Future Natural Products

doi: 10.34172/fnp.2022.03 2022;8(1):7-14 http://futurenatprod.skums.ac.ir

Original Article

Phytochemical composition profile of *Scutellaria bornmuelleri* methanolic extract using GC-MS analysis

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Abstract

Background and aims: *Scutellaria bornmuelleri* Hausskn. ex Bornm. ssp. *mianensis* Rech.f is one of the species of *Scutellaria* genus, *Lamiaceae*, that have long been used in traditional medicine. The aim of this study was to analyze the methanol extract of aerial parts of the plant.

Methods: Chemical composition of methanol extract of *S. bornmuelleri* aerial parts was determined using gas chromatography/mass spectrometry (GC/MS).

Results: A total of 113 compounds were identified in the methanol extract of *S. bornmuelleri* shoot. The main compounds were organic acids (23.649%), aldehydes (14.516%), ketones (11.353%), alcohols (5.439%), carbohydrates (3.85%), methyl esters (3.713%), sugars (1.953%), acetates (1.933%), terpenes (2.522%), metal related compounds (1.229%), amides (1.169%) and other compounds (9.423%). Pentacosane (1.021%) was identified as the main carbohydrate, *n*-hexadecanoic acid (4.1%) as the major acid, 5-hydroxymethylfurfural (10.063%) as the main aldehyde, 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- (2.289%) as the main ketone, syringol (2.395) as the main alcohol, phytol (0.66%) as the main terpene, isopropyl acetate (0.728%) as the main acetate, propanoic acid, 2-oxo-, methyl ester (1.298%) as the main methyl ester, 9-octadecenamide, (Z)- (0.685%) as the main amide, d-glycero-d-ido-heptose (0.532%) as the main sugar, and (4H)1,3,2-dioxaborin, 4-ethenyl-4, 6-diethyl-5-(1-methylethyl)- (0.514%) as the main metal related compound.

Conclusion: The presence of different compounds with known bioactivities can make this plant a suitable medicinal plant for supplementary medication for various diseases.

Keywords: Gas chromatography/mass spectrometry, *Scutellaria bornmuelleri*, Methanol extract, *n*-Hexadecanoic acid

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Received: 5 February 2022
Accepted: 17 April 2022
ePublished: 30 June 2022

Introduction

Scutellaria is the second largest genus in the Lamiaceae family with over 470 species distributed worldwide (1), of which 70 species are used traditionally as plant medicines in China, Korea, and Japan (2). In folk medicine, *Scutellaria* extracts and the medicines derived from it are more commonly used by people for inducing diuresis, blood circulation and reducing edema (2). Flavonoids and neoclerodan diterpenoids of this genus, which have been isolated from several species, are important with respect to pharmacological activities (3-6). The main flavones of *Scutellaria* species are baicalin, baicalein, wogonoside and wogonin (7).

In some *in vitro* investigations, baicalin (5,6-dihydroxy-2-phenyl-4H-1-benzopyran-4-one-7-O-d-β-glucuronic acid) and its aglycone baicalein (5,6,7-trihydroxyflavone) exhibited potent cytotoxic effects on human leukemia T-ALL cell lines, human promyelocytic leukemia HL-60, bladder cancer, and colorectal cancer cells (8-12). Wogonin (5,7-dihydroxy-8-methoxyflavone) is an o-methylated flavone extracted from *Scutellaria* species with several bioactive characteristics such as anticancer, antiviral, antioxidant and anti-inflammatory (13). It has been known that wogonin and its glycoside, which is called wogonoside, exert cytotoxic effects against various cancer cells (14-18).

Scutellaria bornmuelleri Hausskn. ex Bornm. ssp. *mianensis* Rech.f, which is also called *Scutellaria orientalis* subsp. Bornmuelleri, is an annual herb that is native to Iran (19). The plant is used in Iranian Traditional Medicine to treat several diseases (20). *S. bornmuelleri* often grows on steep lands at an altitude of 1000-1500 m with an annual rainfall of 200-250 mm (Figure 1).

A total of 89 compounds belonging to different types of volatile oil constituents were identified in the essential oil of *S. bornmuelleri* (21). The main compounds identified in the essential oil from the leaf and stem of *S. bornmuelleri* were as follows: Steroids, with 14-b-H-pregna (32.9%) as the main compound; hydrocarbons (aliphatic, aromatic, and cyclic hydrocarbons; 22.5%); sesquiterpenes (11.4%); acids (5.4%); monoterpenes (4.6%); and ketones (3.5%). Among

Figure 1. *Scutellaria bornmuelleri* in nature.

the identified sesquiterpene and monoterpene compounds, hydrocarbon sesquiterpenes (7.1%) and hydrocarbon monoterpenes (3.7%) were the main compounds (21). The methanol extract of *S. bornmuelleri* root, containing the flavonoids apigenin-7-glucoside, pratensein, tricin, tenaxin II, skullcapflavone II, 5,8-dihydroxy-6,7-dim ethoxyflavones and isorhamnetin, exhibits cytotoxic effects on the growth of colorectal cancer cell lines including HCT-116 and SW-480 cells (22). *S. bornmuelleri* contains a wide variety of flavones, especially flavonoids, including aglycones (scutellarein, baicalein and wogonin), glycosides (scutellarin, baicalin and wogonoside), and methylated derivatives (4,23). In addition, using HPLC-DAD-ESI/MSn analysis, four phenylethanoid glycosides consisting of martynoside, acteoside, allysonoside and verbascoside were identified from the root and shoot of *in vitro* cultured *S. bornmuelleri* (4).

Due to remarkable anticancer activity and cytotoxicity of methanol extract of *S. bornmuelleri* and its usage in folk medicine, we decided to identify the bioactive compounds of methanol extract of this valuable medicinal plant. To the best of our knowledge, there are no reports on the chemical composition of methanol extract of *S. bornmuelleri* shoot.

