



in vitro Antioxidant Activity and Total Phenolic Content of Various Extracts of *Satureja hortensis* L. Collected from Turkey

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The antioxidant capacities of the acetone, ethanol and water extracts of leaves and flowers of *S. hortensis* were estimated using different antioxidant tests, including lipid peroxidation, reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, iron chelation, superoxide anion radical scavenging and hydrogen peroxide scavenging. The contents of antioxidant components in these extracts were also determined. Results showed that the highest contents of these components including phenols and flavonoids were found in water extract. This study verified that the water extract has strong antioxidant activities which were correlated with its high level of phenolics and flavonoids. This extract can be used as a source of potential antioxidant or functional food material.

Key Words: *Satureja hortensis*, Antioxidant, Phenol, Flavonoid, DPPH.

INTRODUCTION

Free radicals have been claimed to play an important role in affecting human health by causing several diseases including cancer, hypertension, heart attack and diabetes. Dietary intake of phenolic compounds correlates with reduced coronary heart disease, cancer mortality and protective in many health-related properties, such as antioxidant, anticancer, antiviral and antiinflammatory activities¹⁻³. Plants and their products are rich sources of phytochemicals and have been found to possess a variety of biological activities including antioxidant potential^{4,5}.

Satureja hortensis L. contains high levels of carvacrol, paracymene, ascorbic acid, tannin, borneol, monoterpene, diterpene, sesquiterpene and essential oils. It is an herbaceous perennial plant of the Lamiaceae family and a grassy plant with pink flowers and small green to yellow seeds, which grows in Trakya region of Turkey and South Europe. *Satureja hortensis* is generally known under the names "çibrika or geyikotu" and is widely used as spice in Turkey. *S. hortensis* herbs are used to treat stomach ache in Turkish folk medicine. In addition, this herb is used as diuretic, savory and for quinsy. Furthermore, it had an analgesic effect.

This study can help in food industry as a natural compound for antioxidant activity, which might be used as an alternative to synthetic antioxidants since it is environmentally friendly and safe for consumption.

EXPERIMENTAL

Linoleic acid, α -tocopherol, potassium persulfate, nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, pyrocatechin, gallic acid and 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Ammonium thiocyanate, ferrous chloride, polyoxy-ethylenesorbitan monolaurate (Tween-20), trichloroacetic acid (TCA), ethanol (EtOH) and acetone were purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma-Aldrich or Merck.

Fresh leaves and flowers of *S. hortensis* were purchased from a traditional market at Edirne City (Edirne, Turkey). Plant materials were washed with distilled water and dried at room temperature. The dried plant was stored in -20 °C until used.

Preparation of extracts: For water extraction, dried plant (25 g) was put into a fine powder in a mill and was mixed with 500 mL boiling water by magnetic stirrer for 15 min. Then, the extract was filtered over Whatman No. 1 paper. The filtrates were frozen and lyophilized in lyophilizator at 5 μ m Hg pressure at -50 °C (Labconco, Freezone 1 L). Similarly, dried plant (25 g) was put into a fine powder in a mill and was mixed with 500 mL acetone or ethanol. The residue was re-extracted

until extraction solvent became colourless. Each of the extracts obtained were dried using a rotary evaporator (Buchi R-200, Switzerland). The dry extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dries weight of plant material. All the extracts were kept at -20 °C and were dissolved in water or solvent before use.

Determination of total phenolic compounds: Total phenolics in *S. hortensis* extracts were determined with Folin-Ciocalteu reagent, according to the method of Slinkard and Singleton⁶. Briefly, 1 mL of the *S. hortensis* extracts (20-100 µg/mL) was transferred into test tubes and their volumes made up to 46 mL with distilled water. After addition of 1 mL Folin-Ciocalteu reagent and 3 mL 2 % Na₂CO₃ solution, tubes were vortexed and then allowed to stand for 2 h intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (Shimadzu UV-1601, Japan). The total phenolic compounds in the *S. hortensis* extracts were calculated as mg of catechol equivalent from the calibration curve and as mg catechol equivalents per g of extracts. The data were presented as the average of triplicate analyzes.

