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Research Article

Antioxidant Properties, Phenolic Profile and Nutritional Value for *Sorbus umbellata* Fruits from Turkey

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Abstract

Sorbus umbellata fruits are a wild edible natural food source with a special taste and aroma. *S. umbellata* fruits were particularly consumed by human and birds, and other livings. An analysis of *S. umbellata* fruits was performed by means of ultra-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS). In the present study, total phenolic and flavonoid contents, individual phenolic profiles, related antioxidant activities (β-carotene linoleic acid, $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radical scavenging) and nutritional value were examined. *S. umbellata* fruits contained gentisic acid, protocatechuic acid as a major constituent; the others were ferulic acid, chlorogenic acid, caffeic acid, syringic acid, vanillic acid, 3,4-dihydroxybenzaldehyde. Total phenolic and flavonoid contents of *S. umbellata* fruits were evaluated and the results were in accordance with LC individual phenolic content. *S. umbellata* fruits, picking by gatherers, can be consumed as traditional-handmade jam, juice, ice-cream and liquor. Thus, the natural wild edible *S. umbellata* fruits may show up a value and importance in commercial food product and a source of natural healthy product with significant ingredients.

Keywords: *Sorbus umbellata;* Phenolic compounds; Antioxidants; Nutrients; UPLC-ESI-MS/MS

Introduction

Sorbus umbellata is a species in the genus *Sorbus* which includes approximately 99 to 207 species and belongs to the family of the Rosaceae. In the various region of Turkey, *S. umbellata* fruits were grown as a natural food source for hunter-gatherers at the autumn. In addition to fruit ingestion as a nutritional source, from the health point of view, the consumption of fruits in a healthy diet provides the reduction of cardiovascular diseases and degenerative pathologies [1-4].

Although there were studies devoted to the investigation of phenolic acids, sugars and minerals in berries, apples, peaches, papayas and avacados [4-7], while, to our knowledge, no data are reported in literature on phenolic compounds and antioxidant or antiradical activity in *S. umbellata* fruits from Turkey.

Phenolic compounds protect the plants against insects and animal pests. Thousands of phenolic compounds are found in plants and their products, because of remarkable structural differences of phenolic compounds. Phenolic compounds can contribute the taste and aroma of plant origin foods. Phenolic compounds also serve as natural antioxidants. They prevent diseases such as cancer, heart and lung diseases by stopping or inhibiting reactions caused by free radicals. A large group of flavonoids are responsible for colors of the foods. Anthocyanins, a group of the flavonoids, are natural coloring materials and they are the reason of pink, red and purple colors of vegetables, fruits, fruit juice and vine [8].

The phenolic compounds in medicinal pills and foods are in the main parts of secondary metabolites that are derived from phenylalanine or tyrosine of plant tissues [9,10]. Chemically, phenolics are defined as the compounds which have one or more hydroxyl groups and aromatic ring and their functional derivatives. Their existence in animal tissues and non-plant materials generally depends on digestion of the plant origin foods. Some of phenolic compounds are effective in forming flavor of fruit and vegetables. Especially, the reason of bitterness and astringency feelings in the mouth is phenolic compounds. The main reason of evaluation of fruits as functional foods is their health promoting effects. These effects are caused by antioxidant and antimicrobial properties of phenolic compounds [11].

Phenolic compounds are natural antioxidants and considered to have a preventive role in the development of cancer and heart disease [12]. Investigations related to biological and pharmacological features have also been reported for phenolic compounds, including free radicals scavenging, apoptosis of cancer cells [13,14] anti-herpetic, anti-Human Immunodeficiency Virus (HIV) reverse transcriptase and anti-HIV activity [15,16].

In this study authors aimed to reveal the total phenolic and flavonoid content with individual phenolic ingredients in terms of the antioxidant activity by means of oxidative inhibition of *S. umbellata* fruits from Turkey.

