https://doi.org/10.31925/farmacia.2023.1.12 **ORIGINAL ARTICLE**

PHYTOCHEMICAL SCREENING OF *SATUREJA KITAIBELII* WIERZB. EX HEUFF. EXTRACTS BY GC/MS AND TLC

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Abstract

The Balkan endemic species *Satureja kitaibelii* Wierzb. ex Heuff. is known in the folk medicine as herbal remedy, spice and natural food preservative. It has many biological properties such as antimicrobial, antioxidant, anti-inflammatory, etc. The purpose of this study was the GC/MS and TLC analyses of *Satureja kitaibelii* extracts of Bulgarian origin. Th plant surface exudate, the methanolic extract and a fraction prepared by alkaline hydrolysis were obtained the aerial parts. Phenolic acids, flavonoid aglycones (methyl derivatives of flavones), triterpene acids, terpenoids and fatty acids were identified in the plant surface exudate. Carvacrol, oleanolic acid, ursolic acid and 4-hydroxybenzoic acid were determined as main compounds in the plant surface exudate. Phenolic acids as quinic and rosmarinic acids, and carvacrol and palmitic acid prevailed in the methanolic extract. The most prevalent methanol insoluble phenolic acids further extracted with ethyl acetate after alkaline hydrolysis were caffeic and 4-hydroxycinnamic acids, followed by ferulic, syringic, 4-hydroxybenzoic and 3,5-dihydroxybenzoic acids. The investigated *S. kitaibelii* extracts showed a high content of important biologically active substances.

Rezumat

Specia endemică balcanică *Satureja kitaibelii Wierzb. ex Heuff.* este cunoscută în medicina populară ca remediu empiric, ca și condiment sau conservant alimentar natural. Are multe proprietăți biologice, cum ar fi efectul antimicrobian, antioxidant, antiinflamator, etc. Scopul acestui studiu a fost analiza GC/MS și TLC a extractelor de *Satureja kitaibelii* din Bulgaria. S-au obținut exudatul de suprafață al plantei, extractul metanolic și o fracțiune preparată prin hidroliză alcalină din părțile aeriene. În exudatul de suprafață au fost identificați acizi fenolici, agliconi flavonoizi (derivați metilici ai flavonelor), acizi triterpenici, terpenoizi și acizi grași. Carvacrolul, acidul oleanolic, acidul ursolic și acidul 4-hidroxibenzoic au fost determinați ca și compuși principali în exudatul de suprafață al plantei. Acizii fenolici, precum acizii chinic și rosmarinic, carvacrolul și acidul palmitic au predominat în extractul metanolic. Cei mai răspândiți acizi fenolici insolubili în metanol extrași cu acetat de etil după hidroliză alcalină au fost acizii cafeic și 4-hidroxicinamic, urmați de acizii ferulic, siringic, 4-hidroxibenzoic și 3,5-dihidroxibenzoic. În concluzie, extractele de *S. kitaibelii* investigate au arătat un conținut ridicat în substanțe biologic active.

Keywords: GC/MS, TLC, extract composition, external flavonoids, *Satureja kitaibelii*

Introduction

The species *Satureja kitaibelii* Wierzb. ex Heuff. is one of the five most common wild species of the genus in the Balkans [1-3]. Its distribution includes the territories of the former SFR Yugoslavia, Bulgaria and a small part of Romania [1, 4, 5]. The above-ground parts of the plants are used in traditional cuisine and medicine in the whole range of species distribution, but most widely in Serbia. As a folk remedy they are mainly used for diarrhoea, nausea, indigestion, respiratory and infectious diseases, cramps, muscle pain, infertility and menstrual disorders. In Serbia, the stalks are offered as a commercial product under the name Rtanj tea [5]. In the recent years many valuable properties of the species such as antioxidant, anti-inflammatory, antimicrobial, hypoglycaemic and antitumour activity were reported [6-8]. The purpose of the present study was the GC/MS and TLC analysis of *Satureja kitaibelii* extracts in order to identify the bioactive compounds of the specie endemic in Bulgaria.

