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RESEARCH ARTICLE

Rapid Detection of Adulteration in *Boswellia* Extracts with Citric Acid by UPLC–HRMS and ^{1}H NMR

Péter Püski[a], Tímea Körmöczi[b], Róbert Berkecz[b], Anita Barta[a], Ákos Bajtel[a] and Tivadar Kiss[a]

[a]Institute of Pharmacognosy, University of Szeged, Szeged, Hungary; [b]Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary

ABSTRACT

Boswellia serrata ole-gum-resin extracts (BSEs) are commonly used as food supplements, especially in osteoarthritis management. The quality standard is established by determining 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-boswellic acid (AKBA) content using high-performance liquid chromatography (HPLC) or assessing the total boswellic acid (TBA) content by titrimetry. The limited geographical distribution of *Boswellia* species and increasing industrial demand could increase the risk of adulteration in *Boswellia*-containing products. In this study, 14 BSEs from commercial sources, used in food supplements, were analyzed in comparison with a USP Reference Standard extract. The KBA and AKBA content was determined by HPLC, whereas the TBA content was determined by titration. Targeted UHPLC-high-resolution mass spectrometry (HRMS) was applied to identify the carboxylic acid content in the samples. The ^{1}H NMR spectra of extracts were also analyzed. Only two products met the criteria for KBA and AKBA content. Although, the TBA content complied with the expected amount, 10 extracts contained citric acid levels of 6–11% even though citric acid is not a characteristic component of BSEs. Our results suggest undeclared addition of citric acid to comply with declared contents of TBA when using titration methods. Incorporation of citric acid to industrial samples – in order to alter the outcomes of the titration analysis – was demonstrated for the first time.

KEYWORDS

Adulteration; *Boswellia*; citric acid; food supplement; frankincense; total boswellic acid content

Introduction

Frankincense is a natural product that has been traditionally used for centuries. The use of the exudate obtained from different *Boswellia* species via incision of the bark has deep roots in religions and medicine. *Boswellia* species are commonly used as incense in various religious ceremonies and practices, especially in Christianity, Judaism, and Islam. This incense is believed to have purifying and cleansing effects, and it is often used to promote feelings of peace and relaxation (Moussaieff and Mechoulam

CONTACT Tivadar Kiss kiss.tivadar@szte.hu Institute of Pharmacognosy, University of Szeged Eötvös u. 6, H-6720 Szeged, Hungary.

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2009). In addition to religious application, a number of contemporary studies have found potential benefits of *Boswellia serrata* Roxb. in the treatment of inflammatory bowel disease, arthritis, peritumoral edema, and asthma (Kimmatkar et al. 2003; Madisch et al. 2007; Binns et al. 2018). *B. serrata* or its main constituents (i.e. 11-keto-β-boswellic acid (KBA) and 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA)) have been investigated as treatments in clinical trials. In addition, *Boswellia serrata* extract (BSE) is globally available in food supplements with various indications.

The increasing demand for BSE has led to concerns about its adulteration. The loose regulation of food supplements is a major issue in Hungary. In addition, food supplements are not subjected to rigorous analytical assessment prior to marketing in the European Union (Binns et al. 2018; Czepielewska et al. 2018). Thus, a large number of currently marketed products might be adulterated. Moreover, attempts to deceive analytical methods detecting botanical ingredients have been detected (Gafner et al. 2023).

Usually, *Boswellia* extracts are standardized according to the total boswellic acid (TBA) content using analytical methods based on simple acid–base titration. However, the exudate of *B. serrata* as the most marketed species is characterized in the European Pharmacopoeia (Ph. Eur. 11.0) by its KBA and AKBA content (EDQM 2023). It should be noted that BSE is not defined in Ph. Eur. 11.0. The European Food Safety Authority (EFSA) has characterized BSE as an off-white to cream powder with a characteristic odor and taste. Based on guidelines, BSE should contain a minimum TBA content of 65% as determined by titrimetry and 2–5% KBA and 2–5% AKBA content as determined by high-performance liquid chromatography (HPLC) (Bampidis et al. 2022). Moreover, the United States Pharmacopeia – National Formulary (USP-NF) has a clear definition for the resin and extract. Specifically, the sum of the AKBA and KBA content must be not less than 1% (United States Pharmacopeia 2023a, 2023b). A review of botanical, chemical, and genetic characterization methods of oleo-gum-resin was published in a laboratory guidance document by the Botanical Adulterants Prevention Program. The document thoroughly collected analytical methods for the unambiguous identification of *B. serrata*-based drugs and extracts; however, the possible chemical adulteration of *Boswellia* extracts was not discussed.

The boswellic acid profile varies greatly among the 28 *Boswellia* species. Moreover, the ratio of different boswellic acids as well as the TBA content varies widely among the exudates of species of different geographical origin (Schmiech et al. 2019).

This study screened the quality of commercial *Boswellia* extracts meant for food supplement production using acid–base titration and marker compound quantification as described by Ph. Eur. 11.0 (EDQM 2023). The aim of this study was to evaluate the effectivity of TBA as quality describing parameter. Further aim was to screen commercial *Boswellia* extracts for their quality and possible adulteration applying UPLC-PDA, UPLC–HRMS, and ^{1}H NMR spectroscopy.

Materials and methods

Boswellia extracts and reference compounds

Boswellia extracts were randomly selected and purchased online from China (**1**–**12**), Italy (**13**), and Spain (**14**). For comparison, a USP grade reference standard extract of *B. serrata* (**15**) was purchased (USP Reference Standard). The reference standards for

AKBA and KBA were purchased from Phytolab (Vestenbergsgreuth, Germany). Carboxylic acids (benzoic acid, citric acid, malic acid, oxalic acid, and tartaric acid) were purchased from Merck KGaA (Darmstadt, Germany).

The organoleptic assessment of extracts was performed by simple sensory testing (color, odor, and taste).