Materials and Methods

Sample

Sorbus umbellata fruits originating from southwestern Turkey were collected in 2013. Taxonomic identification was provided from Department of Biology, Faculty of Science, Muğla Sıtkı Koçman

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University, Muğla (Turkey). Samples were divided into two portions: one portion was lyophilized (Christ Freeze Dryer Alpha 1-4 LD plus, Germany) and reduced to a fine dried powder, other portion was stored at -18°C without the application of any process.

Standards and reagents

Phenolic standards (pyrogallol, homogentisic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), gentisic acid, pyrocatechol, galanthamine, *p*-hydroxy benzoic acid, 3,4-dihydroxybenzaldehyde, catechin hydrate, vanillic acid, caffeic acid, syringic acid, vanillin, epicatechin, catechin gallate, *p*-coumaric acid, ferulic acid, rutin, *trans*-2-hydroxy cinnamic acid, myricetin, resveratrol, *trans*- cinnamic acid, luteolin, quercetin, naringenin, genistein, apigenin, kaempferol, hesperetin, and chrysin) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Folin&Ciocalteu's phenol reagent (FCR), β-carotene, tween-40, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), potassium persulfate ($K_2S_2O_8$), neocuproine, ferrin, 5,5'-Dithiobis(2-Nitrobenzoic acid (DTNB), Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), Acetylcholine Iodine (AcI), Butyrylcoline Iodine (BuI), potassium acetate, aluminum nitrate, ammonium formate, ammonium acetate, Butylated Hydroxytoluene (BHT), α-tocopherol, Butylated Hydroxyanisole (BHA) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals and solvents were of analytical grade and purchased from usual suppliers. Water used in the studies was treated in a Milli-Q water purification system (Advantage A10 Millipore, Pure Water Systems, Molsheim, France).

Nutritional value

The samples were analyzed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures [17]. The crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash amount was determined by burning at 650 ± 15°C. Total carbohydrates were calculated by difference. Energy was calculated according [18] to Eq. (1).

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}) \quad \text{Eq. (1)}$$

Phenolic compound extraction, identification and quantification

Phenolic compounds were analyzed according to previously described method with slight modifications [19]. Briefly, samples were frozen -first with liquid nitrogen then lyophilized for 12 hours. 3 g of dried sample was extracted by 30 mL of 80% acetone at 25°C for 6 h. Then it was placed in ultrasonic bath for 15 min and the sample was centrifuged at 4000 rpm for 10 min at 20°C, and filtered using Whatman No 4. The residue was then extracted with three additional 30 mL portions of 80% (v/v) acetone, as described earlier. The solvent in the combined extracts were evaporated at 40°C and redissolved in 80% (v/v) methanol and filtered through a PTFE-20/25 filter disk for LC analysis. Phenolic compounds were analyzed by UPLC-MS/MS (Waters Acquity Ultra Performance LC, Xevo TQ-S MS/MS, Waters Co., Milford, MA, USA). The chromatographic and mass spectrometry conditions were previously given elsewhere [19]. Detection was carried out with a tandem mass spectrometry, using multiple reactions monitoring (MRM) mode. Mass spectra were acquired in positive Electrospray Ionization (ESI) mode. Data analysis and quantitation was executed using the Waters MassLynx and TargetLynx software (Waters Corp.). The MRM was applied to monitor the transitions of quantifier ion to qualifier ions (the parent > daughter ions transitions, *m/z*). Confirmation of compounds was achieved through two or more daughter ions. The phenolic compounds present in the samples were characterized according to their mass to charge (*m/z*) ratio with those of commercial standards. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of different concentration of standards.

