





Phenolic profile, antioxidant and enzyme inhibitory activity of the ethyl acetate, methanol and water extracts of *Capparis spinosa* L.

Bulent Kirkan ^{1,*}, Olcay Ceylan ¹, Cengiz Sarikurkcu ¹, Bektas Tepe ¹

¹Suleyman Demirel University, Water Institute, TR-32260, Isparta, TURKEY

²Mugla Sitki Kocman University, Faculty of Science, TR-48000, Mugla, TURKEY

³Afyonkarahisar Health Sciences University, Faculty of Pharmacy, TR-03100, Afyonkarahisar, TURKEY

⁴Kilis 7 Aralik University, Faculty of Science and Literature, TR-79000, Kilis, Turkey

Abstract: In this study, it was aimed to determine the phytochemical compositions and biological activities of ethyl acetate (EtOAc), methanol (MeOH) and water extracts obtained from the aerial parts of *Capparis spinosa* L. As a result of spectrophotometric analyzes, MeOH extract was found to be richer in terms of both phenolics and flavonoids compared to other extracts [81.45 mg GAEs (gallic acid equivalent)/g and 36.57 mg RE (rutin equivalent)s/g, respectively], while chromatographic analyzes showed that the extract in question contains a significant amount of hesperidin (72927.48 µg/g), quercetin (1335.88 µg/g), hyperoside (1227.73 µg/g), and 4-hydroxybenzoic acid (924.08 µg/g). Phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging, Cupric Reducing Antioxidant Power (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) reducing and ferrous ion chelating activity tests resulted in superiority of MeOH extract [371.0, 44.93, 56.46, 91.77, 52.61 mg TEs (trolox equivalent)/g and 14.85 mg EDTAEs/g, respectively]. On the other hand, EtOAc extract exhibited higher activity than other extracts in acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-amylase, and α-glucosidase inhibitory activity tests [3.29, 2.12 mg GALAEs (galanthamine equivalent)/g, 541.01 and 1584.20 mg ACEs (acarbose equivalent)/g, respectively]. The tyrosinase inhibitory activity test resulted in the superiority of MeOH extract [41.90 mg KAEs (kojic acid equivalent)/g]. A strong correlation was determined between the phenolic and flavonoid contents of the extracts and their antioxidant activities.

ARTICLE HISTORY

Received: Aug. 10, 2021

Revised: Sept. 30, 2021

Accepted: Oct. 20, 2021

KEYWORDS

Capparis spinosa,
LC-ESI-MS/MS,
Antioxidant,
Enzyme inhibitory,
Chemical composition,

1. INTRODUCTION

Plants can be used in industries such as medicine, pharmacy, food, cosmetics, etc., due to their pharmacological/biologically active phytochemicals, and therefore, new plants are attracting the attention of researchers every day (Orphanides *et al.*, 2016). Researchers have revealed that many phytochemicals such as polyphenols, flavonoids, flavonoids, etc. can be used as critical functional compounds in the treatment of many metabolic diseases (Ng *et al.*, 2012). Since

*CONTACT: Bektas TEPE ✉ bektastepe@yahoo.com 📍 Kilis 7 Aralik University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, Kilis, Turkey

plants have low cost and are sustainable sources of phytochemicals, it is of great importance to use those with proven biological/pharmacological activities for industrial use as an alternative to synthetic compounds (Samy *et al.*, 2005; Ng *et al.*, 2020).

Foods deteriorate over time due to lipid oxidation and lose their quality (Yanishlieva & Marinova, 2001). The deterioration of foods with high lipid content can be delayed by the addition of compounds that prevent oxidation during processing. The most effective way to control lipid oxidation is to benefit from antioxidant compounds (Shahidi & Zhong, 2015). These compounds can be found widely in plants and animals, as well as chemically synthesized. Polyphenols and tocopherols are among the most potent antioxidant compounds and are abundant in many vegetables, fruits and grains. In addition, there are various reports of compounds with antioxidant effects in fish, algae and shellfish (Shahidi & Amarowicz, 1996; Amarowicz *et al.*, 1999; Athukorala *et al.*, 2003). In the last decades, researchers have focused on many plant species for the discovery of new and more effective natural compounds that can be used instead of synthetic antioxidants to extend the shelf life of foods. As a result of these studies, many antioxidant phytochemicals have been identified (Liyana-Pathirana *et al.*, 2006; Shahidi & Zhong, 2007; Cumby *et al.*, 2008).

Plants are of particular interest to researchers because they contain phytochemicals with enzyme inhibitory activity as well as antioxidant activities. In the enzyme inhibitory activity studies intensified in recent years, it has been reported that some plant species or some phytochemicals found in these species exhibit inhibitory activity such as cholinesterase (Hung *et al.*, 2008; Loizzo *et al.*, 2010; Pinho *et al.*, 2013), α -amylase/ α -glucosidase (Liu *et al.*, 2017; Rasouli *et al.*, 2017; Tan *et al.*, 2017), tyrosinase (Kubo & Kinoshita, 1999; Likhitwitayawuid, 2008; Maisuthisakul & Gordon, 2009), etc.

Capparis spinosa L. is an industrial plant species distributed in western and central Asia and along the Mediterranean coastline (Trombetta *et al.*, 2005; Rahimi *et al.*, 2020). This herb has been traditionally used by people for many years in the treatment of various diseases (gout, rheumatism, etc.) (Romeo *et al.*, 2007; Aliyazicioglu *et al.*, 2013; Zhang *et al.*, 2018). Local people living in countries bordering the Mediterranean coastline also frequently benefit from *C. spinosa*'s analgesic properties (Fu *et al.*, 2008). In studies conducted by researchers, it has been reported that the aerial parts, roots or seeds of the plant exhibit many biological/pharmacological activities (anti-allergic, immunomodulatory, anti-inflammatory, antimicrobial, anti-histaminic, antiviral, etc.) (Trombetta *et al.*, 2005; Tlili *et al.*, 2011; Kulisic-Bilusic *et al.*, 2012).

The aim of this study was to determine the chemical compositions of ethyl acetate (EtOAc), methanol (MeOH) and water extracts obtained from *C. spinosa* by qualitative and quantitative chromatographic methods, *in vitro* antioxidant and to document their inhibitory activities on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α -amylase, α -glucosidase, and tyrosinase.

2. MATERIAL and METHODS

2.1. Plant Material and Extract Preparation

Aerial parts of *C. spinosa* was collected from Camlibel village, Kavaklidere, Mugla-Turkey (780 m., 37° 24 849'N 28° 27 688'E) (Herbarium number: O.1196). Dr. Olcay Ceylan (Mugla Sitki Kocman University) performed the identification of the plant material. Aerial parts of the plants were used as the study material to obtain EtOAc, MeOH and water extracts [extract yields: 10.20, 11.31 and 21.58% (w/w), respectively]. Details of the extraction procedure can be found in supplementary file.

2.2. Determination of the Phenolic Compositions of the Extracts

Details of the spectrophotometric and chromatographic analysis were given in supplementary file (Zengin *et al.*, 2017; Cittan & Çelik, 2018).

2.3. Biological Activity

Details of the antioxidant (Apak *et al.*, 2006; Tepe *et al.*, 2011; Kocak *et al.*, 2016; Zengin *et al.*, 2017; Sarikurkcu *et al.*, 2020) and enzyme inhibitory activity (Ozer *et al.*, 2018) tests were given in supplementary file.

