum-resin is comprised of over 200 different natural products (30-60% resin, 5-10% essential oils, and polysaccharides such as galactose, arabinose, and xylose). In Islamic traditional medicine in Arabian countries, frankincense is mentioned in Avicenna's (Ibn Sina's) Canon of Medicine and is used to treat tuberculosis, amnesia, infections, bruises, diarrhea, burns, stomach issues, and eye sores. It is also referenced in Chinese traditional medicine, where frankincense-based pills are used to treat bronchial, nasopharyngeal, or pancreatic carcinoma [4]. The frankincense is also a part of Ayurvedic system of medicine because of its anti-inflammatory properties [7,8], and used in treatment of diarrhoea, dysentery, ringworm, boils, fevers (antipyretic), skin and blood diseases, cardiovascular diseases, mouth sores, bad throat, bronchitis, asthma, cough, vaginal discharges, hair-loss, jaundice, hemorrhoids, syphilitic

diseases, irregular menses and stimulation of liver [5]. The powdered olibanum form is used to relieve toothache [9].

The content of secondary metabolites in resin varies significantly. Some species are rich in monoterpenes, while others contain diterpenes or triterpenes [10]. The qualitative and quantitative composition of chemical compounds in the resins of different *Boswellia* species depends on geographical location, climatic conditions and the timing of resin collection [11]. An overview of the main chemotypes of *Boswellia* species is given in Table 1.

Table 1. Overview of the main chemotypes of *Boswellia* species based on the analysis of essential oils obtained by hydrodistillation of the resin [12]

<i>Boswellia</i> species	Major chemotypes	Reference
<i>B. ameero</i>	α -Thujene, cosmene	[12]
<i>B. carteri</i>	α -Pinene, α -thujene	[12]
<i>B. dalzielii</i>	α -Pinene, myrcene	[12]
<i>B. dioscoridis</i>	α -Thujene	[12]
<i>B. elongata</i>	α -Thujene, α -pinene	[12]
<i>B. frereana</i>	α -Pinene, α -Thujene	[12]
<i>B. nana</i>	α -Thujene	[12]
<i>B. neglacta</i>	α -Pinene, α -thujene	[12]
<i>B. papyrifera</i>	Octyl acetate	[12]
<i>B. popoviana</i>	α -Thujene	[12]
<i>B. rivaie</i>	Limonene, α -pinene	[12]
<i>B. sacra</i>	α -Pinene, limonene	[12]
<i>B. serrata</i>	α -Thujene, myrcene, sabinene	This work
<i>B. socotrana</i>	α -Pinene, α -thujene	[12]
<i>B. thurifera</i>	α -Pinene	[12]

The resin obtained from the Indian frankincense tree contains pentacyclic triterpenoids and boswellic acids (BAs) as dominant compounds (25–35% [13]. Six major boswellic acids were reported: α and β -boswellic acids are the leading contenders (BA, 10–21%), followed by Acetylated α and β -Boswellic acids (ABA, 0.05–6%), 11-keto- β -boswellic acid (KBA, 2.5–7.5%) and 3-O-acetyl-11-keto- β -boswellic acid (AKBA, 0.1–3%) [14]. BA and their derivatives are *in-vitro* non-redox inhibitors of 5-lipoxygenase (5-LOX), an enzyme in neutrophils responsible for the conversion of endogenous arachidonic acid into leukotrienes that cause vasoconstriction, bronchospasm and chemotaxis [15]. These acids possess numerous activities including anti-inflammatory, antitumor, immunomodulatory and in the treatment of inflammatory bowel diseases [8,14].

The essential oil and resin of frankincense are used in treatment of many diseases. Interest in this plant is growing [7] due to the therapeutic potential of its isolates [16]. The various activities demonstrated by the isolates (essential oil, extracts) of *B. serrata* resin have led to the development of different formulations in the pharmaceutical and cosmetic industry e.g. 50 ml of “Eau de Parfum” containing *B. serrata* extract is sold for 165\$ [17]. In “Western medicine” frankincense was introduced in the early 20th century to treat various inflammation diseases and is listed in the 7th supplement of the European Pharmacopoeia [4]. Standardized preparations of *Boswellia serrata* extract (BSE) are commercially available and are used to treat inflammatory diseases such as: rheumatoid arthritis, Crohn's disease, ulcerative colitis and inflammatory bowel disease [18].