Determination of the antioxidant activity with the β-carotene bleaching method

The total antioxidant activity was evaluated using *β*-carotene-linoleic acid test system based on the detection of inhibition of conjugated dien hydroperoxides due to oxidation of linoleic acid with slight modifications [20,21]. This method is based on bleaching of *β*-Carotene. *β*-Carotene (0.5 mg) dissolved in 1 mL of chloroform was added into mixture of 20 µL of linoleic acid and 200 mg of Tween 40 emulsifier. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen was added by vigorous shaking. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a 96-well microplate reader. The absorbance of the emulsion was read again at the same wavelength after the incubation of the plate for 120 min at 50°C. Methanol was used as a control. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHT, BHA and *α*-tocopherol were used as antioxidant standards for comparison of the activity [21]. The bleaching rate (R) of *β*-carotene was calculated according to Eq. (2).

$$R = [ln(a/b)]/t \quad \text{Eq. (2)}$$

where: *ln* = natural log, a = Absorbance at time zero, b = absorbance at time t (120 min). The antioxidant activity was calculated in terms of percent inhibition relative to the control, using eq. (3)

$$\text{Antioxidant activity (\%)} = (R_{Control} - R_{Sample}) / R_{Control} \times 100 \quad \text{Eq. (3)}$$

DPPH free radical scavenging activity

The free radical scavenging activity of fruit extract was determined using DPPH free radical with slight modification [22]. In its radical form, DPPH free radical absorbs at 517 nm. Briefly, the reaction was initiated by the addition of 4 mL of DPPH free radical (0.4 mM) prepared in ethanol into 1 mL sample solution. After thirty minutes at room temperature, the absorbance was measured at 517 nm. Ethanol was used as a control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability of scavenging the DPPH free radical was calculated by using Eq. (4).

$$\text{DPPH radical scavenging effect (\%)} = (A_{Control} - A_{sample})/A_{Control} \times 100 \quad \text{Eq. (4)}$$

where $A_{Control}$ is the initial concentration of the DPPH· and A_{Sample} is the absorbance of the remaining concentration of DPPH

free radical in the presence of the extract and positive controls. The extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated from the graph of DPPH radical scavenging effect percentage against extract concentration. BHT, BHA and α-tocopherol were used as antioxidant standards for comparison of the activity [12].

ABTS cation radical decolorization assay

The spectrophotometric analysis of $ABTS^{\cdot+}$ scavenging activity was determined according to the previously described method, with slight modifications [12,23]. The $ABTS^{\cdot+}$ was obtained by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Oxidation of ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical cation was stable in this form for more than 2 days in storage in the dark at room temperature. Before usage, the $ABTS^{\cdot+}$ solution was diluted to get an absorbance of approximately 0.700 at 734 nm with ethanol. Then, 160 µL of $ABTS^{\cdot+}$ solution was added to 40 µL of sample solution in ethanol at different concentrations. After 10 min the absorbance was measured at 734 nm by using a 96-well microplate reader. The percentage inhibitions were calculated for each concentration relative to a blank absorbance (ethanol). The scavenging capability of ABTS cation radical was calculated using Eq. (5).

$$\text{ABTS scavenging effect (\%)} = (A_{Control} - A_{sample})/A_{Control} \times 100 \quad \text{Eq. (5)}$$

where $A_{Control}$ is the initial concentration of the ABTS cation radical and A_{Sample} is the absorbance of the remaining concentration of ABTS cation radical in the presence of sample. The extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated from the graph of ABTS cation radical scavenging effect percentage against extract concentration. BHT, BHA and α-tocopherol were used as antioxidant standards for comparison of the activity [12].

Determination of total phenolic compounds

The concentrations of total phenolic content in ethanol extract were expressed as microgrammes of Pyrocatechol Equivalents (PEs), determined with Folin-Ciocalteu Reagent (FCR), according to the method of Slinkard and Singleton [13]. One milliliter of the solution (contains 1 mg) of the extract in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 min, 3 mL of sodium carbonate (2%) were added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of total phenolic content was calculated according to Eq. (7) that was obtained from the standard pyrocatechol graph:

$$\text{Absorbance} = 0.0073 \times X\ (\mu g)\ \text{pyrocatechol} - 0.1665\ (r^2 : 0.9976) \quad \text{Eq. (7)}$$

Determination of total flavonoid concentration

Measurement of total flavonoid concentration of the extract was based on the method [24] with a slight modification and results were expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg of extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate and 4.8 mL of ethanol. After 40 min at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard. The concentrations of flavonoid compounds were calculated according to Eq. (8) that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.0082 \times X\ (\mu g)\ \text{quercetin} - 0.0073\ (r^2 : 0{:}9998) \quad \text{Eq. (8)}$$

Statistical analysis

Three replicates of sample were prepared for each analysis. The results are expressed as mean values ± standard deviation (SD). Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Student's *t*-test. $p<0.05$ was regarded as significant.

