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First Report on Bio-accessibility, Anti-oxidant Activity and Total Phenolic Compounds From *Stachys thirkei* C. Koch Using A Simulated In Vitro Digestion System

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Research Article

Abstract − It is of great importance to detect the anti-oxidant features of plants, particularly those used for food, pharmacology and medicinal purposes. *Stachys thirkei* C. Koch belonging to Lamiaceae family is utilised as a medicinal aromatic plant in Turkey. It was aimed to investigate the total phenolic content (TPC), anti-oxidant activity and bio-accessibility of *S. thirkei* C. Koch. The TPC was evaluated by Folin-Ciocalteu colorimetric proce-dure and antioxidant activity to determine four distinctive assays (ABTS•+, CUPRAC, DPPH• and FRAP). The experimental analysis showed that, the levels of hydrolysable phenolics (1538.99 mg of GAE/100g) approximately three and a half fold higher than extractable phenolics (422.96 mg of GAE/100g). The TPC of *S. thirkei* C. Koch was determined to be 1961.95 mg of GAE/100g. Moreover, the bio-accessible fractions and phenolic bio-accessibility of *S. thirkei* C. Koch were found to be 1766.72 µmol Trolox/g and 90.05 %, respectively. At the same time, the antioxidative bio-accessibility of *S. thirkei* C. Koch was found to be higher in FRAP method (1164.29 µmol Trolox/g) and also the bio-accessibility (%) of *S. thirkei* C. Koch was found to be higher in CUPRAC method (93.41%). Present investigation is the primary report to investigate the bio-accessibility of the extracts from *S. thirkei* C. Koch. The results of the present may be strong scientific evidence to use *S. thirkei* C. Koch as a favorable source of antioxidant and the researches can be further extended to investigate whether they exhibit similar activities in in vivo systems.

Keywords − *ABTS$^{\bullet+}$, Biological Activity, CUPRAC, DPPH$^{\bullet}$, FRAP, Medicinal Plant*

1. Introduction

Free radical formation occurs uncontrollably due to various uncontrollable environmental and physiological factors and therefore causes cell damage. Observation of damage is defined as oxidative stress (Aydemir & Sarı, 2009). The free radicals are cause to aging, tissue damage, and various diseases, such as Parkinson, Alzheimer, diabetes mellitus and cardiovascular diseases (Umeno, Biju & Yoshida, 2017). Correlatively, in several studies reported that free radicals have been causes numerous disorders (Biswas, Das, & Banarji, 2017; Khan, Garg, Singh, & Kumar, 2018; Sharifi-Rad, et al., 2020).

Anti-oxidants serve a function in avoiding the creation of and scavenging of free radicals and other possible toxic oxidizing species (Alpay, Dulger, & Karabacak, 2017). Free radicals are constituted in human body by several systems. In some situation this free radicals' ratio can rise over the ability of

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the control by the human body and hereby, the oxidative stress appear (Alkadi, 2020). The antioxidants are deactivate harmful free radicals (Dey et al., 2012) and prevent the body from diseases. Intake of natural antioxidants in daily diet is mostly connected with decrease the risk of cancer (Háznagy, Czigle, Zupkó, Falkay, & Máthé, 2006). As a consequence, the antioxidant capacity has been widely studied due to the properties to avoid or treat the cancer in human (Alpay et al., 2017). Therefore; determining the capacities of active ingredients of plants used especially for food, pharmacology and medicinal purposes have become increasingly common. Studies have shown that new methods have been developed for antioxidants and removal of free radicals. Among these methods, free radical removal methods such as the 2,2-diphenyl-2-picrylhydrazyl (DPPH•), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Cupric-Reducing Antioxidant Capacity (CUPRAC), ferric reducing antioxidant activity (FRAP) are the most frequently used methods (Gulcin, 2006). These antioxidant determination methods have been used due to their easy use, sensitivity, applicability of analyses in a short time and their economic advantages (Bursal, 2009). Furthermore, phenolic compounds found in most of the plants reflected one of the broadest groups of phytochemicals and are critical compounds for human health (Silinsin, 2016). It has been stated that phenolic compounds protect body tissues against oxidative stress that prevent or delay the initiation or progression of oxidizing chain reactions (Prior, 2003; Amarowicz et al., 2010). Due to the toxic effects/reactions of artificial anti-oxidants/drugs used in the food/pharmacology industry, there is a growing interest to the phenolic compounds. This has led scientists to find naturally sourced food supplements and pharmaceutical active ingredients.

