

Evaluation of the Antioxidant Capacity and Phenolic Content of *Agriophyllum pungens* Seed Extracts from Mongolia

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ABSTRACT: Antioxidants are an important group of medicinal preventive compounds as well as being food additives inhibiting detrimental changes of easily oxidizable nutrients. The present investigation has been carried out to evaluate the antioxidant properties of different solvent extracts of *Agriophyllum pungens* seeds by various *in vitro* systems. The antioxidative activities of these samples were determined using four methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, ferric-reducing antioxidant potential (FRAP), and hydroxyl (OH) radical scavenging activities. Additionally, total flavonoids and phenolic contents (TPC) were also determined. Yield of extracts varied widely among solvents and was the highest for water extract (5.642% based on dry weight basis), while ethyl acetate extract exhibited the highest total phenolic content (0.149 mg/mL), total flavonoid content (0.111 mg/mL), and antioxidant activities ($P < 0.05$). The ABTS radical scavenging activity of *A. pungens* seeds occurred in the following order: ascorbic acid (92.9157%) > BHA (90.1503%) > α -tocopherol (87.7527%) > APEA (83.9887%) > APWR (75.5633%); the antioxidant activity of the extracts might be attributed to the presence of these phenolics. This suggests that *A. pungens* seed extract is a potential source of natural antioxidants, which could be added to dietary supplements to help prevent oxidative stress.

Keywords: *Agriophyllum pungens*, Mongolian Sand-rice, antioxidant activity, phenols, flavonoid

INTRODUCTION

Reactive oxygen species (ROS) and free radicals, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, are constantly formed in the human body by normal metabolic action. Their action is opposed by a balanced system of antioxidant defenses, including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death (1). Recently, much attention has been given to naturally occurring antioxidants, which may play an important role in inhibiting both free radicals and oxidative chain reactions within tissues and membranes (2). Moreover, ROS are a predominant cause of qualitative decay of foods, which leads to rancidity, toxicity, and destruction of biomolecules important in physiologic metabolism (3). Therefore, the evaluation of antioxidant activities of extracts and fractions is considered an important step prior to the isolation of antioxidant phytochemicals they contain (4). Many phenolic compounds, particularly flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, anti-

allergic, anti-thrombotic, and vasodilatory actions (5). The health promoting effect of antioxidants from plants is thought to arise mainly from their protective effects by counteracting reactive oxygen species, which are believed to play a significant role in the etiology and pathogenesis of various chronic diseases, premature ageing, and the oxidative deterioration of cosmetics, foods, and pharmaceutical preparations (6). In plants, antioxidants prevent lipid peroxidation and oxidative modification of low-density lipoproteins (7). To find new natural sources of active compounds, we studied the antioxidant potential of various extracts from *Agriophyllum pungens* seeds.

Agriophyllum pungens (Vahl) Link ex A. Diterich is a native Mongolian medicinal plant belonging to the Chenopodiaceae family, which is widely distributed in Middle Khalkha, Khovd, East-Mongolia, Depression of Great Lakes, Valley of Lakes, Eastern Gobi, Trans-Altai Gobi, Dzungarian Basin semi-desert and Alashan Gobi. In Mongolia, this plant commonly known as "*Tsulihir*, and *Sand-rice*", is widely used in local foods. The seeds are an ingredient in most traditional milk products and mainly used to prepare flour and tea material. This special-tast-

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ing flour is mixed with milk and eaten for breakfast. Traditionally, the seeds have been used in the treatments of cough, and kidney and liver diseases. The seeds also showed preventive effects on hepatitis, hyperlipidemia, and fatty liver *in vivo*. *A. pungens* seeds contain high levels of crude protein with essential amino acids (lysine, threonine, and methionine), fatty acids, and polysaccharides (8). However, information on the antioxidant activity of *A. pungens* seeds is scanty.

Based on the highly acclaimed properties of this plant, the present investigation was carried out to evaluate the antioxidant properties of various extracts of *A. pungens* seeds by different *in vitro* models.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), sodium bicarbonate, 2-deoxy-D-ribose, trichloroacetic acid (TCA), Folin & Ciocalteu's phenol reagent, naringin, L-ascorbic acid, α -tocopherol, and butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Tannic acid was obtained from Yakuri Pure Chemicals Co., Ltd. (Kyoto, Japan). All organic solvents and chemicals used in this study were of analytical grade.

