ORIGINAL ARTICLE

A C G publications *J. Chem. Metrol*. 17:2 (2023) 199-214

journal of chemical metrology

Quantification of main secondary metabolites of *Satureja icarica* P.H. Davis (Lamiaceae) by LC-HRMS and evaluation of antioxidant capacities

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Abstract: In this study, we determined antioxidant activity of *Satureja icarica* (Lamiaceae) which is used as a spice by the local population against 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), N,N-dimethyl-p-phenylenediamine radicals (DMPD$^{\bullet+}$), and 2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS$^{\bullet+}$) as well as their ability to reduce Fe^{3+}, Fe^{3+}-TPTZ, and Cu^{2+}. The antioxidant activities of both extracts were similar to those of standards in all assays. For instance, IC_{50} of *S. icarica* were for scavenging DPPH was 10.19 µg/mL. The results of liquid chromatography-high resolution mass spectrometry (LC-HRMS) revealed that the main secondary metabolites in *Satureja icarica* were found to be as rosmarinic acid and luteolin-7-*O*-rutinoside.

Keywords: *Satureja icarica*; LC-HRMS; seconder metabolite; antioxidat activity; rosemarinic acid.

1. Introduction

The genus *Satureja* L. (Lamiaceae) has a total of seventeen species in Turkey, seven of which are endemic [1]. However, some species of *Satureja* are known locally as “kekik” (thyme), “kılıç kekiği” (sword thyme), or “taş kekiği” (rock thyme) and are used in cooking or as medicinal plants [2]. In addition, aerial parts of *Satureja* species such as flower stems and leaves are used by the public in the

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The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology July-December 2023 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.2311.2956
Available online: December 14, 2023

treatment of different ailments such as cramps, nausea, muscle pains, indigestion, infectious diseases, and diarrhea. It has also been reported that *Satureja* species have antispasmodic properties in the literature [2-5].

Reactive oxygen species (ROS) occur in normal aerobic metabolism, with some environmental and physiological factors such as excessive drug use, smoke, radiation, or malnutrition [6-8]. Excessive amount of ROS attack membrane enzymes, proteins, DNA, lipids, and carbohydrates, which results in various pathologic and physiologic diseases [9, 10]. On the other hand, antioxidants are natural or synthetic compounds consisting of a large number of exogenous or endogenous chemicals, especially phenolic and polyphenolic compounds, which have great health benefits and reduce the risk of developing different diseases [11-13]. Also, they are crucial agents, which protect organisms from hazardous damages caused by oxidative stress induced by ROS and free radicals [14,15]. Synthetic antioxidants have been severely limited in their use in food and pharmaceutical products due to their carcinogenic effects [16]. For this reason, interest in synthetic antioxidants has decreased considerably and interest in natural antioxidants has increased considerably recently. Therefore, it has gained great importance to investigate the antioxidant potentials of plants rich in phenolic compounds. As an inevitable result of this, natural products have now become an important potential in drug discovery development processes [17, 18]. Recently, the number of studies on traditional and medicinal plants with economic value has increased considerably [19, 20]. These plants containing biologically functional products have been reported to significantly reduce the risk of neurodegenerative diseases, atherosclerosis, inflammation, cardiovascular diseases, cancer, and diabetes. The most important feature of plants, which are natural and potential drug sources, is that they have lower toxicity than synthetic drugs [21, 22]. In addition, it is known that natural products obtained from different plant sources have more positive effects on human health than synthetics [23-25].

In this study, it was aimed to determine the antioxidant capacity of the *S. icarica* P.H. Davis species, which is widely used among the public in Türkiye, and to develop an analytical method based on the LC-ESI-HRMS technique for the quantification of major bioactive secondary metabolites the species.

2. Experimental

2.1 Chemicals

2,2-Azino-bis3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 2,9-dimethyl-1,10-phenanthroline (neocuproine), N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD), 2,9-dimethyl-1,10-phenanthroline (neocuproine), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, trichloroacetic acid (TCA), and α-tocopherol were purchased from Sigma-Aldrich Corp., St. Louis MO, USA) and (Steinheim, Germany). Caffeic acid (≥98% Sigma-Aldrich), luteolin-7-*O*-rutinoside (>97% Carbosynthlimited), vanilic acid (≥97% Sigma-Aldrich), naringin (≥90% Sigma-Aldrich), luteolin 7-*O*-glucoside (>97% TRC Canada), hesperidin (≥98% J&K), rosmarinic acid (≥96% Sigma-Aldrich), naringenin (≥95% Sigma-Aldrich), acacetin (>97% TRC Canada), dihydrocapsaicin (≥97% Sigma-Aldrich) were used.

