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SHORT COMMUNICATION

Modern green approaches for obtaining *Satureja kitaibelii* Wierzb. ex Heuff extracts with enhanced biological activity

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Abstract: Modern trends in phytochemical extraction from alternative crops support the use of alternative technologies, such as ultrasound- and microwave--assisted extractions. Considering the reduction of toxic solvents, rapid and effective extraction process, the above-mentioned technologies have become the foundation of green chemistry approaches in a wide range of applications. These extractions have not been studied on *Satureja kitaibelii* Wierzb. ex Heuff, which is a highly potent plant when considering its aromatic and medicinal properties. This preliminary study presents an *in vitro* evaluation of biological activities of ultrasound- and microwave-assisted extracts of *S. kitaibelii*, for the first time. Furthermore, it offers a totally green, modern, fast and reproducible method for extraction of phytochemicals from *S. kitaibelii* herba (Rtanj Mountain, Serbia). This short communication suggests that the applied microwave-assisted extraction, using only water as the solvent, can be a promising approach for obtaining green products with commercial potential.

Keywords: ultrasound-assisted extraction; microwave-assisted extraction; *in vitro* antioxidant analysis; in vitro antimicrobial analysis; Lamiaceae.

INTRODUCTION

Satureja kitaibelii Wierzb. ex Heuff. or Rtanj tea is an endemic Lamiaceae species, mainly spread across the Balkan Peninsula.[1] *Satureja kitaibelii* is well known for its aromatic and medicinal properties; hence it is used as a culinary herb in Mediterranean dishes, in aromatherapy, or in traditional medicine to treat various ailments.[2] The extraction process is a crucial step in the valorisation of

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the plant sources; different extraction techniques and extracting solvents can influence the final phytochemical composition and bioactive potential of the obtained extracts. After reviewing scientific-relevant literature it was found that the articles regarding *S. kittaibelii* usually include conventional extraction approaches, essential oil, and with two non-conventional method (subcritical water extraction and ultrasound-assisted extraction).[1,3–5] Conventional extraction techniques have disadvantages like thermal degradation of bioactive compounds, or the use of a large quantity of organic solvents with toxic impact on the environment or on human health. Recent trends in phytochemical extraction from plants recommend exploring the use of modern technologies; ultrasound-assisted (UA) and microwave-assisted extractions (MA) which become popular due to the fact that these techniques reduce the consumption of toxic solvents, increase the speed and extraction efficiency, thus being compliant to the principles of green chemistry.[6]

UA extracts (UAE) and MA extracts (MAE) of *S. kittaibelii* have not been studied until now. Thus, the aim of this study was to investigate the effects of UAE and MAE on the extraction of phytochemicals from this plant, in order to obtain valuable information regarding possible application in food and pharmaceutical industries. The impact of these modern extraction technologies on the phytochemical composition and biological activity (antioxidant and antimicrobial) was evaluated by varying different green extraction solvents.

EXPERIMENTAL

Plant material

The *Satureja kitaibelii* herb was collected on the Rtanj Mountain, Serbia (43◦46′34″ N; 21◦53′36″ E) in July 2020. Voucher specimens (BUNS 2-1373) were used for the identification of species. The collected aboveground flowering parts were dried naturally in shade at ambient temperature. Constant weight was gained after one week of drying. Dry plant material was placed in a soft paper bag until further analysis.

Ultrasound-assisted extraction

Ultrasound-assisted (UA) extraction was carried out in an ultrasonic bath (Iskra, Slovenia) by placing samples in the proximity of the ultrasound source. Ground sample (5 g) was extracted at room temperature with 100 ml of solvent (70% methanol or distilled water) for 30 min. The extracts were filtered (Whatman paper No. 1) and stored at 4 °C until further analysis.

Microwave-assisted extraction

Microwave-assisted (MA) extraction was carried out in an adapted microwave oven described previously by Švarc-Gajić *et al.*[7] Ground sample was extracted maintaining the same sample-to-solvent ratio, extraction time, and solvent type as in the case of UA extraction, for comparison reasons. The extraction was carried for 30 min applying magnetron power of 450 W. After completing the extraction process, the extracts were filtered (Whatman paper No. 1) and stored at 4 °C until further analysis.