Sample preparation

Agriophyllum pungens seed were collected from fresh plants in the East Gobi located in the Southeastern regions of Mongolia from September to October in 2012. The plant specimens were authenticated and deposited in the Botany Herbarium of the Department of Biology, Ulaanbaatar University of Mongolia. The plants were cleaned and air-dried under shade. The dried samples were stored at -70°C until used.

Preparation of extract

The dried and powdered *Agriophyllum pungens* (AP) seeds

(3 kg) were exhaustively extracted with 9 L of 80% methanol for 30 days at room temperature. Finally, the material was macerated using 80% ethanol and water (1,000 mL) with constant stirring at room temperature for 21 days. The residual materials were re-extracted by the same procedure (3 times). The extracts were filtered over filter paper (No. 3, Whatman International Ltd., Kent, England), and the filtrate was evaporated by a rotary evaporator (N11; Yamato Co., Tokyo, Japan) under reduced pressure at a temperature lower than 40°C . The residue was dissolved in distilled water (200 mL) and then extracted with ethyl acetate (1 L) using a separating funnel. The ethyl acetate layer was collected and evaporated to dryness in a vacuum to yield the ethyl acetate (21.1 g) fractions. The dried samples were dissolved in respective solvents and filtered with a $0.45\ \mu\text{m}$ membrane filter (Millipore, Billerica, MA, USA). The dried extract obtained with each solvent was weighed. The percentage yield was calculated in terms of the air-dried plant material. The extract obtained was used for the assessment of various antioxidant assays and for further analyses. The positive controls (α -tocopherol, BHA, butylated hydroxyanisole, ascorbic acid) and each solvent extract (APME, APEA, APWR, APET) were dissolved in methanol to the concentration of 100 mg/mL as stocks. All samples were placed in a glass bottle and stored at 4°C until further analyses. The extraction procedure is shown in Fig. 1.

Extract recovery percentage

The amount of crude extract recovered after successive extraction was weighed and the percentage of yield was calculated by the following formula,

$$\text{Extract recovery (\%)} = \frac{\text{Extract+container (g)} - \text{Empty container (g)}}{\text{Amount of plant sample (g)}} \times 100$$

Determination of total phenolics

Total phenolic contents in each extract were determined

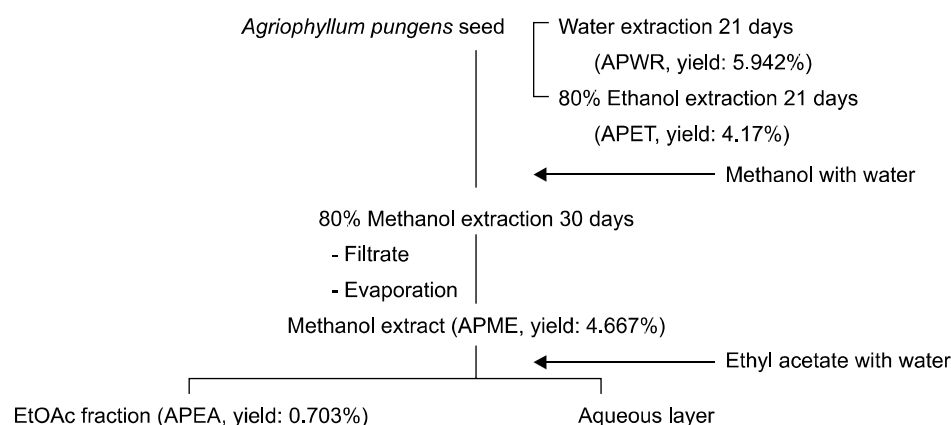


Fig. 1. Procedure to obtain different solvent extracts (APME, APEA, APET, and APWR) of *Agriophyllum pungens* seed.

with Folin-Ciocalteu reagent according to the method of Singleton (9). The plant sample was dissolved using 20 mM phosphate buffered saline (PBS, pH 7.4), to a final concentration of 100 mg/mL. An aliquot (0.33 mL) of the extract was added to a test tube containing 2.5 mL of distilled water, vortexed, and then mixed with 0.16 mL of Folin-Ciocalteu reagent. After 5 min, 0.3 mL of 10% sodium bicarbonate solution was added. The mixture was allowed to stand 30 min at room temperature in darkness, and the absorbance was measured at 760 nm (DU650, Beckman Coulter, Brea, CA, USA). A standard curve was prepared to express the results as tannic acid equivalents, i.e. the quantity of tannic acid (mg/mL).