Bio-accessibility is the in vitro determination of the soluble and accessible part of food components after passing through the gastrointestinal simulation system (Konak, Ates, & Sahan, 2017). Bioavailability is an important factor in nutrition due to differences in environments related to different foods, food ingredients and the digestive system. As reported by Usal & Sahan (2020), data on nutrition do not indicate the biological activities of foods, due to the inability to absorb all the digested nutrients. For this reason, it is notable to investigate the bio-accessibilities of food and drinks. Bio-accessibility can be defined as a bio active component that is absorbed and stocked after physiological activities in living organisms (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009; Rebellato, Pacheco, Prado, & Pallone, 2015).

Apart from well recognized and traditionally consumed natural antioxidants that obtained from beverage and foods (Schuler, 1990), there were so many various plants have been evaluated in the search for alternative anti-oxidants seeking (Chu, Chang, & Hsu, 2000; Oke & Hamburger, 2002). Some of these plants have an important place in human life are known as medicine-spice plants and most of them are also known as medicinal and aromatic plants because they have aromatic properties and are used for medicinal purposes (Beyzi, 2011). As reported in previous studies, the family of Lamiaceae is rich family in medicinal and aromatic properties (Alan, Ozkan, & Tuncer, 2010; Raja, 2012; Carović-Stanko et al. 2016). These unusual plant species belong to the Stachys, one of the broadest genera of the Lamiaceae. Although the Stachys L. is concentrated in the moderate areas of the Southwest Asia and Mediterranean, it stands out in South and North and Africa (Leblebici, 2011) and it also has a wide distribution in Turkey. It has been shown in scientific studies that many species belonging to this genus have biological activities such as antibacterial, antifungal, antitumor (Skaltsa, Demetzos, Lazari, & Sokovic, 2003; Farjam, Khalili, Rustayian, Javidnia, & Izadi, 2011; Saeedi, Morteza-Semnani, Mahdavi, & Rahimi 2008; Yousefi, Gandomkar & Habibi, 2012). In addition to, the ground surface parts of the *S. thirkei* are boiled such as tea and used in the treatment of gastrointestinal diseases in Turkey (Unsal, Vural, Sariyar, Ozbek, & Otuk, 2010).

Rasgele & Dulger (2021) indicated that the anti-mutagenic effect of the ethanolic extract of *S. thirkei* was 26.79% and 44.03%. Askun, Tekwu, Satil, Modanlioglu, & Aydeniz (2013) determined that different extracts of *S. thirkei* showed no activity against *Mycobacterium tuberculosis*. However, Tunali Erkan & Dulger (2016) reported that different extracts of *S. thirkei* were effective against microbial infections such as Staphylococcus aureus ATCC 6538P, Candida glabrata ATCC 90030. Goren et al. (2011) and Askun et al. (2013) stated that 29 and 12 compounds were found in essential oil of *S. thirkei* C. Koch. But there is no direct study related to TPC, antioxidant activity and bio-accessibility of *S. thirkei* C. Koch. In this respect, TPC, anti-oxidant activity and bio-accessibility of *S. thirkei* C. Koch was evaluated in this study. The TPC

was appointed by Folin-Ciocalteu colorimetric procedure and antioxidant activity determined four distinctive procedures. In addition to these, the bio-accessible phenolics of *S. thirkei* C. Koch were determined via simulated digestion system.

2. Materials and Methods

2.1. Plant Material

S. thirkei C. Koch plants were collected between July and August 2019 from Konuralp (40°54'14.0"K, 31°10'30.8"D) town of Duzce in Turkey and taxonomically identified by Assoc.Prof. Ersin KARABACAK, senior taxonomist from the Department of Biology, Canakkale Onsekiz Mart University and desiccated under suitable herbarium conditions.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the extract of *S. thirkei* was carried out in Düzce University (DUBIT) Laboratory. An Agilent 7890A GC System connected to an Agilent 5975C inert MSD with Tri-Axis Detector was used in the study. Separation of components was performed with an Agilent HP5-MS (30 m x 0.25 mm x 0.25 µm) GC column. The oven temperature was held at 40 °C for 5 min., then ramped at 5 °C/min. to 100 °C for 5 min., then ramped at 20 °C/min. to 225 °C and held at this temperature for 8 min. The total run time was 33,25 min. The injector temperature was fixed at 200 °C and splitless mode was used with helium carrier gas. The ion source was electron ionization and the MS source temperature was set at 230 °C. The injection volume was 1.0 µL.