Determination of total flavonoid content: Total flavonoid content was determined by using a method described by Wang *et al.*⁷ with minor modification using catechin as standard flavonoid compound. Briefly, 10 mL of each extract (1000 µg/mL) or (+)-catechin standard solution (20-100 µg/mL) was mixed with 1 mL of a 5 % sodium nitrite solution. After 6 min, 1 mL of a 10 % aluminium nitrate solution was added and the mixture was allowed to stand for a further 6 min before 10 mL of a 4.3 % NaOH was added. The mixture was brought to 25 mL with distilled water and mixed well. After 15 min, the absorbance was measured at 510 nm using a spectrophotometer. Results were expressed as the average of triplicates. The results were expressed as the mean (± SD) mg of (+)-catechin equivalents per g of extract.

DPPH radical scavenging activity: The DPPH (1,1-diphenyl-2-picrylhydrazil) radical scavenging activity of the *S. hortensis* extracts was measured according to the procedure described by Shimada *et al.*⁸. Briefly, 0.1 mM solution of DPPH[•] in ethanol was prepared and 1 mL of this solution was added to 3 mL of *S. hortensis* extracts at different concentrations (20-100 µg/mL). The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 0.5 h. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm against ethanol. All measurements were made in triplicate and averaged. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

Reducing power: The reducing power of the *S. hortensis* extracts was determined according to the method described by Oyaizu⁹. Different amounts of extracts (20-100 µg) in 1 mL of distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer (pH = 6.6) and 2.5 mL potassium ferricyanide (1 %) and then the mixture was incubated at 50 °C for 0.5 h. After-

wards, 2.5 mL of trichloro acetic acid (10 %) was added to the mixture to stop the reaction, then the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL FeCl₃ (0.1 %) and then absorbance was measured at 700 nm in a spectrophotometer. The reducing power of the tested samples increased with the absorbance values.

Iron(II) chelation activity: Ferrous ion (Fe²⁺) chelation by *S. hortensis* extracts was estimated by the method of Dastmalchi *et al.*¹⁰. Briefly, extracts (20-100 µg/mL) were added to 0.05 mL of 2 mM FeCl₂ solution. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe²⁺ complex formation was calculated using the formula given below:

$$\text{Metal chelating activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

H₂O₂ scavenging activity: The hydrogen peroxide scavenging ability of *S. hortensis* extracts was determined according to the method of Ruch *et al.*¹¹. 40 mM H₂O₂ solution was prepared in phosphate buffer (pH = 7.4) and 0.6 mL of this solution was added to extracts (20-100 µg/mL) in distilled water. The absorbance value of the reaction mixture was recorded at 230 nm. The percentage of H₂O₂ scavenging of extracts and standard compounds was calculated as:

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

Superoxide anion radical scavenging activity: Measurement of superoxide anion scavenging activity of *S. hortensis* extracts was based on the method described by Liu *et al.*¹² with slight modification. Briefly, the superoxide radicals were generated in 3 mL of 16 mM Tris-HCl buffer (pH = 8) containing 1 mL of 50 µM NBT solution, 1 mL of 78 µM NADH solution and sample solution of *S. hortensis* extracts (20-100 µg/mL) in water. The reaction was started by adding of 1 mL of 10 µM phenazine methosulphate solution to the mixture. The reaction mixture was incubated at 25 °C for 5 min and then absorbance was measured at 560 nm. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

Inhibition of lipid peroxidation: The total antioxidant activity of *S. hortensis* extracts was determined according to the thiocyanate method¹³. For stock solutions, 10 mg of each *S. hortensis* extracts was dissolved in 10 mL water (or solvent).

Then, the solution, which contains the same amount of *S. hortensis* extracts or standard samples (60 µg/mL) in 2.5 mL of 0.04 M potassium phosphate buffer (pH = 7) was added to 2.5 mL of linoleic acid emulsion in 0.04 M potassium phosphate buffer (pH = 7). Fifty millilitres linoleic acid emulsion contained 175 µg Tween-20, 155 µL linoleic acid and 0.04 M potassium phosphate buffer (pH = 7). On the other hand, 5 mL control was composed of 2.5 mL linoleic acid emulsion and 2.5 mL of 0.04 M potassium phosphate buffer (pH = 7). The mixed solution (5 mL) was incubated at 37 °C in a glass flask. The peroxide level was determined by reading the absorbance at 500 nm. This step was repeated every 10 h until the control reached its maximum absorbance value. The per cent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Statistical analysis: Results were expressed as mean \pm standard deviation of triplicate analyses. The correlation coefficient (r^2) between the parameters tested was established by regression analysis.

