Trop J Nat Prod Res, July 2022; 6(7):1113-1118 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Total Polyphenolic Content and Antioxidant Activity of Different Extracts from *Sideritis scardica*

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ARTICLE INFO

Article history:
Received 27 May 2021
Revised 30 June 2022
Accepted 26 July 2022
Published online 03 August 2022

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ABSTRACT

Sideritis scardica is an endemic plant in Bulgaria traditionally used for its therapeutic properties. It has a wide composition of biologically active substances such as polyphenolic compounds, flavonoids, and organic acids. Its antioxidant activity has been well documented in recent decades and is considered a possible principle in the treatment and prevention of inflammatory and neurodegenerative diseases. The influence of the solvent and extraction approach on the yield, total polyphenolic, and flavonoid content, and antioxidant activity in *Sideritis scardica* extracts were evaluated. Water, acetone, and water-ethanol mixtures (20%, 50%, and 70% ethanol) were used as solvents to extract bioactive compounds from the plant material. The applied extraction procedures included infusion, decoction, maceration, as well as green extraction techniques such as ultrasonic and microwave irradiation. The highest values for total polyphenols and total flavonoids were demonstrated in 70% ethanol extracts, followed by other water-ethanol mixtures and water decoctions. The microwave and ultrasonic irradiations reduced the extraction time, as the value of phenolic content in these extracts was the highest. The results showed that the highest extraction yields are obtained with 50% and 70% ethanol maceration, followed by aqueous infusion. The highest antioxidant potential was found in extractions with water and 70% ethanol solution, where the amount of polyphenols was also sufficient. A positive correlation between polyphenol content and antioxidant activity was observed. Therefore, extracts of *Sideritis scardica* are a rich source of polyphenols with pronounced antioxidant properties.

Keywords: *Sideritis scardica*, Lamiaceae, Antioxidant activity, Polyphenolic content.

Introduction

Mentioned for the first time in the Materia medica by Pedanios Dioscorides, *Sideritis scardica* has been well known for its prolific anti-inflammatory properties for centuries. The name derives from the Greek word "sideros", meaning iron. Ancient soldiers used the plant to help the healing process of cut wounds caused by iron weapons. The European Medical Agency Committee on Herbal Medicinal Products points to its traditional use for the relief of cough, associated with cold and gastrointestinal disorders.[1] Other described biological activities of the herb include anti-glioma, cytotoxic, antimicrobial, and antirheumatic.[2-5] Belonging to the Lamiaceae Family, *Sideritis scardica* has become more popular outside the Balkan Peninsula in the last decades. Different studies have confirmed its positive effect on cognitive and neurodegenerative diseases.[6-8] The phytochemical profile of the plant reveals many polyphenolic compounds – phenylethanoid glycosides, acetylated and non-acetylated flavonoids, and several organic phenolic acids.[2,3,9,10]
With this composition, preparations from *Sideritis scardica* and other herbs belonging to the same genus in the Mediterranean display prominent antioxidant activity. Oxidative stress has long been linked with neurodegenerative processes, whether as a cause or part of a pathological cascade induced by other factors.

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Citation: Yanchev N, Petkova N, Ivanov I, Delev D. Total Polyphenolic Content and Antioxidant Activity of Different Extracts from *Sideritis scardica*. Trop J Nat Prod Res. 2022; 6(7):1113-1118. doi.org/10.26538/tjnpr/v6i7.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The use of antioxidants and polyphenol-rich phyto preparations has emerged as a promising approach to combat and prevent neuronal cell dysfunction and cell death – a central paradigm in the pathology of globally relevant diseases like Alzheimer's disease and Parkinson's disease.[11,12]
Utilizing the diverse potential of polyphenol-rich *Sideritis scardica* extracts and expanding it towards possible new indications goes along with the summarization of the main properties of the preparations, acquired via different methods of extracting the biologically active compounds from the plant matrix. The present work aims to create a wider systematization of the antioxidant activity and polyphenolic content of preparations obtained by a plethora of technological approaches, including classic aqueous and alcoholic infusions, decoctions, and macerations, as well as modern "green" methods like microwave-assisted and ultrasound-assisted extraction.