2.2. Chemicals

All chemicals were used to analytical-grade purity. Trolox (CAS No: 53188-07-01) was purchased from Aldrich (Aldrich Chemicals Company, Steinheim, Germany), $ABTS^{\bullet+}$ (CAS No: 30931-67-0), neocuproine (CAS No: 484-11-7), sodium hydroxide (CAS No: 1310-73-2), concentrated hydrochloric acid (CAS No: 7647-01-0) and concentrated sulfuric acid (CAS No:7664-93-9), methanol (CAS No: 67-56-1), ethanol (CAS No: 64-17-5), bile salts (Pcode: 101738600) and DPPH• (CAS No:1898-66-4) were purchased from Sigma (St. Louis, MO, USA). Gallic acid (CAS No: 149-91-7), potassium chloride (CAS No:7447-40-7), pepsin (CAS No:9001-75-6), sodium chloride (CAS No: 7647-14-5), sodium carbonate (CAS No: 144-55-8), ammonium acetate (CAS No: 631-61-8), copper(II)chloride (CAS No: 10125-13-0) and Folin-Ciocalteau phenol reagent (CAS no: HC56273201) were purchased from Merck (Darmstadt, Germany). Pancreatin (CAS No: 8049-47-6) was purchased from AppliChem (Darmstadt, Germany).

2.3. Preparation of Extractable, Hydrolysable, and Bio-accessible Fractions

The extractable, hydrolysable, and bio-accessible fractions of *S. thirkei* C. Koch sample was extracted according to procedure developed by Vitali, Dragojevic, & Sebecic (2009) with some alterations. The analysis was performed in triplicate.

To determine the extractable phenolic (EP), 1.0 g dry weight (dw) *S. thirkei* C. Koch was blended with $HCl_{conc.}$/methanol/water (1:80:10, v/v/v) in a definite value and swashed with rotary shaker (Heidolph Multi Reax; Germany) at 250 rpm (2 h, 20°C) and centrifuging was performed at 3500 rpm (10 min, 4°C) (Eppendorf, 5430R-USA). The obtained liquid phase of EP compounds was stored at -20°C prior to analysis.

For hydrolysable phenolic (HP), the residue EP was blended with methanol/$H_2SO_{4conc.}$ (10:1, v/v) in a volume of 20 mL, was rinsed in water bath (20 h, 85°C). Then, before applying the centrifuge (at 3500 g, 10 min, 4°C), the obtained mixture was left for a while at room temperature to decrease the temperature. The separated supernatants of HP compounds were kept at -20°C prior to analysis.

In vitro digestive enzymatic extraction assay was performed to evaluate the fraction of bio-accessible phenolics. This *in vitro* method mimics the gastrointestinal tract system (Vitali et al., 2009; Sahan et al.,

2019) with minor modifications. Briefly, the sample was treated with pure water and pepsin and the pH was adjusted to 2.0, using 5 mol/L HCl. The shaking water bath (37°C, 1h) was used for incubation. At the end of the period, to emulate the intestinal digestion, pH was adjusted neutral and bile/pancreatin mixture and NaCl/KCl were inserted to the sample and then the sample was shaked. And then, the sample was centrifuged to obtain the supernatant (3500g, 10 min).

2.4. Measurement of TPC

To determine the all TPC fractions, the Folin-Ciocalteu colorimetric assay was used, as described by Xu et al., (2009) with minor changes. After incubation for half an hour at 20-24°C, the absorbance value of the extracts was detected at UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 750 nm. The calibration curve drawn with gallic acid and methanol was used as a blank. The obtained data were stated as gallic acid equivalents per gram of *S. thirkei* C. Koch. TPC was computed as the total amount of extractable and hydrolysable fractions. The study was repeated three times for all extracts.