Materials and Methods

Plant material

Aerial parts of *S. kitaibelii* plants in full flowering were harvested in August 2020 from the protected area "Kailaka", near the town of Pleven, Middle Danube plain, Bulgaria. The bedrock in this area is limestone and the altitude is 200 m a.s.l. The sample material was air-dried without exposure to direct sunlight. A voucher specimen (CO 1414) was deposited at the Herbarium of Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM).

Reagents

Methanol, acetone, chloroform, ethyl acetate, sodium hydroxide, hydrochloric acid, pyridine (HPLC grade,

Alfa Aesar) were purchased from Valerus, Bulgaria. N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, Sigma-Aldrich, product no. 15222), 3,5-dichloro-4-hydroxybenzoic acid (Sigma-Aldrich, product no. D64007), 2-aminoethyldiphenyl borate (Sigma-Aldrich, product no. 42810), TLC silica gel plates Kieselgel 60 F254, TLC DC-Alufolien Polyamid 11 F254 plates, TLC mobile phases toluene/dioxan/acetic acid (95:25:4, v/v/v) and toluene/methylethylketon/methanol (60:25:15, v/v/v) were purchased from Merck through the local distributor FOT Ltd. (Bulgaria). Apigenin, scutellarein-6,7-dimethyl ether (cirsimaritin), luteolin, naringenin and eriodictyol were purchased from Biosynth, Slovakia. Apigenin 6,7,8-trimethyl ether (xanthomicrol) was obtained from Sigma–Aldrich.

Acetone extraction of plant surface compounds

Dried, whole aerial parts (3 g) of *S. kitaibelii* were briefly (2 - 3 min) rinsed with acetone at room temperature to dissolve the lipophilic compounds (exudate) accumulated on the plant surface. The acetone solution was then dried using a rotary-evaporator.

Methanolic extract

100 mg powdered plant material was macerated with 1 mL of methanol, in 2 mL Eppendorf tubes. After 24 h of extraction at room temperature an aliquot of 500 µL was transferred into a glass vial and dried.

Alkaline hydrolysis of residue plant material

The plant material remaining after methanol extraction was hydrolysed using 2 M sodium hydroxide for 4 h at room temperature. 50 µL of 3,5-dichloro-4-hydroxy-benzoic acid (1 mg/mL) were used as internal standard. After acidification to pH 1 - 2 with concentrated hydrochloric acid, the phenolic compounds were extracted with ethyl acetate, dried with anhydrous sodium sulfate and then the ethyl acetate was allowed to evaporate at room temperature.

Derivatization

100 µL of pyridine and 100 µL of N,O-bis-(trimethyl-silyl) trifluoroacetamide were added to the dried samples of the plant surface exudate, the methanolic extract and the plant residue after alkaline hydrolysis, and heated at 70°C for 2 h. After cooling, 300 mL of chloroform were added and the samples were analysed by GC/MS.

GC/MS analysis

GC/MS analysis of the plant surface exudate, methanolic extract and the fraction obtained by alkaline hydrolysis was recorded on a Thermo Scientific Focus GC coupled with Thermo Scientific DSQ mass detector operating in EI mode at 70 eV. A DB-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The following program sequence was used: 100 - 180°C at 15°C/min, 180 - 300°C at 5°C/min and 10 min hold at 300°C. The injector temperature was set at 250°C. The carrier gas was helium. The flow rate was 0.8 mL/min. The split ratio was 1:10. 1 µL of the solution was injected. The GC-MS spectra of the compounds in the extracts were denoised by AMDIS 2.64 software (National Institute of Standardization and Technology (NIST), Gaithersburg, MD) before comparing with the standards. The components were identified based on their mass spectra and retention indices (RI) *versus* standards in the National Institute of Standards and Technology (NIST) spectra library. The response ratios were calculated for each metabolite relative to the internal standard using the calculated areas for both components.