Phytochemical analysis

Phenolic quantification was performed using Shimadzu Prominence HPLC, connected to an SPD-20AV UV/Vis detector (Shimadzu, Kyoto, Japan). Separation was performed on a Luna C-18 RP column, 5 μm, 250 mm×4.6 mm (Phenomenex, Torrance, CA, USA) with a C18 guard column, 4 mm×9 mm×30 mm (Phenomenex, Torrance, CA, USA). The filtered extracts were examined by HPLC reverse phase analysis as described by Aćimović *et al.*[4]

In vitro *antioxidant analysis*

Antioxidant activity of extracts was investigated using four *in vitro* assays, as outlined by Aćimović *et al.*:[4] 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), superoxide anion (SOA) and reducing power (RP). The antioxidant activities were expressed as μmol of Trolox equivalents per g of dry plant material.

In vitro *antimicrobial analysis*

Antimicrobial activity of *S. kitaibelii* extracts was determined against the following ATTC referent strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes* (bacteria), *Saccharomyces cerevisiae*, *Candida albicans* (yeasts), and *Aspergillus brasiliensis* (fungi). Disk diffusion and microdilution methods for *in vitro* evaluation of antimicrobial activity as well as for testing minimal inhibitory concentration were performed by methods defined by Mićić *et al.*[8]

Statistical analysis

Statistical analyses were carried out using Origin v. 8.0 SRO software. Significant differences were calculated by ANOVA ($p < 0.05$). Results are presented as mean value ± standard deviation ($n = 3$).

RESULTS AND DISCUSSION

The phenolics profiles of the analyzed extracts are presented in Table I. They possess obviously different phenolics contents depending on the applied technique and solvent, which could be related to different extraction mechanisms and polarity of the presented compounds. The highest concentration of phenolic compounds was found in the extracts prepared by MAE; in terms of used solvent, significantly higher phenolic content was noted in water extract. In extracts prepared by UAE, the concentration of phenolic compounds was lower, but not their number. In this case, 70 % methanol exhibited better efficiency for phenolic extraction. The obtained results were in correlation with the literature data; Mašković *et al.*[6] reported that MAE ethanol extract of summer savory (*Satureja hortensis* L.) was richer in phenolic compounds than UAE. In general, microwaves induce a sudden increase in temperature inside the cellular structure, which leads to rupturing of cell walls and fast release of phytochemicals into extracting medium. The efficiency of microwave-assisted extraction lies in the fact that the energy of microwaves is directly converted to heat, by instantaneous absorption, *i.e.*, by rapid alignment of sample dipoles with the frequency of microwaves, thus generating heat inside the matrix.[10] Consequently, an induced sudden increase in temperature inside cells causes rupture of cell walls and fast release of phyto-

chemicals into the extracting medium. Apart from solvent selectivity towards the analyte, the dielectric constant is a significant factor for obtaining high-quality extracts as well. According to Vladić *et al.*,[9] the chosen solvent should possess a high dielectric constant and strongly absorb the microwave energy; water has the highest dielectric constant, followed by methanol and ethanol. Conversely, in the case of the UAE, the cavitation phenomenon and free-radical formation can cause degradation of phytochemicals.[10]

TABLE I. HPLC analysis of phenolic compounds in extracts (c / mg g^{-1}) obtained by ultrasound-assisted (UAE) and microwave-assisted extraction (MAE); values in rows with different superscripts are significantly different at $p < 0.05$