2.5. Determination of Anti-oxidant Activity

Due to the complexity form of plants and possible reactions between them, the anti-oxidant activity should not be detected just sole assay (Valadez-Carmona et al., 2016). Therefore, in this study, anti-oxidant activity of extractable, hydrolysable and bio-accessible fractions were evaluated using four distinctive methods ($ABTS^{\bullet+}$, CUPRAC, $DPPH^{\bullet}$ and FRAP). In present study, the spectrophotometric analysis was performed by using a UV-1800 spectrophotometer. To obtain more reliable results, all antioxidant activity tests were proceed in triple, and the obtained data were stated as µmol Trolox equivalent per 1.0 g dw of specimen. The mean values ± standard deviations were recorded.

$ABTS^{\bullet+}$ and CUPRAC anti-oxidant activities were conducted according to methodologies as described by Apak, Guclu, Ozyurek, & Karademir (2004) and Apak et al., (2007), respectively. Absorbance's were read at 734 nm for $ABTS^{\bullet+}$; at 450 nm for CUPRAC.

$DPPH^{\bullet}$ and FRAP assays were developed from Brand-Williams, Cavalier, & Berset (1995) and Benzie & Strain (2002) with minor differences, at 517 and 595 nm, respectively.

2.6. Statistical Analysis

In present study, to perform the statistical analyses, the JMP IN 7.0.0 software was used. All of the data obtained from three replicates and mean values were reported. The least significant difference was used to specify the different groups ($p \leq 0.05$).

3. Results and Discussion

The present research was reported for the first time bio-accessibility, TPC and anti-oxidant performance of *S. thirkei* C. Koch using four distinctive methods.

According to GC-MS analysis, as seen in Table 1, the principal compounds in *S. thirkei* were malic acid (27.703%), butanedioic acid (10.73%), palmitic acid (8.814%), propanedioic acid (8.616%), p-xylene (7.843%), m-dimethylbenzene (6.634%) and o-xylene (5.558%).

Table 1

Compounds of hydrolysable fractions of *S. thirkei*.

RT (min)	Compounds	Rate of Similarity (%)	Ratio of Distribution %
5.830	Furfural	49	0.317
5.882	Oxalic acid, dimethyl ester	56	2.069
6.110	Ethylbenzene	94	0.652
6.318	o-Xylene	93	5.558
6.463	p-Xylene	97	7.843
6.884	m-Dimethylbenzene	97	6.634
7.563	Propanedioic acid, dimethyl ester (Dimethyl malonate)	91	8.616
8.196	3,3-dimethoxy- Propanoic acid, methyl ester	83	0.268
8.378	Ethyl (E)-2-(hydroxymethyl)but-2-enoate	47	0.280
8.435	2-hydroxy-3-methyl-Pentanoic acid, methyl ester	56	1.045
8.772	Methyl fumarate	91	1.221
8.845	dl-Limonene	98	0.330
8.892	Butanedioic acid, dimethyl ester	90	10.738
9.006	3,5-dimethyl-4-[2-(pyrrolidin- 1-yl)ethyl]-2,5- Heptadien-4-ol	35	0.338
9.442	4,4-Dimethoxy-butanoic acid, methyl ester	59	0.602
9.561	Benzoic acid, methyl ester	70	0.274
9.857	dl-Malic acid, dimethyl ester	83	27.703
10.308	Benzeneacetic acid, methyl ester	95	1.292
10.381	(Z)-2-Dodecene	94	0.137
10.448	Dodecane	80	0.163
10.656	(2,2-diethoxyethyl)- Benzene	35	0.495
10.796	Allyl isovalerate	47	0.390
11.891	(E)-2-Tetradecene	95	0.286
12.259	Octanedioic acid, dimethyl ester (Dimethyl suberate)	91	0.172
12.420	3-Hydroxy-3-methoxycarbonyl-pentanedioic acid dimethyl ester	78	2.917
12.612	6,6-Dimethoxy-Octanoic acid, methyl ester	50	0.445
12.762	Di-t-butyl-phenol	90	0.214
12.980	Nonanedioic acid, dimethyl ester (Azelaic acid, dimethyl ester)	95	0.809
13.333	1-Hexadecene	98	0.209
13.385	1,1-dimethoxy- Octadecane (Stearaldehyde, dimethyl acetal)	50	0.559
13.821	Aromadendrene	95	0.183
13.961	.gamma.-Muurolene	87	0.211
14.293	cis-tetrahydro-2,5-Thiophenedicarboxylic acid, dimethyl ester	46	0.250
14.609	12-methyl-Tridecanoic acid, methyl ester (Methyl isomyristate)	96	0.417
15.725	Benzoic acid, hexyl ester	50	0.475
15.896	Benzoic acid, pentyl ester	50	0.402
16.327	L-Isoleucine	43	0.668
16.363	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	98	8.814
16.586	Phytol	70	0.183
16.768	2-Methyl-3-phenyl-1,2-propanediol	3	0.839
18.138	(Z,Z,Z)-9,12,15 -Octadecatrienoic acid, methyl ester	99	2.494
17.126	trichloro-Acetic acid, 3-phenylpropyl ester	64	0.135
17.723	2,7-dimethyl-2,6-octadien-4-ol	53	0.229
18.060	(Z,Z)-9,12-Octadecadienoic acid, methyl ester (Methyl linoleate)	99	1.240
18.407	Octadecanoic acid, methyl ester (Stearic acid, methyl ester)	99	0.882

