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Phenolic content, antioxidant potentials of *Saponaria prostrata* endemic plant

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ABSTRACT
Saponaria prostrata (S. prostrata) is an endemic and medicinal plant that contains secondary metabolites such as flavonoids, phenolic compounds, fatty acids, and triterpenoids. This study was carried out to evaluate the antioxidant potentials, and phenolic composition of S. prostrata. Antioxidant properties of the ethanol and water extracts of S. prostrata were evaluated by three different in vitro bioanalytical methods including CUPRAC and FRAP reducing antioxidant methods and DPPH radical scavenging antioxidant method. Effective antioxidant potentials of the plant extracts were found especially in the CUPRAC method. Rutin (36.3 µg/g extract) and hesperidin (32.7 µg/g extract) were characterized as major phenolic compounds of S. prostrata using an advanced HPLC technique.

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Introduction

Traditional medicine is a major part of human healthcare in many parts of the world including developed countries [1]. Due to the resistance and the side effects of the microorganisms against antibiotics, researchers have been focused on plant-derived bioactive compounds used in herbal medicine [2, 3]. The researchers have focussed on plant phytochemicals to find new ways in the treatments of some diseases including cataract treatment [4], cancer metastasis [5], and metabolic inflammation [6]. Some phytochemicals such as hesperidin [7], astragalus polysaccharides [8], and allicin [9] are the key factors in cancer metastasis. Phenolic and flavonoid compounds can be used in the new drug development process[10]. Therefore, the high majority of plants have been recognized as having commercial values [11].

Saponaria prostrata WILLD subsp. *prostrata* (*S. prostrata*) is an endemic and medicinal plant in the flora of Turkey [12] that belongs to *Caryophyllaceae* family [13]. This plant species might be annual, biennial, or perennial plants that branched with

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decumbent prostrate or ascending branches with spreading white hairs [14]. *S. prostrata* contains secondary metabolites such as flavonoids, phenolic compounds, fatty acids, triterpenoids, different saponins, and some other natural sources [15]. These main components are known for their medicinal properties [16]. Phenolic compounds have various biological benefits and have been related to antioxidant properties [17]. Flavonoids have been noted as strength free radical scavengers and demonstrated antioxidant activities [18]. They have preventive effects against the metal chelating properties, hydrogen donor functions, and reducing activities [19]. Due to their natures, green plants, fruits, and vegetables are often being used as primary natural antioxidant sources. The phytochemicals are accepted to reduce the risks of brain dysfunction, cardiovascular diseases, cataracts, and cancers [20]. Compare to the synthetics, consumption of natural antioxidants is better for human health because synthetics have numerous carcinogenic effects. Recently, due to the undesired effects, there is a remarkable request for new and safe natural antioxidants sources [21]. The human body involves both enzymatic and non-enzymatic antioxidant systems, that regulate the balance between antioxidants and reactive oxygen species (ROS) [22]. In this research, we reported phenolic composition and the antioxidant properties of *S. prostrata*.

Experimental

Chemicals

Reagent we used in antioxidant and HPLC methods were obtained from Sigma-Aldrich and Merck.

Identification and collection of the plant material

S. prostrata plant were collected from rocky slopes of Haserek mountain at 1800-1850 m altitude from Bingol, Turkey by Dr. Ömer Kılıç (herbarium number: 4764). according to the “The Flora of Turkey and East Aegean Islands, Volume 5” the taxonomic description was made [23].

Plant extraction

Extraction and purification of active plant ingredients materials demand a proper process [24]. *S. prostrata* plant were extracted considering our previous study[25]. For this purpose, 10 gram air-dried powdered-plant leaves were mixed with 100 mL ethanol and distilled water (1/10:w/v) homogenized with a magnetic stirrer (about 24 hours),

and filtered using filter papers. Using lyophilizer (Labconco, Freezone 1L) the frozen water solvent was lyophilized at -50 °C and 5 mm Hg. Using a rotary evaporator (Heidolph 94200, Bioblock Scientific) the ethanol solvent was evaporated. All of the samples were stored at -30 °C.