Determination of total flavonoid contents

According to the method of Davis (10), total flavonoid contents were determined by preparing 0.4 mL of extracts in 4.0 mL of 90% diethylene glycol and 40 mL of 1 N NaOH. The mixture was shaken thoroughly, and allowed to stand for 1 h at room temperature. The absorbance of the solution was measured at 420 nm. Total flavonoid contents were determined using a standard curve obtained from various concentrations of naringin.

DPPH radical scavenging activity

The free radical scavenging activity was carried out according to the Blois method (11) with slight modifications. Methanol solution (200 μ L) of sample at various concentrations (0.1 ~ 10 mg/mL) was added to 0.1 mM methanolic solution of DPPH (4 mL) and shaken vigorously. The reaction mixture was allowed to stand for 30 min at room temperature in the dark. The absorbance of the mixture was determined using a spectrophotometer (DU650, Beckman Coulter) at 517 nm. The antioxidant activity of plant extracts was expressed as IC_{50} , which was defined as the concentration of extract required to inhibit the formation of DPPH radicals by 50%. This activity is given as percent DPPH scavenging and calculated as: $[(\text{control absorbance} - \text{extract absorbance}) / (\text{control absorbance})] \times 100$. BHA, α -tocopherol and ascorbic acid were used as references.

Ferric-reducing antioxidant power (FRAP) assay

The antioxidant capacity of samples was determined according to the Benzie and Strain method (12) with some modifications. The method is based on the reduction of the Fe^{3+} -TPTZ complex to the ferrous form at low pH. The fresh working solution (FRAP reagent) was prepared by mixing 25 mL of 0.3 M acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution, and then warmed (preheated) at 37°C before used. A properly diluted sample (0.1 mL) was added to 4.0 mL of FRAP reagent to form a mixture. The mixture was incubated at 37°C for 10 min in the dark, and the absorbance

was measured at 593 nm against a blank that was prepared using distilled water. The results, obtained from triplicate analyses, were expressed as aqueous solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), and derived from a calibration curve of the standards (20 ~ 1,000 μ M). The FRAP values of BHA, α -tocopherol and ascorbic acid were obtained by the same procedure.

ABTS radical scavenging activity assay

This assay was based on the ability of different substances to scavenge the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts radical cation ($ABTS^{\bullet+}$) according to the methods of Re et al. (13) with some modifications. The $ABTS^{\bullet+}$ stock solution was diluted with ethanol (about 1:88 v/v) to an absorbance of 0.70 ± 0.02 at 734 nm (Beckman Coulter DU650) for measurement, and equilibrated at 30°C. Aliquots (0.1 mL) of the samples were mixed with 2.9 mL of diluted $ABTS^{\bullet+}$ solution. After incubation at room temperature for 20 min, the absorbance at 734 nm was measured. Appropriate solvent blanks were run in each assay. BHA, α -tocopherol and ascorbic acid were employed as positive controls. A lower absorbance of the reaction mixture indicates higher ABTS radical scavenging activity. The activity is given as percentage ABTS radical scavenging, which is calculated with the equation:

$$\% \text{ ABTS radical scavenging activity} = [(\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance}] \times 100$$