The amount of extractable, hydrolysable, bio-accessible fraction and TPC of *S. thirkei* C. Koch were presented in Table 2.

Table 2

Different fractions of TPCs of *S. thirkei* C. Koch

Sample	Total Phenolic Contents				
	Extractable Phenolics*	**Hydrolysable Phenolics***	**Total Phenolic Content[a]***	**Bio-accessible Phenolics (μmol Trolox/g)**	**Phenolic Bio-accessibility[b] (%)**
S. thirkei	422.96±4.70	1538.99±4.57	1961.95±4.46	1766.72±2.96	90.05±1.50

[a] It was computed as the total of EPs and HPs.
[b] Bio-accessibility was computed as the percentage of TPC.
*mg of GAE/100g
Data are stated as X ± SD (n = 3)

According to the results, the level of HPs (1538.99±4.57 mg of GAE/100g) approximately three fold higher than EPs (422.96±4.70 mg of GAE/100g). The TPC of *S. thirkei* C. Koch was determined to be 1961.95±4.46 mg of GAE/100g. Besides, the bio-accessible fractions and phenolic bio-accessibility of *S. thirkei* C. Koch were found to be 1766.72±2.96 μmol Trolox/g and 90.05±1.50%, respectively.

Results showed that the bio-accessibility of TPC of *S. thirkei* C. Koch is quite high. The studies conducted with *S. thirkei* are limited in literature to compare the total phenolic contents and consist of antimutagenicity, content analyses and antimicrobial activity of different extracts against bacteria and yeast cultures (Goren et al., 2011; Askun et al., 2013; Tunali Erkan & Dulger 2016; Rasgele & Dulger, 2021). Therefore, all results were collated with the results of the researches conducted with former species belonging to the *Stachys* genus (Table 3). According to the results in the table, it can be observed that the TPC value is in the range of 16.59-1200.94. The TPC result obtained from our study was 1961.95 ± 4.46, which is quite higher than the results obtained from previous studies. This dissimilarity may be due to the extraction method, differences of species and the phytogeographic conditions in which the plant grows.

In our study, the anti-oxidant performance of *S. thirkei* C. Koch was determined using four methods, namely $ABTS^{\bullet+}$, CUPRAC, $DPPH^{\bullet}$ and FRAP assay. Antioxidant activity results obtained from *S. thirkei* C. Koch extracts were presented in Figure 1.