KBA and AKBA analysis by HPLC

The samples were analyzed using the method of Ph. Eur. 11.0 (EDQM 2023). Samples were prepared by dissolving 1.0 g of each extract in 90 mL of methanol followed by 10 min of sonication. During this procedure, the mixtures were rigorously shaken, diluted to 100 mL with methanol, and centrifuged for 5 min (5000 rpm, room temperature). Finally, a mixture of 16 volumes of mobile phase A (0.1% [v/v] phosphoric acid aqueous solution) and 84 volumes of mobile phase B (acetonitrile with 0.1% [v/v] phosphoric acid) was used to dilute 1.0 mL of the clear solution to 10.0 mL.

Experiments were performed on a Shimadzu Nexera X2 ultra-high-performance liquid chromatography (UHPLC) system (Shimadzu, Kyoto, Japan) equipped with a diode array detector. The analysis was performed at 25 °C using a LiChrospher C18 column (25 × 4 mm, 5 µm, Phenomenex, Torrance, CA). All solvents were of analytical reagent grade. The solvents used for HPLC analyses were acetonitrile (HiPerSolv Chromanorm, VWR International, Radnor, PA) and ultrapure water (Direct-Q 3 UV Water Purification System).

Mobile phase A was 0.1% (v/v) phosphoric acid, and mobile phase B was acetonitrile with 0.1% (v/v) phosphoric acid in water. Gradient elution was applied for 12.5 min from 84% B to 94% B, and then the gradient was set to 100% B for 1 min and held until 28 min. This was followed by a 7-min equilibration period prior to the next injection. The flow rate and injection volume were 1.0 mL/min and 20 µL, respectively. Detection was conducted over the range of 190–400 nm, and the chromatographic profile was registered at 250 nm. All measurements were performed in triplicate.

Screening and quantification of organic acids by targeted UHPLC–HRMS methods

The matrix solution was prepared by adding 1 mL of acetonitrile to 1 mg of maltodextrin (excipient in food supplements) and the mixture was vortexed and centrifuged for 15 min at 15,000 rpm. The supernatant was collected and used to prepare calibration solutions.

Benzoic acid was selected as an internal standard to quantify citric acid content. For the working solution, the 1 mg/mL benzoic acid stock solution in water/acetonitrile (50%/50% [v/v]) was diluted with water/formic acid (95%/5% [v/v]), obtaining a final concentration of 50 µM.

The citric acid calibration series (0, 3.91, 7.81, 15.6, 31.3, 62.5 125, 250, 500, and 1000 µM) was prepared by serial dilution of the 1 mg/mL citric acid stock solution using the matrix solution.

Boswellia extracts were uniformly dissolved in acetonitrile, obtaining a concentration of 1 mg/mL organic solution, followed by centrifugation for 15 min at 15,000 rpm. The

upper layer was collected and diluted 10-fold twice with the working solution as described for the calibration solutions. Samples were diluted 100-fold before measurement.

The targeted UHPLC-high-resolution mass spectrometry (HRMS) measurement was performed using a Waters Acquity I-Class UPLC system (Waters, Milford, MA) coupled to Thermo Scientific Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific, Waltham, MA).

Chromatographic separation was performed using an Acquity UPLC BEH C18 column (2.1 × 30 mm, 1.7 µm, Waters, Milford, MA) with a guard column (Waters, Milford, MA). For UHPLC, mobile phase A was 0.2% formic acid in water, and mobile phase B was 0.2% formic acid in acetonitrile. The gradient, which was delivered at a flow rate of 0.3 mL/min, was as follows: start at 3% B, hold for 0.3 min, ramp up to 100% B within 0.7 min, hold for 2 min, return to the initial conditions within 0.2 min, and column equilibration for 0.8 min. The column temperature was 25 °C, and a 5-µL sample was injected.

The mass spectrometer was operated in the negative heated electrospray ionization (HESI) mode. The HESI source settings were as follows: ion transfer tube temperature, 325 °C; vaporizer temperature, 350 °C; S-Lens RF level, 70; spray voltage, 3.5 kV; sheath gas flow, 45; sweep gas flow, 1; and auxiliary gas flow (arbitrary units), 19. To screen carboxylic acids and their structural reinforcement, the mass spectrometer was operated in the full scan and parallel reaction monitoring (PRM) modes, whereas the selected ion monitoring (SIM) mode was selected for the quantitative analysis of citric acid. The HCD collision energy was optimized for each deprotonated acid by direct infusion (Table 1) (Seligson et al. 2003; Schmiech et al. 2019; Rashan et al. 2020; Orhan et al. 2022). For PRM and SIM, the resolution was 15,000 (FWHM), the AGC setting was defined as 100%, and the maximum IT was set to 50 ms. The width of the isolation window of the precursor ion was 0.5 Da.

MassLynx 4.1 (Milford, MA) software was used to control the UHPLC system, and Xcalibur 4.5 software (Waltham, MA) was used for data acquisition. For the quantitative evaluation of UHPLC-HRMS data, raw files were processed using the integrated processing setup of Xcalibur software. The quantitative results were obtained by an external calibration approach using an internal standard. The correlation coefficient was higher than 0.995 for citric acid in the used concentration range.

TBA determination by acid–base titration

TBA content was determined by acid–base titration. The stock solution was prepared using 500 mg of the extract dissolved in 50 mL of ethanol. The 10 mL of the stock

Table 1. The main UHPLC–HRMS (SIM and PRM) parameters of organic acids.

Name	$[M-H]^-$ (*m/z*)	HCD collision energy (eV)	Fragment ions (*m/z*)	Retention time (min)
Malic acid	133.01425	9	115.00368 71.01385	0.27
Benzoic acid	121.02950	10	77.03967 57.03459	1.12
Tartaric acid	149.00916	11	87.00877 72.99312	1.12
Citric acid	191.01973	10	111.00877 87.00877	0.38

solution were used as the sample, to which five drops of phenolphthalein solution were added, followed by titration with 0.1 M sodium hydroxide aqueous solution to a light red color. The titration was performed in triplicates. The titration results were corrected with an ethanol blank sample (Gupta et al. 1984; Lakshmi and Rajendran 2013).