RESULTS AND DISCUSSION

Extract yield (amount of total extractable compounds) and contents of total phenolics and flavonoids: Water, EtOH and acetone extracts prepared from 25 g of *S. hortensis* gave a yield of 0.185, 0.048 and 0.036 g extractable compounds (EC) per gram of DW, respectively (Table-1). Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Polyphenols are marker of the nutritional quality of foods. Polyphenols are known for their antioxidant activity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular disease, other pathologies¹⁴. Plants containing high phenolic compounds can be a good source of antioxidants. For this reason, this information has led to the determination of the total phenolic content of the sample under study.

TABLE-1
TOTAL EXTRACTABLE COMPOUNDS (EC), TOTAL PHENOLIC COMPOUNDS (AS CATECHOL EQUIVALENTS) AND TOTAL FLAVONOIDS (AS CATECHIN EQUIVALENTS) IN WATER, ETHANOL AND ACETONE EXTRACTS OF *S. hortensis*

Extract	EC ^a (g/g DW)	Total phenols (mg catechol/g extract)	Total flavonoids (mg catechin/g extract)
Water	0.185	183 \pm 0.67	45.5 \pm 0.24
Ethanol	0.048	116 \pm 1.85	31.7 \pm 2.6
Acetone	0.036	83 \pm 1.24	41.7 \pm 1.8

Values were the means of three replicates \pm standard deviation.
^aExtractable compounds; DW = Dry weight.

Extracts contained high amounts of phenolic compounds. The content of total phenolic compounds is given in Table-1. The mean of the total phenolic content per gram of crude extract of water extract (183 \pm 0.67 mg catechol equivalent/g extract) was found to be higher than that of the EtOH (116 \pm

1.85 mg catechol equivalent/g extract) and acetone (83 \pm 1.24 mg catechol equivalent/g extract) extracts. Thus, it was concluded that water was more efficient at extracting phenolic compounds from the plant than was ethanol and acetone.

Flavonoids are natural phenolic compounds and well known antioxidants. Therefore dietary intake of flavonoid-containing foods was suggested to be of beneficial for the preservation from free radical damage. The concentration of flavonoids in the extracts was expressed as mg of catechol equivalents per g of the extract (Table-1). In our investigation, the water extract of *S. hortensis* exhibited the highest total flavonoids content (45.5 \pm 0.24 mg catechin equivalent/g extract), whereas the contents obtained with EtOH (31.7 \pm 2.6 mg catechin equivalent/g extract) or acetone (41.7 \pm 1.8 mg catechin equivalent/g extract) extracts were smaller. These amounts were comparable with results described in the literature for other extracts of plant products^{15,16}.

DPPH radical scavenging activity: Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance¹⁷. The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in relatively short time as compared to other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The DPPH radical scavenging activities of all the extracts of *S. hortensis* increased with increasing concentration. With regard to the *S. hortensis* extracts, Fig. 1 illustrates that the sequence for DPPH radical-scavenging ability was EtOH extract > ascorbic acid \approx BHA > BHT > water extract > acetone extract > α -tocopherol. At 60 µg/mL, the scavenging abilities on DPPH radicals were 65.36 \pm 1.65, 63.39 \pm 1.48, 59.48 \pm 5.93, 52.94 \pm 2.26, 49.67 \pm 1.69, 44.44 \pm 3.17 and 35.29 \pm 1.53 % for the BHA, ascorbic acid, EtOH, acetone and water extracts, α -tocopherol and BHT, respectively. Though the DPPH radical scavenging activities of extracts were less than those of ascorbic acid and BHA, the study revealed that extracts have free radical scavengers or inhibitors, acting possibly as primary antioxidants. It was reported that phenolics and flavonoids reduced DPPH radical by their hydrogen donating ability¹⁸.

Table-2 shows that the EC₅₀ values of DPPH radicals scavenging for the acetone, EtOH and water extracts were 33.57 \pm 1.19, 16.97 \pm 2.31 and 15.94 \pm 1.62 µg/mL, respectively.