Compound	UAE 70 % methanol	UAE water	MAE 70 % methanol	MAE water
Vanilic acid	3.15±0.01^{b}	–	1.13±0.00^{a}	–
Epicatechin gallate	7.94±0.01^{d}	0.15±0.01^{a}	0.40±0.00^{b}	7.31±0.05^{c}
Syringic acid	31.03±0.03^{b}	5.17±0.04^{a}	58.62±0.07^{c}	59.76±0.02^{d}
Coumarin acid	1.65±0.00^{c}	0.36±0.01^{a}	0.81±0.00^{b}	2.50±0.02^{d}
Caffeic acid	4.26±0.03^{d}	0.55±0.00^{a}	0.96±0.00^{b}	2.45±0.01^{c}
Gentisic acid	–	1.61±0.01^{a}	2.95±0.03^{b}	–
Sinapic acid	4.01±0.02^{c}	1.14±0.02^{a}	1.45±0.01^{b}	4.65±0.02^{d}
Rosmarinic acid	4.44±0.03^{c}	1.45±0.00^{a}	2.23±0.06^{b}	8.63±0.03^{d}
Ferulic acid	4.01±0.01^{b}	3.12±0.02^{a}	7.97±0.03^{c}	17.96±0.1^{d}
Rutin	0.04±0.00^{a}	0.45±0.00^{b}	0.95±0.00^{c}	4.25±0.02^{d}
Luteolin	–	–	0.05±0.00^{a}	–
Total phenolic compounds	60.53±0.14^{b}	14.00±0.11^{a}	77.52±0.20^{c}	107.51±0.28^{d}

HPLC analysis showed that the dominant compound in all extracts was syringic acid, which ranged from 5.17 m/g (UAE water extract) to 59.76 mg/g (MAE water extract). The highest content of syringic acid was earlier confirmed in *S. kitaibelii* subcritical water extract.[4] Ćetković *et al.*[3] have also classified syringic acid among the most main phenolic compounds in *S. kitaibelii* extracts obtained by a conventional extraction technique and different organic solvents.

In general, a single assay method is not sufficient for *in vitro* assessment of antioxidant activity of endogenous phytochemicals. Antioxidant molecules differ in polarities, thus they can act by different mechanisms. Antioxidant activity of *S. kitaibelii* extracts was challenged by four methods (Table II); significant antioxidant potential was found in water and 70 % methanol extracts, obtained by MAE. More precisely, the antioxidant potential of tested samples decreased respectively: MAE water > MAE 70 %methanol > UAE 70 % methanol > UAE water.

There were considerable differences noted in antimicrobial effects against tested microorganisms between UAE and MAE extracts (Table III). The UAE extracts did not show antimicrobial effect, with the exception of water extract which showed low inhibition potential against *A. brasiliensis*. Consequently, the

defined minimal inhibitory concentration is above the initial concentration of extract, and further antimicrobial potential of the concentrated extracts is required. However, the antimicrobial effect was observed in the case of MAE extract, especially in the one prepared by using water as a solvent.

TABLE II. *In vitro* antioxidant activity (μmol TE/g) of *Satureja kitaibelii* extracts obtained by ultrasound (UAE)- and microwave-assisted extraction (MAE); values in rows with different superscripts are significantly different at $p < 0.05$

Antioxidant	UAE 70 % methanol	UAE water	MAE 70 % methanol	MAE water
DPPH	78.99±14.33^b	33.64±2.07^a	225.63±11.45^c	385.38±16.56^d
ABTS	744.66±9.74^b	685.30±17.73^a	1757.86±82.45^c	2571.12±76.58^d
RP	153.20±2.99^b	133.50±1.64^a	336.71±5.68^c	414.93±20.07^d
SOA	3730.81±20.57^b	1993.66±45.94^a	4417.17±15.30^c	4506.69±0.53^d

TABLE III. *In vitro* antimicrobial activity of *Satureja kitaibelii* extracts obtained by ultrasound (UAE)- and microwave-assisted extraction (MAE)

Test organism	UAE 70 % methanol	UAE water	MAE 70 % methanol	MAE water
Inhibition zone, mm[a]				
E. coli ATCC 25922	nd	nd	13.33± 0.57	40.00±0.00
P. aeruginosa ATCC 27853	nd	nd	nd	29.00±0.00
S. Typhimurium ATCC 13311	nd	nd	nd	nd
B. cereus ATCC 11778	nd	nd	nd	28.00±0.00
S. aureus ATCC 25923	nd	nd	27.33±0.57	27.00±1.00
E. faecalis ATCC 19433	nd	nd	nd	nd
L. monocytogenes ATCC 35152	nd	nd	24.00±1.00	17.33±0.57
S. cerevisiae ATCC 9763	nd	nd	nd	nd
C. albicans ATCC 10231	nd	nd	nd	18.33±0.57
A. brasiliensis ATCC 16404	nd	11.00±0.0	nd	nd
Minimal inhibitory concentration, mg/mL[b]				
E. coli ATCC 25922	> 50	> 50	> 50	0.78
P. aeruginosa ATCC 27853	> 50	> 50	> 50	1.56
S. Typhimurium ATCC 13311	> 50	> 50	> 50	> 50
B. cereus ATCC 11778	> 50	> 50	> 50	0.78
S. aureus ATCC 25923	> 50	> 50	12.5	0.78
E. faecalis ATCC 19433	> 50	> 50	> 50	> 50
L. monocytogenes ATCC 35152	> 50	> 50	25	> 50
S. cerevisiae ATCC 9763	> 50	> 50	> 50	> 50
C. albicans ATCC 10231	> 50	> 50	> 50	> 50
A. brasiliensis ATCC 16404	> 50	> 50	> 50	> 50