Results and Discussion

Dietary patterns are thought to influence the onset and progression of chronic and degenerative diseases [25]. Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body [25-28]. Fruits and vegetables are a primary food source providing essential nutrients for sustaining life; they also contain a variety of phytochemicals such as phenolics and flavonoids, which provide important health benefits [29].

In this study, the results of the nutritional composition and obtained energetic value of a wild edible fruits, *S. umbellata*, were shown in Table 1. *S. umbellata* fruits has carbohydrate and protein in high levels. However, the fruit studied in this paper had low fat content (2.02%), energetic value (350.82 kcal/100 g) was high. The average moisture content was 79.42% (value was not significantly different ($p = 0.05$)).

Total phenolic and total flavonoid contents of *S. umbellata* fruits were displayed in Table 2. The determination of total phenolic content was included in this study because strong correlations between total phenolic content and antioxidant activity in various kinds of fruits were found in previous studies [30,31].

The results are in accordance with the individual phenolic ingredients. The fact is that phytochemicals occurring in food and natural products play a significant role in disease prevention and health promotion. This led to an ever-growing interest in nutraceutical products [32]. As it was previously reported in the literature that the total phenolic contents of blackberry, blueberry, strawberry and sweet cherry were also significantly high [33]. This work has clearly shown

Table 1: Nutritional composition and obtained energetic value of *S. umbellata*.

Parameter	*S. umbellata*
Moisture (g/100 g fw)	79.42 ± 2.47
Ash (g/100 g dw)	14.99 ± 1.08
Carbohydrate (g/100 g dw)	66.04 ± 2.87
Proteins (g/100 g dw)	17.12 ± 0.98
Fat (g/100 g dw)	2.02 ± 0.13
Energy (kcal/100 g dw)	350.82 ± 3.98

Abbreviations: fw: Fresh Weight; dw: Dry Weight

Table 2: Total phenolic and total flavonoid content of *S. umbellata*.

	Total phenolic µg PEs/mg extract	Total flavonoid µg QEs/mg extract
S. umbellata	213.56±3.07	18.56±1.43

Values expressed are means ± S.D of three parallel measurements, $p<0.05$.

Table 3: Antioxidant activities of the extract of *S. umbellata* fruits sample by *β*-Carotene-linoleic acid, DPPH· and $ABTS^{\cdot+}$ assays.

	Antioxidant activity		
	β-Carotene-linoleic acid assay IC_{50} (μg mL^{-1})	DPPH · assay IC_{50} (μg mL^{-1})	$ABTS^{\cdot+}$ assay IC_{50} (μg mL^{-1})
S. umbellata	16.42±0.98	62.09±1.41	20.17±0.94
BHA	4.08±0.10	57.71±0.55	8.83±0.19
BHT	4.14±0.09	62.04±1.02	8.22±0.12
α-Tocopherol	6.67±0.17	9.77±0.28	7.57±0.56
IC_{50} values represent the means ± SD of three parallel measurements ($p<0.05$)			
BHA Butylated hydroxyanisole, BHT Butylated hydroxytoluene			

that the Turkish *S. umbellata* fruit have high phenolic and flavonoid contents.

Antioxidant activities of the extracts of *S. umbellata* fruit sample by *β*-Carotene-linoleic acid, DPPH· and $ABTS^{\cdot+}$ assays were evaluated in Table 3. Results were found to be statistically significant ($p<0.05$) when compared with the control. In the extract of *β*-carotene/linoleic acid assay, *S. umbellata* possessed the inhibition value (IC_{50}) 16.42±0.98 μg/ mL. Studied extract indicated lower lipid peroxidation inhibition activity than BHA, BHT, α-tocopherol.