Materials and Methods

Collection of the plant material

The shoots of *Scutellaria bornmuelleri* were collected from Bostanabad, Tabriz, Iran during the summer season (July, 2018). The plant was identified by Dr. Talebpour (Plant Taxonomy Department, University of Tabriz, Tabriz, Iran) and a voucher specimen was deposited in the herbarium of the East-Azarbaijan Agricultural and Natural Resources Research and Education Center, Tabriz, Iran (No. 14748) for it.

Extraction of plant material

Plant material (leaves, 10 g) was extracted with 30 mL of methanol at room temperature using percolation for 48 hours. The methanol extract was filtered through Whatman filter paper No. 1. The filtrate was evaporated to dryness at 80°C and stored until further analysis. Methanol extract (1 µL) was injected for gas chromatography-mass spectrometry (GC/MS) analysis.

Gas chromatography-mass spectrometry analysis

The methanol extract of *S. bornmuelleri* shoot was subjected to GC-MS analysis on a Hewlett-Packard (HP, Palo Alto, CA) 7890A gas chromatograph system. Helium (99.999%) was utilized as the carrier gas at the constant flow of 1 mL/min. The injection port temperature was set at 240°C; column temperature was firstly set at 40°C for 1 minute, and then slowly risen to 240°C at the rate of 3°C/min. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 50 minutes.

Identification of compounds

Interpretation of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectra of the unknown compounds were compared with those of the known compounds stored in the NIST library. The chemical compounds of the methanol extract were determined by comparing their mass spectra with those of GC/MS library (WILEY 7n D. 04.00 and NIST). The relative percentage of methanol extract compounds was quantified based on GC peak areas.

Results

A total of 111 compounds including acids (27.925%), aldehydes (14.507%), ketones (11.353%), alcohols (5.439%), hydrocarbons (3.85%), methyl esters (3.713%), sugars (1.953%), acetates (1.933%), terpenes (2.522%), metal related compounds (1.229%) and amides (1.169%) were identified in the aerial parts of *S. bornmuelleri* by GC-MS analysis. The peaks indicated the presence of bioactive constituents. The GC-MS chromatograms of the identified compounds are shown in Figure 2. The bioactive compounds were identified, characterized and interpreted on mass spectrum GC–MS conducted using the database of NIST which is having more than 62 000 patterns. The bioactive principles with their molecular formulae and peak areas (%) are shown in Table 1.

Comparatively, the main compounds in terms of their relative abundance were 5-hydroxymethylfurfural, n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, syringol, 4H-pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- and isovanillic acid (10.063%, 4.1%, 2.776%, 2.395%, 2.289%, and 2.153%, respectively). Similarly, the amounts of minor compounds ranged 0.03-0.5%. Specifically, 1,6:3,4-Dianhydro-2-O-acetyl-beta-d-talopyranose, tetracosane, acetic acid, hydroxy-, methyl ester and 2-isopropyl-5,6-dimethyl-1,3-oxathiane were the compounds with the lowest amounts among the compounds present in methanol extract of *S. bornmuelleri* shoot. The obtained results revealed the presence of

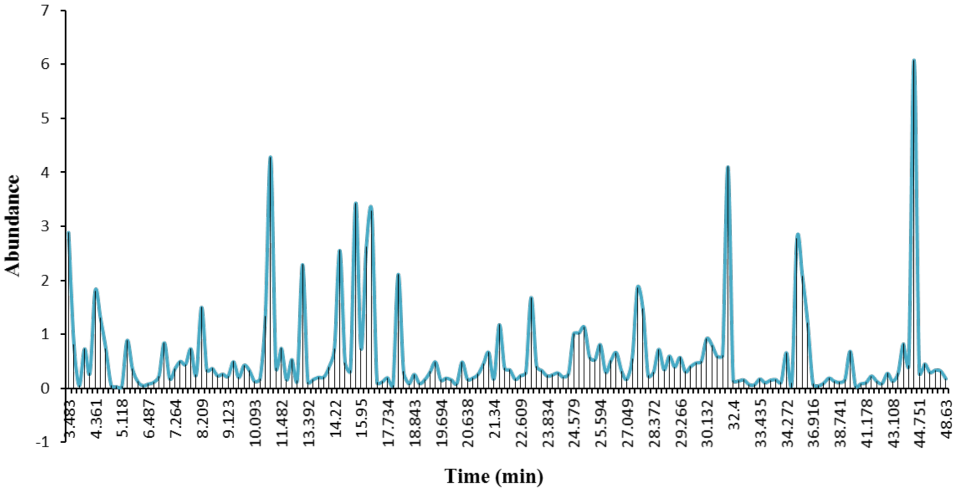

Figure 2. Gas chromatography-mass spectrometry chromatogram of methanol *Scutellaria bornmuelleri* shoot extract.

Table 1. The chemical composition of methanol extract of *Scutellaria bornmuelleri* shoot

No.	Hydrocarbons	Formula	3.85%
1	2-Hexen-4-yne, 2-methyl-	C_7H_{10}	0.397
2	Naphthalene, 1,2-dihydro-1,1,6-tri methyl-	$C_{13}H_{16}$	0.054
3	Naphthalene, 1,2,3,4-tetrahydro-1, 6,8-trimethyl-	$C_{13}H_{18}$	0.302
4	3-Methylhexacosane	$C_{27}H_{56}$	0.093
5	9-Hexacosene	$C_{26}H_{52}$	0.191
6	Tetracosane	$C_{24}H_{50}$	0.041
7	Octadecane, 2,6,10,14-tetramethyl-	$C_{22}H_{46}$	0.108
8	Triacontane	$C_{30}H_{62}$	0.128
9	1-Tricosene	$C_{23}H_{46}$	0.13
10	Nonadecane	$C_{19}H_{40}$	0.769
11	Tricosane	$C_{23}H_{48}$	0.616
12	Pentacosane	$C_{25}H_{52}$	1.021
	Acids		23.649%
13	Acetic acid	CH_3COOH	2.885
14	Isobutyric acid	$C_4H_8O_2$	0.733
15	2-Butenoic acid, 2-methyl-	$C_5H_8O_2$	0.98
16	Phenol	C_6H_6O	1.836
17	Squaric acid	$C_4H_2O_4$	0.367
18	Octanoic acid, 7-oxo-	$C_8H_{14}O_3$	0.485
19	2-Butenedioic acid, 2,3-dihydroxy-, (E)-	$C_4H_4O_6$	0.19
20	2- Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	0.259
21	Isovanillic acid	$C_8H_8O_4$	2.153
22	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.867
23	Palmitoleic acid	$C_{16}H_{30}O_2$	0.606
24	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	4.1
25	Heptadecanoic acid	$C_{17}H_{34}O_2$	0.172
26	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	2.776
27	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	2.082
28	Octadecanoic acid	$C_{18}H_{36}O_2$	1.206
29	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	$C_{18}H_{26}O_3$	0.131