Based on current data, BSE can be considered as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs) [19] due to less pronounced gastrointestinal and cardiovascular side effects [20]. Unlike NSAID, which are well known to disrupt glycosaminoglycan synthesis and lead to joint damage in arthritis, it has been shown that BA significantly reduces the decomposition of this polysaccharide [21]. Studies have also shown that extracts from this species can be effective against malignant breast diseases and may prevent the spread of certain forms of leukemia and brain tumors, thanks to the presence of BA [22]. The essential oil of Indian frankincense exhibits a wide range of pharmacological activities, including antioxidant, antimicrobial, anticancer, anti-inflammatory, neuroprotective, and antiulcer activity [23,24,25]. The determination of the chemical composition of *B. serrata* essential oil is based on a method that combines gas chromatography (GC) with mass spectrometry (MS) [26,27]. Due to the presence of pinene, frankincense essential oil modulates antibiotic resistance by reducing the minimum inhibitory concentration (MIC) of gentamicin, erythromycin and triclosan up to 512 times [28], and in addition, thujene, camphene, β -Pinene, myrcene, limonene also exhibit antimicrobial activity [29]. Of all BA, AKBA is the most potent antibacterial compound against oral cavity pathogens [14]. The essential oil of Indian frankincense is used as an antiseptic agent in mouthwashes, and to treat asthma and coughs [30], and because of all above mentioned benefits, it is highly valuable and widely commercialized.

The aim of this paper is to provide a comparative analysis of the chemical composition, antimicrobial properties and antioxidant activity of *B. serrata* essential oils isolated from commercially available frankincense resin and the one that is commercially available on the Serbian market.

2. Materials and Methods

Plant material

Commercial essential oil of Indian frankincense was used in this work (Probotanic D.O.O., Belgrade, Serbia) bought in local drugstore (Leskovac, Serbia) and *B. serrata* frankincense resin bought in specialized store (Belgrade, Serbia). Just before the essential oil was isolated, the Indian frankincense resin was grounded to a fine, brown powder.

Reagents and chemicals

The following reagents and chemicals were used: ethanol, 96% (Centrohem Zemun, Serbia), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, alkane standard solution C₈–C₂₀ (~40 mg/L each, in hexane), alkane standard solution C₂₁–C₄₀ (Sigma Chemical Company, St. Louis, USA) and diethyl ether (J.T. Baker, New Jersey, USA).

Isolation of Indian frankincense resin essential oil

The essential oil from frankincense resin was isolated using Clevenger's distillation. Precisely, 50.0 g of resin was weighed and grounded by a pestle in mortar, quantitatively transferred to the boiling flask, and 500.0 mL of distilled water was added afterwards (hydromodule 1:10, m/v). The distillation continued up to 4 h, after

which a light yellow essential oil was obtained and therefore stored in a refrigerator at 4°C up to analysis.

Gas chromatography-mass spectrometry (GC/MS) of isolated and commercial *B. serrata* resin essential oils

The GC/MS analysis of essential oils was performed on a gas chromatograph instrument Agilent Technologies 7890B coupled with inert, selective 5977A mass detector of same company. Isolated and commercial essential oil was dissolved in diethyl ether and 1 µL of prepared solution was injected in split (40:1) injector operation mode at temperature 220°C.

As a carrier was used helium of purity 99,999% at a constant flow rate of 1 mL/min. The components were separated on a non-polar, silica capillary column HP-5MS (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). The oven temperature was increased linearly starting from 60°C to 246°C at speed of 3°C/min. The total time of the analysis was 62 min. The eluate was by the MSD transfer temperature line 250 °C transferred to quadrupole mass spectrometer with electron ionization (ion source and quadrupole mass analyzer temperatures were 230°C and 150°C, respectively, and electron energy was 70 eV) and analysed in Scan mode, in *m/z* range of 41-415 Da. Analysis was performed in triplicate.

Data analysis was done by MSD ChemStation Data Analysis in combination with AMDIS (*Automatic Mass Spectral Deconvolution and Identification System*) and NIST MS Search softwares (Agilent Technologies, USA).

For definitely identification of the components of the mixture were used experimentally determined retention indices by using a series of homologous n-alkanes C₈-C₂₀ as a standard. The identification of the components is based on the comparison of their retention indices (RI^{exp}) to values from literature (RI^{lit}), on the comparison of their mass spectra with spectra from Willey, NIST and RTLPEST library (MS) and when was possible, by co-injecting of responding standard (C₀-I).

Quantitative analysis was done by applying the surface normalization method GC/FID of signal without any corrections.

Antimicrobial activity of isolated and commercial Indian frankincense essential oils

Antimicrobial activity of isolated and commercial Indian frankincense essential oil was tested against 9 microorganisms, four Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* in house strain, *Bacillus subtilis* ATCC 6633, *Listeria monocytogenes* ATCC 15313; four Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Klebsiella pneumoniae* ATCC 700603 and fungus *Candida albicans* ATCC 2091.

