

Total phenolic and flavonoid content in *Boswellia serrata* Roxb. resin extracts obtained with subcritical water

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Boswellia serrata Roxb. is a tree that is mainly found in the dry regions of India. Its oleoresin, known internationally as Indian frankincense, is used in Ayurvedic, traditional Arabic and Chinese medicine. This gum resin contains 15-20% of boswellic, lupeolic and other pentacyclic triterpenic acids, of which the boswellic acids (beta-boswellic acid, keto-beta-boswellic acid and acetyl-11-keto-beta-boswellic acid) have been shown to have anti-inflammatory, anticancer and antidiabetic properties and are used in the modern pharmaceutical industry. Besides its ability to prevent and treat various diseases (rheumatoid arthritis, osteoarthritis, Chron's disease, ulcerative colitis and asthma), other biological functions of *B. serrata* resin should not be neglected.

The aim of this study was to analyze, for the first time, extracts of *B. serrata* resin obtained with subcritical water at different temperatures (110–190 °C) for their phenolic and flavonoid content. The total phenolic content (TPC) was determined by UV-spectrophotometry using the Folin-Ciocalteu method. The total flavonoid content (TFC) was also determined by UV-spectrophotometry using a simple method with AlCl₃.

With increasing extraction temperature, the TPC increased from 3.76 mg GAE/g DW at 110 °C to 13.78 mg GAE/g DW at 190 °C. The highest TFC was observed in the extract obtained at 170 °C (8.56 mg RE/g DW). The results of this study suggest that extracts of *B. serrata* resin obtained with subcritical water are a rich source of bioactive compounds that can be used in pharmaceuticals, dietary supplements and functional foods.

Keywords: *Boswellia serrata* Roxb. resin; subcritical water extraction; total phenolic content; total flavonoid content

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1. INTRODUCTION

Frankincense is an aromatic resin obtained from trees of various species of the genus *Boswellia* by making incisions in the trunk of the trees. The resins obtained from the cuts of these trees play a role of a natural defense against wounds made in the bark, to repel herbivores and to attract animals to spread their pollen or seeds (Guta et al., 2024). The *Boswellia* genus is commonly cultivated in the dry regions throughout Africa, South Asia and the Arabian Peninsula (Camarda et al., 2007). It is used as a perfume, flavoring agent for cosmetics, in aromatherapy and as a traditional medicine to treat various diseases in Ayurvedic, traditional Arabic and Chinese medicine. Even the ancient Romans, Egyptians and Greeks recognized the therapeutic importance of frankincense (Al-

Harrasi et al., 2019). More recently, frankincense has been used in European countries to treat various chronic inflammatory problems such as arthritis, chronic bowel diseases and asthma (Guta et al., 2024). The chemical profile of the oil and resin of the various *Boswellia* species is very different, which is reflected in the great chemical diversity of the various *Boswellia* species (Al-Harrasi et al., 2019). The resins of *Boswellia* species are rich in pentacyclic triterpenes (boswellic acids) and other triterpenoids (lupeol and lupeolic acids), which target pro-inflammatory signals and are responsible for its biological activity. Boswellic acids bind to IκB kinase (IKK) and inhibit its activity, leading to inhibition of the proinflammatory transcription factor NF-κB (Nuclear Factor Kappa B), while acetyl-

lupeolic acid (ALA) inhibits the kinase AKT, which also influences inflammation and oxidative stress. Inhibition of NF- κ B by boswellic acids significantly reduces the expression of the important proinflammatory cytokine TNF- α in human monocytes. This provides a rational basis for the traditional use of *Boswellia* and suggests that the use of drugs containing *Boswellia* extracts may provide therapeutic benefit in inflammatory conditions (Schmiech et al., 2024).