2.2. Plant Material

Satureja icarica P.H. Davis species were collected from Gökçeada district of Çanakkale province in August 2018 (38°24'21.1″N, 42°06'01.8″E). The botanist Dr. Tuncay Dirmenci (Balıkesir University, Necatibey Education Faculty, Department of Biology Education) identified the plant material. A voucher specimen (TD 5166) was deposited at the Herbarium of Department of Biology Education at Balıkesir University.

2.3. Extraction

After the above-ground parts of the *Satureja icarica* plant were dried thoroughly in the shade. The 50 g of powdered aerial parts of *S. icarica* species were further subjected to 3-day maceration directly

with methanol (MeOH). Then, the plant parts were separated from the solvents by filtering through the filter paper. Solvents were evaporated under low vacuum in a rotary evaporator (Heidolph Hei-VAP HL, Germany) and dried. Yield of the extraction was determined as 6% from the dry plant. The 3 g extract was stored at +4 °C until further experiments.

2.4. Antioxidant Activity

Antioxidant capacity evaluation of the *S. icarica* extract were carried out based on the following assays such as ferric ions (Fe^{3+}) reducing ability assay, cupric ions (Cu^{2+}) reducing ability assay (CUPRAC), DPPH radical scavenging assay, ABTS radical scavenging assay and DMPD radical scavenging assay. Details of these tests are given in the following sections.

2.4.1. Ferric Ions (Fe^{3+}) Reducing Ability Assay

The abilities of *Satureja icarica* to reduce Fe^{3+} were determined using the Oyaizu method [26] as described previously [27, 28]. The basis of this method is defined as the reduction occurring in the $Fe^{3+}(CN^{-})_6$ complex. After the adding excess ferric ions (Fe^{3+}) to $Fe^{3+}(CN^{-})_6$ complex, the Perls' Prussian blue complex was formed [29]. First, 0.75 mL of *Satureja icarica* extract, which including different concentrations (15–45 g/mL) were mixed with $K_3Fe(CN)_6$ (1%, 1.25 mL) and buffer (1.25 mL, 0.2 M, pH 6.6) solutions. The mixture was then incubated for 30 min at 50 °C. Then, the mixture was combined with 1.25 mL of trichloroacetic acid (TCA, 10%) and 0.5 mL of $FeCl_3$ (0.1%) before the absorbance was recorded at 700 nm [30].

2.4.2. Cupric İons (Cu^{2+}) Reducing Ability Assay

The CUPRAC reducing ability assay was used to assess the Cu^{2+} reducing capabilities of *Satureja icarica* [31, 32]. Neocuproine was used as a chromogenic oxidizing agent in this method [33-35]. For this aim, 1 mL of acetate buffer (1.0 M), neocuproine (7.5 mM), and $CuCl_2$ solution (10 mM) were transferred to test tubes and vortexed. Then, the samples were added to tubes at concentrations (15 to 45 µg/mL). With distilled water, the tubes were filled to 1 mL. The samples were maintained at 25 °C for 30 min, and the absorbance was recorded at 450 nm [36].

2.4.3. DPPH Radical Scavenging Assay

The DPPH free radical scavenging capacity of was assessed according to Blois method [37] as described in detail [38]. This technique, frequently used in research, is based on the ability of antioxidants to scavenge DPPH free radicals [39-44]. The samples of standards and *Satureja icarica* extracts were prepared at concentrations ranging from 15 to 45 µg/mL. The 0.5 mL of DPPH (0.1 mM) was added to tubes including samples. Then, these tubes were kept in the dark at 25 °C for 30 min. Finally, their absorbances were recorded at 517 nm.

2.4.4. ABTS Radical Scavenging Assay

$ABTS^{\bullet+}$ scavenging method, which is frequently used in research [45-48], was used to evaluate the radical scavenging capacity of *Satureja icarica* extract [49]. First, an ABTS radical cation was generated. For this experiment $K_2S_2O_8$ (2.45 mM) and ABTS (7.0 mM) were interacted. The solution's absorbance was adjusted to 0.750±0.025 at 734 nm with buffer solution (pH 7.4, 0.1 M). Then, *Satureja icarica* at varying concentrations (15-45 µg/mL) were transferred to one mL of $ABTS^{\bullet+}$ solution. Finally, their absorbances were assessed at 734 nm after 30 min incubation [49,50]. The decrease in absorbance reflects the sample's capacity for radical scavenging [51].