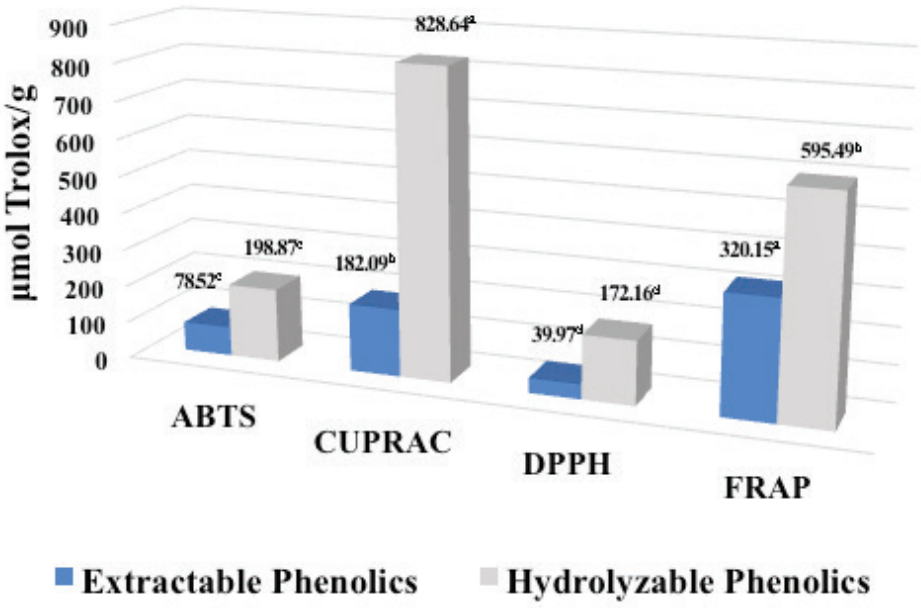

Figure 1. Antioxidant activity of *S. thirkei* C. Koch

Table 3

The TPC and TFC studies conducted with other species belonging to the Stachys species.

	Extract	Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)	References
S. byzantine	Methanol	18.64 mg/g dw	11.1 mg QE/ mg dw	Sytar, Hemmerich,, Zivcak, Rauh, & Brestic, 2018
S. lavandula		16.59 GAE/g dry matter (dm)	4.48 mg QE/g dm	Khoigani, Rajaei, & Goli, 2017
S. guyoniana	n-Butanol	354.91 mg/g	-	Ferhat et al., 2016
	Ethyl acetate	300.50 mg/g	-	
S. parviflora	Ultrasonic	20.89 mg GAE/g dm	6.22 mg QEs/g dm	Bashi et al., 2016
S. officinalis	Methanol	61.2 mg GAE/g dry ext	-	Šliumpaitė, Venskutonisa, Murkovic, & Ragažinskienė, 2013
	Acetone	82.3 mg GAE/g dry ext	-	
S. byzantine	Methanol	46.00 mg ChAE/100 g ext	-	Hajimehdipoor, Gohari, Ajani, & Saeidnia, 2014
S. iberica		44.01 µg GAEs/ mg ext	5.97 µg QEs/ mg ext	Tepe, Degerli, Arslan, Malatyali, & Sarikurkcu, 2011
S. pinardii		600.74 mg GAE/100 g dm	-	
S. cretica subsp. mersinaea	Methanol	1200.94 mg GAE/100 g dm	-	Ozkan, Gokturk, Unal, & Celik, 2006
S. aleurites		900.61 mg GAE/100 g dm	-	

In regard to all methods, the HPs and EPs was found to be statistically ($p \leq 0.05$) different. With the hydrolysis process, the bound phenolic compounds were also transformed into an extractable form. According to the results, HP fractions were higher than the EP fractions. The EPs of *S. thirkei* C. Koch ranged from 39.97±0.88 to 320.15±3.77 µmol Trolox/g. On the other side, the HPs of *S. thirkei* C. Koch changed between 172.16±9.78 to 828.64±1.45 µmol Trolox/g. The $ABTS^{\bullet+}$ anti-oxidant activity of EPs and HPs were found to be 78.52±1.99 and 198.87±1.25 µmol Trolox/g, respectively. The CUPRAC anti-oxidant activity values of the EPs and HPs were found to be 182.09±1.16 µmol Trolox/g and 828.64±1.45 µmol Trolox/g, respectively. The $DPPH^{\bullet}$ antioxidant activity of EPs was found to be 39.97±0.88 µmol Trolox/g, while that of HPs was found to be 172.16±9.78 µmol Trolox/g. The FRAP antioxidant activity of the EPs and HPs were found to be 320.15±3.77 µmol Trolox/g and 595.49±5.05 µmol Trolox/g, respectively. Due to the complexity of the composition of *S. thirkei* C. Koch and possible reactions between them, the antioxidant activity can be determined in different ratios in between the methods.