Table 1. Continued.

No.	Hydrocarbons	Formula	3.85%
30	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O$	0.821
	Aldehydes		14.516%
31	Furfural	$C_5H_4O_2$	1.805
32	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	0.804
33	Acetaldehyde semicarbazone	$C_3H_7N_3O$	0.235
34	5-Hydroxymethylfurfural	$C_6H_6O_3$	10.063
35	Vanillin	$C_8H_8O_3$	0.793
36	Benzaldehyde, 4-hydroxy-3,5-dimethoxy- (syringaldehyde)	$C_9H_{10}O_4$	0.807
	Ketones		11.353%
37	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	0.812
38	2-Propanone, 1-(acetyloxy)-	$C_5H_8O_3$	0.365
39	Butyrolactone	$C_4H_6O_2$	0.112
40	2(5H)-Furanone	$C_4H_4O_2$	0.227
41	2-Cyclopenten-1-one, 2-hydroxy-	$C_6H_8O_2$	0.843
42	6-Octen-2-one	$C_8H_{14}O$	0.261
43	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	$C_6H_8O_2$	0.491
44	2-Pentanone, 5-methoxy-	$C_6H_{12}O_2$	0.3
45	2(3H)-Benzofuranone, hexahydro-4,4 ,7a-trimethyl-	$C_{11}H_{18}O_2$	0.161
46	Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1H-inden-4-yl)-	$C_{13}H_{16}O$	0.154
47	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	$C_6H_8O_4$	2.289
48	Ethanone, 1-(2-methylphenyl)-	$C_9H_{10}O$	0.114
49	Damascenone	$C_{13}H_{18}O$	0.665
50	Ethanone, 1-[4-(methylthio)phenyl]	$C_9H_{10}OS$	1.177
51	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	0.168
52	Vanillyl methyl ketone	$C_{10}H_{12}O_3$	0.235
53	3,3-Dimethoxy-6,6-dimethyl-cyclohexa-1,4-diene	$C_{10}H_{16}O_2$	0.296
54	Megastigmatrienone	$C_{13}H_{18}O$	1.006
55	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	$C_{10}H_{12}O_4$	0.552

Table 1. Continued.

No.	Hydrocarbons	Formula	3.85%
56	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	0.396
57	Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, semicarbazone	$C_{11}H_{19}N_3O$	0.571
58	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.158
	Alcohols		5.439%
59	2-Furanmethanol	$C_5H_6O_2$	0.885
60	Creosol	$C_8H_{10}O_2$	0.205
61	Syringol	$C_8H_{10}O_3$	2.395
62	Ethanol, 2-(tetradecyloxy)-	$C_{16}H_{34}O_2$	0.501
63	1-Nitro-2-acetamido-1,2-dideoxy-d-glucitol	$C_8H_{16}N_2O_7$	1.092
64	Estra-1,3,5(10)-trien-17.beta.-ol	$C_{23}H_{26}O_2$	0.361
	Terpenes		2.522%
65	Bicyclo[2.2.1]heptane, 1,3,3-trimethyl-	$C_{10}H_{18}$	0.085
66	2,4,6-Octatrien-1-ol, 3,7-dimethyl -(E,E)-	$C_{10}H_{16}O$	0.17
67	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	$C_{15}H_{24}$	0.45
68	Phytol	$C_{20}H_{40}O$	0.66
69	6-O-Acetyl-1-[[4-bromophenyl]thio]-.beta.-d-glucoside S,S-dioxide	Diterpeneoid	0.56
70	Neophytadiene	$C_{20}H_{38}$	0.597
	Acetates		1.933%
71	Isopropyl acetate	$C_5H_{10}O_2$	0.728
72	Betaine	$C_5H_{11}NO_2$	0.649
73	*E*-8-Methyl-7-dodecen-1-ol acetate	$C_{15}H_{28}O_2$	0.309
74	*E*-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	0.144
75	1-Docosanol, acetate	$C_{24}H_{48}O_2$	0.103
	Methyl esters		3.713%
76	Acetic acid, hydroxy-, methyl ester	$C_3H_6O_3$	0.049
77	Pentanoic acid, 2-hydroxy-, methyl ester	$C_6H_{12}O_3$	0.286
78	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	1.298
79	3-Ethoxy-3-(methylamino)-2-propenoic acid methyl ester	$C_7H_{12}NO_3$	0.74
80	Benzoic acid, 2,5-dihydroxy-, methyl ester	$C_8H_8O_4$	0.104
81	Benzoic acid, 4-hydroxy-3-methoxy- , methyl ester	$C_9H_{10}O$	0.339
82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	0.587
83	9,12-Octadecadienoic acid (Z,Z)- ,methyl ester	$C_{19}H_{34}O$	0.15
84	*cis*-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O$	0.16
	Metal related compounds		1.229%
85	Hexane, 1-fluoro-	$C_6H_{13}F$	0.056
86	2-Isopropyl-5,6-dimethyl-1,3-oxathiane	$C_9H_{18}OS$	0.049
87	(4H)1,3,2-Dioxaborin, 4-ethenyl-4, 6-diethyl-5-(1-methylethyl)-	$C_{12}H_{21}BO_2$	0.514
88	1-Bromo-4-bromomethyldecane	$C_{11}H_{22}Br_2$	0.29
89	1-Bromo-11-iodoundecane	$C_{11}H_{22}BrI$	0.32
	Amides		1.169%
90	Propanamide, N-acetyl-	$C_5H_9NO_2$	0.162
91	Benzamide, 2-amino-N-(2-oxo-3-pipe ridyl)-	$C_{12}H_{15}N_3O_2$	0.322
92	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	0.685
	Sugars		1.953%
93	1,6,3,4-Dianhydro-2-O-acetyl-.beta .-d-altropyranose	$C_8H_{10}O_5$	0.029
94	*d*-Glycero-d-ido-heptose	$C_7H_{14}O_7$	0.532
95	*dl*-Threitol	$C_4H_{10}O_4$	0.15
96	Lactose	$C_{12}H_{22}O_{11}$	0.53
97	*d*-Mannitol, 1,4-anhydro-	$C_6H_{12}O_5$	0.308
98	.*beta*.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	$C_{12}H_{22}O$	0.404
	Other compounds		9.423%
99	1-Pyrrolidinecarbonitrile	$C_5H_8N_2$	0.126
100	Oxime-, methoxy-phenyl-	$C_{10}H_{13}NO_2$	0.079
101	Piperazine, 1-methyl-	$C_5H_{12}N_2$	0.497
102	N-[Dimethylaminomethyl]aziridine	$C_5H_{12}N_2$	0.202
103	Oxirane, (ethoxymethyl)-	$C_5H_{10}O_2$	0.329
104	*dl*-Alanyl-dl-asparagine	$C_7H_{13}N_3O_4$	0.378
105	1-Tetradecanamine	$C_{16}H_{35}NO$	0.157
106	Bicyclo[3,3,1]non-2-ene, 7-oxa-2,8 ,9-trimethyl-5-acetoxymethyl-	$C_{14}H_{22}O_3$	0.12
107	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	0.372
108	1H-1,5-Benzodiazepine, 2,3,4,5-tetrahydro-2,2,4-trimethyl-	$C_{12}H_{16}N_2$	0.411
109	(E)-2,6-Dimethoxy-4-(prop-1-en-1-y l)phenol	$C_{11}H_{14}O_3$	0.661
110	2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine	$C_{12}H_{14}N_4O_5$	0.721
111	2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine	$C_{10}H_{15}N_5O_5$	0.918
112	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	0.176
113	Phenol, 2-methoxy-	$C_8H_{10}O_4$	4.276
	Total		80.749%