Disk-diffusion method was applied for antimicrobial analysis. The substrates were sterilized in an autoclave, 15 minutes at temperature 120 °C and pressure 110 kPa. Microorganism suspensions were prepared by the method of direct suspension of colonies. Colonies were suspended directly from the plate into 10 mL of sterile 0,8% physiological solution. The turbidity of the starting suspension was adjusted by comparing with the turbidity of 0.5 McFarland standard solution so that suspension contains 10⁸ CFU/mL of bacteria, that is 10⁶ CFU/mL

yeast cells. Bacterial suspensions were seeded onto the surface of the Mueller hinton plates containing agar („Torlak”, Belgrade, Serbia), while the suspension of yeas was seeded onto the surface of the Sabouraud malt agar („Torlak”, Belgrade, Serbia). On the hardened inoculated substrate, sterile disks of diameter 9 mm (Schleicher&Schuell) were placed, which are impregnated with 50 µL of essential oil. The samples were incubated during 24 h at 37°C for bacteria and 48 h at 25 °C for yeast. After incubation, the zones of inhibition of microorganisms growth were measured by the diameter measuring of the inhibition zone together with the disc and expressed in millimeters. The presence of a transparent inhibition zone was a confirmation of the antimicrobial activity of the tested sample. DMSO was used as a negative control, while commercial disks (6 mm) of Ciprofloxacin 5 µg (Carl Roth, Karlsruhe, Germany) and antimycotic Nystatin 100 I.U. (Carl Roth, Karlsruhe, Germany) were used as positive control.

Antioxidative activity of isolated and commercial Indian frankincense essential oils – DPPH test

The antioxidative activity of isolated and commercial Indian frankincense essential oils were determined spectrophotometrically using the DPPH test. The solutions of essential oil in ethanol concentration of 0.5 mg/mL were prepared. After that, a series of solutions of different concentrations was made (0.0078-0.0625 mg/mL). Ethanolic solution of DPPH radical was added into 2.5 mL of prepared solutions of essential oil. Absorbance was measured at 517 nm after 20 and 40 minutes of incubation at a room temperature. Also, absorbance was determined for ethanolic solution of DPPH radical (1 mL of DPPH radical and 2.5 mL of ethanol) as for ethanolic solution of essential oils (2.5 mL of essential oil and 1 mL of ethanol). The free radical scavenging capacity was calculated by formula [31]:

$$\text{DPPH radical scavenging capacity (\%)} = 100 - [(A_S - A_B) \times \frac{100}{A_C}]$$

A_S = Absorption of the „sample“ at 517 nm.

„Sample“ - ethanolic solution of samle treated with DPPH radical (2.5 mL of sample + 1 mL of DPPH radical); *A_B* = Absorption of the „blank“ at 517 nm. „Blank“ - ethanolic solutionof sample which was not treated with DPPH radical (2.5 mL of sample + 1 mL of ethanol); *A_C* = Absorption of the „control“ at 517 nm. „Control“ - diluted ethanolic solution of DPPH radical (2.5 mL of sample + 1 mL of DPPH radical).

3. Results

Qualitative and quantitative composition of isolated and commercial Indian frankincense essential oils

The appearance of frankincense resin as well as the isolated and commercial essential oils is shown in Figure 1.

The GC/MS method was used to determine the chemical composition of the isolated Indian frankincense essential oil, and the results are presented in Table 2.

The chemical composition of the commercial Indian frankincense essential oil was determined in the same manner, and the results of the analysis are presented in Table 3.

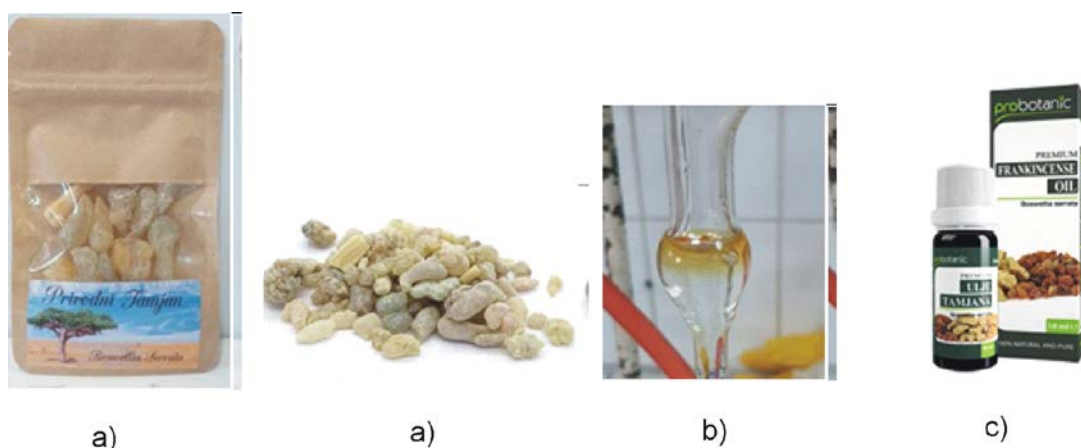


Figure 1. The frankincense resin (a), isolated essential oil (b), commercial essential oil (c)

Table 2. Chemical composition of isolated Indian frankincense essential oil (*B. serrata*)