2.3. Statistical Analysis

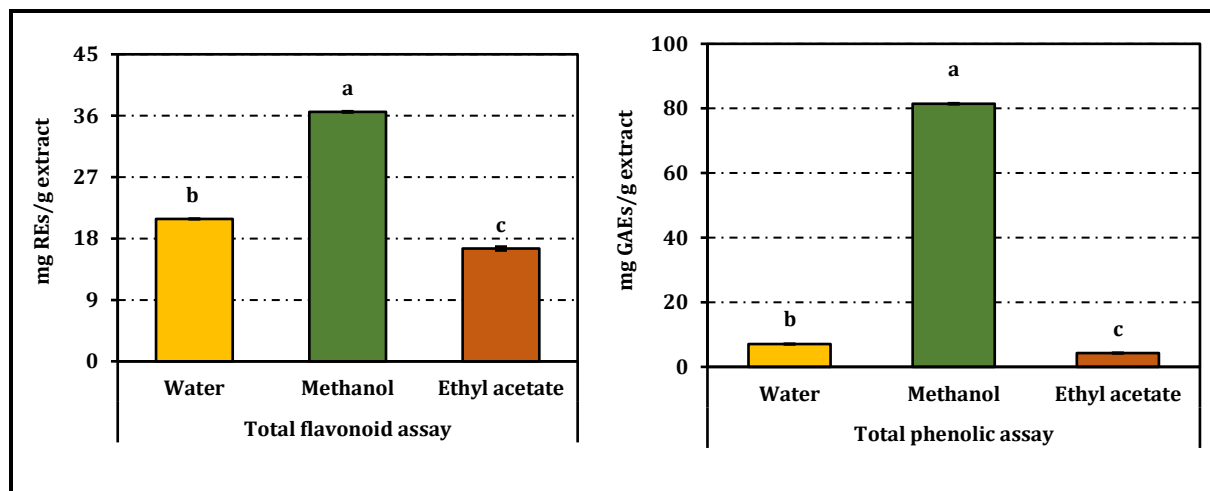
Details of the relative antioxidant capacity index (RACI) (Sun & Tanumihardjo, 2007) and statistical analysis can be found in the supplementary file.

3. RESULTS / FINDINGS

3.1. Chemical Composition

The total amounts of phenolic and flavonoid compounds of the extracts are given in Figure 1. According to the data obtained by spectrophotometric method, MeOH extract was found to be rich in both phenolics and flavonoids. The total amount of phenolic and flavonoid compounds of this extract was 81.45 mg GALAEs/g and 36.57 mg REs/g, respectively. Although the amounts of phenolic and flavonoid compounds of EtOAc and water extracts were close to each other, the chemical compositions of these extracts were statistically different from each other ($p < 0.05$).

Figure 1. Amounts of total flavonoids and phenolics in the extracts of *C. spinosa*. Different letters (a, b, c) on the bars show that the relevant data are statistically different from each other ($p < 0.05$).



The chemical composition data of the extracts obtained by chromatographic methods are given in Table 1. According to the data in the table, it was understood that none of the extracts contained (+)-catechin, luteolin 7-glucoside, apigenin 7-glucoside, pinoselin, kaempferol and luteolin. Chromatographic analyses showed that hesperidine was present in high amounts in the MeOH extract (72927.48 $\mu\text{g/g}$). Quercetin (1335.88 $\mu\text{g/g}$), hyperoside (1227.73 $\mu\text{g/g}$), 4-hydroxybenzoic acid (924.08 $\mu\text{g/g}$) were also found in the MeOH extract. In addition to these phytochemicals, *p*-coumaric acid was also found in high amounts in EtOAc and water extracts.

3.2. Antioxidant Activity

The total antioxidant activities, reducing powers, radical scavenging and chelating capacities of the extracts are given in Figure 2. On the figures, the statistical relationship between the antioxidant activities of the extracts and each other is also indicated with small letters.

In all antioxidant test systems presented in Figure 2, the MeOH extract exhibited significantly higher activity than the others. The activity value of this extract in phosphomolybdenum, DPPH and ABTS radical scavenging, CUPRAC and FRAP reducing and ferrous ion chelating assays were 371.0, 44.93, 56.46, 91.77, 52.61 mg TEs/g and 14.85 mg EDTAEs/g, respectively. In the radical scavenging and ferrous ion chelating activity tests, the MeOH extract was followed by the water extract (18.80, 35.36 mg TEs/g and 9.97 mg EDTAEs/g, respectively), while the EtOAc extract ranked second in the phosphomolybdenum and CUPRAC reducing assays (329.40 and 48.34 mg TEs/g, respectively). In the FRAP reducing assay, however, no statistically significant difference was found between the antioxidant activities of water and EtOAc extracts.

Table 1. Concentrations of selected phenolic compounds in the extracts of *C. spinosa* ($\mu\text{g/g}$ extract).

Compound	EtOAc	MeOH	Water
Gallic acid	3.84 \pm 0.02 ^b	8.46 \pm 0.40 ^a	3.80 \pm 0.10 ^b
Protocatechuic acid	646.52 \pm 0.37 ^a	179.25 \pm 2.86 ^b	13.36 \pm 0.29 ^c
3,4-Dihydroxyphenylacetic acid	13.36 \pm 0.43 ^a	14.28 \pm 0.37 ^a	14.76 \pm 0.19 ^a
Pyrocatechol	26.39 \pm 0.14 ^b	42.93 \pm 2.92 ^a	33.11 \pm 1.04 ^b
(+)-Catechin	nd	nd	nd
Chlorogenic acid	4.33 \pm 0.01 ^a	4.06 \pm 0.10 ^b	4.19 \pm 0.01 ^{ab}
(-)-Epicatechin	2.61 \pm 0.09 ^b	3.08 \pm 0.13 ^a	2.41 \pm 0.02 ^b
2,5-Dihydroxybenzoic acid	16.93 \pm 0.62 ^a	11.75 \pm 0.90 ^b	14.51 \pm 1.32 ^{ab}
4-Hydroxybenzoic acid	648.78 \pm 1.04 ^b	924.08 \pm 2.26 ^a	106.19 \pm 3.28 ^c
Vanillic acid	215.30 \pm 17.44 ^b	305.77 \pm 14.43 ^a	138.24 \pm 5.15 ^c
Caffeic acid	12.91 \pm 0.85 ^b	24.96 \pm 1.84 ^a	14.13 \pm 0.43 ^b
Syringic acid	9.74 \pm 0.06 ^b	53.98 \pm 6.66 ^{aa}	5.65 \pm 0.10 ^b
3-Hydroxybenzoic acid	10.64 \pm 0.60 ^a	11.74 \pm 0.77 ^a	11.39 \pm 0.23 ^a
Vanillin	7.85 \pm 0.22 ^c	19.42 \pm 2.16 ^b	31.79 \pm 1.21 ^a
Verbascoside	5.78 \pm 0.08 ^a	6.04 \pm 0.13 ^a	5.90 \pm 0.03 ^a
Taxifolin	7.11 \pm 0.16 ^c	15.60 \pm 0.11 ^a	8.77 \pm 0.26 ^b
<i>p</i> -Coumaric acid	144.54 \pm 0.56 ^b	693.59 \pm 10.15 ^a	34.32 \pm 2.24 ^c
Sinapic acid	4.97 \pm 0.13 ^c	33.80 \pm 0.14 ^a	6.06 \pm 0.04 ^b
Ferulic acid	71.80 \pm 1.21 ^b	176.20 \pm 4.65 ^a	13.39 \pm 2.38 ^c
Luteolin 7-glucoside	nd	nd	nd
Hyperoside	4.53 \pm 0.19 ^b	1227.73 \pm 16.22 ^a	9.32 \pm 0.29 ^b
Hesperidin	260.27 \pm 3.47 ^a	72927.48 \pm 659.21 ^b	155.34 \pm 1.26 ^a
Rosmarinic acid	16.83 \pm 1.01 ^b	28.82 \pm 0.63 ^a	25.49 \pm 1.89 ^a
Apigenin 7-glucoside	nd	nd	nd
2-Hydroxycinnamic acid	3.02 \pm 0.09 ^b	2.06 \pm 0.07 ^c	3.54 \pm 0.05 ^a
Eriodictyol	9.56 \pm 0.12 ^b	46.83 \pm 2.88 ^a	13.64 \pm 0.11 ^b
Pinoresinol	nd	nd	nd
Quercetin	5.42 \pm 0.15 ^c	1335.88 \pm 6.51 ^a	100.11 \pm 1.39 ^b
Kaempferol	nd	nd	nd
Luteolin	nd	nd	nd