However, in the $DPPH^{\cdot}$ assay, the extract of *S. umbellata* exhibited the similar inhibition activity (IC_{50}) 62.09±1.41 μg /mL with that of standards, BHA and BHT, and lower than *α*-tocopherol.

In the $ABTS^{\cdot+}$ assay, the extract of *S. umbellata* displayed lower cation radical scavenging activity with inhibition values (IC_{50}) 20.17±0.94 μg/mL with that of standards, BHA, BHT and *α*-tocopherol. Thus, according to the antioxidant activity data presented in Table 3, the results obtained with the *β*-carotene/linoleic acid, the $DPPH^{\cdot}$, and the $ABTS^{\cdot+}$ assays supported each other.

Identification of the phenolic compounds was carried out by comparing retention times and mass spectra with those of authentic standards. Individual phenolic results were explained in Table 4. *S. umbellata* fruits contained 102.87±1.29μg/g gentisic acid, 74.45±1.07μg/g protocatechuic acid as a major constituent; the others were 7.67±1.02μg/g ferulic acid, 7.51±0.99μg/g chlorogenic acid, 3.03±0.48μg/g caffeic acid, 2.91±0.78μg/g syringic acid, 2.52±0.56μg/g vanillic acid, 2.44±0.18μg/g 3,4-dihydroxybenzaldehyde. Total ion chromatograms of determined phenolic compounds in *S. umbellata* fruits using UPLC-ESI-MS/MS were displayed in Figure 1. These results agree with those reported by other authors in berries, chlorogenic, caffeic, p-hydroxybenzoic,ferulic, and p-coumaric acids were identified [34]. However, the results were correlated with total phenolic and flavonoid contents, and antioxidant activity of *S. umbellata* fruits.

Conclusion

In the present study, mainly, the involvement of the studied fruit in the daily diet may provide health benefits, owing to its antioxidant properties and nutrients. Furthermore, the fruits could be used in nutraceutical or pharmaceutical industries.

The method used in the determination of individual phenolic compounds provides high accuracy and robustness owing to UPLC-ESI-MS/MS instrument. As a result, *S. umbellata* fruits are a highly

Table 4: Phenolic contents (μg/g dry weight ± standard deviation) of *S. umbellata* fruits.

No	Compounds	t_R (min)	*S. umbellata*
1	Pyrogallol	0.97	nd
2	Homogentisic acid	1.47	nd
3	Protocatechuic acid	2.38	74.45±1.07
4	Gentisic acid	2.38	102.87±1.29
5	Pyrocatechol	2.38	nd
6	Galantamine	2.68	nd
7	*p*-hydroxy benzoic acid	3.87	1.41±0.13
8	3,4-dihydroxybenzaldehyde	4.68	2.44±0.18
9	Catechin hydrate	3.45	nd
10	Vanillic acid	6.34	2.52±0.56
11	Caffeic acid	5.43	3.03±0.48
12	Syringic acid	6.67	2.91±0.78
13	Vanillin	4.50	nd
14	*p*-coumaric acid	4.65	nd
15	Ferulic acid	7.86	7.67±1.02
16	Epicatechin	6.97	0.38±0.06
17	Catechin gallate	5.91	nd
18	Rutin	8.36	1.81±0.38
19	*trans*-2-hydroxy cinnamic acid	6.32	nd
20	Myricetin	10.61	0.38±0.09
21	Resveratrol	7.23	nd
22	*trans*-cinnamic acid	8.71	0.16±0.04
23	Luteolin	10.65	0.59±0.07
24	Quercetin	8.96	1.30±0.10
25	Naringenin	9.29	0.26±0.03
26	Genistein	9.22	nd
27	Apigenin	10.95	0.11±0.05
28	Kaempferol	10.98	0.22±0.07
29	Hesperetin	10.62	0.14±0.04
30	Chrysin	11.39	0.12±0.01
31	Chlorogenic acid	5.44	7.51±0.99
32	Gallic acid	1.96	n.d.