2.4.5. DMPD Radical Scavenging Assay

The $DMPD^{\bullet+}$ scavenging effect of *Satureja icarica* was performed according to the method of described by Gülçin [52]. For this purpose, an aliquot (1 mL) of $DMPD^{\cdot+}$ solution (0.1 M) was transferred to acetate buffer (1 mL, pH 5.25, 0.1 M) containing different concentration of *Satureja icarica* (10–30 µg/mL). Then, 0.25 mL of ferric chloride ($FeCl_3$, 50 mM) was added to this mixture. Then, the absorbances of mixtures were spectrophotometrically measured at 505 nm.

2.5. IC_{50} Value Determination

The IC_{50} value is often used in biochemistry to compare biological activities [53]. The IC_{50} values clearly describe the removal effect quantitatively. A lower IC_{50} value indicates higher removal effect. So, the IC_{50} value is the most practical way to evaluate removal affinities [54]. The IC_{50} value of a pure drug or herbal extract is known as the concentration required to half maximum scavenging concentration. This value is derived from the graph of the percent radical scavenging of *S. icarica* versus increasing concentrations [55,56].

2.6. LC-HRMS Analysis

2.6.1. Sample Preparation for LC-HRMS

200 mg of the extracts was weighed and dissolved in 5 mL methanol. It was kept in an ultrasonic bath until it became a clear solution. Then, the solution was filtered through (0.45 µm Millipore Millex-HV filter). 30 µL of internal standard dihydrocapsaicin solution from 1000 ppm stock solution in methanol was added to each extract solution. The sample (1 mL) was placed in the vials to be ready for LC-ESI-HRMS measurements.

2.6.2. Standard Solutions

Standard solution mixtures were dissolved in methanol and prepared at 10 different concentrations (0.01, 0.05, 0.1, 0.3, 0.5, 1, 3, 5, 7 and 10 mg/L). 1000 mg/L of stock solution of dihydrocapsaicin (purity 97%) in methanol was used as an internal standard.

2.6.3. Instruments, Optimization and Chromatographic Conditions of LC-ESI-HRMS

The major secondary metabolites of *S. icarica* were screened firstly from the database of the ILMER at Bezmialem Vakif University, then the most abundant metabolites were quantified by LC-ESI-HRMS using an ORBITRAP Q-EXACTIVE mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a Troyasil C18 column (150 x 3 mm 5 µ particle size). Optimization of the method was carried out by injection of the standard compounds to the mass spectrometry instrument and ionizaiton pattern and target ions were determined. The standards and spiked solutions were measured accordingly. While the the Mobil Phase A composed of from 1% formic acid – water mixture, the Mobile Phase B composed of from 1% formic acid – methanol. At the end of many trials from the several type of columdns such as Fortis Universil C18, Phenomenex Luna C18 and Troyasil C18 HS, we decided to use Troyasil C18 HS (150 x 3 mm 5 µ column) which achieved the most successful resolution and symmetric peaks in the developed method. The gradient programs were 50% A and 50% B for 0–1 min, 100% B for 1–6 min, and 50% A and 50% B for 6–10 min. The column oven was used and set to 25 °C and flow rate of the mobile phase was 0.35 mL/min. The environmental conditions for temperature nad humidity of the laboratory were recorded as 22.0 ± 5.0 °C and 50 ± 15%, respectively. The MS conditions were used as following: sheath gas flow rate 45, auxiliary gas flow rate, 10, spray voltage, 3.80 kV, capillary temperature, 320 °C, auxiliary gas heater temperature 320 °C, S-lens RF level 50. Compounds were identified by comparing the retention times and target ions of the compounds in LC-ESI-HRMS [57-59].

2.6.4. Method Validation of LC-HRMS

The applied method was validated according to the EURACHEM CITAC Guide and in our previous reports [60-62]. Method validation parameters were selected as specificity, accuracy, linearity, LOD and LOQ.

2.6.4.1. Specificity

Specificity is the observation of only analyte peaks at the retention time of the target analyte in the presence of other components (impurities, matrix components and degradation products). The target analyte is precisely measured and accurately identified in the matrix without any interference. The specificity of the developed LC-ESI-HRMS method was determined by direct analysis (blind) of the entire prepared sdifferent solvents, *S. icarica* extract and added target analytes. The LC-HRMS method was preferred in order to achieve the required selectivity and sensitivity in the matrix and to eliminate the negative effects of the interventions.