The extremely valuable essential oil of *Boswellia serrata* Roxb., commonly known as the Indian frankincense tree, is used in the food, flavoring and perfume industries. This essential oil is rich in boswellic acid, lupeolic acid and other pentacyclic triterpenic acids (15–20%), of which beta-boswellic acid, keto-beta-boswellic acid and acetyl-11-keto-beta-boswellic acid are the most important (Tironi de Castilho et al., 2023). They have been described to inhibit elastase in leukocytes, inhibit proliferation, induce apoptosis and inhibit topoisomerases (Baliga et al., 2013) and therefore they are considered to have biological activities such as anti-cancer, anti-inflammatory, anti-diabetic and antimicrobial. The pure natural substances of these acids are used in the modern pharmaceutical industry (Gupta et al., 2022). In addition to the therapeutic effects of *B. serrata* essential oil, the other biological functions of *B. serrata* resin as a whole should not be neglected, as this resin contains numerous bioactive compounds.

In general, *B. serrata* resin consists of 60–85% water insoluble pentacyclic triterpenes, 6–30% polysaccharides, and 5–9% essential oil, which is a mixture of monoterpenes and sesquiterpenes (Guta et al., 2024).

In order to fully utilize the biomedical potential of a plant material, it is very important to use a suitable extraction method (Radovanović et al., 2022). Subcritical water extraction (SWE) is suitable for the production of natural extracts due to its environmental friendliness and the safety of the final extracts by using water in the subcritical state (below the critical temperature of 374.15 °C and the critical pressure of 22.1 MPa) instead of synthetic organic solvents used in most conventional extraction processes. This technique can shorten the operating time and provides better yield and quality of the extract (Radovanović et al., 2022; Švarc-Gajić and Morais, 2022). Water is the safest and most cost-effective solvent that can change its properties by changing the temperature in SWE processes. Temperature plays a crucial role in determining extraction efficiency and selectivity (as the temperature changes, the dielectric constant, surface tension and viscosity of the water also change, altering the polarity and other properties of the solvent) (Plaza and Marina, 2023). Under subcritical conditions, it becomes an excellent solvent that can even dissolve compounds that are poorly-soluble in water at room temperature, making this technique particularly suitable for extracting compounds from the resin of *B. serrata*. On the other hand, the solubility of polar and moderately polar compounds does not change significantly.

In the scientific literature, extraction of bioactive compounds from *B. serrata* resin was performed with petroleum ether and ethyl alcohol (Alshafei et al., 2023; Sharma et al., 2016), and methanol (Gupta et al., 2021, 2022; Katragunta et al., 2019). Guta et al. (2024) performed selective extraction with subcritical and supercritical CO₂ methods from *Boswellia papayrifera* resin. To our knowledge, extraction with subcritical water has never been performed on any *Boswellia* species. With its exceptional properties of adjustable polarity, subcritical water could provide new insights into the chemical characterization of this complex material, which is insoluble in water at ambient conditions.

The aim of this study was to investigate, for the first time, the total phenolic content (TPC) and total flavonoid content (TFC)

of *B. serrata* resin extracts obtained with SWE at different temperatures (110–190 °C). This study is the first step towards the characterization of *B. serrata* resin extracts obtained by SWE.

2. MATERIALS AND METHODS

2.1. Plant material and reagent

The plant material used in this study, *B. serrata* gum (Gond Kondru, Gond Kundru or Shallaki gum), was a commercial product imported from India (Sanchar Vihar Colony, Uttar Pradesh).

The sample was ground with a laboratory grinder and the obtained powder was stored in a plastic container until extraction. Gallic acid and rutin trihydrate were purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Folin-Ciocalteu reagent was purchased from Lachner (Neratovice, Czech Republic). Sodium carbonate and aluminum chloride hexahydrate were purchased from Alpha Aesar GmbH & Co KG (Karlsruhe, Germany). Nitrogen under pressure (99.999%) was supplied by Messer (Bad Soden, Germany). All other chemicals were of analytical reagent grade.

2.2. Subcritical water extraction (SWE)

The SWE of the *B. serrata* resin was performed in a homemade subcritical water extractor/reactor with a high-pressure stainless steel process vessel of a total volume of 1.7 liters (Švarc-Gajić et al., 2017). The resin powder and distilled water were added to the process vessel in a ratio of 1:20. After closing, the extraction vessel was pressurized to constant pressure of 15 bar with nitrogen via the gas inlet valve installed in the lid of the vessel. The extraction vessel was placed on a heating/vibration platform. The heating rate was approximately 10 °C/min and the frequency of vibration was maintained at 3 Hz. After the operating temperature was reached (110–190 °C), the extraction time (30 min) was measured. After extraction, the process vessel was cooled to 20 ± 2 °C in a flow-through water bath and the pressure was released by opening the valve. The extracts obtained were filtered through a grade 1 Whatman filter paper, and stored in polyethylene bottles in a refrigerator (4 °C) for further analysis.