Antioxidant activity

FRAP assay

By the reduction of Fe^{3+} to Fe^{2+}, the reducing antioxidant effect of *S. prostrata* was determined as described previously [26]. Potassium ferricyanide (1%), phosphate buffer (0.2 M, pH 6.6), and $FeCl_3$ (0.1%) solutions were mixed. 10-30 μg/mL concentrations of extracts of the ethanol and water were added. Acidified with trichloroacetic acid (10%). Incubation was done at 50 ^oC for 20 min. The absorbance was measured at 700 nm.

CUPRAC assay

Cupric ions (Cu^{2+}) reducing ability of S. prostrata plant extract was carried out considering a well-known previous study. [27]. Acetate buffer (1.0 M), ethanolic neocuproine (7.5 mM), and $CuCl_2$ solution (10 mM) were mixed. 10-30 μg/mL of concentrations of the ethanol and water were added to mixture completed with with distilled water (2 ml). Then, the test tubes Incubation was done for 30 min at room condition. Absorbance was calculated at 450 nm.

DPPH assay

$DPPH^{\bullet}$ scavenging effect of *S. prostrata* was detected considering previously described work [28]. For this, 0.5 mL, 0.1 mM of DPPH solution and 1.5 mL of sample ethanol and water solution (10-30 μg/mL) mixed. Mixture left in dark for 30 min. Then absorbance was recorded at 517 nm.

HPLC assay

The phenolic composition of *S. prostrata* plant was analyzed by an advanced HPLC technique. Initially, plant extract was prepared to run on HPLC instrument. In a flask, 10 mg of S. prostrata extract was dissolved adding 1 mL of acetonitrile-water (v/v, 50:50) solution and homogenized 3 min with vortex. To separate and quantify the phenolic composition of the S. prostrata plant sample HPLC instrument was used. Each standard compound was prepared at a 10 mg/mL concentration and used for the standard graphs. To prepare stock solutions, acetonitrile and acetic acid (1%) were

mixed (1:9), then methanol was added to the mixture (1:1). For gradient elution, 1% acetic acid was used as solvent A and 100 % acetonitrile as solvent B [29].

Results and Discussion

Antioxidant activity

There are many different *in vitro* methods in the literature for the determination of antioxidant potentials of plants. These methods are commonly used to calculate food and plant antioxidant properties [30]. In the present study, we used DPPH assay to determine the radical scavenging activities of the extracts. By donating hydrogen, antioxidant substances reduce radicals and antioxidant scavenging abilities can be measure using spectrophotometric methods [31]. The radical scavenging level of samples demonstrates their antioxidant potential.

DPPH radical scavenging capacities of *S. prostrata* extracts were compared to the standard antioxidants. According to the DPPH method, the water extract of *S. prostrata* did not show significant scavenging activity on DPPH free radicals. However, the ethanol extract of *S. prostrata* showed considerable free radicals scavenging activity when compared to the standards used. It was observed in Figure 1, the free radical scavenging properties of the plant extracts and standards used increased with their concentration amounts. DPPH scavenging percentages of extracts and standards at 30 µg/mL concentration ordered as: water extract (2.2±0.2) < ethanol extract (26.3±4.0 %) < BHT (48.9±4.9 %) < BHA (85.2±5.9 %) < ascorbic acid (88.9±5.7 %). Also, the IC_{50} values of extracts and standards were found as; 108.6±9.7 µg/mL for water extract 36.8±14.6 µg/mL for ethanol extract, 11.5±5.2 µg/mL for BHA, 19.9±8.3 µg/mL for BHT, and 11.1±5.2 µg/mL for ascorbic acid.

The reducing power antioxidant activities of *S. prostrata* and standards were evaluated by both FRAP and CUPRAC reducing methods.

FRAP assay is a prevalent way to evaluate the reducing antioxidant ability. The antioxidant substance can change ferric (Fe^{3+}) ions into ferrous (Fe^{2+}). As seen in Figure 1, the reducing ability of the extracts was lower when compared with the standard reducing levels. The ethanol extract showed higher activity than the water extract. At certain concentrations, the reducing antioxidant capacities decreased as BHA > ascorbic acid > BHT > *S. prostrata* ethanol > *S. prostrata* water.