NMR analysis

Extracts (**1**–**15**) and reference compounds were dissolved in methanol (1:10 ratio) followed by 10 min of sonication and centrifuged for 5 min (5000 rpm, room temperature). Supernatants were filtrated and the solvents were evaporated using nitrogen flow. For NMR experiments, the dry residues, AKBA, KBA, and citric acid were dissolved in CD_3OD and the concentration was set up to 10–20 mg/mL.

1D ^1H NMR spectra were recorded in CD_3OD on Bruker Avance DRX 500 spectrometer (Bruker, Billerica, MA) at 500 MHz. Signal of the deuterated methanol was taken as reference. The chemical shift values (δ) were given in ppm. The ^1H NMR spectra were analyzed using MestReNova 9.0 software.

Statistical analysis

The normality of the data distribution was checked by the Shapiro–Wilk test, and one-way analysis of variance was used to determine the differences between investigated extracts. In cases of significance ($p < .05$), pairwise comparison was performed using the post hoc Bonferroni test. Statistical analyses were performed using R (version 4.0.3, The R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org).

Results

Extracts **2**, **5**, **7**, **10**, and **12** were white in color; extracts **1**, **3**, **4**, **6**, **8**, **11**, and **15** had a characteristic pale yellow color; and extracts **9**, **13**, and **14** were yellow in color (Figure 1). The taste and odor of the USP reference standard (**15**) were considered characteristic of frankincense. Extracts **1**–**8** and **12** were sour, **9** and **11** were sweet, **10** had no specific taste, and **13** and **14** were similar in taste to the reference standard. Extracts **1**–**7** and **10**–**12** had no odor, whereas the odor of extracts **8** and **9** was not characteristic of frankincense. Only extracts **13** and **14** had an odor characteristic of frankincense.

Acid–base titration of ethanolic solutions of the extracts allowed the estimation of the TBA content (Table 2). The TBA content of the reference extract was 69.56%. The technical data sheets (TDSs) of nine products (**1**–**8**, **14**) contained information regarding the TBA content. The results of titrimetry supported the TDS claims about the TBA content of the examined extracts. The TBA content was not declared for extracts **9**–**13**. The lowest acidic content of 2.96% was detected in extract **11**. The TBA content of extracts **1** and **2** did not significantly differ from that of **15**. The TBA content in extracts **3**–**8**, **10**, and **12** was significantly higher than that of **15** (all $p < .001$), whereas the content was lower in extracts **9** ($p < .001$), **13** ($p < .001$), and **14** ($p < .01$).

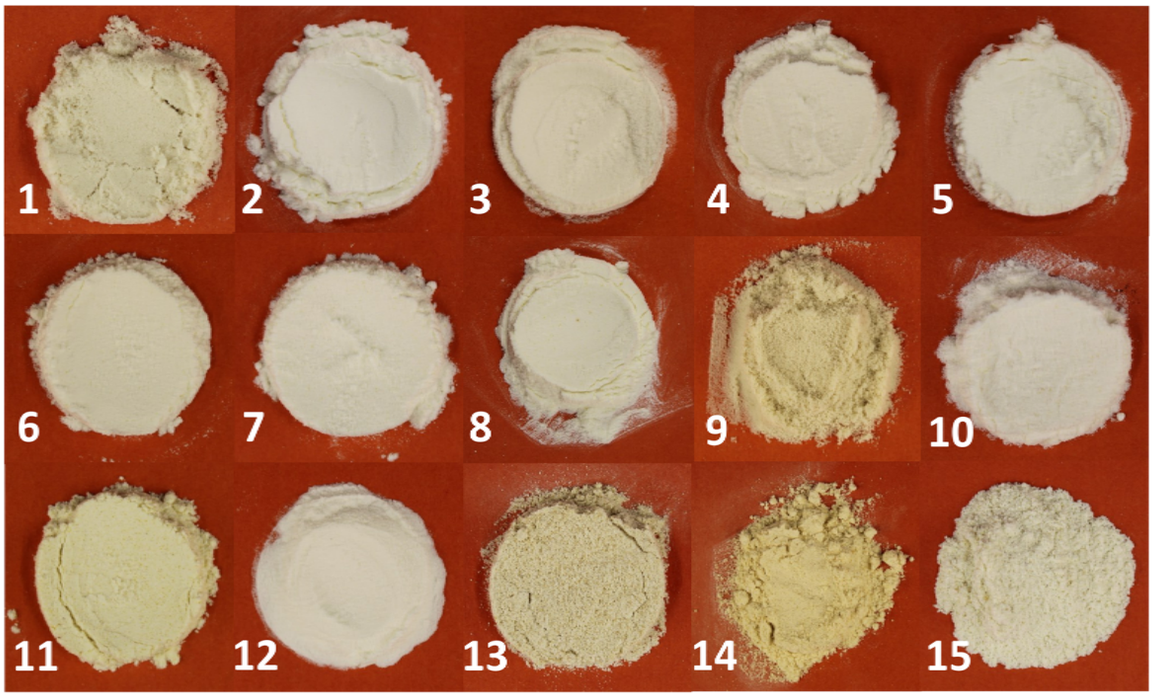

Figure 1. Color and powder characteristics of the investigated *Boswellia* extracts.

Table 2. Chemical characteristics of *Boswellia* extracts.