Thin layer chromatography (TLC)

Dry plant surface exudate of *S. kitaibelii* was dissolved in 200 mL methanol and 50 µL of the solution was applied on TLC plates. Flavonoid aglycones solutions were used as reference compounds on the same TLC plates. Two TLC sorbents and mobile phases were used to identify the flavonoid aglycones – A: stationary phase silica gel plates Kieselgel 60 F254 (10 x 20 cm, 0.2 mm layer) and mobile phase toluene/dioxan/acetic acid (95:25:4, v/v/v); B: stationary phase DCAlufolien-Polyamid 11 F254 (10 x 20 cm, 0.15 mm layer) plates and mobile phase toluene/methylethylketon/methanol (60:25:15, v/v/v). The migration distance was 90 mm. The chromatograms were viewed in UV light at 336 nm before and after spraying with 1% solution of Naturstoffreagenz A (2-Aminoethyl diphenylborinate) in methanol.

Results and Discussion

The phytochemical composition of surface exudate and methanolic extract of *Satureja kitaibelii* obtained through GC/MS are presented in Table I. Oleanolic and ursolic triterpene acids, carvacrol and free fatty acids like caprylic and palmitic acids were the main metabolites of the plant surface exudate. Oleanolic and ursolic acids are relatively non-toxic triterpenoids and have many beneficial effects, notably hepato-protective, anti-inflammatory, antitumour and anti-hyperlipidemic [9-10]. Previous studies showed that carvacrol was present only as a minor component in the essential oil of just one of 16 chemotypes of *S. kitaibelii* originated from Serbia [11]. As far as we know the first study of the essential oil composition of *S. kitaibelii* from Bulgaria was conducted with plant material, originated from West Bulgaria [12]. The main components of the essential oil were limonene, *p*-cymene and germacrene. Recent comparative study on some Bulgarian and Serbian species of *S. kitaibelii* showed carvacrol as one of the predominant essential oil compounds which is characteristic for Western Balkan Mountain species [13]. Since the subject of our research was the analysis of *S. kitaibelii* extract phytochemical composition, further studies of its essential oil should reveal if the high content of carvacrol is one of the unique characteristics of this population from our geographical area. Caprylic acid, which was also among the main metabolites of the plant surface exudate, is reported in the literature as an effective antimicrobial agent with potent application

in the food industry [14-15]. In the perspective of potential palmitic acid use, recent findings showed that its physiological concentrations could be beneficial for the prevention of oxidative stress in vascular endothelium [16]. The amount of this essential fatty acid in the samples of *S. kitaibelii* could be due to an adaptive response of the plants to adverse environmental conditions. In support of this are some findings which state that under cold, drought or salinity stress, plant membrane lipids accumulate higher amounts of palmitic acid while some other membrane lipid components increase their polarity [17]. Indeed, the geographical area from where the plant material was harvested is very dry most of the year, with cold winters, so the high amount of palmitic acid may be due to plants' adaptation to cold and drought stress.

Table I

Phytochemical composition of plant surface exudate and methanol extract of *Satureja kitaibelii* by GC/MS

Original data for *S. kitaibelii* extract composition from the present study				**Previous data for *S. kitaibelii***	**Previous data for *S. montana***	**Previous data for other *Satureja* sp.**
Compounds	**RI**	**Plant surface exudate % area**	**Methanol extract % area**			
Terpenes						
Borneol	1224	1.3		[13, 18, 19]		
Carvacrol	1339	4.4	1.1	[11]	[20-23]	[23]
Caryophyllene oxide	1583	0.9		[11]	[22]	
Phenolic compounds						
4-hydroxybenzoic acid	1625	0.7	0.2		[24]	[25]
Vanillic acid	1776	0.2		[6]	[21, 23, 24, 26]	
Quinic acid	1843		1.9	[27]		[25]
Caffeic acid	2141	0.4		[6, 28]	[21, 23, 24, 26]	[25, 29, 30]
Rosmarinic acid	3642		1.1	[28]	[23-24]	[25, 29, 30]
Fatty acids						
Caprylic acid	1557	3.2				
Palmitic acid	1929	3.4	1.4			[23]
Carbohydrates						
Fructose	1803	10.8	15.4			
Glucose	1889	6.3	10.5			
Sucrose	2628	13.5	22.2			
Triterpenes						
Oleanolic acid	3739	7.6	0.3			[31]
Ursolic acid	3786	11.2	0.5			
Sterols						
ß-Sitosterol	3389		0.3			[23]