Reducing power: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity¹⁹. Fig. 2 shows, the extent of the reduction, in terms of absorbance values at 700 nm. The reducing power of water extract (0.176 \pm 0.003), EtOH extract (0.110 \pm 0.009) and acetone extract (0.086 \pm 0.004) was concentration dependent and was found to be below those of ascorbic acid (0.552 \pm 0.077) and BHA (0.184 \pm 0.002) at 100 µg/mL. Extracts showed higher reducing power than tocopherol (0.061 \pm 0.005) and BHT (0.04 \pm 0.003) at the same concentration. Reducing power of extracts and standards decreased in order of ascorbic acid > BHA > water extract > EtOH extract > acetone extract

TABLE-2
EC₅₀ VALUES OF DPPH RADICAL-QUENCHING ACTIVITY, REDUCING POWER, CHELATING CAPACITY, HYDROGEN PEROXIDE AND SUPEROXIDE RADICALS OF *S. hortensis* EXTRACTS

Plant extracts	EC ₅₀ (µg/mL)				
	Scavenging ability on DPPH radicals	Scavenging ability on superoxide	Scavenging ability on hydrogen peroxide	Reducing power	Chelating power
Water	15.94 ± 1.62	11.37 ± 1.66	13.81 ± 4.28	42.63 ± 3.26	14.03 ± 1.03
Acetone	33.57 ± 1.19	40.09 ± 0.17	24.46 ± 3.51	29.28 ± 3.15	24.46 ± 1.92
Ethanol	16.97 ± 2.31	58.56 ± 1.04	18.77 ± 1.74	32.09 ± 1.64	18.35 ± 2.44
BHA	10.43 ± 0.17	12.66 ± 0.04	12.09 ± 1.12	40.02 ± 0.03	14.93 ± 0.25
BHT	56.71 ± 1.82	11.51 ± 2.41	12.05 ± 2.13	15.54 ± 1.17	20.14 ± 1.09
α-Tocopherol	25.02 ± 4.26	19.85 ± 1.74	11.99 ± 3.26	14.96 ± 0.52	16.01 ± 0.06
Ascorbic acid	11.21 ± 0.29	40.29 ± 0.03	12.07 ± 0.05	46.91 ± 2.84	17.09 ± 0.7

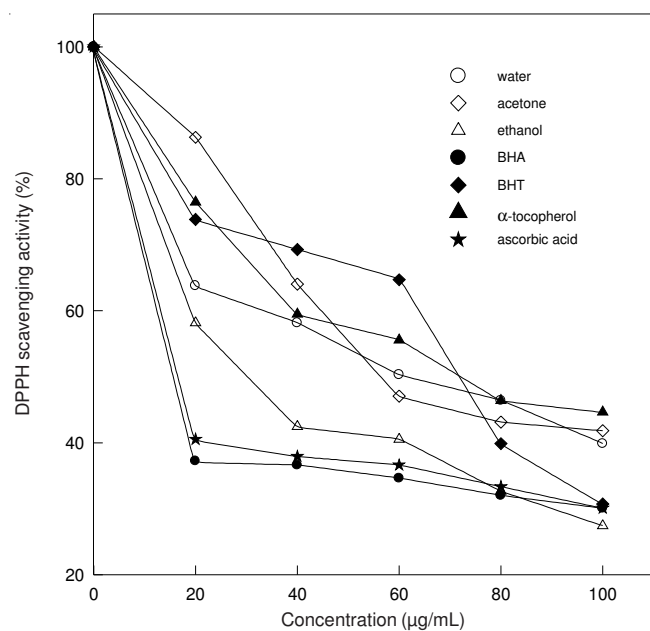


Fig. 1. DPPH radical scavenging activity of *S. hortensis* extracts. α-Tocopherol, ascorbic acid, BHA and BHT were used as reference antioxidants. Values are means ± SD (n = 3)

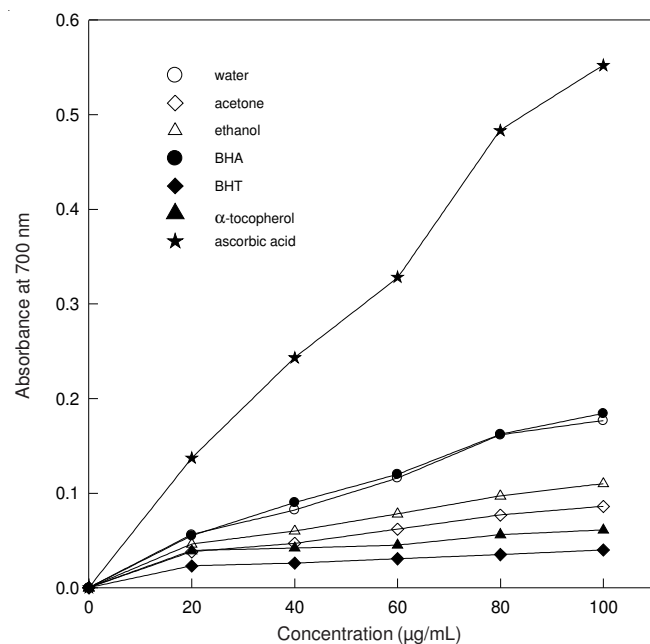


Fig. 2. Reducing power of the *S. hortensis* extracts. α-Tocopherol, ascorbic acid, BHA and BHT were used as reference antioxidants. Values are means ± SD (n = 3)