Material and Methods

Plant material
Dry *Sideritis scardica* Griseb. was purchased from the producer NV Health Ltd., Bulgaria, harvested in August 2019, voucher specimen number SOM 1308 was obtained from the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences. The air-dried plant material was finely ground in a laboratory mill Munro SM-450 (Munro, United Kingdom). The ground sample was stored in a tightly closed glass vessel before being used.

Chemicals and reagents
Folin-Ciocalteu reagent, $Al(NO_3)_3$, Trolox (6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine), gallic acid, quercetin, ethanol, methanol, and acetone were purchased from

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Sigma-Aldrich (Steinheim, Germany). All other reagents were of analytical grade.

Determination of moisture content
The moisture content was determined by a moisture analyzer KERN DAB 100-3 (Kern, Germany).

Preparation of extracts
Infusion
The infusions were prepared as previously described by Irakli *et al.* [13] as 1 g of dry plant material was poured into 100 ml of boiling water (85-90°C) and after 10 minutes it was filtered. The final volume was reported. The extract was used for further studies.

Decoction
Dry *Sideritis scardica* (1 g) was poured into 100 ml of boiling water (85-90ºC) and then heated on a magnetic stirrer for 30 minutes at 95-98ºC. Then it was filtered. The volume was taken into account and further analyses were performed.

Maceration
Dried and finely ground *Sideritis scardica* (5 g) was poured into 200 ml of solvent (distilled water, acetone, 20, 50, and 70% ethanol, respectively). The samples were allowed to stand for 24 hours. Then the extracts were filtered through a paper filter, the volume was checked and samples were used for further analyses.

Ultrasonic extraction
The ultrasonic extraction was performed in two different power ultrasonic baths: 1: SIEL UST 5.7-150 bath (Gabrovo, Bulgaria) with an ultrasonic frequency of 36 kHz, power 240 W, and bath 2: VWR (Malaysia) with frequency 45 kHz, power 30W. The extraction of *Sideritis scardica* was performed in three centrifuge tubes with a screw and a capacity of 50 ml, in which 0.5 g of sample was pre-weighed. To each sample were added 10 ml of solvent - 20, 50, and 70% ethanol, acetone, and water. Ultrasonic extraction was performed at 50°C for 20 minutes. Each of the extracts obtained was filtered through a paper filter with a pore diameter of 0.45 µm. The extraction process was repeated three times. The final volume was checked and extracts were used for analyses.

Microwave extraction
The microwave extraction was performed in a CROWN microwave oven with a power of 700 W and frequency of 2450 MHz for 4 min at an average power of 541 W. The extraction process was again performed in three centrifuge tubes with the same solvents as described in the above-mentioned procedure. After the extraction, the samples extracted with the appropriate solvent were removed and filtered through a paper filter with a pore diameter of 0.45 µm. The extraction process was performed in duplicate. After completion of the extraction with the appropriate solvent carried out under microwave or ultrasonic conditions, the total extract obtained for each solvent was reported.
The yield of the *Sideritis scardica* extracts was determined gravimetrically after the solvent was evaporated. The results were expressed in % and g/g dry extracts.

Determination of total phenols
The amount of total phenols in the obtained extracts was determined by the Folin – Ciocalteu method.[14] To *Sideritis scardica* extracts (0.2 ml) was added 1 ml of Folin-Ciocalteu reagent (diluted 5 times) and 0.8 ml of 7.5% Na_2CO_3. After 20 minutes at room temperature, the absorbance was measured at 765 nm against a blank.[15] The results are presented as milligram equivalents of gallic acid per gram (mg GAE / g dry plant material and mg GAE/ g extract).

Determination of total flavonoids
The content of total flavonoids in the *Sideritis scardica* extracts was also determined using Al $(NO_3)_3$.[16] The results are presented as milligram equivalents of mg quercetin (mg QE)/g dry plant material and for g extract.

Determination of antioxidant activity
DPPH method
Sideritis scardica extracts (0.15 ml) was added to 2.85 ml of freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) (0.1 mM in methanol). The reaction mixture was incubated in the dark for 15 minutes at 37°C. The reduction in absorbance was read spectrophotometrically at 517 nm.[17] Percentage inhibition was calculated (I%), using the following equation: % inhibition= A0 - A1/A0 × 100, where A0 was the absorbance of the control and A1 was the absorbance of the sample. The results were substituted in the equation of the linear regression, mMTE/ml=102.06.I%+0.7954, and antioxidant activity was presented as mM TE per g of dry plant material[14] and dry extract (mM TE/g).