several bioactive compounds.

Among several bioactive compounds belonging to several chemical groups, pentacosane (1.021%), *n*-hexadecanoic acid (4.1%), 5-hydroxymethylfurfural (10.063%), 4H-pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl- (2.289%), syringol (2.395%), phytol (0.66%), isopropyl acetate (0.728%), propanoic acid, 2-oxo-, methyl ester (1.298%), (4H)1,3,2-dioxaborin, 4-ethenyl-4, 6-diethyl-5-(1-methylethyl)- (0.514%), d-glycero-d-ido-heptose (0.532%) and 9-octadecenamide, (Z)- (0.685%) were identified as main hydrocarbon, acid, aldehyde, ketone, alcohol, terpene, acetate, methyl ester, metal related compound, sugar and amide, respectively.

The phenol compounds identified in the methanol extract of *S. bornmuelleri* could be classified as syringol-type including ethanone, 1-(2-methylphenyl)- (0.114%), ethanone, 1-[4-(methylthio)phenyl] (1.177%), and ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (0.552%), guaiacol-type including 2,4-di-tert-butylphenol (0.372%), and (E)-2,6-Dimethoxy-4-(prop-1-en-1-y l)phenol (0.661%), and benzenediol-type including Syringaldehyde (0.807%), 2(3H)-benzofuranone, hexahydro-4,4,7a-trimethyl- (0.161%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (0.168%), benzoic acid, 2,5-dihydroxy-, methyl ester (0.104%), benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (0.339%), benzamide, 2-amino-N-(2-oxo-3-pipe ridyl)- (0.332%), and 1H-1,5-benzodiazepine,2,3,4,5-

tetrahydro-2,2,4-trimethyl- (0.411%).

Figures 3-8 show the mass spectrograms of some of the main chemical bioactive compounds including estra-1,3,5(10)-trien-17β-ol, 9,12-octadecadienoic acid (Z,Z)-, phytol, syringaldehyde, 5-hydroxymethyl-furfural, β-D-glucopyranose, 4-O-β-D-galactopyranosyl, 2-butenoic acid, 2-methyl and 2(5H)-furanone, respectively.

Discussion

The GC-MS analysis of methanol extract of *S. bornmuelleri* demonstrated the presence of 113 bioactive phytochemical compounds (Table 1), which are isolated from leaf and stem. These compounds included twelve 12 (3.85%), 18 acids (23.649%), six aldehydes (14.516%), 22 ketones (11.353%), six alcohols (5.439%%), six

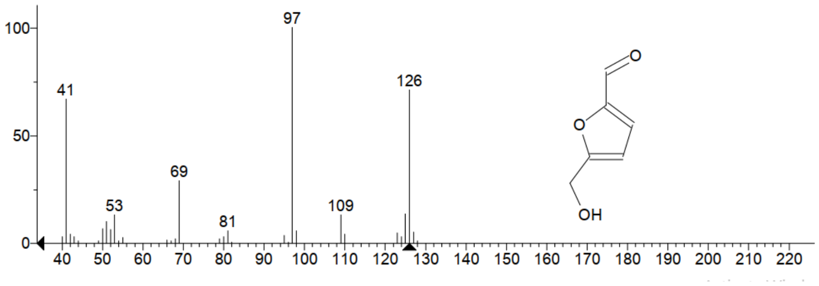

Figure 3. Mass spectrum of 5-hydroxymethylfurfurale (10.063%, RT 15.848).