Hydroxyl radical scavenging activity

Hydroxyl radical (OH^{\bullet}) scavenging assay was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} /ascorbate/EDTA/ H_2O_2 system (14). Hydroxyl radicals were generated by direct addition of iron (II) salts to the reaction mixture (15). The reaction mixture was prepared with 0.2 mL of 10 mM $FeSO_4 \cdot 7H_2O$ containing 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM 2-deoxy-D-ribose solution (0.2 mL), sample solution (0.15 mL) or 0.1 M phosphate buffer (1.0 mL pH 7.4). The reaction was initiated by addition of 10 mM H_2O_2 (0.2 mL), followed by incubation at 37°C for 4 h. After incubation, 1 mL of 2.8% trichloroacetic acid (TCA) and 1.0% thiobarbituric acid (TBA) were added, and the mixture was heated in boiling water for 10 min. Finally, the reaction mixture was cooled in ice water and centrifuged at $800 \times g$ for 10 min. The absorbance of the supernatant was measured at 532 nm. Each assay was performed in triplicate. Hydroxyl radical scavenging activity was expressed as 50% inhibitory concentration (IC_{50}), which is the concentration to reduce the absorbance of treated samples by 50% with reference to the control. α -tocopherol, ascorbic acid, and BHA were used as the

positive controls.

Statistical analysis

The results are expressed as the mean \pm standard deviation (SD). All statistical analyses were performed using the SPSS 12.0 (SPSS Inc., Chicago, IL, USA) software. The differences among groups were evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple range tests. A level of $P < 0.05$ was used as the criterion for statistical significance.

RESULTS AND DISCUSSION

Recovery percent, total phenolics, and flavonoids

The extraction yields were 4.667, 0.703, 5.942, and 4.170 in 80% methanol, ethyl acetate, water and 80% ethanol extracts, respectively (Table 1). The highest extraction yield was recorded for the water seed extract of AP (APWR), whereas the lowest was for the ethyl acetate extract. However, no significant difference was observed between 80% methanol and ethanol extracts. Different solvent systems have been used for extraction of polyphenols from plant material (16), but the reason for choosing these solvents has not been justified. Generally known, the yield of chemical extraction depends on the type of solvents with varying polarities and pH, extraction time, and temperature as well as on the chemical compositions and physical characteristics of the sample (17). Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidative effect via interactions with the phenol ring and its resonance stabilization effect (18). Since plant-de-

rived phenolic compounds have shown various physiological activities, levels of phenolic and flavonoid contents from *A. pungens* seed were first assessed (Table 1).

The ethyl acetate extract of AP contained significantly ($P < 0.05$) higher amounts of total phenolics (0.149 mg/mL) than methanol (0.119 mg/mL), water (0.138 mg/mL) and ethanol (0.060 mg/mL) extracts. Previously, Zhou et al. (19) reported the presence of chemical constituents (*p*-(acetylamino)phenol, ayamenin A, irisone, and salicin) of the water and ethyl acetate extracts of *Agriophyllum squarrosum*. Gómez-Caravaca et al. (20) reported on the phenolic composition of *Chenopodium* species. Miranda et al. (21) noted the presence of phenolics in quinoa seeds. Our results are also similar to Gennari et al. (22) in that *Beta bulgaris* seeds had higher amounts of phenolic compounds when compared to other plants within the family Chenopodiaceae.

Flavonoids are also potent antioxidants, free radical scavengers, and metal chelators and they inhibit lipid peroxidation. This activity is believed to be mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (23). The total flavonoid contents in methanol, ethyl acetate, water and ethanol extracts were 0.073, 0.111, 0.098, and 0.036 mg/mL, respectively (Table 1). In a recent study by Li et al. (24), flavones (quercetin) and coumarins were reported in butanol extracts and ethyl acetate extracts of the aerial parts of *Agriophyllum squarrosum*. The flavonol conjugates quercetin and kaempferol oligomeric glycosides have previously been identified in the quinoa seeds (25). Awaad et al. (26) found that the ethanol, diethyl ether, chloroform, ethyl acetate, and *n*-butanol extracts of the Chenopodiaceous plant (*Artiplex lentiformis*) had 985 and 895 μ mol Trolox equivalent/g and flavonoid contents, respectively. Similarly, Ranilla et al. (27) and Kumar et al. (28) had also reported antioxidant activity and flavonoid content in quinoa (*Chenopodium quinoa* Willd), Kaniwa (*Chenopodium pallidicaule* Aellen) and *Chenopodium album*. Plants containing flavonoids have been reported to possess strong antioxidant activities (29). Moreover, flavonoids are known to possess a wide range of therapeutic uses due to their antimutagenic and anticarcinogenic properties and the potential to decrease cardiovascular complications (30). Since the phenolic compounds are considered to be major contributors to the antioxidant capacities of plants, the relatively large phenolic and flavonoid contents in the ethyl acetate extract and water extract was suggestive for further research on isolating antioxidant compounds. This study supports our prediction that *Agriophyllum pungens* seeds are a good source of natural antioxidants such as phenolic and flavonoid compounds.