Different letters (a, b, c) within the same row show that the relevant data are statistically different from each other ($p < 0.05$). nd: Not detected.

The relative antioxidant capacity index (RACI) data (Figure 3), in which extracts were compared with each other according to their activity potentials, taking into account all the activities obtained from the antioxidant activity tests, confirmed the data obtained from the antioxidant activity tests. According to the data presented in the figure, the MeOH extract ranked first with a RACI value of 1.20. It was followed by water and EtOAc extracts with RACI values of -0.55 and -0.62, respectively.

Figure 2. Antioxidative capacity of the extracts of *C. spinosa*. Different letters (a, b, c) on the bars show that the relevant data are statistically different from each other ($p < 0.05$).

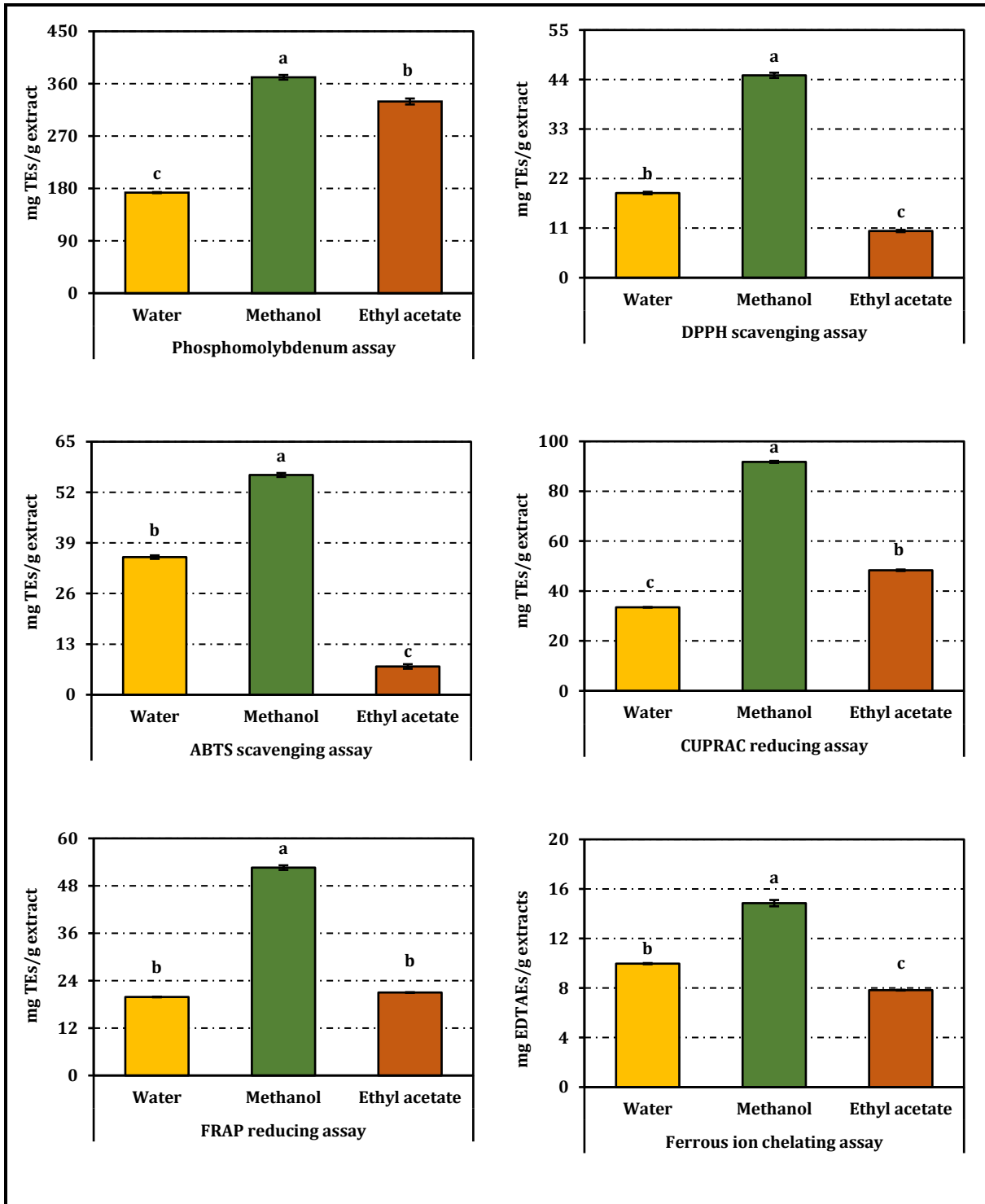
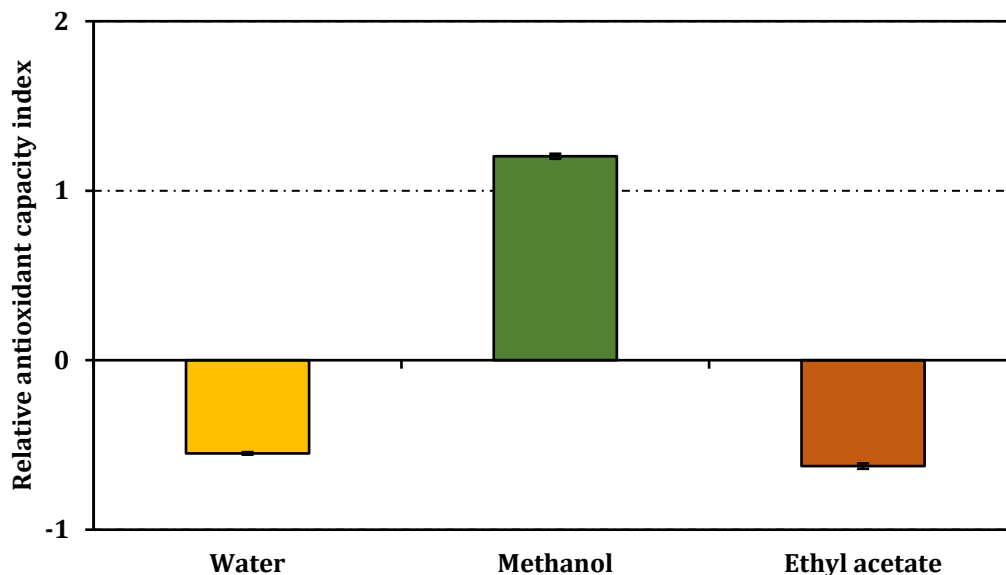


Figure 4 shows the correlation between the antioxidant activities of the extracts and their RACI values. A high correlation was found between the antioxidant activities of the extracts and the RACI values in all tests except the phosphomolybdenum assay. However, in the phosphomolybdenum test, the correlation between the total antioxidant activity of the EtOAc extract and the RACI value was found to be lower than in the other test systems.