ABTS method
The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical was prepared by mixing equimolar amounts of it (7 mM in H_2O) and potassium persulfate (2.45 mM in water). After 16 hours in the dark, 2 ml ABTS radical was dissolved in methanol (1:30 v/v). *Sideritis scardica* extracts (0.15 ml) were added to 2.85 ml of freshly prepared ABTS radical solution. After 15 min at 37ºC, the adsorption reduction was recorded at 734 nm.[15] Inhibition % (I%) was calculated as mentioned above and substituted using the following equation: antioxidant activity, mM/ml=148.24I%-0.2277. The results were presented as mM Trolox equivalents per g dry plant material[15] and dry extract (mM TE/g).

FRAP method
The reagent contains 0.3 M acetate buffer with pH 3.6; 20 mM $FeCl_3 \times 6H_2O$ and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in a ratio of 10:1:1 v/v/v. The *Sideritis scardica* extracts (0.1 ml) were added to 3 mL FRAP reagent. After 5 min the absorption was measured at a wavelength of 593 nm.[18] The results of antioxidant activity were calculated using an equation for antioxidant activity and were expressed as mM TE per g dry plant material and dry extracts (mM TE/g). [15]

Statistical analysis
Statistical analysis was performed using MS Excel 2010. The data were presented as mean values ± standard deviation (SD) from three replications. Statistical analysis was done using ANOVA, with Tukey's range statistically significant at $p < 0.05$.

Results and Discussion

Extraction
Moisture content of the plant material was evaluated as 9.70±0.07%.
The percentage yields of all *Sideritis scardica* extracts obtained after five different extraction methods were compared, as illustrated in Table 1. Among the distinct extraction methods, the highest yield was found for maceration with 50 and 70% ethanol – 16%. Water infusion and microwave-assisted water extraction delivered a yield of 15%. Extraction with 70% ethanol, obtained after ultrasonic and microwave irradiation also showed yields in the range of 14-15%. The lowest extraction yield was detected when acetone was used as a solvent. Therefore, heating for 4 min using microwaves increased the yield and it is the best approach to reduce the time for extraction in comparison to maceration. The obtained yield for *Sideritis scardica* extracts using water and water-ethanol mixtures was higher in comparison with the results reported by Alipieva *et al*.[19] Furthermore, the yields (11-15%), obtained using ultrasound irradiation were comparable to those stated by Alipieva *et al*.[19] The sonication process shortened the extraction time and increased the yield.
The total polyphenols in different solvents obtained after various extraction procedures varied from 4.0 to 32.2 mg GAE/g dry plant material (Figure 1). The total flavonoid content in *Sideritis scardica* aerial parts was in the range of 1.33 to 5.14 mg QE/g dry plant material. In general, the lowest values for total phenols and flavonoids were found in acetone extracts. The highest values of total polyphenols were detected in the water infusion extraction and ethanol-water mixtures, obtained after microwave and ultrasonic

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

irradiation. Some authors explained that the solubility of polyphenolic content increased with infusion temperature, because these treatments accelerate mass transfer, as an infusion for 10 min was the most effective[12]. In the present case, cavitation, resulting from the ultrasonic waves, seems to improve the solubility and increased the values of phenolic content in water-ethanol mixtures (Figure 1). The frequency of 45 kHz had a better impact on the solubility of polyphenols in water-ethanol mixtures. Moreover, microwave treatment also improves extraction yields and led to higher values of polyphenols in water and water-ethanol mixtures from 22 to 27 mg GAE/g dry plant material.