2.6.4.2. Accuracy

Accuracy is a parameter that expresses the closeness or estimation of the measurement results to the real quantity. The % recovery value is one of the checked parameters for accuracy. This value was calculated based on LC-ESI-HRMS data for each analyte according to the following formula (Table 1).

Recovery % = Recovered concentration /injected concentration x 100

2.6.4.3. Linearity

In this study, a calibration curve was determined to make the quantitative determination of the quantified secondary metabolites by LC-ESI-HRMS. For this purpose, six repetitive mesaurements were carried out from the solutions at different concentrations and a calibration curve was obtained by using the nominal values of the analytes. The regression coefficient (R^2) and linear regression equation were obtained from the determined curve (Table 1).

Table 1. LC-ESI-HRMS and validation parameters for quantified compounds

Compounds	m/z	Ionisation mode	Linear range	R^2	Linear regression equation	LOD/ LOQ	Recovery (%)
Caffeic acid	179.0350	Negative	0.01-10	0.9989	y=0.0304x + 0.00366	0.08/0.27	95
Luteolin-7-*O*-rutinoside	593.1512	Negative	0.01-10	0.9987	y=0.00879x + 0.000739	0.01/0.03	96
Vanilic acid	167.0350	Negative	0.30-10	0.9997	y=0.00133x+0.0003456	0.1/0.33	99
Naringin	579.1719	Negative	0.01-10	0.9991	y=0.00576x-0.000284	0.01/0.03	102
Luteolin-7-*O*-glycoside	447.0933	Negative	0.01-10	0.9955	y=0.0162x + 0.00226	0.01/0.03	96
Hesperidin	609.1825	Negative	0.01-10	0.9994	y=0.00423x + 0.0000138	0.01/0.03	96
Rosmarinic acid	359.0772	Negative	0.01-10	0.9991	y=0.00717x + 0.0003067	0.01/0.03	99
Naringenin	271.0612	Negative	0.01-10	0.9992	y=0.0281x + 0.00182	0.01/0.03	87
Acacetin	283.0612	Negative	0.01-7	0.9995	y=0.04597x+0.0001951	0.01/0.03	88

2.6.4.4. Detection and Quantitation Limits

Limits of detection (LOD), limit ofquantification (LOQ), reproducibility, recovery, and linearity were the characteristics used to validate the method. Using the following equation, the LODs of the

approach for distinct substances were determined: LOD or LOQ = κSDa/b, where LOQ is 3 and κ = 3 for LOD [60-62] (see Table 1).

2.11.9. Measurement Uncertainty Assessment

The uncertainty parameter was determined as uncertainty from purity of standard ($u_{standard}$), weighing ($u_{weighing}$), precision ($u_{recovery}$) and calibration curve ($u_{linearity}$) of the applied method. GUM methodology was applied in accordance with the EURACHEM CITAC and ISO Guide 35 for the estimation of the uncertainty measurement [63-68]. The combined uncertainty ($u_{Combined}$) was calculated as follows:

$$u_{Combined} = \sqrt{(u_{standard})^2 + \left(u_{weighing}\right)^2 + \left(u_{recovery}\right)^2 + (u_{curve})^2} \quad (3)$$

The expanded uncertainty (u_{Expanded}) calculated using a coverage factor of 2 giving a confidence level of approximately 95% was calculated as follows:

$$u_{Expanded} = u_{Combined} \times k \quad (4)$$

Deatiled calculation methodologies can be found our earlier reports and uncertainty data of the measurements are sumamrized in Table 2.

Table 2. The quantity of phenolic compounds of MEOH extract of *S. icarica* quantified by LC-ESI-HRMS (mg/kg)

Compound	Aerial parts	U %
Caffeic acid	1231.6	3.8
Luteolin-7-*O*-rutinoside	29972.0	3.1
Vanilic acid	1411.9	3.5
Naringin	1425.8	4.5
Luteolin 7-*O*-glucoside	1178.9	4.1
Hesperidin	6658.5	3.8
Rosmarinic acid	37910.1	3.9
Naringenin	1439.9	4.2
Acacetin	726.2	4.0

3. Results and Discussion

With the isolation of many useful drugs from plants, natural products have gained great importance in medicine [64, 65]. In this study, the ferric ions and copric ions reducing (CUPRAC) (Figure 1) capacities and DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and DMPD$^{\bullet+}$ radicals were used to evaluate the antioxidant activity of *S. icarica* (Table 3, Figure 2).