Figure 3. RACI of the extracts of *C. spinosa*.



3.3. Enzyme Inhibitory Activity

Figure 5 shows the inhibitory activity potentials of *C. spinosa* extracts on AChE, BChE, α -amylase, α -glucosidase and tyrosinase.

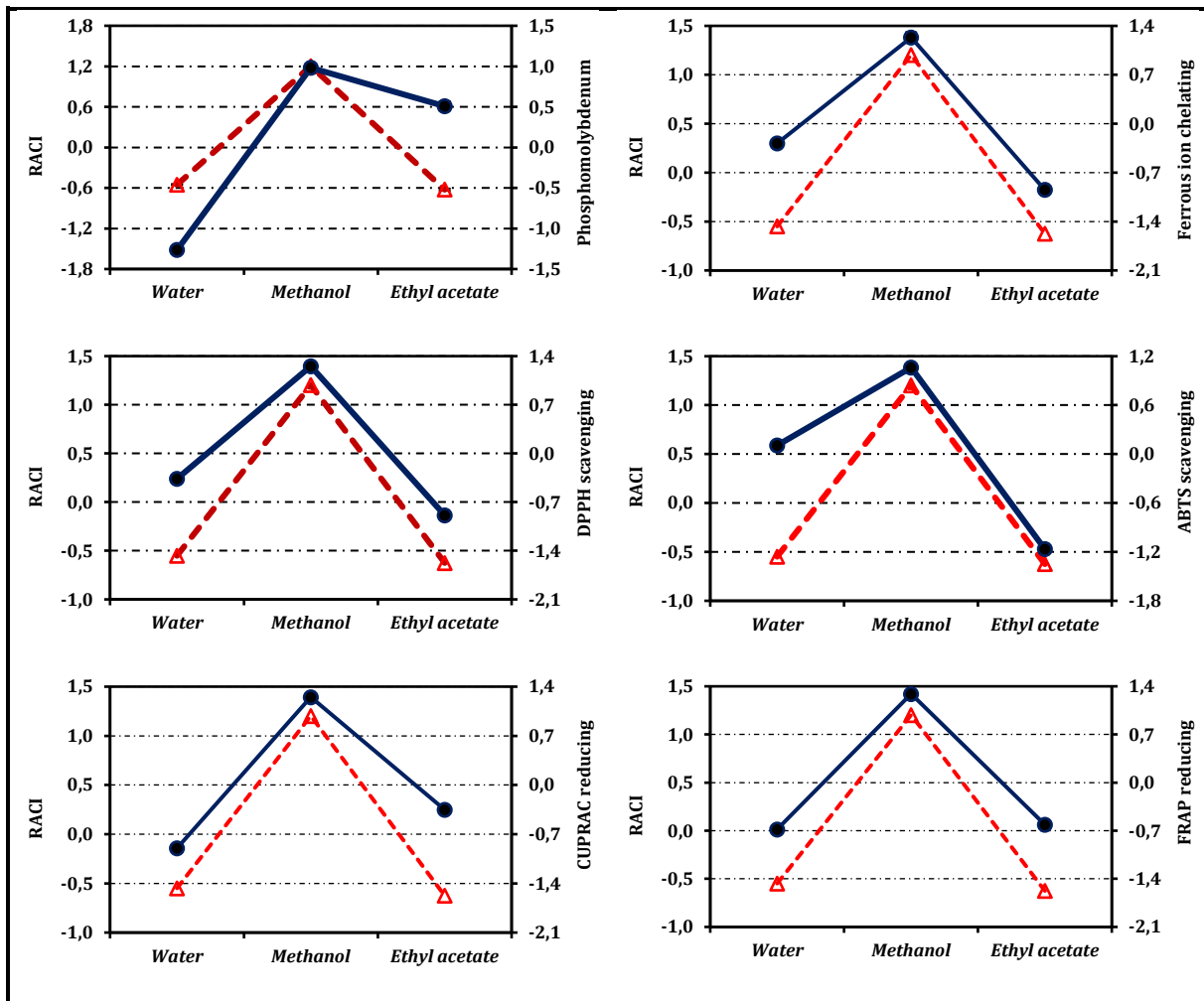
As can be seen from Figure 5, EtOAc extract exhibited higher inhibitory activity than the others in all test systems except the tyrosinase inhibitor activity test. The inhibitory activities of the extract in question in AChE, BChE, α -amylase and α -glucosidase tests were 3.29, 2.12 mg GALAEs/g, 541.01, and 1584.20 mg ACEs/g, respectively. The tyrosinase inhibitory activity test resulted in the superiority of MeOH extract (41.90 mg KAEs/g). In this assay, no statistical difference was found between the activity potentials of the water and EtOAc extracts. While water and MeOH extracts were not active in the BChE inhibitory activity tests, the water extract remained inactive in the α -glucosidase inhibitor activity assay.

3.4. Correlation Coefficients

Table 2 shows the correlation between the biological activity data of the extracts in the tests given above and their chemical compositions.

According to the correlation coefficients given in Table 2, there was a strong correlation between the phenolic and flavonoid contents of the extracts and their antioxidant activities (correlation coefficients were above 0.9). The relationship between these compounds and tyrosinase inhibitory activity was also found to be high. In addition, the relationship between protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, ferulic acid, hesperidin, hyperoside, and quercetin contents of the extracts and their antioxidant and tyrosinase inhibitor activities were also found statistically significant.

Figure 4. Antioxidant activity (solid dark blue line with circle) and RACI (dashed red line with triangle) of the extracts of *C. spinosa*.



4. DISCUSSION and CONCLUSION

There are some data on the chemical composition of *C. spinosa* in the literature. According to these data, the presence of some tannins, saponins, alkaloids and flavonoids has been detected in this plant so far (Anwar *et al.*, 2016; Snoussi *et al.*, 2017). It is of course possible to elaborate on these studies. However, it is seen that some phytochemicals specific to this species come to the fore in some studies. In a study by Fu *et al.* (2007), cappariloside A and stachydrin were found to be the main components, while in some other studies, it was reported that rutin, which is a flavonoid, is found in high amounts in the plant (Stefanucci *et al.*, 2018; Mollica *et al.*, 2019). A review by Anwar *et al.* (2016) documented the flavonoids, alkaloids and essential oil components identified so far in *C. spinosa*. However, none of these studies included the presence of hesperidine and hyperoside, which were identified as the main compounds in the current study. Therefore, the presence of these compounds in *C. spinosa* was brought to the literature for the first time with this study.

Figure 5. The capacity of the extracts of *C. spinosa*. to inhibit some enzymes. Different letters (a, b, c) on the bars show that the relevant data are statistically different from each other ($p < 0.05$).