> α-tocopherol > BHT. The results demonstrate the electron donor properties of *S. hortensis* extracts for neutralizing free radicals by forming stable products. The results were in accordance with Prasad *et al.*¹⁶. They reported that the antioxidant activity of *Cinnamomum* species may involve other mechanisms in addition to those of reductones.

Table-2 shows that the EC₅₀ values of reducing power for the acetone, EtOH and water extracts were 29.28 ± 3.15, 32.09 ± 1.64 and 42.63 ± 3.26 µg/mL, respectively.

Iron(II) chelation activity: An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydrogen peroxide decomposition and Fenton-type reactions. Therefore, it was considered of importance to screen the iron(II) chelating ability of extracts²⁰. All the extracts had high levels of ferrous ion chelating activity (Fig. 3). Water, EtOH, acetone extracts, ascorbic acid, α-tocopherol, BHA and BHT chelated ferrous ions by 93.09 ± 1.14, 90.65 ± 0.33, 91.46 ± 1.63, 91.46 ± 0.06, 87.39 ± 0.37, 90.65 ± 0.12 and 89.02 ± 1.29 % at 100 µg/mL, respectively. The sequence for chelating power was water extract > acetone extract ≈ ascorbic acid > EtOH extract ≈ BHA > BHT > α-tocopherol. The iron chelating data measured at different concentrations (20-100 µg/mL) suggested that ferrous ion chelating effects of all the extracts of *S. hortensis* would be somewhat beneficial to protect against oxidative damage. Similarly, Ozsoy *et al.*²¹, reported that water, methanol and ethyl acetate extracts of Amaranth vegetable showed excellent chelating ability.

From the estimated EC₅₀ values, defined as the concentration of extract required to chelate 50 % of the available iron(II), it can be seen that the most effective iron(II) chelating extract was water (14.03 ± 1.03 µg/mL), followed by the EtOH (18.35 ± 2.44 µg/mL) and acetone extracts (24.46 ± 1.92 µg/mL), in decreasing order. The acetone extract showed less chelation activity than BHA (14.93 ± 0.25), BHT (20.14 ± 1.09), α-tocopherol (16.01 ± 0.06) and ascorbic acid (17.09 ± 0.7).

Hydrogen peroxide radical scavenging activity: Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells, since it may give rise to hydroxyl radicals inside the cell²². Extracts from *S. hortensis* were capable of scavenging hydrogen peroxide in a concentration-dependent manner (20-100 µg/mL). The hydrogen peroxide scavenging activities of water extract (78.89 ± 1.3 %), EtOH extract (81.77 ± 3.19 %) and acetone extract (66.22 ± 0.04 %) were lower than that of BHA (88.87 ± 0.07 %), ascorbic acid

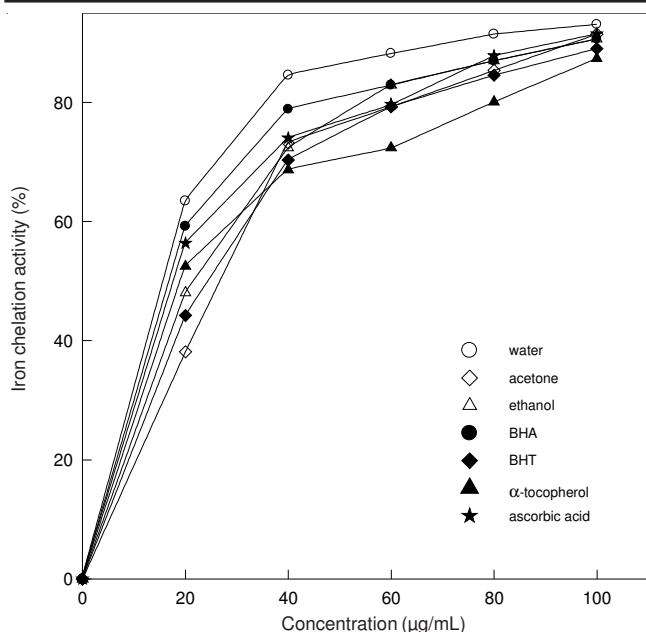


Fig. 3. Metal chelating effect of different concentrations of *S. hortensis* extracts. α -Tocopherol, ascorbic acid, BHA and BHT were used as reference antioxidants. Values are means \pm SD (n = 3)