Table 1: Yield, expressed as % per gram plant material

Extraction method	Yield,%
Infusion water	15.41±0.46^{a}
Decoction water	12.28±0.56^{c}
US 35 kHz water	11.72±0.40^{c}
US 35 kHz acetone	3.30±0.16^{f}
US 35 kHz 20 % EtOH	9.46±0.56^{d}
US 35 kHz 50 % EtOH	9.30±0.35^{d}
US 35 kHz 70 % EtOH	12.69±0.51^{c}
US 45 kHz water	8.73±0.06^{d}
US 45 kHz acetone	2.00±0.06^{g}
US 45 kHz 20 % EtOH	6.80±0.46^{e}
US 45 kHz 50 % EtOH	8.71±0.36^{d}
US 45 kHz 70 % EtOH	14.82±0.50^{b}
MW water	15.31±0.35^{a,b}
MW acetone	1.05±0.06^{g}
MW 20 % EtOH	15.19±0.16^{b}
MW 50 % EtOH	11.15±0.21^{c}
MW 70 % EtOH	14.92±0.32^{b}
Maceration water	7.45±0.12^{d}
Maceration acetone	1.36±0.23^{g}
Maceration 20 % EtOH	11.40±0.32^{c}
Maceration 50 % EtOH	16.20±0.20^{a}
Maceration 70 % EtOH	16.20±0.45^{a}

Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

The obtained result for water infusion was 32.2 mg GAE/g dry plant material, which was in accordance with the reported by Irakli *et al.*[12] values of total phenolic content from the aerial parts of *S. scardica* – 29.20 GAE/g dry plant material and similar to those reported by Alipieva *et al.*[19] for methanol extracts from *S. scardica* acquired via ultrasonic irradiation.

According to Tsibranka *et al.*[10] the use of mixed ethanol-water solvents using 24 h extraction led to the maximum extraction values of total phenols and flavonoids. In the present study, the highest values for total polyphenols were obtained using water infusion, followed by 70% ethanol ultrasonic extraction at 45 kHz (Figure 1). A tendency opposite to the observation of Tsibranka *et al.*[9] for maceration extracts was recorded - the increase of ethanol as a solvent led to a rise in the values of total phenols.

The results of antioxidant activity of *Sideritis scardica* extracts, mM Trolox equivalents per g dry plant material were summarized in Table 2. The highest antioxidant activities were found in the microwave-assisted water extraction, as 70% ethanol green method extractions also delivered high values. Additionally, total phenolic content, total flavonoids, and antioxidant activity were recalculated and presented for g extract (Table 3). There are many reports, describing the antioxidant properties of other extracts from this herb via DPPH and FRAP methods.[19-23] The current study demonstrated the detailed evaluation of the antioxidant potential of *Sideritis scardica* extracts gained after different extraction approaches. Green methods such as microwave and ultrasonic irradiation led to extracts with high antioxidant potential evaluated by radical scavenging activities (DPPH and ABTS) and electron transfer (FRAP method). In general FRAP method demonstrated higher results than other applied methods.

The yields obtained after the evaporation of extracts were listed in Table 3. The highest yields were found in water-ethanol mixtures, obtained after maceration, microwave-, and ultrasonic extractions. The lowest yield was measured after acetone extraction – 0.02-0.08 g/g dry material. The yield of 70 % ethanol extract (0.09 g/g) coincided with data reported by Tsibranka *et al*.[9] for pure ethanol extract. The highest values of total phenolic content in extracts were found in 70 % EtOH extract after 24 h maceration - 99.29 mg GAE/g extract and water infusion extract - 92.1 mg/g extract. Total flavonoid content varied between 0.44 to 41 mg QE/g extract, as the highest values were found in 70 % EtOH extract after maceration. According to Akbaba[24] the results for classical extraction and microwave-assisted extraction methods were found to be 51 and 55 mg/g for TPC, 16.3 and 22 mg/g for TFC, 93 and 103 mg/g for FRAP. The current results for antioxidant activity, quantified via the FRAP method, were higher, especially for microwave-assisted extracts obtained with 70 % ethanol - 1089.0 mM TE/g extract.