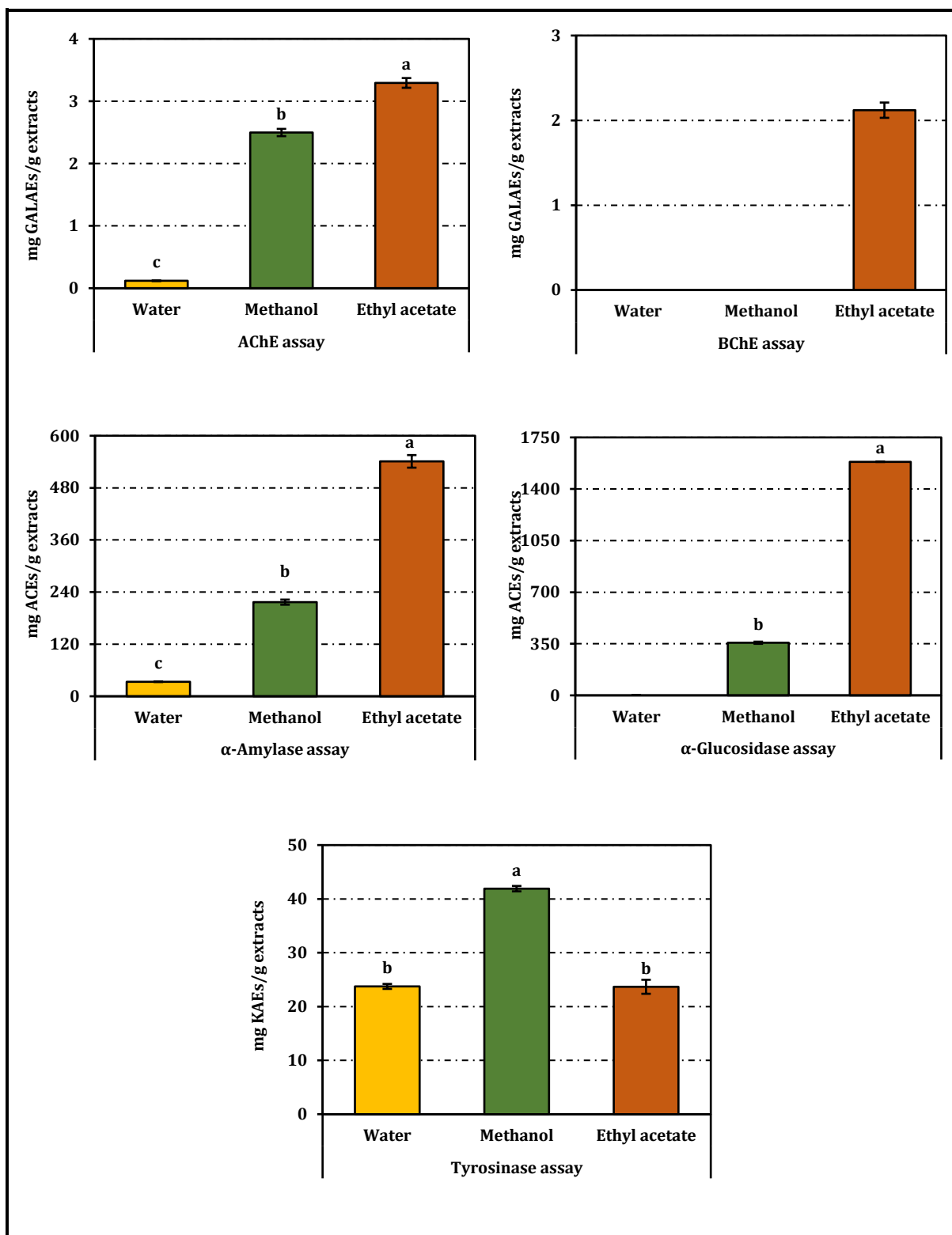


Table 2. Correlations among chemical composition and assays.

	22	1	2	3	4	5	7	8	9	10	11
1	0.986										
2	0.989	0.999									
3	0.990	0.999	0.999								
4	0.987	0.999	0.999	0.999							
5	0.985	0.999	0.999	0.999	0.999						
6	0.988	0.999	0.999	0.999	0.999	0.999					
7	-0.976	-0.992	-0.991	-0.993	-0.991	-0.989					
8	-0.993	-0.999	-0.999	-0.999	-0.999	-0.999	0.987				
9	0.991	0.997	0.998	0.997	0.998	0.998	-0.981	-0.999			
10	-0.974	-0.998	-0.996	-0.996	-0.997	-0.997	0.996	0.993	-0.989		
11	-0.989	-0.999	-0.999	-0.999	-0.999	-0.999	0.993	0.999	-0.997	0.997	
12	0.989	0.999	0.999	0.999	0.999	0.999	-0.991	-0.999	0.998	-0.996	-0.999
13	0.989	0.999	0.999	0.999	0.999	0.999	-0.993	-0.999	0.997	-0.997	-0.999
14	0.986	0.999	0.999	0.999	0.999	0.999	-0.991	-0.999	0.997	-0.997	-0.999
15	0.987	0.999	0.999	0.999	0.999	0.999	-0.993	-0.999	0.997	-0.997	-0.999
16	0.980	0.997	0.997	0.995	0.997	0.998	-0.979	-0.996	0.997	-0.992	-0.995
17	0.986	0.999	0.999	0.999	0.999	0.999	-0.992	-0.999	0.997	-0.997	-0.999
18	0.989	0.999	0.999	0.999	0.999	0.998	-0.996	-0.998	0.995	-0.997	-0.999
19	0.989	0.999	0.999	0.999	0.999	0.999	-0.993	-0.999	0.997	-0.997	-0.999
20	0.989	0.999	0.999	0.999	0.999	0.999	-0.993	-0.999	0.997	-0.997	-0.999
21	0.989	0.999	0.999	0.999	0.999	0.999	-0.993	-0.999	0.997	-0.997	-0.999

1: DPPH, 2: ABTS, 3: CUPRAC, 4: FRAP, 5: FICA, 6: RACI, 7: AChEIA, 8: BChEIA, 9: TIA, 10: AAIA, 11: AGIA, 12: Total flavonoid, 13: Total phenolic, 14: Protocatechuic acid, 15: 4-Hydroxybenzoic acid, 16: Vanillic acid, 17: p-Coumaric acid, 18: Ferulic acid, 19: Hesperidin, 20: Hyperoside, 21: Quercetin, 22: TAP

As detailed in Part 3, the MeOH extract of *C. spinosa* showed remarkable antioxidant activity. There are many studies on the antioxidant activity of *C. spinosa* in the literature (Nadaroglu *et al.*, 2009; Tlili *et al.*, 2017; Yu *et al.*, 2017; Al-Azawi *et al.*, 2018). In many of these studies, the plant species in question exhibited remarkable antioxidant and radical scavenging activity. Therefore, the data obtained from the present study confirm the literature data. Also, according to the literature data, quercetin (Selway, 1986; Rauha *et al.*, 2000; Guardia *et al.*, 2001; Williams *et al.*, 2004), 4-hydroxybenzoic acid (Duke *et al.*, 2003; Manuja *et al.*, 2013) and *p*-coumaric acid (Bonina *et al.*, 2002; Ahmad *et al.*, 2006) obtained from this plant may be phytochemicals responsible for the antioxidant activity. However, as mentioned above, the presence of hesperidin and hyperoside in this plant was brought to the literature for the first time with this study. There are some literature data on the contribution of these compounds to antioxidant activity with some other plants or with these compounds themselves (Ku *et al.*, 2014; Hao *et al.*, 2016; Yatao *et al.*, 2018; Gao *et al.*, 2019; He *et al.*, 2019; Kim *et al.*, 2019; Musa *et al.*, 2019; Aggarwal *et al.*, 2020; Huang *et al.*, 2020). These findings support the correlation coefficient data obtained from the present study.