Table 3. Fe^{3+} and Cu^{2+} ions reducing ability of *Satureja icarica* and standards to at the 45 µg/mL

Antioxidants	**Fe^{3+} reducing**		**Cu^{2+} reducing**	
	λ_{700}	**r^2**	**λ_{450}**	**r^2**
BHA	2.347	0.9086	1.649	0.9584
BHT	0.952	0.9154	0.998	0.9834
α-Tocopherol	2.119	0.9586	1.108	0.9910
Trolox	0.957	0.9863	0.693	0.9934
Satureja icarica	0.703	0.9827	1.056	0.9990

The reducing abilities of *S. icarica* and the standard antioxidants decreased in the following order of BHT (λ_{700}:2.347, r^2: 0.9086) > α-Tocopherol (λ_{700}:2.119, r^2: 0.9586) > Trolox (λ_{700}:0.957, r^2: 0.9863) ≈ BHT (λ_{700}:0.952, r^2: 0.9154) > *S. icarica* (λ_{700}:0.703, r^2: 0.9827) (Table 3 and Figure 1a). These results indicated that *S. icarica* has high Fe^{3+} electron donor capacity and reduction abilities to neutralize ROS and free radicals. The EEAC reducing power was close to that of Trolox and BHT and was lower than BHA and α-Tocopherol ($p<0.001$, Table 3).

The abilities of *S. icarica* and the standards to reduce Cu^{2+} ions (45 µg/mL, Table 3 and Figure 1b) were decreased in the following order of BHA (λ_{450}:1.649, r^2: 0.9584) > α-Tocopherol (λ_{450}:1.108, r^2: 0.9910) > *S. icarica* (λ_{450}:1.056, r^2: 0.9990) > BHT (λ_{450}:0.998, r^2: 0.9834) > Trolox (λ_{450}:0.693, r^2: 0.9934). The Cu^{2+} ions reducing ability of *S. icarica* was higher than BT and Trolox and close to α-Tocopherol, however, lower than BHA ($p<0.001$, Table 3).

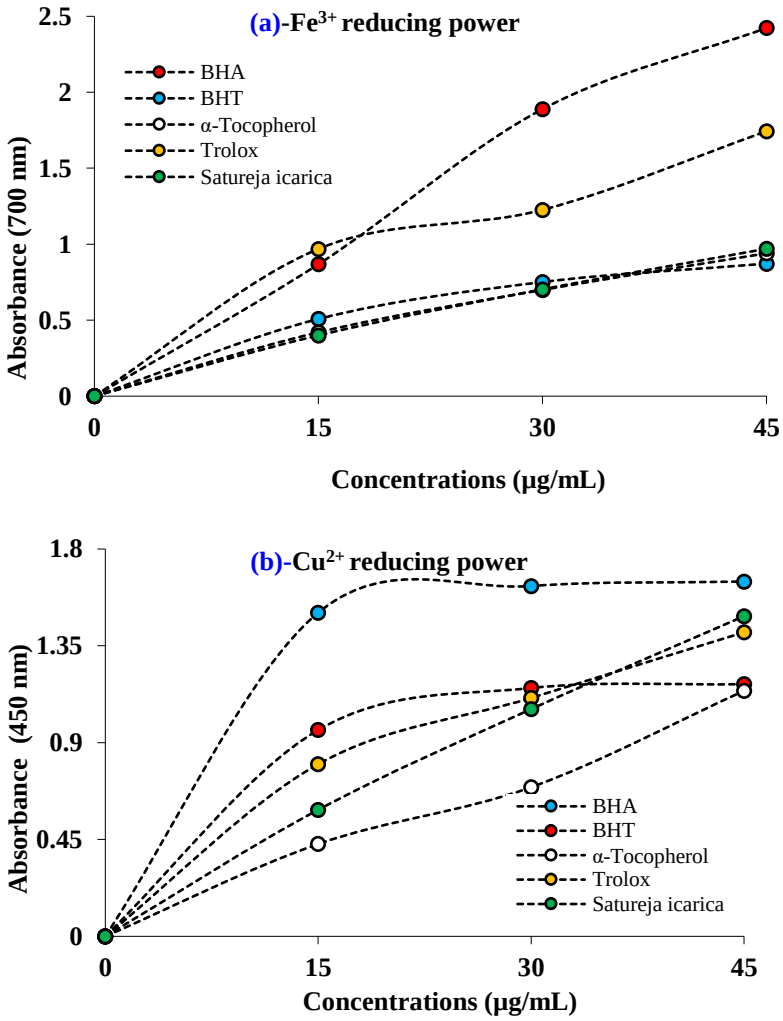

Figure 1. Reducing ability of *Satureja icarica* and standards (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene) (**a**)-Fe^{3+} reducing power (**b**)-Cu^{2+} reducing power