Br.	$t_{ret, min}$	Component	RI^{exp}	RI^{lit}	Identification method	Concentration %
1.	6.70	α -Thujene	914	924	RI, MS	tr
2.	6.92	α -Pinene	922	932	RI, MS, C ₀ -I	tr
3.	7.39	Camphene	937	946	RI, MS, C ₀ -I	tr
4.	8.15	Sabinene	962	969	RI, MS	tr
5.	8.27	β -Pinene	966	974	RI, MS, C ₀ -I	tr
6.	8.68	Myrcene	980	988	RI, MS	tr
7.	9.43	Hexyl acetate	1003	1007	RI, MS	tr
8.	9.56	O-Cresol methyl ether	1007	1005	RI, MS	tr
9.	10.11	Limonene	1022	1024	RI, MS, C ₀ -I	tr
10.	10.41	(Z)- β -Ocimene	1029	1032	RI, MS	tr
11.	10.82	(E)- β -Ocimene	1040	1044	RI, MS	0.5
12.	12.02	n-Octanol	1072	1063	RI, MS	3.8
13.	13.20	Linalool	1103	1095	RI, MS, C ₀ -I	tr
14.	14.05	2-Methyl-2-heptene-6-ol	1123	-	MS	0.8
15.	17.68	Octanol acetate	1210	1211	RI, MS	40.6
16.	20.75	Isobornyl acetate	1283	1283	RI, MS	tr
17.	23.63	(2E,4E)-Decadienal	1325	1315	RI, MS	tr
18.	24.91	Nerylacetate	1342	1359	RI, MS	0.4
19.	32.47	(Z)-Nerolidol	1541	1531	RI, MS	11.9
20.	33.38	Dodecanoid acid	1594	-	MS	1.8
21.	45.08	Cembren	1940	1937	RI, MS	4.0
22.	45.54	(3E)-Cembren A	1955	1947	RI, MS	1.0
23.	46.10	(Z)-Cembren A	1972	1965	RI, MS	7.8
24.	46.52	Sclarene	1984	1974	RI, MS	1.6
25.	47.59	Cembrene S	2018	-	MS	1.5
26.	47.79	Verticila-4(20),7,11-trien	2014	2004	RI, MS	9.9
27.	52.30	Incesole	2164	2158	RI, MS	11.2
28.	52.72	Incesole acetate	2177	2184	RI, MS	3.5
Total identified (%)						100.0

t_{ret} : retention time; RI^{lit} : retention indices from literature (Adams, 2007); RI^{exp} : linear retention indices determined experimentally by using a homologous series of *n*-alkanes(C₈-C₂₀ and C₂₀-C₄₀) on the HP-5MS column; MS: components identified by comparison of mass spectra; RI: components identified by comparison of retention indices; C₀-I: components identified by co-injection of an identical standard; tr: component present in traces < 0,05; results shown are the mean value of three replicates.

Table 3. Chemical composition of commercial Indian frankincense essential oil (*B. serrata*)

Br.	$t_{ret, min}$	Component	RI^{exp}	RI^{lit}	Identification method	Concentration %
1.	7.13	α -Thujene	928	924	RI, MS	75.9
2.	7.32	α -Pinene	934	932	RI, MS, C ₀ -I	5.8
3.	7.60	Thuja-2,4(10)-diene	943	953	RI, MS	0.2
4.	8.49	Sabinene	973	969	RI, MS	3.7
5.	8.63	β -Pinene	978	974	RI, MS, C ₀ -I	0.2
6.	8.99	Myrcene	990	988	RI, MS	1.3
7.	9.51	α -Phellandrene	1006	1002	RI, MS	0.7
8.	9.72	δ -3-Carene	1011	1008	RI, MS	3.5

Br.	t_{ret} , min	Component	RI ^{exp}	RI ^{lit}	Identification method	Concentration %
9.	9.93	α -Terpinene	1017	1014	RI, MS	0.2
10.	10.10	<i>p</i> -Cymene	1021	1020	RI, MS	tr
11.	10.21	<i>o</i> -Cymene	1024	1022	RI, MS	2.5
12.	10.37	Limonene	1028	1024	RI, MS, C ₀ -I	2.5
13.	10.64	(<i>Z</i>)- β -Ocimene	1036	1032	RI, MS	0.2
14.	11.49	γ -Terpinene	1058	1054	RI, MS, C ₀ -I	0.7
15.	12.65	Terpinolene	1089	1086	RI, MS	0.1
16.	13.79	<i>trans</i> -Thujone	1117	1112	RI, MS	0.1
17.	16.33	Terpinen-4-ol	1178	1174	RI, MS, C ₀ -I	1.0
18.	17.18	Methylchavicol	1198	1195	RI, MS	0.8
Total identified (%)						99.4

t_{ret} : Retention time; RI^{lit}: Retention indices from literature (Adams, 2007); RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C₈-C₂₀ and C₂₀-C₄₀) on the HP-5MS column; tr: trace amounts (<0.05%); results shown are the mean value of three replicates.