There are some reports in the literature regarding the cholinesterase inhibitory activity of *C. spinosa*. In a study carried out by Mollica *et al.* (2019), cholinesterase inhibitory activities of extracts obtained from *C. spinosa* by different methods were investigated and it was reported that the highest activity was exhibited by the extract obtained by microwave extraction. In another study by Wojdylo *et al.* (2019), it was reported that extracts obtained from different developmental stages of *C. spinosa*, especially those rich in flavonols (quercetin, kaempferol, myricetin, and isorhamnetin derivatives), showed significant cholinesterase inhibitory activity. Similar findings were also reported by Mekinic *et al.* (2018).

In the current study, the EtOAc extract exhibited the highest cholinesterase inhibitory activity, and according to the data in Table 1, this extract contains high amounts of protocatechuic and 4-hydroxybenzoic acids. There are some reports in the literature that these compounds themselves or some extracts containing high amounts of these compounds exhibit significant cholinesterase inhibitory activity (Szwajgier & Borowiec, 2012; Ertas *et al.*, 2014; Zengin *et al.*, 2017). These reports corroborate the data from the present study.

According to literature data, *C. spinosa* is considered to be a remarkable anti-hyperglycemic agent, in addition to its biological activities given above. In a study by Mollica *et al.* (2017), it was reported that *C. spinosa* leaves or buds normalized biochemical parameters and reversed liver/lung damage in streptozocin-induced diabetic rats. The inhibitory activity of *C. spinosa* phytochemicals on α -amylase and α -glucosidase was also analyzed by *in silico* methods (Ogunwa *et al.*, 2017). In the aforementioned study, it was reported that naringin and rutin show high affinity for α -amylase and α -glucosidase. In the present study, EtOAc extract from *C. spinosa* exhibited the highest inhibitory activity on both enzymes. As can be seen from the data in Table 1, protocatechuic and 4-hydroxybenzoic acids are present in high amounts in this extract. Literature data indicate that both compounds may be responsible for the anti-diabetic activity of the extract (Saltan *et al.*, 2017; Alegbe *et al.*, 2019).

There are also some reports in the literature regarding the tyrosinase inhibitory activity of *C. spinosa*. It was determined that quercetin increased tyrosinase expression in B16 murine melanoma cells treated with *C. spinosa* extract at a concentration of 0.03% (w/v) (Matsuyama *et al.*, 2009). Similar findings have been reported in a different report of the same research group (Matsuyama *et al.*, 2009). In the current study, MeOH extract exhibited the highest tyrosinase inhibitory activity. According to the data in Table 1, it is thought that the main compounds of the MeOH extract contribute significantly to this activity. However, the presence of 1335.88 μ g/g quercetin in the MeOH extract creates a contradiction between the data obtained from the current study and the literature data. Therefore, biological activity-guided fractionation is needed to elucidate the compounds that contribute to the activity.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Bulent Kirkan: Investigation and Resources. **Olca Ceylan:** Resources. **Cengiz Sarikurkcu:** Methodology, Visualization, Software, Formal Analysis and Validation. **Bektas Tepe:** Investigation, Supervision and Writing -original draft.

Orcid

Bulent Kirkan  <https://orcid.org/0000-0003-3462-0681>

Olca Ceylan  <https://orcid.org/0000-0002-4371-2126>

Cengiz Sarikurkcu  <https://orcid.org/0000-0001-5094-2520>

Bektas Tepe  <https://orcid.org/0000-0001-8982-5188>

5. REFERENCES

Aggarwal, V., Tuli, H.S., Thakral, F., Singhal, P., Aggarwal, D., Srivastava, S., Pandey, A., Sak, K., Varol, M., Khan, M.A., & Sethi, G. (2020). Molecular mechanisms of action of hesperidin in cancer: Recent trends and advancements. *Experimental Biology and Medicine*, 245(5), 486-497.

- Ahmad, I., Aqil, F., & Owais, M. (2006). *Modern phytomedicine: Turning medicinal plants into drugs*: John Wiley & Sons.
- Al-Azawi, A.H., Ghaima, K.K., & Salih, H.H. (2018). Phytochemical, antibacterial and antioxidant activities of *Capparis spinosa* L. Cultivated in Iraq. *Bioscience Research*, 15(3), 2611-2618.
- Alegbe, E.O., Terali, K., Olofinson, K.A., Surgun, S., Ogbaga, C.C., & Ajiboye, T.O. (2019). Antidiabetic activity-guided isolation of gallic and protocatechuic acids from *Hibiscus sabdariffa* calyces. *Journal of Food Biochemistry*, 43(7).
- Aliyazicioglu, R., Eyupoglu, O.E., Sahin, H., Yildiz, O., & Baltas, N. (2013). Phenolic components, antioxidant activity, and mineral analysis of *Capparis spinosa* L. *African Journal of Biotechnology*, 12(47), 6643-6649.
- Amarowicz, R., Karamac, M., & Shahidi, F. (1999). Synergistic activity of capelin protein hydrolysates with synthetic antioxidants in a model system. *Journal of Food Lipids*, 6(4), 271-275.
- Anwar, F., Muhammad, G., Hussain, M.A., Zengin, G., Alkharfy, K.M., Ashraf, M., & Gilani, A.H. (2016). *Capparis spinosa* L.: A Plant with High Potential for Development of Functional Foods and Nutraceuticals/ Pharmaceuticals. *International Journal of Pharmacology*, 12(3), 201-219.
- Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., & Erçağ, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Sciences and Nutrition*, 57(5-6), 292-304.
- Athukorala, Y., Lee, K.W., Song, C., Ahn, C.B., Shin, T.S., Cha, Y.J., Shahidi, F., & Jeon, Y.J. (2003). Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *Journal of Food Lipids*, 10(3), 251-265.
- Bonina, F., Puglia, C., Ventura, D., Aquino, R., Tortora, S., Sacchi, A., Saija, A., Tomaino, A., Pellegrino, M.L., & de Capariis, P. (2002). In vitro antioxidant and in vivo photoprotective effects of a lyophilized extract of *Capparis spinosa* L. buds. *Journal of Cosmetic Science*, 53(6), 321-336.
- Cittan, M., & Çelik, A. (2018). Development and validation of an analytical methodology based on Liquid Chromatography–Electrospray Tandem Mass Spectrometry for the simultaneous determination of phenolic compounds in olive leaf extract. *Journal of Chromatographic Science*, 56(4), 336-343.
- Cumby, N., Zhong, Y., Naczek, M., & Shahidi, F. (2008). Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food Chemistry*, 109(1), 144-148.
- Duke, J.A., Bogenschutz-Godwin, M.J., duCellier, J., & Duke, P.-A. (2003). *CRC Handbook of Medicinal Spices*: Boca Raton: CRC Press.
- Ertas, A., Boga, M., Yilmaz, M.A., Yesil, Y., Hasimi, N., Kaya, M.S., Temel, H., & Kolak, U. (2014). Chemical Compositions by Using LC-MS/MS and GC-MS and Biological Activities of *Sedum sediforme* (Jacq.) Pau. *Journal of Agricultural and Food Chemistry*, 62(20), 4601-4609.
- Fu, X.P., Aisa, H.A., Abdurahim, M., Yili, A., Aripova, S.F., & Tashkhodzhaev, B. (2007). Chemical composition of *Capparis spinosa* fruit. *Chemistry of Natural Compounds*, 43(2), 181-183.
- Fu, X.P., Wu, T., Abdurahim, M., Su, Z., Hou, X.L., Aisa, H.A., & Wu, H. (2008). New spermidine alkaloids from *Capparis spinosa* roots. *Phytochemistry Letters*, 1(1), 59-62.
- Gao, Y.T., Fang, L.Y., Wang, X.X., Lan, R.N., Wang, M.Y., Du, G., Guan, W.Q., Liu, J.F., Brennan, M., Guo, H.X., Brennan, C., & Zhao, H. (2019). Antioxidant Activity Evaluation of Dietary Flavonoid Hyperoside Using *Saccharomyces cerevisiae* as a Model. *Molecules*, 24(4), 788.