The DPPH assay can measure of the radical scavenging ability of plants and compounds derived from plants [66, 67]. When antioxidants, such as *S. icarica*, donating an electron to $DPPH^{\bullet}$, results in a change in color change from purple to yellow that can be measured spectrophotometrically [67]. The

IC_{50} values of DPPH free radical scavenging for *S. icarica*, and the standards decreased in the order following of Trolox (IC_{50}:7.05 μg/mL, r^2: 0.9614)> *S. icarica* (IC_{50}:10.19 μg/mL, r^2: 0.9276) = BHA (λ_{450}:10.10 μg/mL, r^2: 0.9015) > α-Tocopherol (IC_{50}:11.31 μg/mL, r^2: 0.9642) > BHT (IC_{50}:25.95 μg/mL, r^2: 0.9221) (Table 4 and Figure 2a). Trolox was found the most powerful $DPPH^{\bullet}$ scavenger. A comparison of all results with standard antioxidants revealed that abilities of *S. icarica* had close $DPPH^{\bullet}$ scavenging activity to Trolox, similar BHA and α-Tocopherol and higher than BHT.

Table 4. Determination of IC_{50} (μg/mL) values for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ removing of methanol extract of *S. icarica* and standard compounds.

Antioxidants	$DPPH^{\bullet}$ scavenging		$ABTS^{\bullet+}$ scavenging		$DMPD^{\bullet+}$ scavenging	
	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
BHA	10.10	0.9015	5.07	0.9356	0.070	0.9465
BHT	25.95	0.9221	6.99	0.9350	0.070	0.9390
α-Tocopherol	11.31	0.9642	8.37	0.9015	-	-
Trolox	7.05	0.9614	6.16	0.9692	0.072	0.9382
Satureja icarica	10.19	0.9276	9.62	0.9324	26.65	0.9794

Another improved assay for the designation of radical scavenging is $ABTS^{\bullet+}$ scavenging ability. For this purpose, ABTS radicals were generated in an ABTS/$K_2S_2O_8$ chemical system [68]. The $ABTS^{\bullet+}$ scavenging ability was descending order of BHA (λ_{450}:5.07 μg/mL, r^2: 0.9356).> Trolox (IC_{50}:6.16 μg/mL, r^2: 0.9692) > BHT (IC_{50}:6.99 μg/mL, r^2: 0.9350).> α-Tocopherol (IC_{50}:8.37 μg/mL, r^2: 0.9015) > *S. icarica* (IC_{50}:9.62 μg/mL, r^2: 0.9324) Figure 2b and Table 4 clearly show that the extract of *S. icarica* scavenged $ABTS^{\bullet+}$ more effectively as similar to standard antioxidants. A lower IC_{50} value indicates higher ABTS scavenging activity as in DPPH free radical scavenging ability. The results clearly demonstrate that *S. icarica* had effective $ABTS^{\bullet+}$ scavenging activity when compared the positive controls.

Antioxidants can act as hydrogen donors for $DMPD^{\bullet+}$, reduce the absorbance at 505 nm of $DMPD^{\bullet+}$ [69, 70]. IC_{50} values for methnaol extract of *S. icarica* and the reference antioxidants including trolox, BHT, and BHA were found as 0.070 mg/mL for BHA (r^2: 0.9465) and BHT (r^2: 0.9390), 0.072 mg/mL for trolox (r^2: 0.9382) and 26.65 mg/mL for *S. icarica* extract (r^2: 0.9794*)* (Table 4 and Figure 2c). A lower IC_{50} value indicates higher $DMPD^{\bullet+}$ scavenging activity as in $ABTS^{\bullet+}$ scavenging.

In order to investigate the sources of the antioxidant capacities shown by the methanol extract of *S. icarica* iron and discussed above, to determine the main components in the methanol extract, firstly, after the preliminary screening measurements made with the Thermo Orbitrap Q-Exactive LC-HRMS system in full scan mode by Using ESI source, the observed main peaks were compared with the standard substances we had. Then, we decided to validate the method for the compounds given in Table 1. Details of method validation procedure were reported in Section 2.6 and the corresponding data for the validation study were given in Table 1. The measurement uncertainty parameters were determined as purity of standard ($u_{standard}$), weighing ($u_{weighing}$), precision ($u_{recovery}$) and calibration curve ($u_{linearity}$) for use LC-HRMS method herein. The combined uncertainty ($u_{Combined}$) values were reported in Table 2.