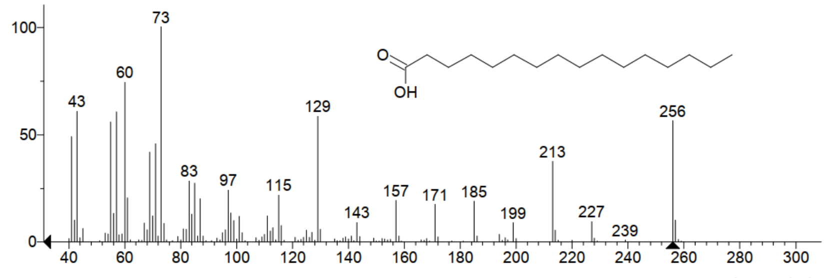

Figure 4. Mass spectrum of *n*-hexadecanoic acid (4.1%, RT 32).

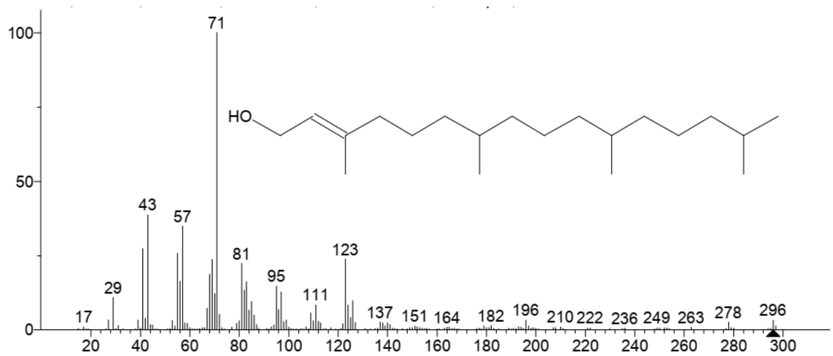

Figure 5. Mass spectrum of phytol (0.66%, RT 34.272).

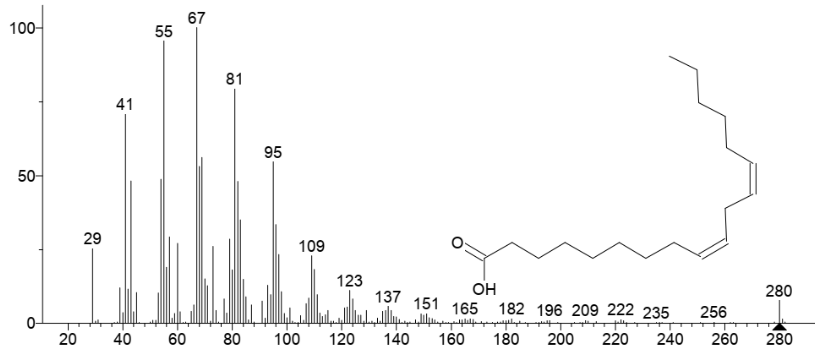

Figure 6. Mass spectrum of 9,12-octadecadienoic acid (Z,Z)- e (2.776%, RT 34.944).

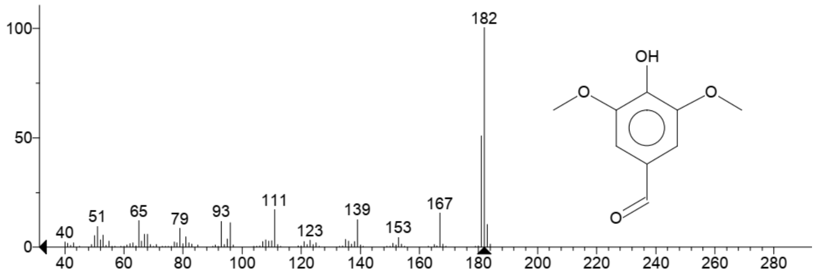

Figure 7. Mass spectrum of syringaldehyde (0.807%, RT 25.594).

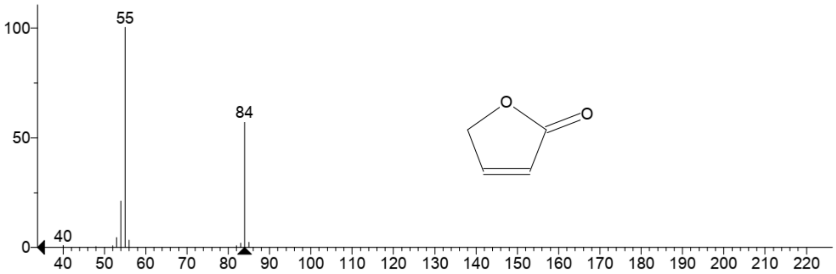

Figure 8. Mass spectrum of 2(5H)-furanone (0.227%, RT 6.631).

terpenes (2.522%), five acetates (1.933%), nine methyl esters (3.713%), five metal related compounds (1.229%), three amids (1.169%), six sugars (1.953%), and 15 other compounds (9.423%). The main compound of methanol *S. bornmuelleri* shoot extract was found to be 5-hydroxymethylfurfural. Hydroxymethylfurfural is an organic compound that exerts different biological effects after conversion to a non-excretable, genotoxic compound called 5-sulfoxymethylfurfural. The compound has several biological effects, such as antioxidative, anti-inflammatory, anti-allergic, anti-sickling, anti-hypoxic, and antihyperuricemic, on human health (24).

n-Hexadecanoic acid, as one of the most abundant acids detected in *S. bornmuelleri* shoots, provides anti-Inflammatory properties through inhibition of phospholipase A2 activity (25). The study of Aparna et al showed that essential oil from *Laminaria japonica*, which is rich in *n*-hexadecanoic acid, exerted potent antibacterial activity against the foodborne pathogens *Staphylococcus aureus* and *Bacillus cereus* (26). The presence of *n*-hexadecanoic acid was reported in the methanol extract and different parts of some plants such as *Rhanterium epapposum* (27), *Leucobryum javense* (28), *Jatropha curcas* L. (29) and *Albizia adianthifolia* (30).