Table 4. Antimicrobial activity of isolated and commercial Indian frankincense essential oils (*B. serrata*)

Microorganisms	Isolated essential oil of Indian frankincense	Commercial essential oil of Indian frankincense	Ciprofloxacin 5 μ g	Nystatin
<i>S. aureus</i> ATCC 25923	10.66 $\pm$ 0.58	12.66 $\pm$ 0.58	26.67 $\pm$ 0.58	n.d.
<i>B. cereus</i> in house strain	16.33 $\pm$ 0.58	11.33 $\pm$ 0.58	28 $\pm$ 1	n.d.
<i>B. subtilis</i> ATCC 6633	/	/	n.d.	n.d.
<i>L. monocytogenes</i> ATCC 15313	/	/	35.67 $\pm$ 0.58	n.d.
<i>E. coli</i> ATCC 25922	11.33 $\pm$ 0.58	10.66 $\pm$ 0.58	40.33 $\pm$ 0.58	n.d.
<i>P. aeruginosa</i> ATCC 27853	/	/	40.67 $\pm$ 0.58	n.d.
<i>P. vulgaris</i> ATCC 8427	/	/	26.33 $\pm$ 0.58	n.d.
<i>K. pneumonia</i> ATCC 700603	/	/	30.33 $\pm$ 0.58	n.d.
<i>C. albicans</i> ATCC 2091	/	12.66 $\pm$ 0.58	n.d.	17.33 $\pm$ 0.58

Zone of inhibition (mm $\pm$ SD); n.d. not detected

Antimicrobial activity of isolated and commercial Indian frankincense essential oils

The antimicrobial activity of isolated and commercial *B. serrata* frankincense essential oils were tested against Gram-negative bacteria, Gram-positive bacteria and fungus. The results obtained are presented in Table 4.

Antioxidant activity of isolated and commercial Indian frankincense essential oils

Antioxidant activity of commercial and isolated essential oils from *B. serrata* frankincense was analyzed by DPPH test and obtained results are shown in Figure 4.

4. Discussion

Qualitative and quantitative composition of isolated and commercial Indian frankincense essential oils

Based on the obtained results (Table 2), the most abundant compounds in the isolated Indian frankincense essential oil are octanol acetate (40.6%), (*Z*)-nerolidol (11.9%) and incesole (11.2%), with their structural formulas presented in Figure 2.

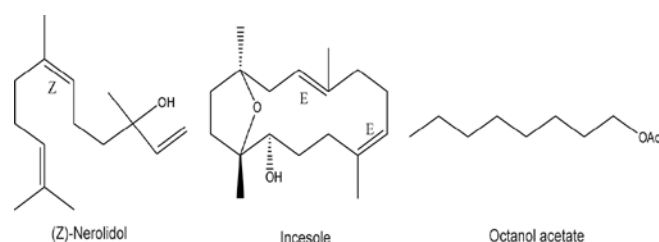


Figure 2. Structural formulas of most abundant components identified from isolated Indian frankincense essential oil

Based on the results of the analysis (Table 3), monoterpenes are dominant compounds in the commercial essential oil comprising 95.0% of the total with α -thujene (75.9%), α -pinene (5.8%), sabinene (3.7%) and δ -3-carene (3.5%). The structures of the most abundant components identified in the commercial essential oil of Indian frankincense are presented in Figure 3.

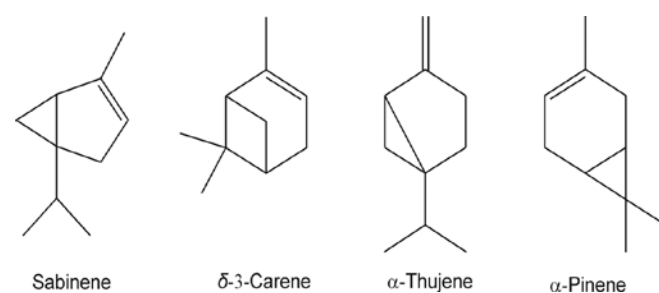


Figure 3. Structural formulas of most abundant components identified from commercial Indian frankincense essential oil

The essential oils of most species in this genus are rich in monoterpenes. The essential oil of *B. carterii* contains α -pinene, α -thujene, limonene, sabinene, myrcene and *p*-cymene [12], while in essential oil of *B. frereana* dominates α -pinene, α -thujene, *p*-cymene and sabinene [32,33,34]. The essential oil of *Boswellia sacra* is rich in α -pinene, myrcene and limonene [33,35,36]. α -thujene can be found in essential oils of the following species: *B. serrata*, *B. ameero*, *B. elongate*, *B. popoviana*, *B. nana* [37]. On the contrary, the essential oil of *B. papyrifera* contains octyl acetate and octanol as its dominant compounds [33,38,39].

Singh et al., 2007 and Camarda et al., 2007 have determined that in the essential oil of Indian frankincense

resin α -thujene is dominant. This is consistent with the results obtained in this study [40,41].

Based on previously published GC/MS analysis results, the most prevalent compounds in commercial Indian frankincense essential oil are from the group of monoterpenes, such as α -thujene [42] and α -pinene [34]. The high content of monoterpenes in the commercial sample analyzed in this study may be the result of long-term storage, as the conversion of oxygenated monoterpenes into their corresponding monoterpene hydrocarbons occurs due to deoxygenation under uncontrolled humidity and temperature conditions [40]. It has been shown that the frankincense resin of *B. serrata* yields a higher content of essential oil (3.30 – 9.37%) compared to *B. sacra* (5.5%), *B. elongate* (2.3%), *B. ameero* (1.8%) and *B. socotrana* (1.2%) [43,44]. Variations in the chemical composition of essential oils may also result from extraction methods and conditions, agroecological factors, storage conditions, seasonal variations, and geographical differences [45,46,47].