There is no antioxidant activity study conducted with *S. thirkei* C. Koch. However, there are lots of studies investigating the anti-oxidant activities of distinct *Stachys* species such as *S. annua* (Alpay et al., 2017), *S. sieboldii* (Yang et al., 2016), *S. glutinosa* (Leporini et al., 2015), *S. lavandulifolia* (Ghaffari, Ghassam, & Prakash, 2012), *S. ocymastrum* (Lakhal et al., 2011). Apart from these, Ferhat et al., (2016) detected the anti-oxidant activity of *S. guyoniana* with different extractions and various assays ($ABTS^{\bullet+}$, CUPRAC and $DPPH^{\bullet}$). The results of analyses were 29.08 (µg/mL) and 21.57 (µg/mL) for $ABTS^{\bullet+}$ assay which were extracted with n-butanol and ethyl acetate respectively; 0.15 (µg/mL) and 2.28 (µg/mL) for CUPRAC assay which were extracted with n-butanol and ethyl acetate respectively; 2.91(µg/mL) and 5.53 (µg/mL) for $DPPH^{\bullet}$ assay which were extracted with n-butanol and ethyl acetate respectively. Carvalho et al., (2015)

stated that *S. brizantina* was the greatest antioxidant activity in the ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ methods. Venditti et al., (2015) reported that in FRAP assay 48.9 (µmol trolox/g) anti-oxidant activity was found for *S. annua* subsp. *annua*. In the same study 178.4 (µmol trolox/g) anti-oxidant activity was found in ABTS$^{\bullet+}$ assay. Tepe et al., (2011) indicated that antioxidant activity of *S. iberica* was quantified as 13.57±0.17 for 0.2 mg/mL; 22.34±2.24 for 0.4 mg/mL; 46.63±0.81 for 1 mg/mL using DPPH$^{\bullet}$ method. Different ecological or geographical origin along with the genetic fractionation, harvest time, climate conditions and/or method of analysis can cause to be differences in chemical composition in plant species.

The antioxidative bio-accessibility of *S. thirkei* C. Koch was shown in Figure 2. As regard to the results, the ABTS$^{\bullet+}$ antioxidant activity of bio-accessible phenolics and bio-accessibility was found to be 389.43±3.26 µmol Trolox/g, 71.23%, respectively. Statistical analyses were applied between the methods. As regard to the results, for bio-accessible phenolics significantly ($p \leq 0.05$) highest results were found to be in FRAP method, followed by CUPRAC, ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ methods. According to the bio-accessibility (%) results, statistically significant ($p \leq 0.05$) differences were determined in CUPRAC method.