Phytol is a valuable diterpene member of the long-chain unsaturated acyclic alcohols and exerts a wide range of biological effects such as cytotoxic, anxiolytic, apoptosis-inducing, metabolism-modulating, antioxidant, anti-inflammatory, antinociceptive, antimicrobial and immunomodulatory (31). Neophytadiene is a diterpene that is 3-methylidenehexadec-1-ene substituted at positions 7, 11 and 15 by a methyl group. It serves as an anti-inflammatory and an antimicrobial agent, and is a plant metabolite and an algal metabolite. It is also an alkene and a diterpene (32).

Isovanillic acid, a phenolic acid isolated from *Scrophularia frutescens* L. var. frutescens, has potential anti-inflammatory activity (33). 9,12-octadecadienoic acid (Z,Z)- is an octadecadienoic acid with two double bonds located at positions 9 and 12. Rossellia et al reported that (Z,Z)-9,12-octadecadienoic acid was the most abundant compound, in both the light petroleum and dichlomethane extracts of *Helleborus bocconei* Ten. subsp. *intermedius* (34). Given these findings, it seems that the better activity of the root extracts can be explained by their greater content of (Z,Z)-9,12-octadecadienoic acid (34). In the current study, (Z,Z)-9,12-octadecadienoic acid was found as one of the most abundant compounds (Table 1). Different studies have revealed its inhibitory action against some bacterial species (35-37).

Benzaldehyde, 4-hydroxy-3,5-dimethoxy-, namely syringaldehyde, belongs to the aromatic aldehyde family with various bioactive properties. It is a naturally occurring unique compound that produces promising antioxidant (38), antifungal/antimicrobial (39,40) and anti-oncogenic effects (41). Syringaldehyde is also used as a redox mediator of bacterial laccase and white radish peroxidase (42,43).

2(5H)-Furanone (2H-furan-5-on, γ-crotonolactone (Figure 1) is a naturally occurring organic compound belonging to a class of α,β-unsaturated lactones (44). Compounds containing 2(5H)-furanone skeleton in their structure are known to display a wide range of biological activities such as anticancer (45,46), antimicrobial (47,48), antiviral (49), antifungal (50), antioxidant (51) and anti-inflammatory (52).

Some compounds including phytol and neophytadiene diterpenoids with well-known bioactivities and also vanillin and damascenone were identified for the first time in methanol *S. bornmuelleri* shoot extract.

Conclusion

In total, 113 metabolites were identified in the methanolic extract of shoot of *S. bornmuelleri*, a native plant to Iran, using GC-MS analysis. The complex phytochemical profile of the extract revealed that it can be a valuable source of bioactive compounds such as acids, aldehydes, ketones, alcohols, hydrocarbons, methyl esters, sugars, acetates, terpenes, and amides, explaining the traditional usage of this species and its application in various herbal formulations. However, it is necessary to undertake *in vivo* studies to *further* confirm its bioactivity and toxicity profile.

Acknowledgments

The authors are grateful to the School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran for assisting us in conducting the study.

Authors' Contribution

Zahra Gharari and Hadiseh Shabani carried out the experiments. Khadijeh Bagheri and Ali Sharafi supervised the study. All authors contributed to the work and read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that there is no conflict of interests.

Ethical Approval

This article was approved by Zanjan University, Faculty of Agriculture, with the letter No. 72464 on April 17th, 2022.