Sample	Country	Declared TBA%	Titration TBA%	KBA%	AKBA%	CA (mg/g)
1	China	65%	67.58 ± 0.56	0.43 ± 0.01	0.87 ± 0.01	69.57 ± 1.03
2	China	65%	69.59 ± 0.68	0.03 ± 0.00	0.08 ± 0.01	79.53 ± 2.13
3	China	90%	92.71 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	117.90 ± 1.35
4	China	90%	94.16 ± 0.65	0.02 ± 0.00	0.06 ± 0.00	97.87 ± 1.16
5	China	70	78.01 ± 0.68	0.02 ± 0.01	0.07 ± 0.00	107.84 ± 0.46
6	China	65	70.67 ± 0.00	0.02 ± 0.01	0.07 ± 0.01	90.78 ± 1.04
7	China	95	95.61 ± 0.00	0.04 ± 0.00	0.09 ± 0.00	107.75 ± 0.92
8	China	65	70.71 ± 0.00	0.08 ± 0.00	0.18 ± 0.01	75.96 ± 0.98
9	China	ND	3.49 ± 0.00	0.02 ± 0.00	0.11 ± 0.00	Not detected
10	China	ND	72.71 ± 0.03	0.05 ± 0.00	0.12 ± 0.00	97.34 ± 1.38
11	China	ND	2.96 ± 0.00	0.01 ± 0.00	0.06 ± 0.00	Not detected
12	China	ND	76.10 ± 0.04	0.02 ± 0.00	0.16 ± 0.00	74.84 ± 1.97
13	Italy	ND	57.17 ± 0.03	3.97 ± 0.12	3.35 ± 0.10	Not detected
14	Spain	65%	67.27 ± 0.00	3.49 ± 0.04	2.48 ± 0.03	Not detected
15	USA	ND	69.56 ± 0.74	8.29 ± 0.07	2.57 ± 0.02	Not detected

KBA: 11-keto-β-boswellic acid; AKBA: 3-*O*-acetyl-11-keto- β-boswellic acid; CA: citric acid; ND: no data.
The data are expressed as the mean ± SD.

The KBA and AKBA levels of the extracts were analyzed by HPLC using method given in Ph. Eur. 11.0 (EDQM 2023). Comparisons of the retention times and UV absorbance with those of the authentic analytical reference compounds allowed us to identify the presence of KBA and AKBA (Figure 2). The boswellic acid derivative content of the extracts was calculated and expressed in a percentage according to the Ph. Eur. 11.0 monograph. The KBA content in the USP Reference Standard (**15**) was 8.29%, whereas its AKBA content was 2.57% (Table 2). Only extracts **13** and **14** had KBA and AKBA content exceeding 1%, whereas the marker compounds were present in insignificant amounts in 12 extracts (**1**–**12**). The AKBA and KBA content of extract

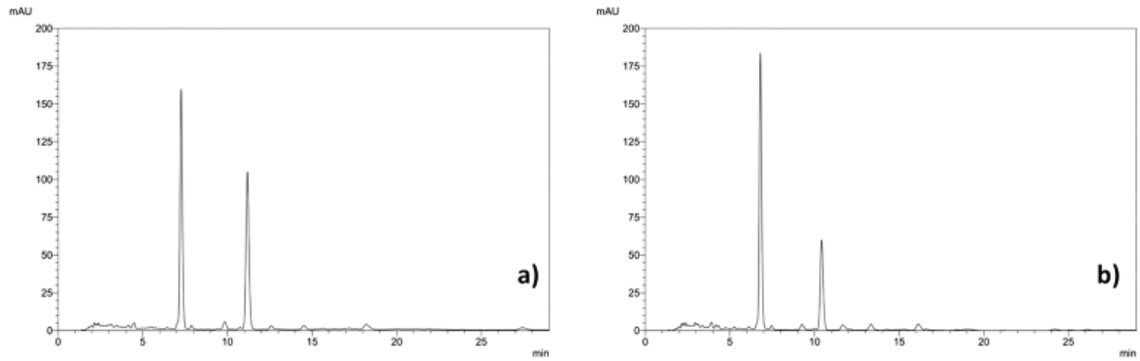

Figure 2. HPLC chromatograms of extract **13** (a) and the reference USP extract (b) detected at 250 nm. The compounds with retention times of 6.7 and 10.2 min were identified as 11-keto-β-boswellic acid and 3-*O*-acetyl-11-keto- β-boswellic acid, respectively.

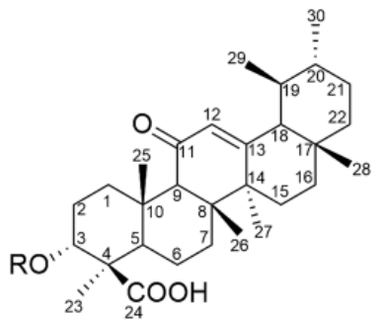

Figure 3. Structure of AKBA (R = CH_3CO) and KBA (R = H).

1 was significantly higher than that of extracts **2**–**12** (all $p < .001$) but significantly lower than that of extracts **13**–**15** (all $p < .001$). Interestingly, the KBA content of the USP standard reference extract was significantly higher than that of all investigated extracts (all $p < .001$); however, the AKBA content of **15** was significantly lower than that of extracts **13** ($p < .001$). HPLC chromatograms of extracts are available in Appendix as supporting information.

In the screening of organic acids, the presence of citric acid was confirmed in the investigated samples based on the UHPLC–HRMS (SIM and PRM) results (Table 1). Interestingly, no other organic acids, such as malic acid, benzoic acid, or tartaric acid, were detected. The targeted quantitative analysis revealed citric acid content in the range of 69.57–117.90 mg/g for **1**–**8**, **10**, and **12**. Conversely, citric acid was not detected in **15**, similarly as observed for extracts **9**, **13**, and **14**.