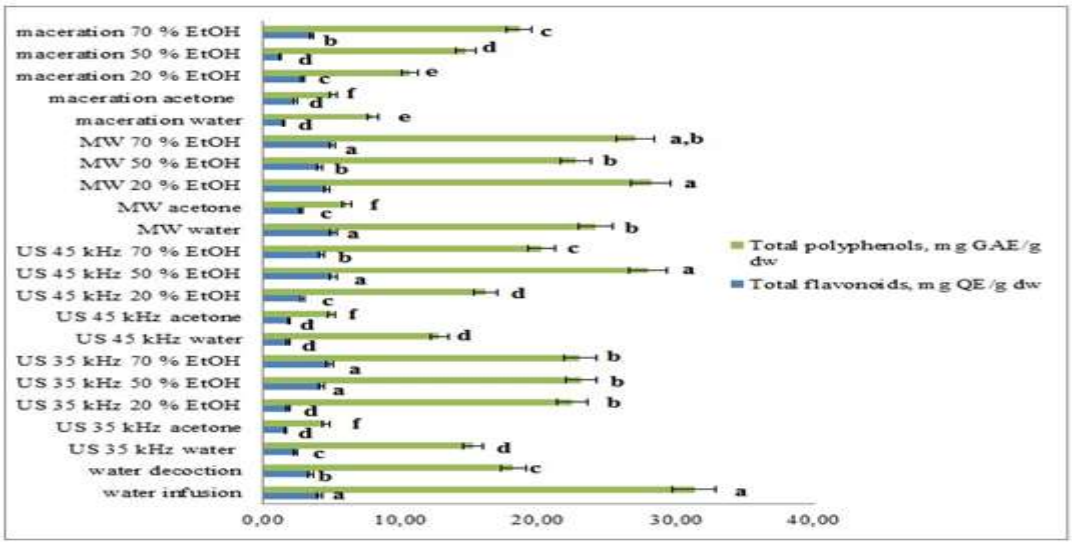

Figure 1: Total polyphenols and total flavonoids in different extracts of *Sideritis scardica*, expressed for g dry plant material
Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The correlation (r^2) between the total antioxidant activity evaluated by DPPH, ABTS, and FRAP methods and total phenolic and total flavonoid contents in *Sideritis scardica* extracts is shown in Table 4. The results showed positive linear correlations between total antioxidant activities evaluated by ABTS and total phenolic contents (coefficient of correlation r = 0.92)., Moreover, a high correlation was observed between total phenols and the antioxidant method based on electron transfer - FRAP. Total flavonoids are weakly correlated with the radical capture method DPPH. Therefore, the radical scavenging activity determined by DPPH and ABTS methods was most directly influenced by the amount of total phenols in *Sideritis scardica* extracts. The correlations between total antioxidant activities (ABTS and FRAP) and total flavonoid content were also documented at a coefficient of correlation of 0.78 and 0.74, respectively. According to the presented data, phenolic and flavonoid content in these extracts demonstrated radical scavenging and metal chelating properties.

Table 2: Antioxidant activity of *Sideritis scardica* extracts, mM Trolox equivalents per g dry plant material

Extraction method	DPPH mM TE/ g dry plant material	ABTS mM TE/ g dry plant material	FRAP mM TE/ g dry plant material
Infusion water	111.1 ± 5.9^{b}	135.7 ± 4.4^{a}	229.5 ± 2.1^{a}
Decoction water	123.3 ± 20.6^{b}	129.4 ± 6.1^{a}	168.5 ± 6.9^{b}
US 35 kHz water	34.4 ± 28.7^{d}	94.0 ± 0.7^{c}	144.4 ± 5.3^{c}
US 35 kHz acetone	10.6 ± 1.6^{g}	56.8 ± 7.9^{d}	96.6 ± 2.2^{d}
US 35 kHz 20 % EtOH	92.6 ± 0.6^{c}	116.0 ± 1.0 ^{a,b}	132.3 ± 3.9^{c}
US 35 kHz 50 % EtOH	68.6 ± 0.3^{d}	119.4 ± 0.8 ^{a,b}	170.5 ± 2.0^{b}
US 35 kHz 70 % EtOH	109.4 ± 25.2^{b}	117.5 ± 2.6 ^{a,b}	154.8 ± 1.2^{c}
US 45 kHz water	22.7 ± 3.1^{f}	85.3 ± 1.9^{c}	119.3 ± 2.3^{d}
US 45 kHz acetone	17.6 ± 1.8^{f}	57.4 ± 3.7^{d}	79.5 ± 3.3^{f}
US 45 kHz 20 % EtOH	20.0 ± 0.3^{f}	99.5 ± 2.4^{c}	123.7 ± 1.2^{d}
US 45 kHz 50 % EtOH	54.1 ± 0.1^{d}	117.1 ± 6.4^{a,b}	172.5 ± 5.7^{b}
US 45 kHz 70 % EtOH	36.5 ± 3.1^{e}	110.5 ± 0.8^{b}	185.7 ± 32.4^{b}
MW water	149.3 ± 6.9^{a}	134.8 ± 2.2^{a}	169.7 ± 2.1^{b}
MW acetone	13.3 ± 6.2^{f}	86.3 ± 6.6^{c}	153.8 ± 11.3^{b}
MW 20 % EtOH	90.5 ± 17.6^{b}	147.2 ± 1.3^{a}	183.9 ± 2.9^{b}
MW 50 % EtOH	59.1 ± 0.3^{d}	104.1 ± 1.5^{c}	144.0 ± 0.2^{c}
MW 70 % EtOH	75.4 ± 1.7^{d}	126.5 ± 2.6 ^{a,b}	159.1 ± 0.3^{b}
Maceration water	9.0 ± 2.3^{g}	49.7 ± 1.8^{f}	60.8 ± 2.0^{g}
Maceration acetone	18.7 ± 5.4^{f}	44.9 ± 4.5^{f}	61.0 ± 1.3^{g}
Maceration 20 % EtOH	12.5 ± 0.2^{f}	59.6 ± 0.2^{f}	74.8 ± 0.9^{f}
Maceration 50 % EtOH	39.6 ± 0.3^{e}	74.2 ± 0.4^{d}	93.8 ± 0.3^{e}
Maceration 70 % EtOH	53.2 ± 3.6^{d}	90.0 ± 6.3^{c}	110.6 ± 0.9^{d}

Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Table 3: Phenolic content and antioxidant activity in *Sideritis scardica* extracts per g extract

Extraction method	Yield, g/g	TPC, mg GAE/g extract	TF mg QE/g extract	DPPH mM TE/g extract	ABTS mM TE/g extract	FRAP mM TE/g extract
Infusion Water	0.18 ± 0.01^{e}	92.1 ± 0.1^{b}	25.2 ± 0.5^{b}	643.5 ± 33.0^{a}	740.2 ± 24.6^{b}	1284.9 ± 5.9^{a,b}
Decoction Water	0.27 ± 0.01^{e}	66.6 ± 0.4^{c}	14.3 ± 0.5^{c}	205.7 ± 15.0^{d}	375.6 ± 22.3^{c}	490.5 ± 1.1^{d}
US 35 kHz water	0.28 ± 0.02^{e}	26.2 ± 0.1^{e}	9.6 ± 1.5^{d}	123.4 ± 2.1^{e}	339.0 ± 2.4^{c}	530.4 ± 9.6^{c,d}
US 35 kHz acetone	0.24 ± 0.02^{e}	9.6 ± 0.4^{f}	7.9 ± 0.8^{d}	45.2 ± 1.3^{g}	213.4 ± 32.9^{d}	405.5 ± 4.5^{d}
US 35 kHz 20 % EtOH	0.32 ± 0.02^{e}	32.4 ± 0.4^{d}	6.6 ± 0.6^{e}	350.0 ± 29.9^{c}	370.0 ± 3.1^{c}	454.1 ± 49,0^{d}
US 35 kHz 50 % EtOH	0.28 ± 0.01^{e}	37.3 ± 0.5^{d}	16.7 ± 0.2^{c}	321.4 ± 10.4^{c}	421.7 ± 2.8^{c}	607.7 ± 3.5^{c}
US 35 kHz 70 % EtOH	0.47 ± 0.05^{d}	24.5 ± 0.4^{e}	11.4 ± 0.3^{d}	259.5 ± 37.9^{d}	246.3 ± 5.5^{d}	330.3 ± 1.3^{e}
US 45 kHz water	0.29 ± 0.04^{e}	20.9 ± 0.8^{e}	7.2 ± 0.3^{e}	78.7 ± 1.1^{f}	286.7 ± 9.6^{d}	407.3 ± 4.0^{d}
US 45 kHz acetone	0.08 ± 0.02^{f}	3.3 ± 0.4^{g}	27.1 ± 0.5^{a,b}	221.1 ± 1.2^{d}	57.2 ± 1.6^{f}	101.4 ± 2.4^{f}
US 45 kHz 20 % EtOH	0.28 ± 0.01^{e}	26.5 ± 0.4^{e}	11.5 ± 0.6^{d}	80.1 ± 11.2^{f}	349.1 ± 13.5^{c}	444.2 ± 2.2^{d}
US 45 kHz 50 % EtOH	0.29 ± 0.02^{e}	49.8 ± 0.8^{d}	19.8 ± 0.3^{b}	206.8 ± 24.3^{d}	416.2 ± 8.4^{c}	611.5 ± 10.0^{c}
US 45 kHz 70 % EtOH	0.44 ± 0.01^{ns}	24.6 ± 0.4^{e}	10.6 ± 0.6^{d}	92.3 ± 13.9^{e}	239.4 ± 14.4^{d}	393.8 ± 36.6^{d}