- Guardia, T., Rotelli, A.E., Juarez, A.O., & Pelzer, L.E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *II Farmaco*, 56(9), 683-687.
- Hao, X.L., Kang, Y., Li, J.K., Li, Q.S., Liu, E.L., & Liu, X.X. (2016). Protective effects of hyperoside against H₂O₂-induced apoptosis in human umbilical vein endothelial cells. *Molecular Medicine Reports*, 14(1), 399-405.
- He, J.T., Li, H.Q., Li, G.F., & Yang, L. (2019). Hyperoside protects against cerebral ischemia-reperfusion injury by alleviating oxidative stress, inflammation and apoptosis in rats. *Biotechnology & Biotechnological Equipment*, 33(1), 798-806.
- Huang, J.Z., Tong, X., Zhang, L., Zhang, Y., Wang, L., Wang, D.G., Zhang, S.J., & Fan, H. (2020). Hyperoside Attenuates Bleomycin-Induced Pulmonary Fibrosis Development in Mice. *Frontiers in Pharmacology*, 11, 550955.
- Hung, T.M., Na, M., Dat, N.T., Ngoc, T.M., Youn, U., Kim, H.J., Min, B.-S., Lee, J., & Bae, K. (2008). Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. *Journal of Ethnopharmacology*, 119(1), 74-80.
- Kim, J., Wie, M.B., Ahn, M., Tanaka, A., Matsuda, H., & Shin, T. (2019). Benefits of hesperidin in central nervous system disorders: a review. *Anatomy & Cell Biology*, 52(4), 369-377.
- Kocak, M.S., Sarikurku, C., Cengiz, M., Kocak, S., Uren, M.C., & Tepe, B. (2016). *Salvia cadmica*: Phenolic composition and biological activity. *Industrial Crops and Products*, 85, 204-212.
- Ku, S.K., Kwak, S., Kwon, O.J., & Bae, J.S. (2014). Hyperoside Inhibits High-Glucose-Induced Vascular Inflammation In Vitro and In Vivo. *Inflammation*, 37(5), 1389-1400.
- Kubo, I., & Kinst-Hori, I. (1999). Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. *Journal of Agricultural and Food Chemistry*, 47(10), 4121-4125.
- Kulisic-Bilusic, T., Schmöller, I., Schnäbele, K., Siracusa, L., & Ruberto, G. (2012). The anticarcinogenic potential of essential oil and aqueous infusion from caper (*Capparis spinosa* L.). *Food Chemistry*, 132(1), 261-267.
- Likhitwitayawuid, K. (2008). Stilbenes with tyrosinase inhibitory activity. *Current Science*, 94(1), 44-52.
- Liu, S., Ai, Z., Qu, F., Chen, Y., & Ni, D. (2017). Effect of steeping temperature on antioxidant and inhibitory activities of green tea extracts against α -amylase, α -glucosidase and intestinal glucose uptake. *Food Chemistry*, 234, 168-173.
- Liyana-Pathirana, C., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as affected by pearling. *Journal of Agricultural and Food Chemistry*, 54(17), 6177-6184.
- Loizzo, M.R., Tundis, R., Conforti, F., Menichini, F., Bonesi, M., Nadjafi, F., Frega, N.G., & Menichini, F. (2010). *Salvia leriifolia* Benth (Lamiaceae) extract demonstrates in vitro antioxidant properties and cholinesterase inhibitory activity. *Nutrition Research*, 30(12), 823-830.
- Maisuthisakul, P., & Gordon, M.H. (2009). Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. *Food Chemistry*, 117(2), 332-341.
- Manuja, R., Sachdeva, S., Jain, A., & Chaudhary, J. (2013). A comprehensive review on biological activities of p-hydroxy benzoic acid and its derivatives. *International Journal of Pharmaceutical Sciences Review and Research*, 22(2), 109-115.
- Matsuyama, K., Villareal, M.O., El Omri, A., Han, J., Kchouk, M., & Isoda, H. (2009). Effect of Tunisian *Capparis spinosa* L. extract on melanogenesis in B16 murine melanoma cells. *Journal of Natural Medicines*, 63(4), 468-472.
- Mekinic, I.G., Simat, V., Ljubenkovic, I., Burcul, F., Grga, M., Mihajlovski, M., Loncar, R., Katalinic, V., & Skroza, D. (2018). Influence of the vegetation period on sea fennel,