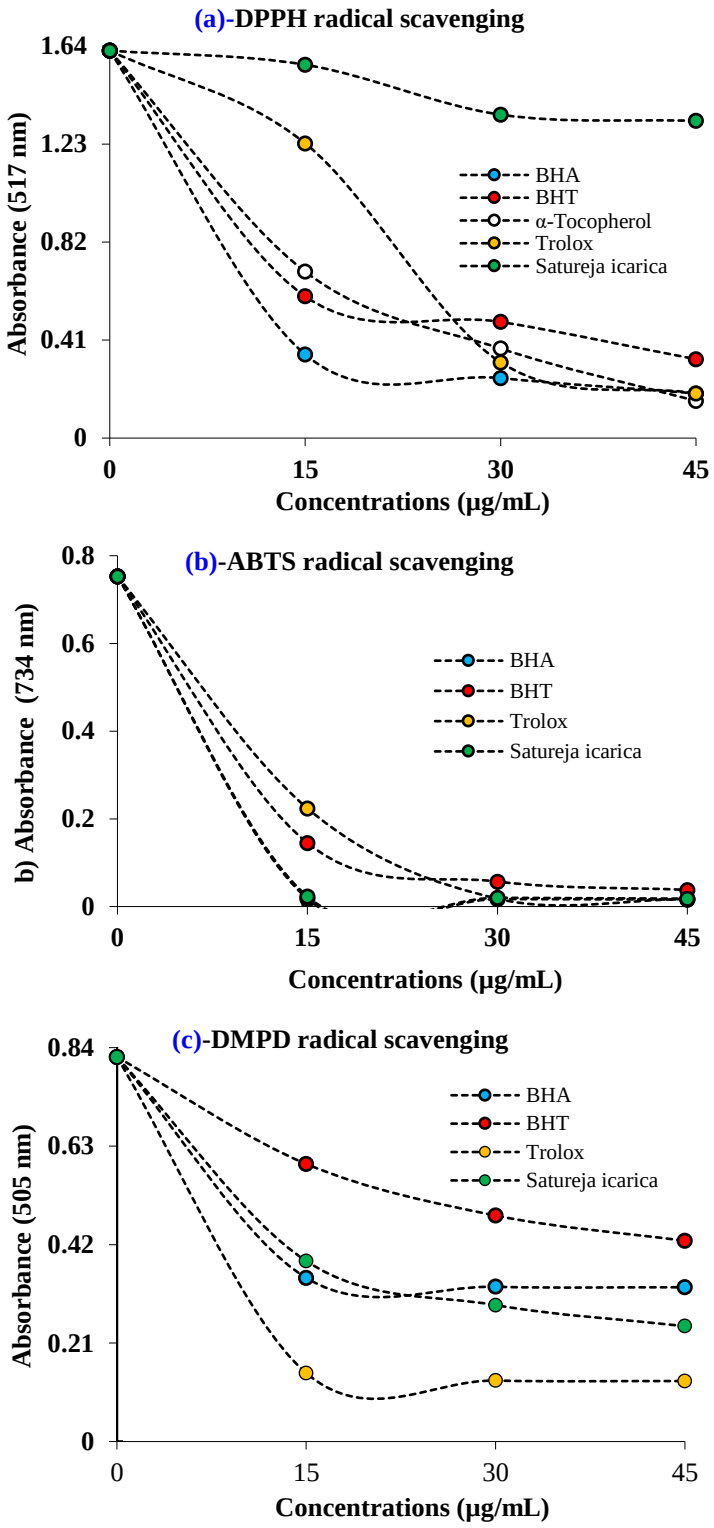

Figure 2. Abilities of methnaol extract of *Satureja icarica* and standard antioxidants to scavenge DPPH, ABTS and DMPD radicals absorbans in **a**) 517 nm, **b**) 734 nm, **c**) 505 nm