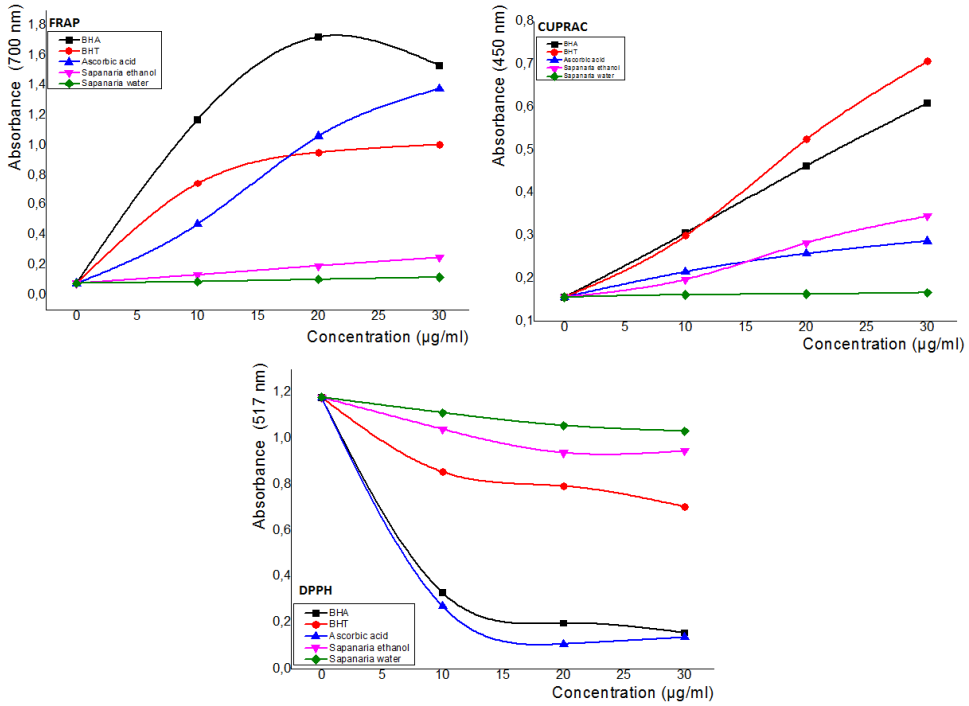

Fig 1 Antioxidant activity of *S. prostrata* and standards by using FRAP, CUPRAC, and DPPH methods

Cupric reducing the potential of *S. prostrata* was calculated using BHA, BHT, and ascorbic acid as standard. In the CUPRAC method, the ethanol extract of *S. prostrata* and standards showed remarkable reducing capacities. Ethanol extract antioxidant activity was higher than ascorbic acid at the 30 µg/mL concentration. However, the water extract showed very low activity. Reducing capacities decreased in the order of BHT > BHA > *S. prostrata* ethanol > ascorbic acid > *S. prostrata* water.

Phytochemical compounds

Polyphenolic compounds of plants have antioxidant and radical scavenging activity properties and are determined by the polymerization degree of the molecule and the number of active functional groups [32].

HPLC technique was used for the identification of the main organic compounds of *S. prostrata* by using different standard phenolic compounds. The results clearly showed the poor amounts of both total phenolic and total flavonoid contents of *S. prostrata.* According to the HPLC experiments, rutin (36.7 µg/g extract) and hesperidin (32.3 µg/g extract) were characterized as two major compounds in *S. prostrata*. Also, the low amounts of hyperoside, malic acid, and quinic acid were identified quantitatively.

The results demonstrated a high amount of rutin in the *S. prostrata* plant extract. It is clear that rutin extensively exists in fruits and vegetables [33, 34]. Due to its antioxidant, anti-inflammatory, and cytoprotective properties rutin is used in the treatment of many diseases [35]. Also, the potent DPPH scavenging ability and lipid peroxidation inhibition properties were reported for rutin as well [33]. As an antioxidant character, rutin could repair the damage of sodium perfluorononyloxy-benzenesulfonate to cell structures, and reduce the death rates of the bacteria under sodium perfluorononyloxy-benzenesulfonate exposure [36].

Conclusions

In this paper, we investigated and reported e phenolic content and antioxidant potential of *S. prostrata*. This work is a pioneer detailed study in describing and evaluating of chemical properties of *S. prostrata*. The results highlighted the potential role of *S. prostrata* to prevent the oxidation process. Therefore, further pharmacological investigations and *in vivo* tests are necessary to characterize and isolate plant active compounds.

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