- Crithmum maritimum* L. (Apiaceae), phenolic composition, antioxidant and anticholinesterase activities. *Industrial Crops and Products*, 124, 947-953.
- Mollica, A., Stefanucci, A., Macedonio, G., Locatelli, M., Luisi, G., Novellino, E., & Zengin, G. (2019). Chemical composition and biological activity of *Capparis spinosa* L. from Lipari Island. *South African Journal of Botany*, 120, 135-140.
- Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Mocan, A., Macedonio, G., Carradori, S., Onaolapo, O., Onaolapo, A., Adegoke, J., Olaniyan, M., Aktumsek, A., & Novellino, E. (2017). Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: In vivo and in vitro evaluation of its nutraceutical potential. *Journal of Functional Foods*, 35, 32-42.
- Musa, A.E., Omyan, G., Esmaily, F., & Shabeeb, D. (2019). Radioprotective Effect of Hesperidin: A Systematic Review. *Medicina-Lithuania*, 55(7).
- Nadaroglu, H., Demir, N., & Demir, Y. (2009). Antioxidant and Radical Scavenging Activities of Capsules of Caper (*Capparis spinosa*). *Asian Journal of Chemistry*, 21(7), 5123-5134.
- Ng, Z.X., Kuppusamy, U.R., Tajunisah, I., Fong, K.C.S., & Chua, K.H. (2012). Investigation of SLC2A1 26177A/G gene polymorphism via high resolution melting curve analysis in Malaysian patients with diabetic retinopathy. *Journal of Diabetes and its Complications*, 26(5), 388-392.
- Ng, Z.X., Yong, P.H., & Lim, S.Y. (2020). Customized drying treatments increased the extraction of phytochemicals and antioxidant activity from economically viable medicinal plants. *Industrial Crops and Products*, 155, 112815.
- Ogunwa, T.H., Adeyelu, T.T., & Fasimoye, R.Y. (2017). Exploring the molecular mechanism of interaction and inhibitory potential of *Capparis spinosa* L. phytoconstituents on diabetes-related targets. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 8(5), 237-248.
- Orphanides, A., Goulas, V., & Gekas, V. (2016). Drying technologies: vehicle to high-quality herbs. *Food Engineering Reviews*, 8(2), 164-180.
- Ozer, M.S., Kirkan, B., Sarikurkcu, C., Cengiz, M., Ceylan, O., Atilgan, N., & Tepe, B. (2018). *Onosma heterophyllum*: Phenolic composition, enzyme inhibitory and antioxidant activities. *Industrial Crops and Products*, 111, 179-184.
- Pinho, B.R., Ferreres, F., Valentão, P., & Andrade, P.B. (2013). Nature as a source of metabolites with cholinesterase-inhibitory activity: an approach to Alzheimer's disease treatment. *Journal of Pharmacy and Pharmacology*, 65(12), 1681-1700.
- Rahimi, V.B., Rajabian, A., Rajabi, H., Vosough, E.M., Mirkarimi, H.R., Hasanpour, M., Iranshahi, M., Rakhshandeh, H., & Askari, V.R. (2020). The effects of hydro-ethanolic extract of *Capparis spinosa* (*C. spinosa*) on lipopolysaccharide (LPS)-induced inflammation and cognitive impairment: Evidence from in vivo and in vitro studies. *Journal of Ethnopharmacology*, 256, 112706.
- Rasouli, H., Hosseini-Ghazvini, S.M.-B., Adibi, H., & Khodarahmi, R. (2017). Differential α -amylase/ α -glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food & Function*, 8(5), 1942-1954.
- Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., & Vuorela, P. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal Of Food Microbiology*, 56(1), 3-12.
- Romeo, V., Ziino, M., Giuffrida, D., Condurso, C., & Verzera, A. (2007). Flavour profile of capers (*Capparis spinosa* L.) from the Eolian Archipelago by HS-SPME/GC-MS. *Food Chemistry*, 101(3), 1272-1278.

- Saltan, F.Z., Okutucu, B., Canbay, H.S., & Ozel, D. (2017). In vitro alpha-Glucosidase and alpha-Amylase Enzyme Inhibitory Effects in *Elaeagnus angustifolia* Leaves Extracts. *Eurasian Journal of Analytical Chemistry*, 12(2), 117-126.
- Samy, J., Sugumaran, M., Lee, K.L.W., & Wong, K.M. (2005). *Herbs of Malaysia : an introduction to the medicinal, culinary, aromatic and cosmetic use of herbs*. Shah Alam, Selangor: Times Editions.
- Sarikurku, C., Locatelli, M., Mocan, A., Zengin, G., & Kirkan, B. (2020). Phenolic Profile and Bioactivities of *Sideritis perfoliata* L.: The Plant, Its Most Active Extract, and Its Broad Biological Properties. *Frontiers in Pharmacology*, 10, 1642.
- Selway, J.W.T. (1986). Antiviral activity of flavones and flavans. *Progress in Clinical and Biological Research*, 213, 521-536.
- Shahidi, F., & Amarowicz, R. (1996). Antioxidant activity of protein hydrolyzates from aquatic species. *Journal of the American Oil Chemists' Society*, 73(9), 1197-1199.
- Shahidi, F., & Zhong, Y. (2007). Measurement of Antioxidant Activity in Food and Biological Systems *Antioxidant Measurement and Applications* (Vol. 956, pp. 36-66): American Chemical Society.
- Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757-781.
- Snoussi, M., Najett, M., Boumediene, M., & Abdelallah, M. (2017). In-vitro and In-vivo antifungal activity of *Capparis spinosa* against eight storage molds, a causal agent of wheat alteration. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 8(6), 13-18.
- Stefanucci, A., Zengin, G., Locatelli, M., Macedonio, G., Wang, C.K., Novellino, E., Mahomoodally, M.F., & Mollica, A. (2018). Impact of different geographical locations on varying profile of bioactives and associated functionalities of caper (*Capparis spinosa* L.). *Food and Chemical Toxicology*, 118, 181-189.
- Sun, T., & Tanumihardjo, S.A. (2007). An integrated approach to evaluate food antioxidant capacity. *Journal of Food Science*, 72(9), R159-R165.
- Szwajgier, D., & Borowiec, K. (2012). Phenolic acids from malt are efficient acetylcholinesterase and butyrylcholinesterase inhibitors. *Journal of the Institute of Brewing*, 118(1), 40-48.
- Tan, Y., Chang, S.K.C., & Zhang, Y. (2017). Comparison of α -amylase, α -glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry*, 214, 259-268.
- Tepe, B., Sarikurku, C., Berk, S., Alim, A., & Akpulat, H.A. (2011). Chemical composition, radical scavenging and antimicrobial activity of the essential oils of *Thymus boveii* and *Thymus hyemalis*. *Records of Natural Products*, 5(3), 208-220.
- Tlili, N., Elfalleh, W., Saadaoui, E., Khaldi, A., Triki, S., & Nasri, N. (2011). The caper (*Capparis* L.): Ethnopharmacology, phytochemical and pharmacological properties. *Fitoterapia*, 82(2), 93-101.
- Tlili, N., Feriani, A., Saadoui, E., Nasri, N., & Khaldi, A. (2017). *Capparis spinosa* leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomedicine & Pharmacotherapy*, 87, 171-179.
- Trombetta, D., Occhiuto, F., Perri, D., Puglia, C., Santagati, N.A., Pasquale, A.D., Saija, A., & Bonina, F. (2005). Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds. *Phytotherapy Research*, 19(1), 29-33.
- Williams, R.J., Spencer, J.P.E., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medicine*, 36(7), 838-849.

- Wojdylo, A., Nowicka, P., Grimalt, M., Legua, P., Almansa, M.S., Amoros, A., Carbonell-Barrachina, A.A., & Hernandez, F. (2019). Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds. *Plants-Basel*, 8(12).
- Yanishlieva, N.V., & Marinova, E.M. (2001). Stabilisation of edible oils with natural antioxidants. *European Journal of Lipid Science and Technology*, 103(11), 752-767.
- Yatao, X., Saeed, M., Kamboh, A.A., Arain, M.A., Ahmad, F., Suheryani, I., Abd El-Hack, M.E., Alagawany, M., Shah, Q.A., & Chao, S. (2018). The potentially beneficial effects of supplementation with hesperidin in poultry diets. *Worlds Poultry Science Journal*, 74(2), 265-276.
- Yu, L., Yang, J.H., Wang, X., Jiang, B., Sun, Y.X., & Ji, Y.B. (2017). Antioxidant and antitumor activities of *Capparis spinosa* L. and the related mechanisms. *Oncology Reports*, 37(1), 357-367.
- Zengin, G., Uren, M.C., Kocak, M.S., Gungor, H., Locatelli, M., Aktumsek, A., & Sarikurkcu, C. (2017). Antioxidant and Enzyme Inhibitory Activities of Extracts from Wild Mushroom Species from Turkey. *International Journal of Medicinal Mushrooms*, 19(4), 327-336.
- Zhang, H., Lei, Z., Tian, R., & Wang, Z. (2018). Polyamidoamine starburst dendrimer-activated chromatography paper-based assay for sensitive detection of telomerase activity. *Talanta*, 178, 116-121.