In the study by Gupta et al. (2016) the chemical composition of essential oils from three geographical locations was compared: essential oil of *B. serrata* resin collected in forest called Šivpurat northwest part of Madhya Pradesh (India) and commercial samples. Based on their GC/MS analysis results, the commercial samples contained a higher percentage of monoterpenes (81.9-88.1%) with α -thujene (61.4-69.8%) as the most abundant compound, which is consistent with the results obtained in this study. On the other hand, the essential oil isolated from resin collected from wild habitats contained a higher percentage of oxygenated monoterpenoids (15.7%) and sesquiterpenes (19.2%). The higher percentage of monoterpene hydrocarbons in commercial samples, particularly α -thujene, α -pinene and δ -3-carene, may be related to their increased antioxidant and antifungal activity [26].

α -thujene is a monoterpene that is also found in eucalyptus, juniper, dill and coriander. Although there are only a few data on the biological activity of α -thujene, it was reported as a potential insecticide, anti-inflammatory and antimicrobial agent [48].

On the other hand, pinenes exhibit numerous biological activities and are used as fungicides, antiviral and antimicrobial agents as well as in fragrances and aromas. It has been shown that they have an inhibitory effect on breast cancer and leukemia. Pinenes are by the US Food and Drug Administration generally recognized as safe [28], which allows their use in various products. α -pinene also exhibits anti-inflammatory, antioxidant, anti-cancer, antibacterial and antinociceptive properties. It has been proven that they are highly versatile in polymer synthesis, with polymers synthesized from pinene demonstrating higher quality than those produced from other sources [49].

Antimicrobial activity of isolated and commercial Indian frankincense essential oils

The antimicrobial activity of various essential oils derived from aromatic plants is extensively studied due to the need for new antimicrobial agents and the increasing resistance of bacteria to conventional antibiotics [8].

The tested essential oils tested in this study exhibited weak to moderate activity against tested microorganisms.

Gram-positive bacteria demonstrated sensitivity to the tested essential oils: *S. aureus* ATCC 25923 and *B. cereus* in house strain, as well as Gram-negative bacteria *E. coli* ATCC 25922. The detected zones of inhibition are significantly smaller compared to the effect of the antibiotic (Table 4). The essential oils of Indian frankincense did not show activity against the other tested bacterial strains. In addition, commercial essential oil of Indian frankincense showed inhibitory effect against fungus *C. albicans* ATCC 2091, while it was not observed when isolated essential oil was tested. To better understand the spectrum of antimicrobial effects of *B. serrata* essential oils, further research is needed, including testing against a larger number of pathogens. The results obtained in this study represent an important contribution particularly in light of the increasing resistance of pathogens to antibiotics, which is one of the greatest threats to global human health. As bacterial resistance to various antibiotics is one of the biggest global challenges, every investigation into new antimicrobial agents of natural origin can contribute to solving this problem [8].

Sadhasivam et al. (2016) tested the antimicrobial activity of nine commercially available essential oils against *Propionibacterium acnes*, *Malassezia* spp., *Trichophyton* spp. and *C. albicans* which primarily cause infections of skin, scalp and nails. Among the nine tested essential oils – *Cyperus scariosus*, *Syngium aromaticum*, *Carum carvi*, *Coriandrum sativum*, *Syngium cumini*, *Elettaria cardamom*, *Occimum sanctum* and *Piper nigrum*, the one of *B. serrata* exhibited superior antimicrobial activity against the tested microorganisms, with the maximum activity observed against *Trichophyton* spp. [48].

The combination of *B. serrata* essential oil with azoles (fluconazole, ketoconazole, posaconazole and voriconazole) showed synergistic activity against the azole-resistant strain of *C. albicans*, highlighting Indian frankincense essential oil as a powerful antifungal agent. However, terbinafine has been shown to exert an antagonistic effect on Indian frankincense essential oil. Additionally, this essential oil exhibited antibiofilm activity against *Staphylococcus epidermidis*, further supporting its use as a potent antimicrobial agent [48]. An experiment was conducted to test the essential oils of *B. papyrifera* and *B. rivae* in relation to bacterial biofilm production. The results indicated that *B. papyrifera* exhibits antimicrobial activity against *S. aureus* and *S. epidermidis*. Similarly, the efficiency of *B. rivae* essential oil was confirmed against *C. albicans* biofilms. These results are significant because staphylococcal biofilms are often resistant to conventional antibiotics at concentrations up to 1,000 times higher than of their MIC [39].