2.3. Spectrophotometric analyses

The content of polyphenols and flavonoids in the resin extracts of *B. serrata* obtained with subcritical water was determined spectrophotometrically.

The TPC was measured using the well-known Folin-Ciocalteu method (Li et al., 2007). The extract/standard solution (400 μ L) was mixed with 2 mL of diluted Folin-Ciocalteu reagent (1:10, v/v). After 4 minutes, 1.6 mL sodium carbonate solution (7.5%, w/w) was added. The blank was prepared with distilled water instead of the extracts. The mixtures were incubated for 90 minutes at room temperature for color development (from yellow to indigo). The absorbance was measured at 765 nm in triplicate for each sample. Gallic acid dissolved in distilled water (0–200 mg/L) was used as a standard. The results were expressed as mg gallic acid equivalent per gramme of resin powder dry weight (mg GAE/g DW) and calculated as mean ± SD.

The TFC was measured using a simple spectrophotometric method with AlCl₃ (Benmerzoug et al., 2020). In brief, 2 mL of AlCl₃ solution (2%) was added to 2 mL of the extract/standard solution. After 10 minutes, the absorbance was measured at 430 nm. Measurements were performed in triplicate for each sample. Distilled water (2 mL) was used to prepare the blank by mixing it with AlCl₃. Rutin-trihydrate dissolved in distilled water (0–125 mg/L) was used as a standard. The results were expressed as mg rutin equivalent per gramme dry weight of resin powder (mg RE/g DW) and calculated as mean ± SD.

2.4. Statistical analysis

Three independent experiments were performed for each analysis. All data were expressed as means \pm standard deviations (SD). A one-way analysis of variance (ANOVA: single factor test) was performed to compare the mean values and to determine significant differences ($P < 0.05$).

3. RESULTS AND DISCUSSION

Five samples of *B. serrata* resin extracts were prepared with subcritical water at five different temperatures (Table 1). The extraction time was 30 minutes, nitrogen pressure was 15 bar, rotation frequency was 3 Hz and the sample to solvent ratio was 1:20 (*w/w*) for all extractions. TPC and TFC were determined in the extracts obtained after appropriate dilution.

Table 1. Extracts of *B. serrata* obtained at different temperatures (110–190 °C) with subcritical water

<i>B. serrata</i> sample	Temperature of SW* (°C)
Extract 1	110
Extract 2	130
Extract 3	150
Extract 4	170
Extract 5	190

*SW – Subcritical Water.

As the most important parameter for extraction efficiency, the influence of temperature on the extraction of *B. serrata* resin, a complex matrix largely composed of water-insoluble compounds (Guta et al., 2024), was investigated over a wide range from 110 °C to 190 °C. As already mentioned, the polarity of water decreases with increasing temperature, as the dielectric constant, surface tension and viscosity decrease. The diffusion properties of water in the subcritical state are improved and the pressure keeps the water in its liquid state. This makes subcritical water an excellent extraction medium for less polar polyphenols and flavonoids.