% area – The values were calculated for each metabolite relative to the internal standard using the calculated areas for both components;
RI - retention indices

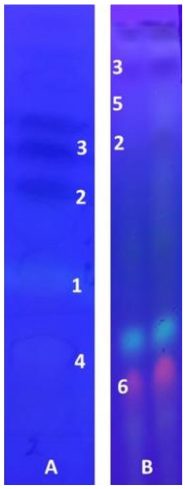

Figure 1.
Flavonoid aglycones in the plant surface exudate of *Satureja kitaibelii* by TLC: Apigenin (**1**); Scutellarein-6,7-dimethyl ether (cirsimaritin) (**2**); Apigenin 6,7,8-trimethyl ether (**3**) (xanthomicrol); Luteolin (**4**); Naringenin (**5**); Eriodictyol (**6**)

Six aglycones of external flavonoids were confirmed in the plant surface exudate of *S. kitaibelii* by TLC analysis (Figure 1). Apigenin, scutellarein-6,7-dimethyl ether (cirsimaritin), luteolin and eriodictyol are known for this species from previous research [27, 28, 32]. As far as we know this is the first report of apigenin 6,7,8-trimethyl ether (xanthomicrol) and naringenin for *S. kitaibelii*, although they are reported for other *Satureja* species [33-35]. External flavonoids are most common in plant species from *Lamiaceae* inhabiting arid and semi-arid territories as they provide protection against UV-B radiation [36].

The main metabolites of the methanolic extract were free phenolic acids as quinic and rosmarinic acids, and the already identified in the plant surface exudate carvacrol and palmitic acid. Both phenolic acids were previously reported for *S. kitaibelii* [27-28]. Quinic and rosmarinic acids are known for remarkable biological activities such as antiviral, antimicrobial, antioxidant, antidiabetic, anticancer, anti-aging, protective, anti-inflammatory, etc. [37-38]. Table II shows the insoluble-

bound phenolic acid composition obtained after alkaline hydrolysis [39]. The most abundant are caffeic acid and 4-hydroxycinnamic acid (*p*-coumaric acid), followed by ferulic, syringic, 4-hydroxybenzoic and 3,5-dihydroxybenzoic acids. The highest amount of caffeic acid, a representative of the class of hydroxycinnamic acids, is in contrast with the prevalence of chlorogenic acid and its derivatives in the Serbian species [27]. According to the scientific data, the extracts of *S. kitaibelii* have shown high antioxidant, antimicrobial and cytotoxic activity and a certain hypoglycaemic potential [6, 27, 28, 32, 40], which can be largely attributed to the complex of phenolic acids [41].

In summary, as a result of the present research we identified some phytomolecules that were not previously reported for the extracts of *S. kitaibelii* such as carvacrol, apigenin 6,7,8-trimethyl ether (xanthomicrol), naringenin, 4-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, hydroxycinnamic acid, 4-hydroxycinnamic acid, oleanolic acid, ursolic acid, caprylic acid, palmitic acid and β-sitosterol (Table I and Table II).