[a]< 22 mm – low; 22–26 mm – intramedier; >26 mm – high antimicrobial activity, nd – not detected; [b]according to the initial concentration of the extracts

Both MAE extracts showed the antimicrobial effect against *E. coli*, *S. aureus* and *L. monocytogenes*, while water extract expressed a similar effect against *P.*

aeruginosa, *B. cereus* and *C. albicans*. These differences can be explained by the phenolic compounds profile of the tested samples. Unlike the UAE samples, the methanolic MAE sample contains syringic acid, while the water MAE sample contains syringic acid, ferulic acid and rutin. All three mentioned phenolic compounds have previously been reported as antimicrobial agents; ferulic acid is an inhibition factor for *P. aeruginosa*, *S. aureus, E. coli* and *L. monocytogenes* growth,[11] syringic acid inhibits the growth of *S. aureus*, while *E. coli*, *P. aeruginosa*, *B. cereus* and *C. albicans* are sensitive to the presence of rutin.[12] The minimal inhibitory concentrations of the *S. kitaibelii* extracts varied according to strain level in the range of 0.78 to 25 mg/mL (Table III). The lower *MIC* values were obtained for the water extract compared with methanolic, which also lead to differences in chemical compositions.

CONCLUSION

In summary, this preliminary study indicates that the extract prepared with MAE and water as solvent exhibited the highest biological activity. Special significance of the presented approach is reflected in a totally green, modern, fast and reproducible process technology. According to the bioactivity screening, this research suggests that the MAE water extract of *S. kitaibelii* could be used as a natural source of antioxidants for developing a wide range of safe and functional products which will be investigated further.

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ИЗВОД

МОДЕРНИ „ЗЕЛЕНИ“ НАЧИНИ ЗА ДОБИЈАЊЕ *Satureja kitaibelii* WIERZB. EX HEUFF ЕКСТРАКТА СА ИЗРАЖЕНОМ БИОЛОШКОМ АКТИВНОШЋУ

ВАЊА ШЕРЕГЕЉ[1], ОЉА ШОВЉАНСКИ[1], ЈАРОСЛАВА ШВАРЦ-ГАЈИЋ[1], ТЕОДОРА ЦВАНИЋ[1], АЛЕКСАНДРА РАНИТОВИЋ[1], ЈЕЛЕНА ВУЛИЋ[1] и МИЛИЦА АЋИМОВИЋ[2]

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Савремени трендови у фитохемијској екстракцији из алтернативних усева подржавају истраживање употребе алтернативних технологија као што су екстракције уз помоћ ултразвука и микроталаса. С обзиром на редукцију токсичних растварача и брз и ефикасан процес екстракције, поменуте технологије су постале темељ приступа “зеленој” хемији у широком спектру примена. С друге стране, ове екстракције нису проучаване на *Satureja kitaibelii* Wierzb. ex Heuff, која је веома значајна ендемична биљка с обзиром на ароматична и лековита својства. Ова прелиминарна студија је по први пут представила *in vitro* процену биолошке активности ултразвучних и микроталасних екстраката *S. kitaibelii*. Нудећи апсолутно “зелену”, модерну, брзу и поновљиву методу за екстракцију фитокемикалија из *S. kitaibelii* (планина Ртањ), ово истраживање сугерише да примењена екстракција уз помоћ микроталаса, користећи само воду као растварач, може бити

перспективан приступ за добијање "зелених" производа са комерцијалним потенцијалом.

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