The ^{1}H NMR assignments for KBA and AKBA (Figure 3) were provided based on our NMR experiments and by comparison of data published in the literature (Badria et al. 2003; Belsner et al. 2003; Csuk et al. 2015):

11-keto-β-boswellic acid (Figure 32S)

(Figure 32S): ^{1}H NMR (500 MHz, methanol-d_4, δ in ppm, J (Hz)): 0.83 (d, 3H, CH_3 (29), J = 6.4 Hz), 0.86 (s, 3H, CH_3 (28)), 0.97 (s, 3H, CH_3 (30), m, 1H, CH (20)),

1.05 (dt, 1H, CH_2 (16b), J = 13.7, 2.5 Hz), 1.14 (s, 3H, CH_3 (25)), 1.20 (s, 3H, CH_3 (26)), 1.26 (s, 3H, CH_3 (27)), 1.28 (m, 1H, CH_2 (15b)), 1.36 (m, 1H, CH_2 (1a), 1H, CH_2 (22a), 1.38 (s, 3H, CH_3 (23)), 1.49 (m, 1H, CH_2 (22b), 1H, CH (5), 2H, CH_2 (21), 1H, CH_2 (7b), 1H, CH (19)), 1.59 (d, 1H, CH (18), J = 11.1 Hz), 1.74 (m, 1H, CH_2 (6b), 1H, CH_2 (7a), 1H, CH_2 (2b)), 1.90 (dd, 1H, CH_2 (15a), J = 14.8, 2.9 Hz), 1.96 (dd, 1H, CH_2 (6a), J = 13.4, 4.5 Hz), 2.17 (dd, 1H, CH_2 (16a), J = 13.6, 4.8 Hz), 2.45 (m, 1H, CH_2 (2a)), 2.39 (dt, 1H, CH_2 (1b), J = 13.2, 3.4 Hz), 2.54 (s, 1H, CH (9)), 3.97 (t, 1H, CH (3), J = 2.7, Hz), 5.51 (s, 1H, CH (12)).

3-O-acetyl-11-keto-boswellic acid (Figure 33S)

^{1}H NMR (500 MHz, methanol-d_4, δ in ppm, J (Hz)): 0.83 (d, 3H, CH_3 (29), J = 6.5 Hz), 0.86 (s, 3H, CH_3 (28)), 0.98 (s, 3H, CH_3 (30), m, 1H, CH (20)), 1.05 (dt, 1H, CH_2 (16b), J = 13.4, 2.5 Hz), 1.16 (s, 3H, CH_3 (25)), 1.20 (s, 3H, CH_3 (26)), 1.21 (s, 3H, CH_3 (23)), 1.29 (m, 1H, CH_2 (1a), 1H, CH_2 (15b)), 1.39 (s, 3H, CH_3 (27)), m, 1H, CH_2 (22a)), 1.48 (m 2H, CH_2 (21), 1H, CH_2 (7b), 1H, CH (19), 1H, CH (5)), 1.56 (dt, 1H, CH_2 (22b), J = 15.6, 3.5 Hz), 1.60 (d, 1H, CH (18), J = 11.5 Hz), 1.74 dd, 1H, CH_2 (2b), J = 13.1, 3.6 Hz), 1.79 (m, 1H, CH_2 (7a)), 1.92 (m, 2H, CH_2 (6)), 1.98 (dd, 1H, CH_2 (15a), J = 13.4, 5.0 Hz), 2.07 (s, 3H, CH_3 (32)), 2.18 (dd, 1H, CH_2 (16a), J = 13.6, 4.8 Hz), 2.25 (m, 1H, CH_2 (2a)), 2.48 (dt, 1H, CH_2 (1b), J = 12.9, 3.3 Hz), 2.50 (s, 1H, CH (9)), 5.26 (t, 1H, CH (3), J = 2.4, 2.3 Hz), 5.52 (s, 1H, CH (12)).

Proton spectra of USP reference standard with chemical shift range of 0.98–5.52 ppm served as fingerprint signal pattern for BSE. NMR spectra of commercial extracts were compared with NMR spectrum of the USP reference standard (Figure 29S) and citric acid (Figure 31S). Neither AKBA nor KBA ^{1}H NMR spectra had non-overlapping signal which would be suitable for identification of these compounds in BS extracts (Figure 4). The USP reference standard BSE has characteristic fingerprint in proton signal pattern, especially in range of 0.80–2.60 ppm. The signal pattern of USP reference standard BSE proton spectrum was compared with proton spectra of commercial samples. Proton signals of methylene groups in citric acid (δ 2.91 [H-β d, J = 15.7]; δ 2.80 [H-β′ d, J = 15.7]) were used for citric acid identification in commercial samples. The comparison of the signal patterns could confirm the presence or absence of BSE, citric acid or both in commercial samples. The proton spectra of samples **1**, **9**, **11**, **13**, and **14** showed high similarity with USP reference standard BSE **15** (Figure 5). These findings might suggest the presence of BSE in commercial samples **1**, **9**, **11**, **13**, and **14**. The characteristic signals of methylene groups in citric acid could be observed in proton spectra of commercial samples **1**–**8**, **10**, and **12**, which might indicate that these samples contain citric acid. These findings were in accordance with our HPLC and UHPLC analysis. The NMR spectra of KBA, AKBA, commercial *Boswellia* extracts, and citric acid are available in Appendix as supporting information.

Discussion

BSE is considered as a potent active ingredient for osteoarthritis management. Placebo controlled, randomized, double blind clinical trials have demonstrated the extracts of *Boswellia serrata* to be superior to placebo based on various indices (e.g. WOMAC