Table II

Alkaline hydrolysable phenolic acids from the methanolic extract of *Satureja kitaibelii*

Alkaline hydrolysable phenolic acids	**RI**	**% area**	**Previous data for *S. kitaibelii***	**Previous data for *S. montana***	**Previous data for other *Satureja* sp.**
4-hydroxybenzoic acid	1637	6.7 ± 2		[24]	[25]
Vanillic acid	1776	0.8 ± 0.4	[6]	[21, 23, 24, 26]	
cis-Hydroxycinnamic acid	1783	1.3 ± 0.3			
Protocatechuic acid	1811	1.4 ± 0.4	[6, 27]	[21, 23, 26]	[25, 29]
cis-Ferulic acid	1863	0.1 ± 0.06	[6]	[21, 24, 26]	[29, 30]
Syringic acid	1888	7.4 ± 1.1	[6]	[21, 24, 26]	
trans 4-hydroxycinnamic acid	1934	23.1 ± 5			
trans 3,5-dihydroxybenzoic acid	2008	6.7 ± 0.5			
trans-Ferulic acid	2063	9.5 ± 2	[6]	[21, 24, 26]	[29-30]
trans-Caffeic acid	2142	94.4 ± 5	[6, 27]	[21, 24, 26]	[25, 29]

% area – The values were calculated for each metabolite relative to the internal standard using the calculated areas for both components (n = 2); RI - retention indices

Conclusions

We hereby report our results regarding the *Satureja kitaibelii* extracts of Bulgarian origin. Our research showed that the plant surface exudate and the methanolic extract of the species possess high contents of biologically active compounds belonging to the groups of phenolic acids, flavonoid aglycones (methyl derivatives of flavones), triterpene acids, terpenoids and fatty acids. Some of these compounds are reported here for the first time in the case of *S. kitaibelii* extract. The studied specie of *S. kitaibelii* has several unique traits in its phytochemical profile compared to the Serbian ones. Research in this area needs to be further investigated.

Conflict of interest

The authors declare no conflict of interest.

References

1. Anchev M, Satureja L.. In Flora *Reipublicae Popularis Bulgaricae*, Velčev V, Ed.; In Aedibus Academiae Scientiarum Bulgaricae: Serdicae, Bulgaricae, 1989; IX; 336-338.
2. Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. www.europlusmed.org.
3. WCSP (2022). World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew; http://wcsp.science.kew.org/.
4. Ciocârlan V, Illustrated Flora of Romania, *Pteridophyta et Spermatophyta*. Ceres: Bucureşti, Romania, 2009; 661-662, (available in Romanian).
5. Đorđević A, Palić I, Stojanović G, Ristić N, Palić R, Chemical profile of *Satureja Kitaibelii* Wierzb. ex Heuff. essential oils: composition of *Satureja Kitaibelii* essential oils. *Int J Food Prop.*, 2014; 17(10): 2157-2165.
6. Ćetković GS, Čanadanović-Brunet JM, Djilas SM, Tumbas VT, Markov SL, Cvetković DD, Antioxidant potential, lipid peroxidation inhibition and antimicrobial activities of *Satureja montana* L. subsp. *kitaibelii* extracts. *Int J Mol Sci.*, 2007; 8(10): 1013-1027.
7. Kundaković T, Milenković M, Zlatković S, Kovačević N, Goran N, Composition of *Satureja kitaibelii* essential oil and its antimicrobial activity. *Nat Prod Commun.*, 2011; 6(9): 1353-1356.
8. Stanojkovic T, Kolundžija B, Ćirić A, Soković M, Nikolić D, Kundaković T, Cytotoxicity and antimicrobial activity of *Satureja kitaibelii* Wierzb. ex Heuff (*Lamiaceae*). *Dig J Nanomater Biostruct.*, 2013; 8(2): 845-854.
9. Liu J, Pharmacology of oleanolic acid and ursolic acid. *J Ethnopharmacol.*, 1995; 49(2): 57-68.
10. Liu J, Oleanolic acid and ursolic acid: Research perspectives. *J Ethnopharmacol.*, 2005; 100(1-2): 92-94.
11. Dodoš T, Rajčević N, Janaćković P, Vujisić L, Marin P, Essential oil profile in relation to geographic origin and plant organ of *Satureja kitaibelii* Wierzb. ex Heuff. *Ind Crops Prod.*, 2019; 139: 111549.
12. Konakchiev A, Tsankova E, The essential oils of *Satureja montana* ssp. *kitaibelii* Wierzb. and