(88.68 ± 4.57 %), α -tocopherol (88.29 ± 3.42 %) and BHT (86.76 ± 2.42 %) at $100 \mu\text{g/mL}$ (Fig. 4). The scavenging abilities on hydrogen peroxide were in descending order of BHA \approx ascorbic acid \approx α -tocopherol > BHT > EtOH extract > water extract > acetone extract.

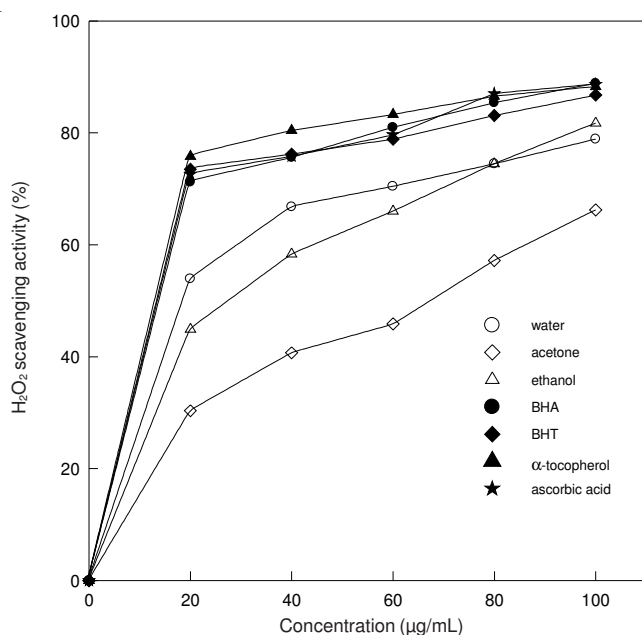


Fig. 4. Hydrogen peroxide scavenging activity of *S. hortensis* extracts. α -Tocopherol, ascorbic acid, BHA and BHT were used as reference antioxidants. Values are means \pm SD (n = 3)

The concentrations of acetone, EtOH and water extracts needed for 50 % inhibition (EC_{50}) were found to be 24.46 ± 3.51 , 18.77 ± 1.74 and $13.81 \pm 4.28 \mu\text{g/mL}$, respectively, whereas $11.99 \pm 3.26 \mu\text{g/mL}$ was needed for α -tocopherol.

Superoxide radical scavenging activity: Superoxide is a reactive oxygen species, which can cause damage to the cells

and DNA leading to various diseases. It was, therefore, proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical²³. *S. hortensis* extracts exhibited excellent superoxide anion scavenging activity. At $100 \mu\text{g/mL}$, the superoxide scavenging activities of water extract, EtOH extract, acetone extract, BHA, BHT, tocopherol and ascorbic acid were 90.83 ± 2.35 , 61.11 ± 0.08 , 80.56 ± 1.96 , 97.22 ± 3.41 , 97.22 ± 0.14 , 84.72 ± 3.12 and 81.94 ± 0.43 , respectively, with BHA \approx BHT > water extract > α -tocopherol > ascorbic acid > acetone extract > EtOH extract (Fig. 5). It was reported that the superoxide anion scavenging activity could be due to the action of a free hydroxyl group of phenolic compounds. Furthermore, flavonoid molecule with polyhydroxylated substitution on ring A or B and a free 3-hydroxyl substitution could present the superoxide scavenging activity²⁴.