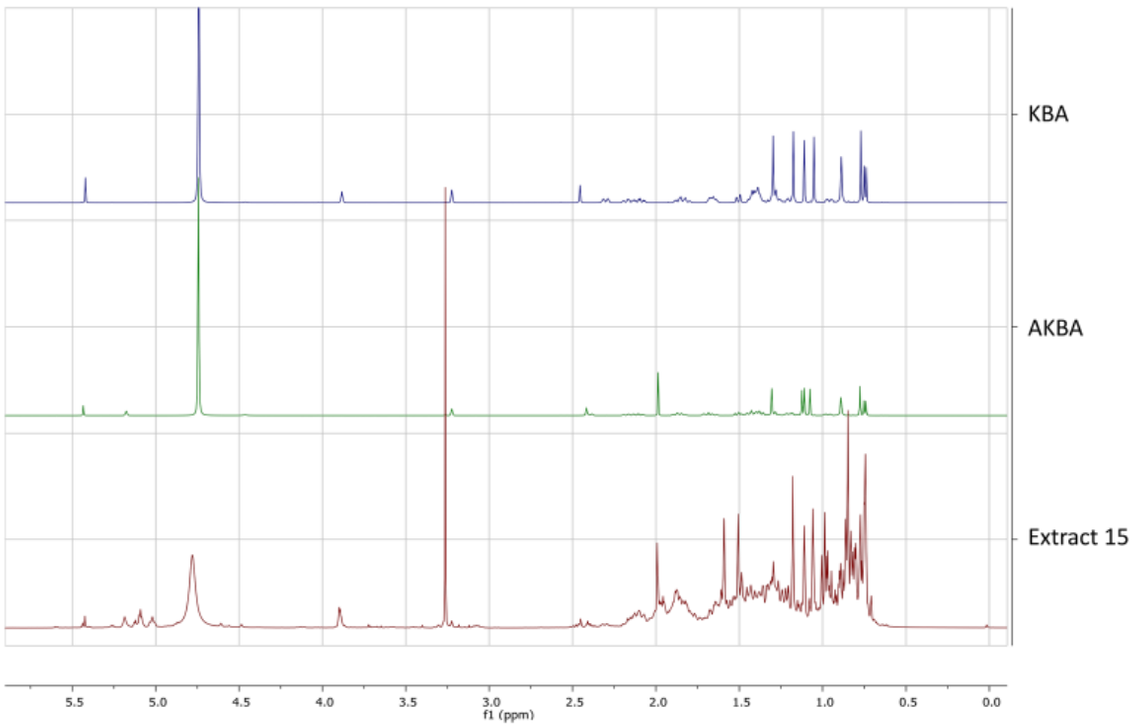

Figure 4. [1]H NMR spectra comparison of USP extract, AKBA and KBA.

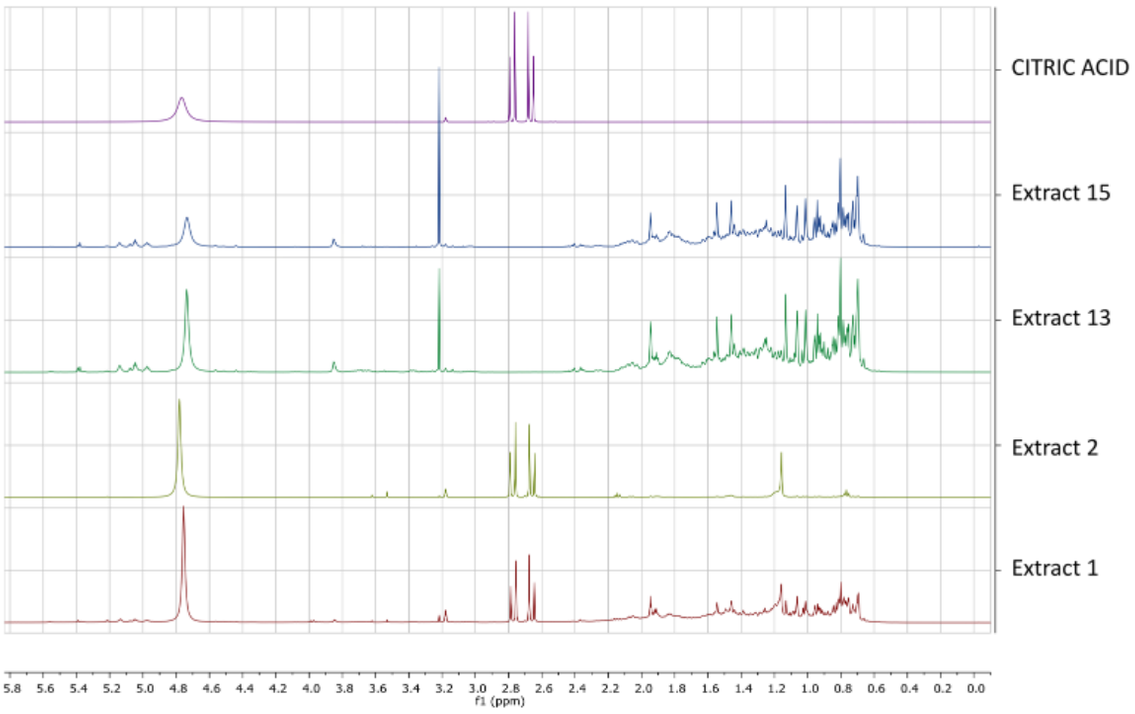

Figure 5. Comparison of [1]H NMR spectra in order to determine the presence of BSE and/or citric acid in commercial extracts.

and Visual Analog Scale (VAS)) and blood biochemical parameters (TNFα, hsCRP, COMP, C2C) (Kimmatkar et al. 2003; Majeed et al. 2019; Kulkarni et al. 2020). However, the study drugs have not been described using rigorous chemical characterization. Majeed et al. have applied Boswellin as study drug, which is standardized to its boswellic acid content as follows: 53.29 mg of AKBA, 20.83 mg of β-boswellic acid, 7.11 mg of KBA, and 6.06 mg of 3-*O*-acetyl-β-boswellic acid. No further information about the extraction method, drug solvent ratio, or other details was provided (Majeed et al. 2019). WokVida and WokVel have been investigated in several clinical trials. These extracts were prepared by extracting *B. serrata* resin with ethanol for 3 h at 70 °C. The extraction process was repeated three times. The ethanolic extract was evaporated using a rotary evaporator, resulting in drug-to-extract ratio (DER) of 5:1. For WokVida, the dry extract was redissolved in ethyl acetate, and stearic acid was added. In the case of WokVel, 333 mg of BSE with 40% boswellic acid were applied (Kimmatkar et al. 2003; Kulkarni et al. 2020). Gokaraju et al. have studied the efficacy of Aflapin, which contains *B. serrata* resin extract enriched in AKBA (min. 20%) combined with *B. serrata* non-acidic resin extract (BNRE) in a 2:1 ratio. BNRE is obtained from the resin in which *Boswellia* oil is distilled. The resin is dispersed in methyl isobutyl ketone and filtrated. The filtrate is extracted with 2% KOH solution to remove the acidic compounds. The acid-free organic phase is then evaporated at 60–70 °C under reduced pressure. The last step of BNRE preparation is solvent evaporation under reduced pressure at 75–85 °C (Gokaraju et al. 2010).