Satureja pilosa var. *pilosa* Velen from Bulgaria. *J Essent Oil Res.*, 2002; 14(2): 120-121.
13. Zheljazkov VD, Semerdjieva IB, Cantrell CL, Astatkie T, Aćimović M, Phytochemical variability of essential oils of two Balkan endemic species: *Satureja pilosa* Velen. and *S. kitaibelii* Wierzb. ex Heuff. (*Lamiaceae*). *Molecules*, 2022; 27: 3153.
14. Hulánková R, Bořilová G, *In vitro* combined effect of oregano essential oil and caprylic acid against *Salmonella serovars*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes*. *Acta Vet Brno*, 2011; 80: 343-348.
15. Drăgan F, Moisa CF, Teodorescu A, Burlou-Nagy C, Fodor KI, Marcu F, Popa DE, Teaha DIM, Evaluating in vitro antibacterial and antioxidant properties of *Origanum vulgare* volatile oil. *Farmacia*, 2022; 70(6): 1114-1122.
16. Palomino OM, Giordani V, Chowen J, Alfonso SF, Goya L, Physiological doses of oleic and palmitic acids protect human endothelial cells from oxidative stress. *Molecules*, 2022; 27: 5217.
17. Zhukov AV, Palmitic acid and its role in the structure and functions of plant cell membranes. *Russ J Plant Physiol.*, 2015; 62(5): 706–713.
18. Mihajilov-Krstev T, Kitić D, Radnović D, Ristić M, Chemical composition and antimicrobial activity of *Satureja kitaibelii* essential oil against pathogenic microbial strains. *Nat Prod Commun.*, 2011; 6(8): 1167-1172.
19. Miladinović DL, Dimitrijević MV, Miladinović LC, Marković MS, Stojanović GS, Seasonal variation in the essential oil of *Satureja kitaibelii* determines chemotypes. *J Essent Oil Res.*, 2022; 25: 1-9.
20. Ćavar S, Maksimović M, Šolić ME, Jerković-Mujkić A, Bešta R, Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chem.*, 2008; 111: 648–653.
21. Silva FVM, Martins A, Salta J, Neng NR, Nogueira JMF, Mira D, Gaspar N, Justino J, Grosso C, Urieta JS, Palavra AMS, Rauter AP, Phytochemical profile and anticholinesterase and antimicrobial activities of supercritical *versus* conventional extracts of *Satureja montana*. *J Agric Food Chem.*, 2009; 57(24): 11557-11563.
22. Trifran A, Aprotosoaie AC, Brebu M, Cioancă O, Gille E, Hăncianu M, Miron A, Chemical composition and antioxidant activity of essential oil from Romanian *Satureja montana* L. *Farmacia*, 2015; 63(3): 413-416.
23. Tepe B, Cilkiz M, A pharmacological and phyto-chemical overview on *Satureja*. *Pharm Biol.*, 2015; 54(3): 375-412.
24. Zeljković SĆ, Topčagić A, Požgan F, Štefane B, Tarkowski P, Maksimović M, Antioxidant activity of natural and modified phenolic extracts from *Satureja montana* L. *Ind Crops Prod.*, 2015; 76: 1094-1099.
25. Aras A, Bursal E, Alan Y, Türkan F, Alkan H, Kılıç Ö, Polyphenolic content, antioxidant potential and antimicrobial activity of *Satureja boissieri*. Iran. *J Chem Chem Eng.*, 2018; 37(6): 209-2019.
26. Ćetković GS, Mandić AI, Čanadanović-Brunet JM, Djilas SM, Tumbas VT, HPLC screening of phenolic compounds in Winter Savory (*Satureja montana* L.) extracts. *J Liq Chromatogr Relat Technol.*, 2007; 30(2): 293-306.