Table 1. Extraction yield and total phenolic and flavonoid contents of different solvent extracts from *Agriophyllum pungens* seeds

Sample ¹⁾	Yield (%) ²⁾	Total phenol (Tannic mg/mL) ³⁾	Total flavonoid (Naringin mg/mL) ⁴⁾
APME	4.667 ²⁾	0.119 \pm 0.001308 ^{b5)}	0.073 \pm 0.00819 ^{ab6)}
APEA	0.703	0.149 \pm 0.01557 ^a	0.111 \pm 0.05808 ^a
APWR	5.642	0.138 \pm 0.01803 ^{ab}	0.098 \pm 0.01752 ^a
APET	4.170	0.060 \pm 0.01411 ^c	0.036 \pm 0.00265 ^b

¹⁾Extracts from AP: APME, 80% methanol; APEA, ethyl acetate; APWR, water; and APET, 80% ethanol.

²⁾Based on weight of aerial parts extracted, Yield (%)=(extract weight/dry weight) \times 100.

³⁾Tannic acid was used as a standard for measuring total phenolic contents.

⁴⁾Naringin was used as a standard for measuring total flavonoid contents.

^{5,6)}Measurements were done in triplicate and values represent mean \pm SD.

^{a-c}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at $P < 0.05$.

DPPH radical scavenging property

Free radicals produced in the body are partly associated with the etiology of cancers and other chronic diseases. Therefore, determining the radical scavenging effect of antioxidants in this seeds is important. Antioxidant activities of the *A. pungens* seed extracts by the different solvents were analyzed by measuring DPPH radical scavenging activities. DPPH[•] assay is widely used for evaluating antioxidant activities in a short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (31). The results on DPPH radical scavenging activities of the methanol (APME), ethyl acetate (APEA), water (APWR), and ethanol (APET) extracts and the positive control reference standards BHA, α -tocopherol, and ascorbic acid are shown in Table 2. According to the results, as the IC₅₀ value decreases, the antioxidant activity increases. The IC₅₀ value of each extract was compared with α -tocopherol (IC₅₀, 0.575 mg/mL), BHA (IC₅₀, 0.570 mg/mL) and ascorbic acid (IC₅₀, 0.553 mg/mL). DPPH radical scavenging activity of APEA was found to be higher than that of APET. Our findings are partially comparable with previous studies by Gorinstein et al. (32) and Nsimba et al. (2) in which some quinoa seeds of various extracts showed high antioxidative activities. Gennari et al. (22) had recently reported that ethyl acetate seed extract of Swiss chard (*B. vulgaris*) has high antioxidant activity. Similar to our result, Wang et al. (33) reported that *Agriophyllum squarrosum* also showed high antioxidant activity. According to a well-established thought, antioxidant activities of various vegetables, fruits, and natural plants are attributed to the contents of phenolic compounds (34). The radical-scavenging activity of polyphenols depends on the molecular structure and the substitution pattern of the hydroxyl groups, the availability of phenolic hydrogens,

and the possibility of stabilization of the resulting phenoxyl radicals via hydrogen donation or by expanded electron delocalization (35). This radical scavenging ability of extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating system. These results indicate that *A. pungens* seeds have significant effects on scavenging free radicals.