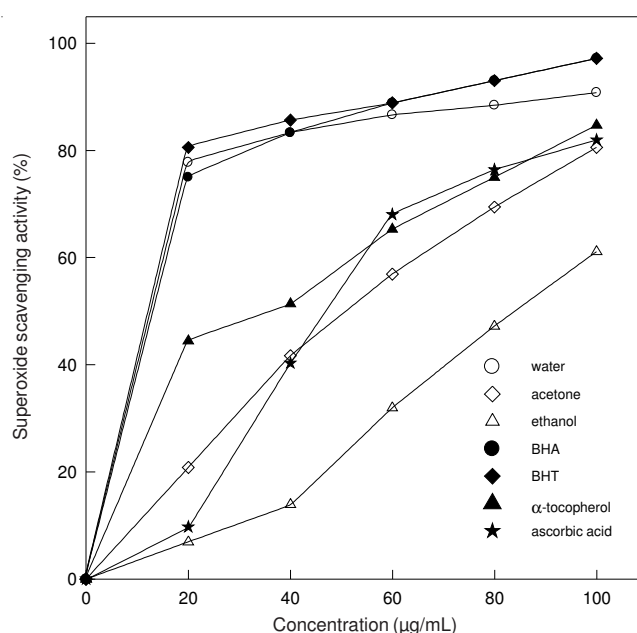


Fig. 5. Superoxide anion radical scavenging activity of *S. hortensis* extracts. α -tocopherol, ascorbic acid, BHA and BHT were used as reference antioxidants. Values are means \pm SD (n = 3)

EC_{50} values of acetone, EtOH and water extracts on superoxide radical scavenging activity were found to be 40.09 ± 0.17 , 58.56 ± 1.04 and $11.37 \pm 1.66 \mu\text{g/mL}$, respectively, whereas the EC_{50} value of α -tocopherol was found to be $19.85 \pm 1.74 \mu\text{g/mL}$ (Table-2).

Activity determination by ferric thiocyanate method:

The ferric thiocyanate method measures the amount of peroxide, which is the primary product of oxidation produced during the initial stages of oxidation. All the extracts of *S. hortensis* showed a significant total antioxidant activity. The total antioxidant activity of water extract (46.7 %), EtOH extract (56.4 %) and acetone extract (54 %) at $60 \mu\text{g/mL}$ was higher than those of α -tocopherol (29 %) and ascorbic acid (30.5 %). The sequence for total antioxidant activity was EtOH extract > acetone extract > water extract > ascorbic acid > α -tocopherol (Fig. 6). The total antioxidant capacity of plant extract may be attributed to their chemical composition and phenolic acid content.

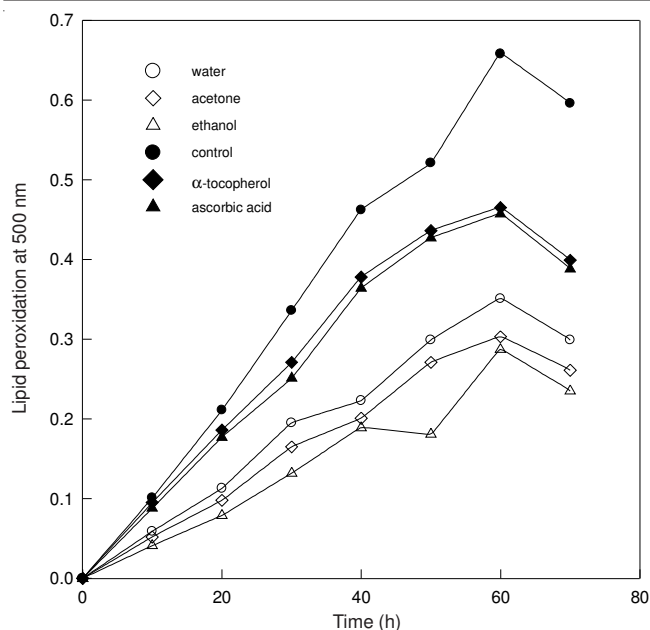


Fig. 6. Inhibition (%) of lipid peroxidation of *S. hortensis* extracts. Ascorbic acid and α -tocopherol were used as reference antioxidants

Conclusion

Water could extract the highest concentration of antioxidant compounds (polyphenols and flavonoids) from *S. hortensis* in this investigation; this extract also showed higher antioxidant activity than those extracted by ethanol or acetone. These results reveal that *S. hortensis* flowers and leaves, generally regarded as a disposable byproduct, has potential for exploitation to promote human and animal health.

Abbreviations

NADH, nicotinamide adenine dinucleotide; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NBT, nitroblue tetrazolium; PMS, phenazine methosulfate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine; Tween-20, polyoxyethylenesorbitan monolaurate; TCA, trichloroacetic acid; EtOH, ethanol.

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