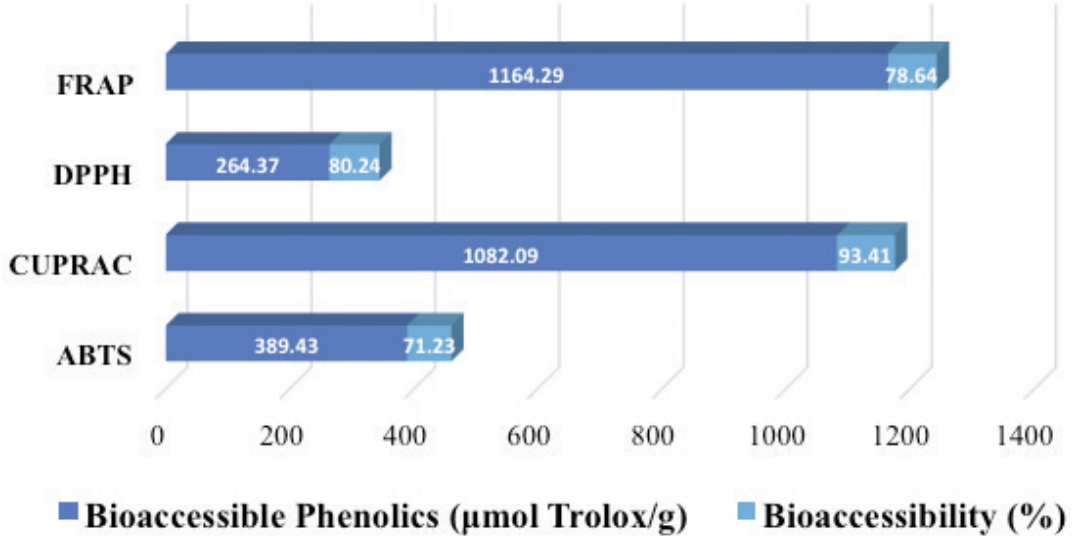

Figure 2. Antioxidative bio-accessibility of *S. thirkei* C. Koch

The CUPRAC antioxidant activity of bio-accessible phenolics and bio-accessibility was found to be 1082.09±1.16 µmol Trolox/g, 93.41%, respectively. The DPPH$^{\bullet}$ antioxidant activity of bio-accessible phenolics and bio-accessibility was found to be 264.37±2.85 µmol Trolox/g, 80.24%, respectively. The FRAP antioxidant activity of bio-accessible phenolics and bio-accessibility was found to be 1164.29±2.20 µmol Trolox/g, 78.64%, respectively.

According to the literature review, our study is the first investigation on the bio-accessibility of *S. thirkei* C. Koch. So, the results of the our research were collated with the results of the studies conducted with other genus belonging to the Lamiaceae. Labanca, Svelander, & Alminger, (2019) pointed that the bio-accessibility of the phenolic and flavonoid compounds of *Salvia hispanica* L. plant were 78.19% and 14.20%, respectively. Also Pellegrini et al., (2018) stated that the bio-accessibility of *Salvia hispanica* was greater in phenolic acids. Gayoso et al., (2018) informed that the bio-accessibility percentages were between 58-98% for *Melissa officinalis,* 36-107% for *Lavandula latifolia* and 41-93% for *Origanum vulgare.* Daly, Jiwan, Obrien, & Aherne, (2010) indicated that carotenoid bio-accessibilities of basil (*Ocimum basilicum*), mint (*Metha* L.) and sage (*Salvia officinalis*) were 6.6-21.3%, 4.8-8.7% and 19.0%. In the same study, the bioavailability of rosemary (*Rosmarinus officinalis*) was found to be 0%. The reasons for these differences between samples are thought to be differences in environmental conditions, climate and diversity of extraction processes (solvent type, plant material, solvent ratio).

4. Conclusion

In presented study the total phenolic compounds, anti-oxidant activity and bioaccessibility of *S. thirkei* C. Koch was determined with four different methods ($ABTS^{\bullet+}$, CUPRAC, $DPPH^{\bullet}$ and FRAP) due to the complexity form of it. Especially, the bio-accessibility and anti-oxidant effect of the extracts from *S. thirkei* C. Koch was reported for the first time with this study. Proximate analysis indicates that the hydrolysable phenolic determined higher than the extractable phenolic. Moreover, the bio-accessible fractions of *S. thirkei* C. Koch was found to be 1766.72µmol Trolox/g and phenolic bio-accessibility of *S. thirkei* C. Koch were found to be 90.05%. The findings of this results showed that *S. thirkei* C. Koch may be a good source of antioxidants due to the main components such as malic acid, butanedioic acid, and palmitic acid. The determined high phenolic compounds may be the evidence of the anti-oxidant activity. In other words, these obtained results may be potent scientific proof to utilise the *S. thirkei* C. Koch as a beneficial source of anti-oxidant patterns. As a result, it was concluded that the *S. thirkei* C. Koch is good source of bio-accessible total phenolic content and antioxidant materials that are noted to be beneficial in many aspects to health and can be used as an alternative supplementary foodstuff. However, further studies are still necessary to identify the *S. thirkei* C. Koch compounds contribute in these pharmacologic properties.

Author Contributions

Emine Aydin: Conceived and designed the analysis; collected data and performed the analysis; performed statistical analysis and wrote the paper; approved the version to be published.

Pinar Goc Rasgele: conceived and designed the analysis; performed the analysis performed statistical analysis and wrote the paper; revised the paper; approved the version to be published.

Gorkem Dulger: Conceived and designed the analysis; approved the version to be published.

Conflicts of Interest

The authors have no conflict of interest regarding the content of this paper.

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