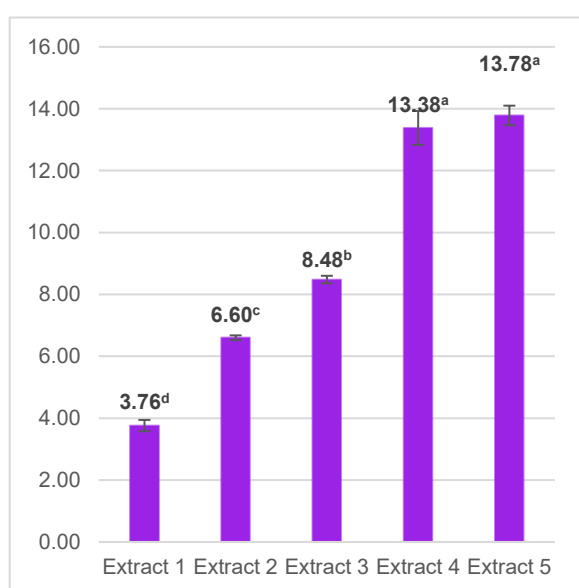


Fig. 1. The TPC (Total Phenolic Content) of *B. serrata* resin extracts obtained with subcritical water (mg GAE/g DW). The error bars indicate standard deviation ($n = 3$). Different letters (a, b, c, d) indicate a significant statistical difference in the observed data ($P < 0.05$).

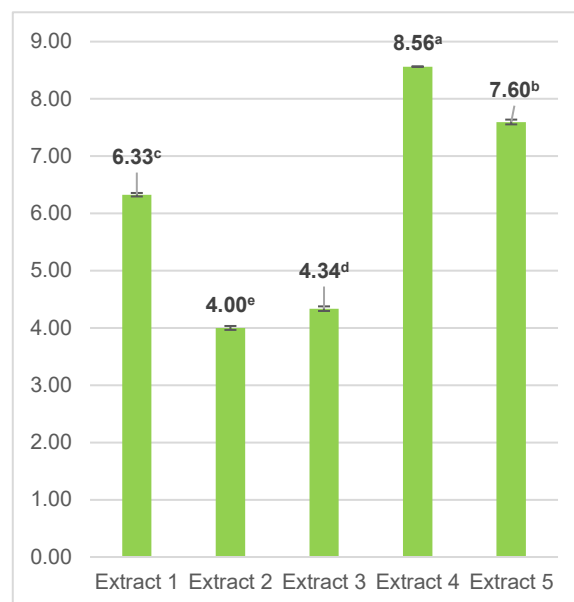


Fig. 2. The TFC (Total Flavonoid Content) of *B. serrata* resin extracts obtained with subcritical water (mg RE/g DW). The error bars indicate standard deviations ($n = 3$). Different letters (a, b, c, d, e) indicate a significant statistical difference in the observed data ($P < 0.05$).

The TPC of *B. Serrata* resin extracts is shown in Figure 1, while the TFC is shown in Figure 2.

According to the statistical analysis (ANOVA), the TPC values differed significantly ($p < 0.05$) for all extracts, except for extract 4 and extract 5. As can be seen from Figure 1, the highest TPC value (13.78 mg GAE/g DW) was obtained for Extract 5, which was close to that of extract 4 (13.38 mg GAE/g DW). These extracts were obtained at high temperatures, 190 °C and 170 °C, respectively. The sample obtained at the lowest temperature tested (Extract 1) had the lowest TPC value (3.76 mg GAE/g DW). In fact, the TPC value gradually increased with increasing temperature. This result contradicts the general knowledge that polyphenols are heat-sensitive compounds. However, in many studies oriented towards investigation of the optimal temperature for SWE, temperatures of up to 200 °C were reported (Antony and Farid, 2022; Correia et al., 2022; Palma et al., 2001; Vergara-Salinas et al., 2012; Vladić et al., 2020). A possible explanation for the results of this study could be the chemical composition of *B. serrata* resin, which consists mainly of polymers of pentacyclic triterpenes and gums consisting of polysaccharides. At elevated temperatures, these macromolecules may be degraded to simpler molecules that react with the Folin-Ciocalteu reagent and increase the TPC. The different extraction temperatures may also have affected the types of polyphenols extracted. At high temperatures, the formation of new compounds known as Maillard reaction and caramelization products can also affect the results of the spectrophotometric assay for polyphenols (Antony and Farid, 2022). These chemical reactions are not always desirable as they often lead to the formation of toxic compounds (Plaza and Marina, 2023).