Boswellic acid and its derivatives are marker compounds for the genus *Boswellia*. Thus, Schmiech et al. used the ratio of pentacyclic triterpenoid acids (i.e. 3-*O*-acetyl-β-boswellic acid, AKBA, and β-boswellic acid) to calculate the *Boswellia* index, which might be useful for the identification of specific species (Schmiech et al. 2019). Interestingly, the levels of two primary markers used for quality analysis in the pharmaceutical industry (i.e. KBA and AKBA) differ across *Boswellia* species. Schmiech et al. detected the highest AKBA content in *B. dalzielii* (6.47%). Meanwhile, *B. sacra* (3.13%) and *B. serrata* (1.18%) contained lower AKBA levels, whereas AKBA could not be detected in *B. frereana* (Schmiech et al. 2019). Consequently, the presence of marker compounds in extracts does not prove that the analyzed sample originated from *B. serrata*. Laboratory guidance published by the American Botanical Council highlighted the possibility of adulteration and described combined botanical and chemical methods for identification (Orhan et al. 2022).

Regulations issued by authorities and scientific reports have not provided clear information about extraction methods. According to USP-NF, the drug should be extracted with isopropanol, ethanol, methanol, hexane, or mixtures of these solvents with a DER of 6:1 (United States Pharmacopeia 2023b). Neither the European Medicines Agency nor EFSA has published a clear definition for *Boswellia* extracts. Patents disclosed methods suitable for AKBA enrichment using saline precipitation from aqueous solution (Seligson et al. 2003; Rashan et al. 2020). Furthermore, the extraction methods are rarely specified for extracts or finished consumer products. Various methods of extraction might have different yields; moreover, the boswellic acid content of the extracts obtained by different methods might also vary considerably. For instance, the boswellic acid content of *B. sacra* extract could be 2.47-fold higher in the acidic fraction (obtained by solvent–solvent partitioning) than in the solvent extraction (obtained

solely by organic solvents, e.g. hexane, methanol) (Schmiech et al. 2019). Numerous extraction methods might be used by manufacturers, such as solvent extraction, solvent extraction followed by solvent–solvent partitioning, extraction of the waste product of hydro-distillation, or the addition of synthetic boswellic acid derivatives to the extract. These methods might result in products with approximately 30–70% TBA content and a wide range of marker compound levels (3–4.7% for KBA and 2.2–2.9% AKBA). Extracts prepared using various conditions might display differences in appearance, taste, and smell (Basch et al. 2004; Abdel-Tawab et al. 2011). Thus, information on the extraction method would be helpful for explaining and understanding the cause of low marker compound levels in products. The marker compounds KBA and AKBA are believed to contribute significantly to the pharmacological effects of the extracts; however, recent studies have found major differences in the pharmacokinetic properties of boswellic acids. The levels of chemical markers used by the pharmacopoeias (AKBA and KBA) do not exceed the effective concentration in human plasma. Meanwhile, the accumulation of boswellic acids lacking the 11-oxo moiety in plasma could be more advantageous in terms of pharmacological effects (Sharma S et al. 2004). A clinical trial comparing 5-Loxin (active ingredient: BSE with 30% AKBA) (Ganga et al. 2004) and Aflapin (active ingredient: BSE and BNRE with 20% AKBA) found that both drugs significantly reduced pain and stiffness versus placebo; however, Aflapin, with its lower AKBA content, displayed superiority to 5-Loxin (Sengupta et al. 2008). Nonetheless, KBA, and AKBA are commonly present in BSEs. Thus, these markers must be present in quantifiable amounts in the extracts. No or extremely low levels of marker compounds in analyzed samples could suggest dilution or adulteration of the extracts.

All of the purchased industrial extracts used in our analysis were labeled with TBA content, which might be measured by the sum of the major boswellic acid or titration (Schmiech et al. 2019). The latter analytical method is undoubtedly simple, fast, and inexpensive, but it is not specific. The boswellic acid content determined by titrimetry and HPLC significantly differs. A possible reason for this difference could be that the content of acids other than boswellic acid derivatives is attributable to 'other organic acids.' According to Sharma et al., these acids might be tirucallic acids present in amounts of 27–43% (Sharma N et al. 2016). Based on a literature review, benzoic acid, citric acid, malic acid, oxalic acid, and tartaric acid are not commonly present in *Boswellia* extracts; moreover, these compounds do not contribute significantly to the acidic character of the extracts. Consequently, neither the scientific literature, nor our analysis supports the presence of citric acid in *Boswellia* extracts. Interestingly, the measured citric acid content of extracts **1**–**8**, **10**, and **12** was highly correlated with the TBA content based on the consideration that 1 g of citric acid is equivalent to 7.13 g of boswellic acid. In addition to organoleptic analysis, the targeted UHPLC–HRMS measurements confirmed the presence of citric acid in the examined extracts (i.e. **1**–**8**, **10**, and **12**).