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MW water	0.46 ± 0.01^{ns}	26.1 ± 0.2^{e}	12.2 ± 0.3^{d}	348.7 ± 37.9^{c}	287.2 ± 4.7^{d}	364.1 ± 2.3^{d}
MW acetone	0.02 ± 0.01^{g}	1.9 ± 0.4^{g}	17.2 ± 0.3c	536.9 ± 13.0^{b}	83.8 ± 6.2^{e}	64.8 ± 8.4^{g}
MW 20 % EtOH	0.46 ± 0.02^{ns}	28.4 ± 0.4^{e}	11.2 ± 0.3^{d}	199.2 ± 3.6^{d}	317.8 ± 2.8^{c}	397.3 ± 3.2^{d}
MW 50 % EtOH	0.41 ± 0.01^{ns}	29.5 ± 0.5^{e}	$11.0 \pm 0.5^{ns,d}$	147.0 ± 5.6^{e}	254.5 ± 3.6^{d}	348.7 ± 0.3^{e}
MW 70 % EtOH	0.60 ± 0.02^{c}	20.6 ± 0.4^{e}	9.4 ± 0.5^{d}	132.7 ± 9.1^{e}	208.9 ± 4.4^{d}	266.4 ± 0.2^{f}
Maceration water	0.06 ± 0.01^{f}	65.1 ± 0.4^{c}	$30.3 \pm 0.5^{a,b}$	157.5 ± 3.9^{e}	902.0 ± 31.6^{a}	1089.0 ± 17^{b}
Maceration acetone	0.01 ± 0.01^{g}	26.8 ± 0.4^{e}	3.6 ± 0.4^{f}	63.5 ± 3.0^{f}	149.1 ± 0.5^{e}	$72.4 \pm .74.0^{g}$
Maceration 20 % EtOH	1.06 ± 0.04^{b}	46.4 ± 0.4^{d}	3.1 ± 0.5^{f}	11.5 ± 0.3^{g}	56.2 ± 0.2^{f}	71.0 ± 0.4^{g}
Maceration 50 % EtOH	2.98 ± 0.05^{a}	22.6 ± 0.6^{e}	0.5 ± 0.1^{g}	13.4 ± 0.1^{g}	25.0 ± 0.1^{g}	31.5 ± 0.1^{g}
Maceration 70 % EtOH	0.09 ± 0.01^{f}	99.3 ± 0.4^{a}	45.9 ± 0.5^{a}	651.0 ± 42.5^{a}	998.5 ± 73.4^{a}	1286.2 ± 0.6^{a}

Notes: Values are mean ± standard deviation of three replications. Different letters within each column show significant differences according to Tukey's test at $p < 0.05$

Table 4: Correlation coefficient (r) between total phenolic content, total flavonoids, and antioxidant activities

	DPPH	ABTS	FRAP	Total flavonoids
Total phenols	0.7769	0.9212	0.7957	0.7857
Total flavonoids	0.6594	0.7867	0.7482	-

Conclusion

The study contributes to the better understanding and utilization of the bioactive compounds in *Sideritis scardica* and successfully reveals how alteration of the extraction method impacts the features of the preparation. Polar solvents like water and water-ethanol mixtures deliver the highest yield, antioxidant activity, and polyphenolic content. Green methods such as sonication and microwave irradiation provide a promising approach towards the acceleration of the extraction process. *Sideritis scardica* emerges as a rich polyphenols herb with pronounced antioxidant properties and could be suggested for indications in the adjuvant therapy of neurodegenerative and other oxidatve stress-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The study was financially supported by the Doctoral and postdoctoral project program of the Medical University of Plovdiv – DPDP-14/2020.

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