As for the TFC of *B. serrata* resin, the ANOVA test showed significant differences between all extracts obtained at different temperatures. The highest value (8.56 mg RE/g DW) was observed for Extract 4 obtained at 170 °C. At a temperature of 190 °C (Extract 5), a slight decrease in the value was observed (7.06 mg RE/g DW), probably due to thermal degradation. These values were higher than the TFC values of the extracts obtained at lower temperatures (Extract 1, 2 and 3) which contradicts the fact that flavonoids as a group of polyphenols are

thermolabile compounds. Due to the chemical structure of the resin of *B. serrata*, a possible explanation for these results could be that the flavonoids are chemically and physically bound in the structure of the macromolecules of the resin. The temperatures of 170 °C and 190 °C are high enough to degrade the structure of the resin and release them during the extraction time of 30 minutes without degrading the flavonoids.

There is limited data in the scientific literature on the TPC and TFC as well as the polyphenolic profile of *B. serrata* resin. Alshafei et al. (2023) found that the TPC of *B. serrata* resin was 540.88 µg GAE/g of the aqueous extract, while the TFC was 0.118 µg QE/g of the aqueous extract of the resin. These results cannot be compared with the data in this paper as different units are used to express the content. The same authors provided a polyphenolic profile of *B. serrata* resin after HPLC analysis of the aqueous extracts and found catechin, rutin, naringenin, kaempferol, taxifolin, pyrocatechin, vanillic acid, caffeic acid, ellagic acid, cinnamic acid, and syringic acid. Gomma et al. (2019) found that the TPC in *B. serrata* resin extracts obtained with petroleum ether and ethanol was 25.84 µg GAE/mg DW, while the TFC of the same extracts was 15.09 µg RE/mg DW. These values are comparable to the results obtained in this study. The GC/MS analysis performed by the same authors confirmed the presence of phenolic compounds, mainly 2,7,8-trimethoxy-3-methyl-5,6-methylenedioxy-naphtho-1,4-quinone (28.43%), terpenoid compounds including monoterpenes, diterpenes and triterpenes, the major terpenes being (E,E,10S)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadiene (18.54%) and boswellic acid (7.5%). The content of alkaloids was 5.02%. On the other hand, the chemical composition of the essential oil of the resin, which is the main carrier of bioactivity, is well studied (Ayub et al., 2018; Camarda et al., 2007). Some papers have investigated the TPC, TFC or antioxidant and anti-inflammatory activities of the leaf's extracts (Afsar et al., 2012; Subhashini Devi et al., 2014) and bark (Rao et al., 2024) of *B. serrata*.

However, to determine the individual phenolic composition of *B. serrata* resin extracts obtained by SWE and the formation of unwanted compounds due to Maillard and caramelization reactions, especially at high temperatures, further analyses with modern analytical instruments are required.

4. CONCLUSION

The present study is the first to address SWE of *B. serrata* resin, which is insoluble in water at ambient conditions, to obtain safe extracts rich in polyphenols and flavonoids. SWE is an environmentally friendly, powerful and safe technique that uses water at subcritical conditions and can extract polar and less polar compounds due to the adjustable polarity depending on the applied temperature. Samples were extracted at different subcritical water temperatures (110–190 °C). The extracts obtained were analyzed spectrophotometrically for TPC and TFC. According to the ANOVA statistical test, almost all TPC values and all TFC values differed significantly between all extracts ($p < 0.05$), indicating a strong influence of temperature on the analyzed parameters. The TPC increased from 3.76 mg GAE/g DW to 13.78 mg GAE/g DW with increasing temperature from 110 – 190 °C, indicating a possible degradation of the resin macromolecule to compounds that react with the Folin-Ciocalteu reagent and increase the TPC. Since the highest TFC was observed for the extract obtained at 170 °C (8.56 mg RE/g DW), these results suggest that flavonoids may be incorporated into the structure of the resin macromolecule and released after its thermal degradation. However, these conclusions should be confirmed by further analyses of the polyphenolic profile and antioxidant activity of the extracts obtained.

The results of this study suggest that extracts of *B. serrata* resin obtained with subcritical water are a rich source of bioactive compounds such as polyphenols and flavonoids and can be considered as potential medicinal materials, dietary supplements and additives in functional foods after further chemical and biological characterization, once their efficacy and safety have been confirmed by standardized tests. In addition, further research could go in the direction of valorizing biowaste: The resin of *B. serrata*, which remains after the production of the essential oil, could be subjected to extraction with subcritical water to conduct further studies on health-promoting compounds.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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