Determination of reducing power

FRAP assay is commonly used to study the antioxidant capacity of plant materials. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron can stimulate lipid peroxidation by the Fenton reaction and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals, which can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The simple and reliable FRAP assay measures the reducing potential of an antioxidant reacting with a ferric-TPTZ (Fe(III)-TPTZ) complex and producing a colored ferrous-TPTZ (Fe(II)-TPTZ) complex by a reductant at a low pH (about 3.6). The ferric-TPTZ complex can be monitored at 593 nm. A higher absorbance power indicates a higher ferric reducing power (36). In the present study, the FRAP values for APME, APEA, APWR and APET are shown in Table 3. The APEA (0.1921 mmol Fe(II)/mg extract) registered significantly higher ($P<0.05$) ferric reducing antioxidant activity than the APET (0.0753 mmol Fe(II)/mg extract), indicating that APEA possesses higher reducing capacity than APET. These observations showed that the reducing characteristics of the plants can act as antioxidants, reducing agents, hydrogen donors and oxygen quenchers (37). Similar to our result, Wang et al. (33) reported that *Agriophyllum squarrosum* showed the strongest anti-

Table 2. DPPH radical scavenging activity of different solvent extracts from *Agriophyllum pungens* seeds

Sample extract ¹⁾	IC ₅₀ : mg/mL ²⁾
APME	4.833±0.39892 ^{3b)}
APEA	2.6487±0.47171 ^{c)}
APWR	2.9982±0.06918 ^{c)}
APET	8.5083±0.71914 ^{a)}
α -Tocopherol ⁴⁾	0.575±0.02879 ^{d)}
BHA ⁴⁾	0.570±0.03568 ^{d)}
Ascorbic acid ⁴⁾	0.553±0.04709 ^{d)}

¹⁾Same extracts as described in Table 1.

²⁾IC₅₀ (mg/mL), concentration for scavenging 50% of DPPH radicals.

³⁾Measurements were done in triplicate, and values represent mean±SD.

⁴⁾ α -Tocopherol, BHA (butylated hydroxyanisole) and ascorbic acid were used as positive controls.

^{a-d)}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at $P<0.05$.

Table 3. Ferric-reducing activities of different solvent extracts from *Agriophyllum pungens* seeds

Sample extract ¹⁾	mmol Fe(II)/mg extract ²⁾
APME	0.096±0.00900 ^{c3)}
APEA	0.1921±0.00301 ^{a)}
APWR	0.1292±0.00857 ^{b)}
APET	0.0753±0.00057 ^{d)}
α -Tocopherol ⁴⁾	1.137±0.02200 ^{a)}
BHA ⁴⁾	1.164±0.04078 ^{a)}
Ascorbic acid ⁴⁾	1.137±0.1506 ^{a)}

¹⁾Same extracts as described in Table 1.

²⁾Concentration of substance having ferric-TPTZ reducing ability expressed as μ mol Fe(II) equivalents.

³⁾Measurements were done in triplicate and values represent mean±SD.

⁴⁾ α -Tocopherol, BHA (butylated hydroxyanisole) and ascorbic acid were used as positive controls.

^{a-b)}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at $P<0.05$.

oxidant activity through the FRAP assay. Paško et al. (38) and Alvarez-Jubete et al. (39) also observed the highest antioxidant capacity in wild plants, especially in the Chenopodiaceae family, using the FRAP assay. According to Oktay et al. (40), a highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plant species. The presence of abundant total phenolics in several medicinal plants resulted in high a FRAP value. The reductive ability of the samples assessed in this study suggests that ethyl acetate and water extracts were able to donate an electron. Hence, the samples should be able to donate electrons to free radicals in actual biological or food systems, making the radicals stable.

Antioxidant activity by the ABTS^{•+} assay

Another stable free radical cation, ABTS, was used to evaluate the antioxidant activity of the methanol, ethyl acetate, water and ethanol extracts from *Agriophyllum pungens*. The ABTS systems have been commonly used to measure the total antioxidative status of various biological specimens by measuring radical scavenging through electron donation (41). The ABTS radical scavenging effect of different solvent extracts from *A. pungens* exhibited a similar trend to the DPPH radical scavenging activity. The results were expressed as $\mu\text{mol Trolox equivalents/g}$ extract. A steady increase was observed in the percentage inhibition of the ABTS radicals by the APME, APEA, APWR and APET (Fig. 2). The APEA exhibited higher radical scavenging activity than the APME when reacted with the ABTS radicals. As seen in Fig. 2, the scavenging effect on ABTS radical by dilution concentrations (2 mg/mL) of APME, APEA, APWR and APET were found as: 63.7083%, 83.9887%, 75.5633%, and 54.5510%,