27. López-Cobo A, Gómez-Caravaca AM, Švarc-Gajic´ J, Segura-Carretero A, Fernández-Gutiérrez A, Determination of phenolic compounds and antioxidant activity of a Mediterranean plant: The case of *Satureja montana* subsp. *kitaibelii*. *J Funct Foods*, 2015; 18(B): 1167-1178.
28. Gopčević K, Grujić S, Arsenijević J, Karadžić I, Izrael-Živković L, Phytochemical properties of *Satureja kitaibelii*, potential natural antioxidants: a new insight. *Plant Foods Hum Nutr.*, 2019; 74: 179-184.
29. Popovici RA, Vaduva D, Pinzaru I, Dehelean CA, Farcas CG, Coricovac D, Danciu C, Popescu I, Alexa E, Lazureanu V, Stanca HT, A comparative study on the biological activity of essential oil and total hydro-alcoholic extract of *Satureja hortensis* L. Exp. *Ther Med.*, 2019; 18(2): 932-942.
30. Taslimi P, Köksal E, Gören A, Bursal E, Aras A, Kiliç Ö, Alwasel S, Gülçin İ, Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS. *Arab J Chem.*, 2020; 13: 4528-4537.
31. Jafari F, Ghavidel F, Zarshenas MM, A critical overview on the pharmacological and clinical aspects of popular *Satureja* species. *J Acupunct Meridian Stud.*, 2016: 9(3): 118-27.
32. Aćimović M, Šeregelj V, Šovljanski O, Šaponjac VT, Gajić JS, Brezo-Borjan T, Pezo L, In vitro antioxidant, antihyperglycemic, anti-inflammatory, and antimicrobial activity of *Satureja kitaibelii* Wierzb. ex Heuff. subcritical water extract. *Ind Crops Prod.*, 2021; 169: 113672.
33. Tomás-Barberán FA, Wollenweber E, Flavonoid aglycones from the leaf surfaces of some *Labiatae* species. *P1 Syst Evol.*, 1990; 173: 109-118.
34. Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D, Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. *J Agric Food Chem.*, 2002; 50(19): 5294-5299.
35. Skoula M, Grayer RJ, Kite GC, Surface flavonoids in *Satureja thymbra* and *Satureja spinosa* (*Lamiaceae*). *Biochem Syst Ecol.*, 2005; 33(5): 541–544.
36. Fattahi M, Cusido RM, Khojasteh A, Bonfill, Palazon J, Xanthomicrol: A comprehensive review of its chemistry, distribution, biosynthesis and pharmacological activity. *Mini-Rev Med Chem.*, 2014; 14(9): 725-733.
37. Nadeem M, Imran M, Gondal TA, Imran A, Shahbaz M, Amir RM, Sajid MW, Qaisrani TB, Atif M, Hussain G, Salehi B, Ostrander EA, Martorell M, Sharifi-Rad J, Cho WC, Martins N, Therapeutic potential of rosmarinic acid: A comprehensive review. *Appl Sci.*, 2019; 9(15): 3139.
38. Benali T, Bakrim S, Ghchime R, Benkhaira N, El Omari N, Balahbib A, Taha D, Zengin G, Hasan MM, Bibi S, Bouyahya A, Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnol Genet Eng Rev.*, 2022; 21: 1-30.
39. Ross KA, Beta T, Arntfield SD, A comparative study on the phenolic acids identified and quantified in

dry beans using HPLC as affected by different extraction and hydrolysis methods. *Food Chem.*, 2009; 113(1): 336-344.

40. Trifonova D, Gavrilova A, Dyakova G, Gavrilov G, Yotova M, Nikolov S, Preliminary in vitro study of anti-oxidant activity and anti-diabetic potential of plant extracts from 4 herbal substances not traditionally used for treatment of diabetes mellitus. *Pharmacia*, 2021; 68(4): 755-762.
41. Vinayagam R, Jayachandran M, Xu B, Antidiabetic effects of simple phenolic acids: A comprehensive review. *Phytother Res.*, 2016; 30(2): 184-99.