The main components in the plant extract were determined as rosmarinic acid (37910.1 mg/kg), Luteolin-7-*O*-rutinoside (29972.0 mg/kg) and hesperidin (6658.5 mg/kg) by LC-HRMS measurements (Figure 3). Other most abundant compounds were determined as caffeic acid, vanillic acid, naringin, Luteolin-7-*O*-glucoside, naringenin and acacetin (Table 2). As natural antioxidants, phenolic compounds have great structural diversity and different chemical compositions among plant-derived metabolites [71-73]. When the necessary scanning is done in the literature, it is observed that rosmarinic acid [74-76], luteolin [77], hesperidin [78,79], caffeic acid [80], and naringin [81,82] molecules are very active biologically, especially in terms of antioxidant activity. It is known that the high antioxidant capacities of these molecules originate from the phenolic and polyphenolic groups found in their structures [83-86]. Phenolic compounds gain active antioxidant properties by increasing the electron density in the hydroxyl group in the ortho or para position, decreasing the oxygen-hydrogen bond energy and increasing reactivity against lipid free radicals. Phenolic compounds have a very limited effect on meta-position. In addition, stoichiometric factors, steric and electronic effects are responsible for the activities of antioxidants [87-89].

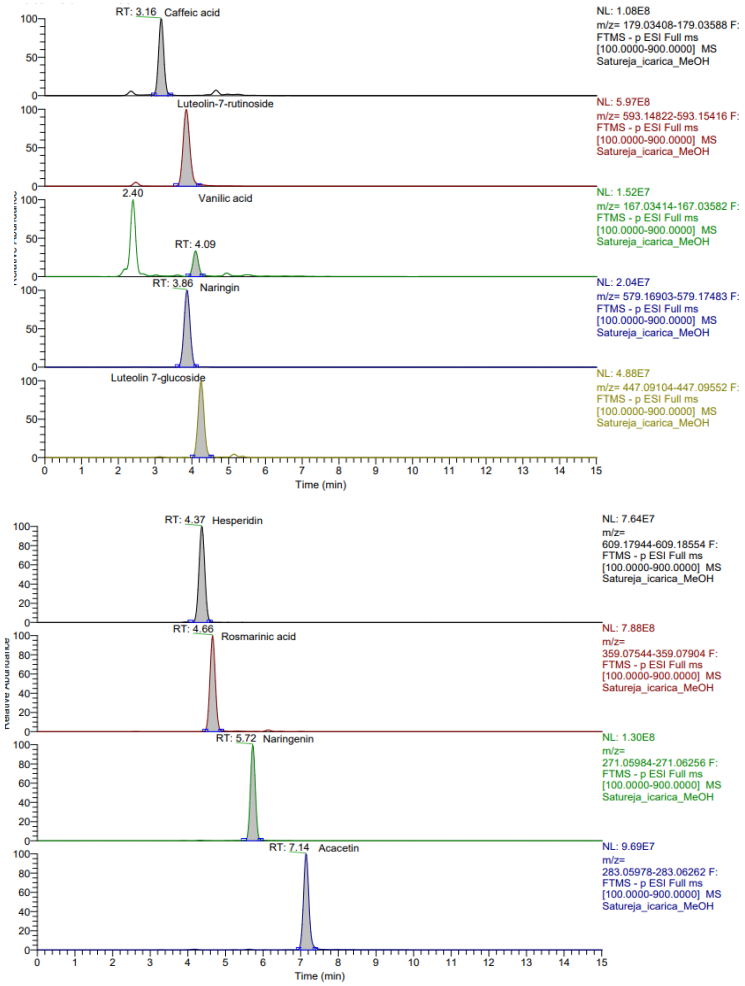

Figure 3. The LC-HRMS chromatogram of quantified compounds of the methanol extract of *S. icarica*

3. Conclusion

In conclusion, we developed and validated anLC-HRMS method for determination of the main compounds of the methanol extractof S. icarica. Measurement uncertainty assessment of the applied methodwas also reported. Rosmarinic acid and Luteolin-7-*O*-rutinoside were found to be the most

abundant secondary metabolites of the methanolic extract of the species. Weconfirmed that Satureja icaricashowed good antioxidant capacity on DPPH, ABTS and DMPD radical scavengingactivity, Fe^{3+} and Cu^{2+} reducing effects. Antioxidant and antiradicalactivities of *Satureja icarica* were observed close to positive controls in the indicated methods. The potentantioxidant and antiradical activities of Si carica should be due to its high phenolic compound concentrations such as rosmarinic acid and luteolin 7-*O*-rutinoside. The *S. icarica* can be used as a new source of rich phenolicsources and antioxidant agents in our daily diets.

Acknowledgments

S. Alwasel would like to extend his sincere appreciation to the Researchers Supporting Project (RSP-2023/59) of King Saud University, Saudi Arabia.

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