Mothana et al. (2011) tested the antimicrobial activity of *B. dioscorides*, *B. elongate* and *B. socotrana* essential oils against two Gram-positive bacteria (*S. aureus* and *B. subtilis*), two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and the fungus *C. albicans*. Gram-positive bacteria were found to be more sensitive to the tested essential oils than Gram-negative bacteria, while the fungus showed resistance. The highest activity against *S. aureus* and *B. subtilis* was observed with *B. socotrana* essential oil, as confirmed by the lowest minimum inhibitory concentration (MIC) values (1.87 mg/mL) [50].

Camarda *et al.* (2007) demonstrated that limonene is responsible for the antifungal activity of essential oils [41]. The absence of limonene in mentioned species examined by Mothana *et al.* (2011) may explain the lack of activity against the tested fungus [50]. In the tested commercial essential oil, the limonene content is 2.5%, and it demonstrated activity against *C. albicans*. This was not the case with the isolated essential oil, where limonene is present only in trace amounts. The results obtained are consistent with the previously mentioned experiments. For the other microbes tested in this study, both essential oils demonstrated activity against the same microbes, exhibiting nearly identical levels of activity despite their differing qualitative and quantitative chemical compositions.

It is important to note that the essential oil of *B. carterii* exhibits significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to penicillin antibiotics (MIC 3.52 $\mu\text{g/mL}$) [41]. The antimicrobial activity exhibited by the essential oils of different species of the *Boswellia* may be the result of the presence of: *E*- β -ocimene, limonene, α -pinene, α -thujene, myrcene, octanol, octyl acetate, *trans*-verbenol and terpinen-4-ol [51]. In fact, α -pinene [52], myrcene [52] and limonene [53] possess antimicrobial activity themselves.

Based on the results obtained from both this study and the experimental work of other authors, it is evident that, despite certain differences in the chemical composition of essential oils from various *Boswellia* sources, these hydro-isolates can be considered serious candidates for the treatment of microbial infections caused by different etiological agents.

Antioxidant activity of isolated and commercial Indian frankincense essential oils

The percentage of antioxidant activity increases linearly with the concentration of essential oils (Figure 4). The incubation time of the samples also influences the neutralization of DPPH radicals. After 20 and 40 minutes of samples incubation at room temperature, the absorbances of the obtained solutions were measured at 517 nm. It was observed that the commercial oil, within a concentration range 0.0078 to 0.0625 mg/mL exhibited a higher antioxidant potential (7.70 – 10.01%) after 40 minutes of incubation. The isolated essential oil, under the same incubation conditions, exhibited lower antioxidant activity (6.90–8.40%) within the tested concentration range (0.0079–0.063 mg/mL). This difference can be attributed to the variation in the chemical composition of the essential oils. The synthetic antioxidant BHT demonstrated an EC_{50} value of 0.021 mg/mL after 20 minutes of incubation with the DPPH radical [54].

Ayub *et al.* (2018) reported that the extraction method used affects both the yield and antioxidant activity of essential oils derived from the resin of *B. serrata*. The essential oil isolated by hydrodistillation exhibited the lowest free radical scavenging potential, while the essential oil obtained through supercritical fluid CO_2 extraction displayed the potential. The difference in antioxidant activity between the essential oils can be attributed to the fact that the oil obtained through supercritical fluid extraction contains significantly more phenolic compounds than the oil obtained by steam distillation. Variations in the antioxidant activity of essential oils are likely due to differences in the chemical composition of the oils isolated by various extraction methods [29].