The global food supplement industry is anticipated to reach an estimated worth of USD 185.16 billion by 2027 (GlobeNewsWire 2020). The global concern around food supplements has been prompted by their increasing popularity and loose regulation (Czepielewska et al. 2018). Consequently, these products are frequently adulterated. The most common methods of adulteration of herbal remedies include substitution

with closely related plants from the same genus, the addition of identical chemicals, and the use of undeclared pharmaceuticals in food supplements (Gafner et al. 2023).

Numerous *Boswellia* species are identified as vulnerable taxa in the IUCN Red List of Endangered Species in 2023 (Iucnredlist 2023). The potential for adulteration of frankincense-containing products is attributable to habitat loss, unsustainable harvesting, and growing demands for the exudates of the species (DeCarlo et al. 2020). It is noteworthy that more than 7% of the global population suffer from osteoarthritis; therefore, they are potentially exposed to falsified frankincense products because the most frequent indications of *Boswellia* extracts are osteoarthritis and inflammatory bowel disease (Jairath and Feagan 2020; Leifer et al. 2022).

Adulteration can occur at different levels of production. It is unquestionable that adulterated frankincense food supplements and veterinary medicines are already on the market (Miscioscia et al. 2019). The source of adulteration could be extract production, as it was in 2019 in a case of saw palmetto extract adulteration (Casetext 2019). In general, analytical methods are seldom used in the Hungarian food supplement industry. Nonetheless, laboratory guidance for frankincense has been published, but advanced methods (e.g. able to distinguish different species) for detecting adulteration of food supplements are not available (Orhan et al. 2022). Hence, it is a common practice to standardize *Boswellia* extracts via titration to ensure that the TBA content is 65%. The acid–base titration method used in this study was not sufficiently specific to authenticate the purchased B*oswellia* extracts. Furthermore, analytical methods for frankincense in pharmacopoeias could not distinguish *Boswellia* species; moreover, the results indicated that BSEs could be adulterated with extracts from other species as other species (e.g. *Boswellia papyrifera* and *Boswellia sacra*) are available in higher quantities (Bongers et al. 2019; Orhan et al. 2022). The boswellic acid profiles of the 28 species of the genus might differ substantially (Schmiech et al. 2019). Thus, an already published analytical method that detects the three major boswellic acids (AKBA, β-boswellic acid, and acetyl-β-boswellic acid) could be beneficial to be applied on food supplements as well, as it requires less standards and is able to distinguish species based on the ratio of boswellic acids could be useful (i.e. *Boswellia* index) (Schmiech et al. 2019). Similar analytical methods have been developed measuring more than three major boswellic acids, but the time-consuming procedures are not suitable for high-throughput screening of a large number of herbal remedies (Katragunta et al. 2019).

Based on our results and data reported in the literature, TBA determination alone is not a sufficient analytical method for quality analysis. Quantification of KBA and AKBA levels as marker compounds and the identification of citric acid or other carboxylic acids as adulterants in *Boswellia* extracts might increase the safety and quality of these specific food supplements.

Our findings suggest that adulterated *Boswellia* extracts might already be on the market. The primary insight of our research is that industrial analytical methods cannot distinguish citric acid from TBA in the extract. No prior research reported the addition of citric acid or other inexpensive acidic compounds to *Boswellia* extracts as a strategy for meeting the criterion of 65% TBA content. Concerning organic acid screening, the UHPLC–HRMS PRM-based method developed in this study confirmed the presence of citric acid in currently marketed extracts. In addition, the targeted

UHPLC–HRMS SIM approach provided quantitative information about their citric acid content. The NMR experiments afforded to confirm the presence of BSE and citric acid in commercial samples. The proton spectra of marker compounds (KBA and AKBA) exhibited substantial overlap with the USP reference standard BSE in terms of signal pattern. As a result, it was unable to detect a proton signal that is exclusive to marker chemicals. Nevertheless, a distinctive pattern was evident in the proton spectra of the USP reference standard BSE within the chemical shift range of 0.80–2.60 ppm. By direct comparison of the proton signal patterns in spectra of commercial samples with the USP standard reference BSE, it was possible to determine if BSE is present or absent. The specific and non-overlapping proton signals of methylene groups of citric acids might be applied to identify the presence of citric acid in commercial samples. Although this study focused solely on qualitative identification of the extract or compounds, it is possible to achieve quantitative evaluation of the extract by applying qNMR. There are several publications emphasizing this method in complex chemical mixtures (Giraudeau 2023) and plant extracts (Rebiai et al. 2022) for quantification.

Although citric acid has a low risk of adverse effects, it is likely to limit the anticipated beneficial effects of counterfeit *Boswellia* extracts (Poerwono et al. 2001). Furthermore, our study revealed the limitations of pharmacopeial methods and proposed potential approaches to develop improved analytical methods for assessing food supplements containing *Boswellia* extracts.

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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About the authors

Péter Püski holds PharmD and is a PhD student at Institute of Pharmacognosy (University of Szeged, Faculty of Pharmacy). His research interests are phytochemistry and analytics of dietary supplements.

Tímea Körmöczi is a researcher at Institute of Pharmaceutical Analysis (University of Szeged, Faculty of Pharmacy). Her research fields are liquid chromatography, mass spectroscopy, and metabolomics.

Róbert Berkecz holds PhD and he is an associate professor at Institute of Pharmaceutical Analysis (University of Szeged, Faculty of Pharmacy). He is the author of 70+ papers in field of analytics.

Anita Barta obtained her PharmD and is in the final stage of her PhD at University of Szeged. Her research field is preparative phytochemistry and NMR spectroscopy of plant secondary metabolites.

Ákos Bajtel PharmD is a clinical pharmacist and researcher at University of Szeged. He is working on completion of his PhD thesis.

Tivadar Kiss holds PhD in pharmacognosy. He is an assistant professor at Institute of Pharmacognosy (University of Szeged, Faculty of Pharmacy). His research interests include phytochemistry, analytics of dietary supplements and science education.

Data availability statement

Data are available for research purpose upon reasonable request to the corresponding author.

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