References

1. Paton A. A global taxonomic investigation of *Scutellaria* (Labiatae). Kew Bull. 1990;45(3):399-450. doi: 10.2307/4110512.
2. Shen J, Li P, Liu S, Liu Q, Li Y, Sun Y, et al. Traditional uses, ten-years research progress on phytochemistry and pharmacology, and clinical studies of the genus *Scutellaria*. J Ethnopharmacol. 2021;265:113198. doi: 10.1016/j.jep.2020.113198.
3. Gharari Z, Bagheri K, Sharafi A. Fractional analysis of dichloromethane extract of *Scutellaria araxensis* Grossh root and shoot by HPLC-PDA-ESI-MSn. Nat Prod Res. 2021:1-5. doi: 10.1080/14786419.2021.1903003.
4. Gharari Z, Bagheri K, Derakhshani B, Sharafi A. HPLC-DAD-ESI/MSn analysis of phenolic components of *Scutellaria araxensis*, *S. bornmuelleri* and *S. orientalis*. Nat Prod Res. 2022;36(9):2440-5. doi: 10.1080/14786419.2020.1837810.
5. Gharari Z, Bagheri K, Danafar H, Sharafi A. Enhanced flavonoid production in hairy root cultures of *Scutellaria bornmuelleri* by elicitor induced over-expression of MYB7 and FNSΙΙ2 genes. Plant Physiol Biochem. 2020;148:35-44. doi: 10.1016/j.plaphy.2020.01.002.
6. Wang L, Chen W, Li M, Zhang F, Chen K, Chen W. A review of the ethnopharmacology, phytochemistry, pharmacology, and quality control of *Scutellaria barbata* D. Don. J Ethnopharmacol. 2020;254:112260. doi: 10.1016/j.jep.2019.112260.
7. Li-Weber M. New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents wogonin, baicalein and baicalin. Cancer Treat Rev. 2009;35(1):57-68. doi: 10.1016/j.ctrv.2008.09.005.
8. Yang B, Bai H, Sa Y, Zhu P, Liu P. Inhibiting EMT, stemness and cell cycle involved in baicalin-induced growth inhibition and apoptosis in colorectal cancer cells. J Cancer. 2020;11(8):2303-17. doi: 10.7150/jca.37242.
9. Singh S, Meena A, Luqman S. Baicalin mediated regulation of key signaling pathways in cancer. Pharmacol Res. 2021;164:105387. doi: 10.1016/j.phrs.2020.105387.
10. Lu HF, Hsueh SC, Ho YT, Kao MC, Yang JS, Chiu TH, et al. ROS mediates baicalin-induced apoptosis in human promyelocytic leukemia HL-60 cells through the expression of the Gadd153 and mitochondrial-dependent pathway. Anticancer Res. 2007;27(1A):117-25.
11. Shieh DE, Cheng HY, Yen MH, Chiang LC, Lin CC. Baicalin-induced apoptosis is mediated by Bcl-2-dependent, but not p53-dependent, pathway in human leukemia cell lines. Am J Chin Med. 2006;34(2):245-61. doi: 10.1142/s0192415x06003801.
12. Chao JI, Su WC, Liu HF. Baicalein induces cancer cell death and proliferation retardation by the inhibition of CDC2 kinase and survivin associated with opposite role of p38 mitogen-activated protein kinase and AKT. Mol Cancer Ther. 2007;6(11):3039-48. doi: 10.1158/1535-7163.mct-07-0281.
13. Gharari Z, Bagheri K, Khodaeiaminjan M, Sharafi A. Potential therapeutic effects and bioavailability of wogonin, the flavone of Baikal skullcap. J Nutr Med Diet Care. 2019;5(2):1-11. doi: 10.23937/2572-3278.1510039.
14. Chen XM, Bai Y, Zhong YJ, Xie XL, Long HW, Yang YY, et al. Wogonin has multiple anti-cancer effects by regulating c-Myc/SKP2/Fbw7α and HDAC1/HDAC2 pathways and inducing apoptosis in human lung adenocarcinoma cell line A549. PLoS One. 2013;8(11):e79201. doi: 10.1371/journal.pone.0079201.
15. Feng Q, Wang H, Pang J, Ji L, Han J, Wang Y, et al. Prevention of wogonin on colorectal cancer tumorigenesis by regulating p53 nuclear translocation. Front Pharmacol. 2018;9:1356. doi: 10.3389/fphar.2018.01356.
16. Wang W, Guo QL, You QD, Zhang K, Yang Y, Yu J, et al. The anticancer activities of wogonin in murine sarcoma S180 both in vitro and in vivo. Biol Pharm Bull. 2006;29(6):1132-7. doi: 10.1248/bpb.29.1132.
17. Chen J, Cao Y, Li Y, Tang L, Yu X, Xia J. Wogonoside exerts potential anti-tumor activity against bladder cancer in vivo and in vitro via regulation of GSK3β/ERK/AKT signaling pathway. Trop J Pharm Res. 2019;18(8):1609-14.
18. Wei C, Jing J, Zhang Y, Fang L. Wogonoside inhibits prostate cancer cell growth and metastasis via regulating Wnt/β-catenin pathway and epithelial-mesenchymal transition. Pharmacology. 2019;104(5-6):312-9. doi: 10.1159/000502400.
19. Ghahreman A, Attar F. Biodiversity of plant species in Iran: Central Herbarium of Tehran University. Faculty of Science. 1999:379 .
20. Zargari A. Medicinal Plants. Tehran University of Medical Sciences; 1997.
21. Gharari Z, Bagheri K, Danafar H, Sharafi A. Essential oil composition of two *Scutellaria* species from Iran. J Tradit Chin Med Sci. 2019;6(3):244-53. doi: 10.1016/j.jtcms.2019.07.003.
22. Gharari Z, Aghajanzadeh M, Sharafi A. *Scutellaria orientalis* subsp. Bornmuelleri: phytochemical composition and biological activities. Nat Prod Res. 2022;36(5):1385-90. doi: 10.1080/14786419.2021.1875463.
23. Gharari Z, Bagheri K, Danafar H, Sharafi A. Simultaneous determination of baicalein, chrysin and wogonin in four Iranian *Scutellaria* species by high performance liquid chromatography. J Appl Res Med Aromat Plants. 2020;16:100232. doi: 10.1016/j.jarmap.2019.100232.
24. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem Cent J. 2018;12(1):35. doi: 10.1186/s13065-018-0408-3.
25. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434-9. doi: 10.1111/j.1747-

0285.2012.01418.x.