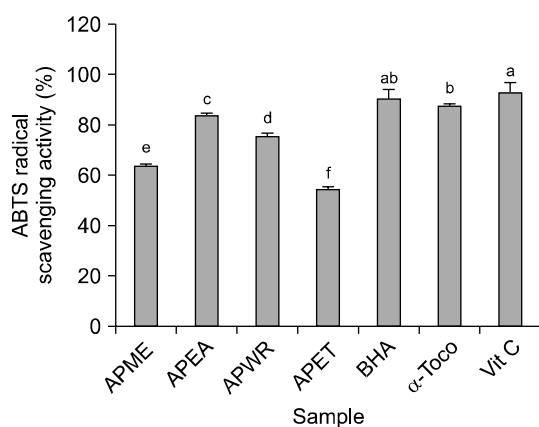


Fig. 2. ABTS radical scavenging activity of different solvent extracts from *Agriophyllum pungens* seed: APME, 80% methanol; APEA, ethyl acetate; APWR, water; and APET, 80% ethanol. Data were presented as mean \pm SD (n=3). Means sharing different letters (a-f) are significantly different ($P<0.05$). Same extracts as created in Fig 1. BHA (butylated hydroxyanisole), α -tocopherol and ascorbic acid at the concentration of 0.5 mg/mL were used as positive controls.

respectively. Quinoa seed extracts have already been reported to exhibit strong ABTS^{•+} scavenging activity (42). Hagerman et al. (15) have reported that the high molecular weight phenolics (tannins) have a greater ability to quench free radicals (ABTS^{•+}) and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of the hydroxyl group substitution than the specific functional groups. The position of hydroxyl groups might be more important for mediating potent total antioxidant activity (43). These results indicated that the APEA contains high amounts of radical scavengers and may be useful as a natural source of antioxidants.

Hydroxyl radical scavenging activity

Hydroxyl radicals can easily cross cell membranes, react readily with most biomolecules including carbohydrates, proteins, lipids, and DNA in cells, and cause tissue damage or cell death. Thus, removing OH[•] is very important for the protection of living systems (44). Hydroxyl radical scavenging activity was estimated by generating hydroxyl radicals using ascorbic acid-ion EDTA. The scavenging abilities of APME, APEA, APWR and APET of *Agriophyllum pungens* are shown in Table 4. The APEA and APWR significantly decreased hydroxyl radical scavenging activity, and IC₅₀ values were 5.8680 and 8.2978 mg/mL, respectively ($P<0.05$). The scavenging ability of the APEA and APWR might be due to the active hydrogen donor ability of hydroxyl substitution. Hydrogen peroxide can be toxic to cells when active. Therefore, removing H₂O₂ and O₂^{•-} is very important for antioxidant defense in cell or food systems.

In conclusion, ethyl acetate extract of *Agriophyllum pungens* seeds possessed good antioxidant activities. Although further research is necessary, our study shows that a supplementation with this plant extract could at least

Table 4. Hydroxyl radical scavenging activity of different solvent extracts from *Agriophyllum pungens* seeds

Sample extract ¹⁾	IC ₅₀ : mg/mL ²⁾
APME	9.426 \pm 3.04409 ^{3)ab}
APEA	5.8680 \pm 1.41030 ^c
APWR	8.2978 \pm 2.18027 ^{bc}
APET	11.5813 \pm 2.32485 ^a
α -Tocopherol ⁴⁾	0.3930 \pm 0.10930 ^d
BHA ⁴⁾	0.4820 \pm 0.18949 ^d
Ascorbic acid ⁴⁾	0.4050 \pm 0.14147 ^d

¹⁾Same extracts as described in Table 1.

²⁾IC₅₀ (mg/mL), concentration for scavenging 50% of hydroxyl radicals.

³⁾Measurements were done in triplicate and values represent the mean \pm SD.

⁴⁾ α -Tocopherol, BHA (butylated hydroxyanisole) and ascorbic acid were used as positive controls.

^{a-d}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at $P<0.05$.

help in preventing or decreasing the damages caused by oxidative stress. Based on these data, additional studies are needed to characterize the bioactive compounds responsible for the antioxidative activities in *Agriophyllum pungens*. Therefore, the study suggests that the whole plant might be a potential source of natural antioxidants.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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