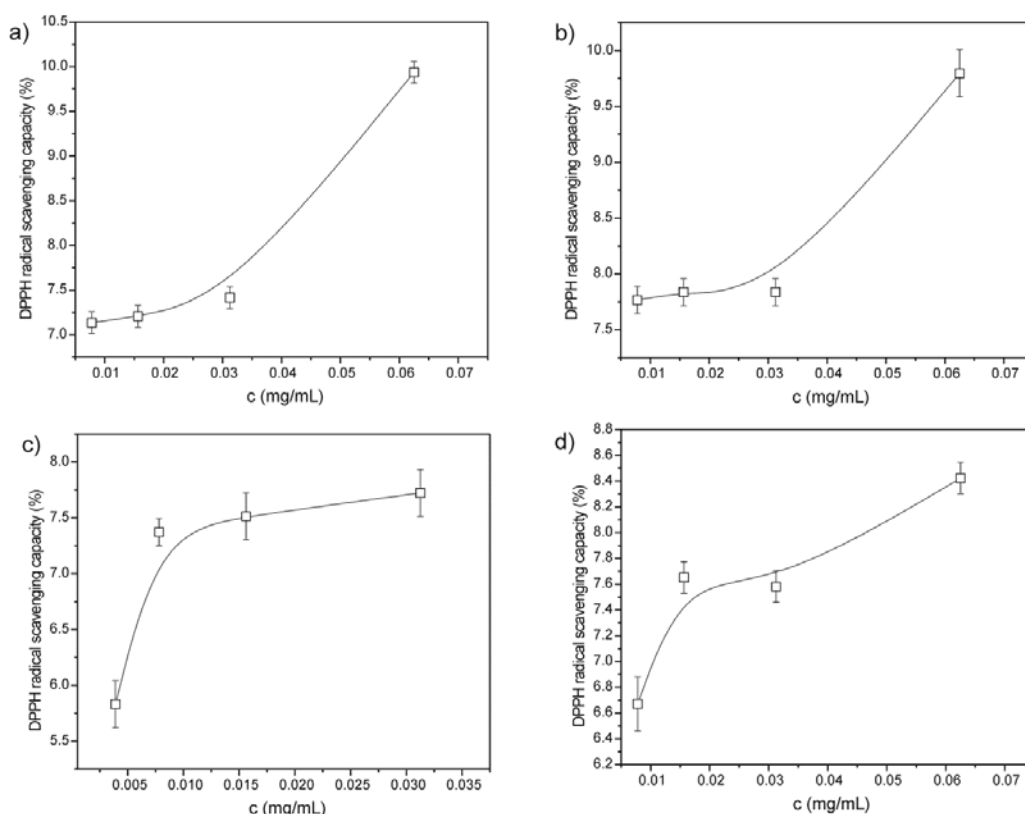


Figure 4. Graphical representation of the dependence of DPPH radical inhibition on the concentration of commercial and isolated essential oil of Indian frankincense (*B. serrata*): (a) commercial essential oil after 20 min of incubation time; (b) commercial essential oil after 40 min of incubation time; (c) isolated essential oil after 20 min of incubation time; (d) commercial essential oil after 40 min of incubation time

On the other hand, the percentage yield of the essential oil isolated by hydrodistillation is significantly higher than that of the oil obtained through supercritical liquid CO₂ extraction. The differences in yield among essential oils obtained by hydrodistillation, steam distillation, and supercritical liquid CO₂ extraction are often attributed to the polarity of CO₂, which is a non-polar solvent with variable insulating power [29].

Ruberto and Barrata (2000) tested the antioxidant activity of three essential oils obtained from *B. dioscorides*, *B. elongate* and *B. socotrana* finding that their free radical scavenging activity was weak (22%, 21% and 28%, respectively) at concentration 1 mg/mL in comparison to the high antioxidant effect of ascorbic acid (96%) [55]. The weak antioxidant activity can be attributed to the lower content of phenolic compounds in the essential oils of the mentioned species, primarily thymol and carvacrol. It is believed that the higher free radical scavenging activity of *B. socotrana* essential oil results from its increased content of oxygenated monoterpenes [44]. Barrata *et al.* (1998) analyzed the antioxidant activity of essential oil from the species *B. thurifera* and concluded that the tested essential oil exhibits good antioxidant activity, comparable to that of α -tocopherol and butylated hydroxytoluene (BHT) [56]. The latest experimental results related to *Boswellia* essential oil (a commercial mixture of *B. carteri*, *B. sacra*, *B. papyrifera*, and *B. frereana*) indicate that at a concentration of 50 μ g/mL, the inhibition (%) was 15.18, compared to 25.22 for ascorbic acid [7].

Compared to other popular essential oils, the essential oil of *B. serrata* exhibited lower free radical scavenging activity than that of *Mentha piperita* and *Melissa officinalis* [57, 58]. However, the results obtained in this study are very similar to the recent findings by Gupta *et al.*, who tested the antioxidant activity of *B. serrata* methanol extracts using the DPPH method [59].

5. Conclusion

In this paper, a comparison of qualitative and quantitative chemical composition of isolated and commercial Indian frankincense essential oil (*B. serrata*) available on Serbian market was given, and it was a basis for interpreting results obtained in antimicrobial and antioxidant activity tests.

By applying GC/MS method it was proved that commercial and isolated essential oils from *B. serrata* resin differ in chemical composition: in the commercial Indian frankincense essential oil monoterpene hydrocarbons are the most abundant (95.0%), followed by aromatic compounds (3.3%) and oxygenic monoterpenes (1.1%), while the essential oil isolated from commercially available *B. serrata* resin contained octanol acetate as dominant compound (40.6%), followed by (*Z*)-nerolidol (11.9%) and incesole (11.2%). The differences in the chemical composition of isolated and commercial essential oils of *B. serrata* are in a relation to their antimicrobial and antioxidant activity.

The commercial essential oil showed better antimicrobial activity against *S. aureus* ATCC 25923 than isolated one. The isolated essential oil showed better antimicrobial

activity against *B. cereus* in house strain and *E. coli* ATCC 25922. Activity against *C. albicans* was shown only by commercial essential oil, while sensitivity of this microbe was not noticed against isolated essential oil.

The commercial essential oil of *B. serrata* resin showed slightly higher antioxidant activity than isolated essential oil. After 40 min of incubation period, commercial essential oil in concentration range from 0.0078 to 0.0625 mg/mL neutralizes DPPH radicals in percentage range from 7.70 to 10.01%, while at same concentration isolated essential oil neutralizes DPPH radicals in slightly lower percentages (from 6.90 to 8.40%).

The Indian frankincense resin and essential oil are valuable source of natural antioxidant and antimicrobial biochemical compounds.

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Statement of Competing Interests

The authors have no competing interests.

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