26. Patra JK, Das G, Baek KH. Chemical composition and antioxidant and antibacterial activities of an essential oil extracted from an edible seaweed, *Laminaria japonica* L. Molecules. 2015;20(7):12093-113. doi: 10.3390/molecules200712093.
27. Azar AW, Rosleine D, Faizal A. Secondary metabolite profiles in the methanolic extract of *Leucobryum javense* isolated from tropical montane forest in West Java, Indonesia. AIP Conf Proc. 2019;2120(1):030027. doi: 10.1063/1.5115631.
28. Rajendrasozhan S, Moll HE, Snoussi M, Romeilah RM, Shalaby EA, Younes KM, et al. Phytochemical screening and antimicrobial activity of various extracts of aerial parts of *Rhanterium epapposum*. Processes. 2021;9(8):1351. doi: 10.3390/pr9081351.
29. Othman AR, Abdullah N, Ahmad S, Ismail IS, Zakaria MP. Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. BMC Complement Altern Med. 2015;15:11. doi: 10.1186/s12906-015-0528-4.
30. Abubakar MN, Majinda RRT. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines (Basel). 2016;3(1):3. doi: 10.3390/medicines3010003.
31. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, et al. Phytol: a review of biomedical activities. Food Chem Toxicol. 2018;121:82-94. doi: 10.1016/j.fct.2018.08.032.
32. Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. Evid Based Complement Alternat Med. 2017;2017:1517683. doi: 10.1155/2017/1517683.
33. Fernández MA, Sáenz MT, García MD. Anti-inflammatory activity in rats and mice of phenolic acids isolated from *Scrophularia frutescens*. J Pharm Pharmacol. 1998;50(10):1183-6. doi: 10.1111/j.2042-7158.1998.tb03332.x.
34. Rossellia S, Maggio A, Formisano C, Napolitano F, Senatore F, Spadaro V, et al. Chemical composition and antibacterial activity of extracts of *Helleborus bocconei* Ten. subsp. intermedius. Nat Prod Commun. 2007;2(6):675-9. doi: 10.1177/1934578x0700200611.
35. Dilika F, Bremner PD, Meyer JJ. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia. 2000;71(4):450-2. doi: 10.1016/s0367-326x(00)00150-7.
36. McGaw LJ, Jäger AK, van Staden J. Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia. 2002;73(5):431-3. doi: 10.1016/s0367-326x(02)00120-x.
37. Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH, Kim WG. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett. 2005;579(23):5157-62. doi: 10.1016/j.febslet.2005.08.028.
38. Bortolomeazzi R, Sebastianutto N, Toniolo R, Pizzariello A. Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential. Food Chem. 2007;100(4):1481-9. doi: 10.1016/j.foodchem.2005.11.039.
39. Kelly C, Jones O, Barnhart C, Lajoie C. Effect of furfural, vanillin and syringaldehyde on *Candida guilliermondii* growth and xylitol biosynthesis. In: Adney WS, McMillan JD, Mielenz J, Klasson KT, eds. Biotechnology for Fuels and Chemicals. Totowa, NJ: Humana Press; 2008. p. 615-26. doi: 10.1007/978-1-60327-526-2_57.
40. Fillat A, Gallardo O, Vidal T, Pastor FIJ, Díaz P, Roncero MB. Enzymatic grafting of natural phenols to flax fibres: development of antimicrobial properties. Carbohydr Polym. 2012;87(1):146-52. doi: 10.1016/j.carbpol.2011.07.030.
41. González-Sarrías A, Li L, Seeram NP. Anticancer effects of maple syrup phenolics and extracts on proliferation, apoptosis, and cell cycle arrest of human colon cells. J Funct Foods. 2012;4(1):185-96. doi: 10.1016/j.jff.2011.10.004.
42. Satar R, Husain Q. Applications of Celite-adsorbed white radish (*Raphanus sativus*) peroxidase in batch process and continuous reactor for the degradation of reactive dyes. Biochem Eng J. 2009;46(2):96-104. doi: 10.1016/j.bej.2009.04.012.
43. Singh G, Capalash N, Goel R, Sharma P. A pH-stable laccase from alkali-tolerant γ-proteobacterium JB: purification, characterization and indigo carmine degradation. Enzyme Microb Technol. 2007;41(6-7):794-9. doi: 10.1016/j.enzmictec.2007.07.001.
44. Lattmann E, Dunn S, Niamsanit S, Sattayasai N. Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5H)-furanones. Bioorg Med Chem Lett. 2005;15(4):919-21. doi: 10.1016/j.bmcl.2004.12.051.
45. Byczek-Wyrostek A, Kitel R, Rumak K, Skonieczna M, Kasprzycka A, Walczak K. Simple 2(5H)-furanone derivatives with selective cytotoxicity towards non-small cell lung cancer cell line A549 - synthesis, structure-activity relationship and biological evaluation. Eur J Med Chem. 2018;150:687-97. doi: 10.1016/j.ejmech.2018.03.021.
46. Wu YC, Luo SH, Mei WJ, Cao L, Wu HQ, Wang ZY. Synthesis and biological evaluation of 4-biphenylamino-5-halo-2(5H)-furanones as potential anticancer agents. Eur J Med Chem. 2017;139:84-94. doi: 10.1016/j.ejmech.2017.08.005.
47. Steenackers HP, Levin J, Janssens JC, De Weerdt A, Balzarini J, Vanderleyden J, et al. Structure-activity relationship of brominated 3-alkyl-5-methylene-2(5H)-furanones and alkylmaleic anhydrides as inhibitors of Salmonella biofilm formation and quorum sensing regulated bioluminescence in Vibrio harveyi. Bioorg Med Chem. 2010;18(14):5224-33. doi: 10.1016/j.bmc.2010.05.055.
48. Gondela E, Walczak KZ. Synthesis and preliminary bioactivity assays of 3,4-dichloro-5-(omega-hydroxyalkylamino)-2(5H)-furanones. Eur J Med Chem. 2010;45(9):3993-7. doi: 10.1016/j.ejmech.2010.05.055.
49. Hashem AI, Youssef AS, Kandeel KA, Abou-Elmagd WS. Conversion of some 2(3H)-furanones bearing a pyrazolyl group into other heterocyclic systems with a study of their antiviral activity. Eur J Med Chem. 2007;42(7):934-9. doi: 10.1016/j.ejmech.2006.12.032.
50. Zapf S, Anke T, Sterner O. Incrustoporin, a new antibiotic from *Incrustoporia carneola* (Bres.) Ryv. (Basidiomycetes). Acta Chem Scand (Cph). 1995;49(3):233-4. doi: 10.3891/acta.chem.scand.49-0233.
51. Uddin MJ, Crews BC, Ghebreselasie K, Tantawy MN, Marnett LJ. [I]-Celecoxib analogues as SPECT tracers of cyclooxygenase-2 in inflammation. ACS Med Chem Lett. 2011;2(2):160-4. doi: 10.1021/ml100232q.
52. Weber V, Coudert P, Rubat C, Duroux E, Vallée-Goyet D, Gardette D, et al. Novel 4,5-diaryl-3-hydroxy-2(5H)-furanones as anti-oxidants and anti-inflammatory agents. Bioorg Med Chem. 2002;10(6):1647-58. doi: 10.1016/s0968-0896(02)00053-6.

Cite this article as: Gharari Z, Shabani H, Bagheri K, Sharafi A. Phytochemical composition profile of methanol *Scutellaria bornmuelleri* extract using GC-MS analysis. Future Nat Prod. 2022;8(1):7–14. doi: 10.34172/fnp.2022.03.