Abbreviations: t_R: Retention time; nd: Not Detected

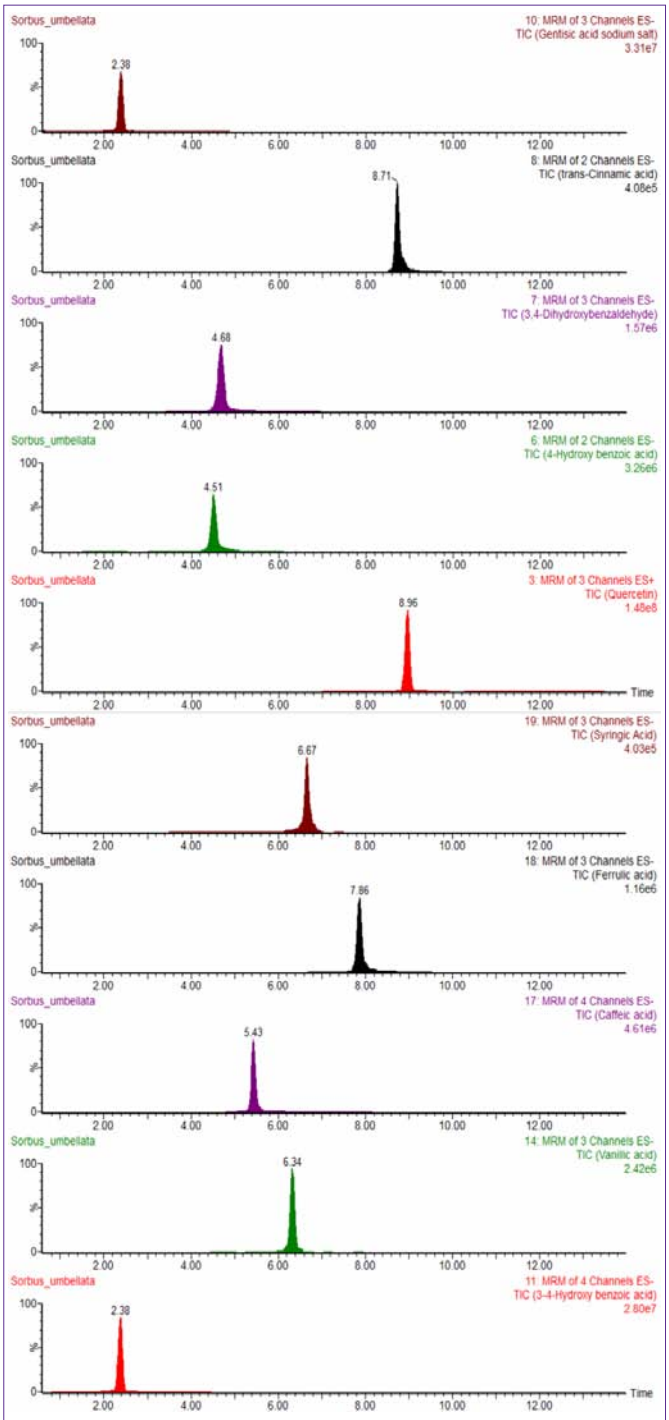

Figure 1: UPLC-MS/MS Total Ion Chromatograms (TIC) of phenolic compounds in *S. umbellata* fruits.

valuable natural product containing phenolic compounds and antioxidant properties.

In the present study, individual phenolic composition obtained by UPLC-ESI-MS/MS analysis and nutritional parameters, antioxidant activities namely, β-carotene-linoleic acid, DPPH· and $ABTS^{\cdot+}$ assays were explored for *S. umbellata* fruits.

S. umbellata fruits have a rich content in terms of phenolic compounds, carbohydrate and protein. According to antioxidant activity data, fruits extract is a valuable natural antioxidant. *S. umbellata* fruits may be used in food industry as a jam, juice, ice-cream and liquor and health industry as a source of natural healthy product with significant ingredients.

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