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POLYPHENOL COMPOSITION AND ANTIOXIDANT CAPACITY FROM DIFFERENT EXTRACTS OF *ASTER SCABER*

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Phenolic contents and antioxidant capacities from different solvent extracts (petroleum ether, ethyl acetate, methanol, butanol and water) of *Aster scaber* leaf were investigated. Antioxidant activity was evaluated by three different methods, namely DPPH radical scavenging activity, reducing power assay and phosphomolybdenum activity. A total of twenty-three polyphenolic compounds were identified and quantified from *A. scaber* leaf extracts, including hydroxybenzoic acids, hydroxycinnamic acids, flavonols and other groups of phenolic compounds. Ultra high performance liquid chromatography (UHPLC) analysis of the leaf extract revealed that myricetin (4850.45 μg/g) was the most dominant flavonols, compared to quercetin and kaempferol. Caffeic acid was the dominant phenolic compound in *A. scaber* leaf extracts, it constituted about 104.20 μg/g, followed by gentisic acid (84.50 μg/g), gallic acid (61.05 μg/g) and homogentisic acid (55.65 μg/g). The total phenolic and flavonoid content was the highest in ethyl acetate extract (322.43 and 6.51 mg/g). The decreasing order of antioxidant activity among the *A. scaber* leaf extracts assayed through all the three methods was found to be ethyl acetate > butanol > methanol > petroleum ether > water extract.

Keywords: Leafy vegetables – *Aster scaber* – antioxidant activity – phenolic compounds – flavonoids

INTRODUCTION

Polyphenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects, including antioxidant activity [13]. Phenolics are aromatic secondary plant metabolites widely spread throughout the plant kingdom and associated with imparting colour, sensory qualities and nutritional and antioxidant properties of food. The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers [2]. Flavonoids are a class of phenolics occurring ubiqui-

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Abbreviations: BHT – butylated hydroxytoluene; DPPH – 1,1-diphenyl-2-picrylhydrazyl; GAE – gallic acid equivalents; QE – quercetin equivalents; UHPLC – ultra high performance liquid chromatography

tously in food plants. They are present as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups [4]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [5, 16]. Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer [7, 23] and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases [6] as well as inflammation and problems caused by cell and cutaneous aging [1]. Earlier studies have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, antibacterial and antiviral effects [27].

Aster scaber Thunb. is a member of the Compositae family, it is widespread and cultivated as an important culinary vegetable in Korea. *Aster* species have been used in traditional Chinese medicine to treat bruises, snakebite, headache and dizziness [15]. Triterpene glycosides and volatile compounds have been reported from *A. scaber* [12]. Four quinic acid derivatives and their inhibitory activities against human immunodeficiency virus-1 (HIV-1) integrase have also been reported [17]. The *A. scaber* therapeutic features have been widely ascribed to the high content of antioxidant compounds such as polyphenols and ascorbic acid which exhibit antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilator actions and are useful in the treatment of arteriosclerosis, cancer, diabetes, neurodegenerative diseases, and arthritis, as well as for the prevention of other diseases [20, 26]. Antioxidant content is variable in plants because their synthesis and storage is a direct consequence of plant-environment interaction. The objectives of this study were to evaluate and compare antioxidant capacity by three common antioxidant activity methods, presented as equivalent antioxidant capacity and total phenolic and flavonoid content, identify and quantify major phenolic compounds present in the *A. scaber* leaf extracts by UHPLC.

MATERIALS AND METHODS

Plant material and sample extraction

Cultivated *Aster scaber* plants were purchased in local vegetable market, Seoul, Korea in May 2012. Taxonomic identification was performed by Dr. Ill-Min Chung, Department of Applied Bioscience, Konkuk University. A voucher specimen (KU-12-001) is deposited in the Department of Applied Bioscience. *A. scaber* leaves were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained, and dried in a hot air oven at 50±2 °C. The dried leaves were finely

powdered. The dried powder (100 g) was extracted with 5 × 2 L methanol for 24 h. After removal of the solvent *in vacuo*, the crude extract was suspended in 0.5 L distilled water and extracted with 0.2 L portions of petroleum ether, ethyl acetate and butanol until the extracts were nearly colorless. Solvents were removed *in vacuo*, and the extracts were obtained, respectively.

Determination of total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu (FC) method [24]. Distilled water (3.16 ml) was mixed with a DMSO solution of the test compound (40 µl). Then, 200 µl of FC reagent was added. After 5 min, 600 µl of 20% sodium carbonate solution was added and the solutions were mixed again. The solutions were left at room temperature for 2 h. Then the absorption of the developed blue color was determined at 765 nm, using a Mecasys Optizen 2120UV plus UV-spectrophotometer (Mecasys, Korea). The concentration of the total phenolic content was determined as mg of gallic acid equivalent (GAE) by using an equation obtained from gallic acid calibration curve. The estimation of phenolic compounds in the fractions was carried out in triplicate and the results were averaged.

Determination of total flavonoid content

Total flavonoid content of *A. scaber* leaf extracts were determined by using the aluminium chloride colorimetric method as described by [30], with some modifications. Extracts (0.5 ml), 10% aluminium chloride (0.1 ml), 1 M potassium acetate (0.1 ml) and distilled water (4.3 ml) were mixed. After incubation at room temperature for 30 min. The absorbance was measured at 415 nm using a Mecasys Optizen 2120UV plus UV-spectrophotometer (Mecasys, Korea). Quercetin was used to make the calibration curve. The estimation of total flavonoids in the extracts was carried out in triplicate and the results were averaged.

Extraction of phenolic compounds for the UHPLC analysis

One gram of dried leaf material was extracted in 10 ml of acetonitrile and 2 ml of 0.1 N hydrochloric acid. The mixture was stirred for 2 h at room temperature. The extract was filtered through No. 42 Whatman filter paper and was concentrated using a vacuum evaporator. The residues were dissolved in 10 ml of 80% aqueous methanol and filtered through a 0.45 µm membrane. The filtrate was used for the UHPLC analysis.

UHPLC analysis of the phenolic compounds

UHPLC was performed using the Thermo Accela UHPLC (Thermo, New York, USA) system. Separation was primarily achieved using a HALO C18 (2.7 μm, 2.1 × 100 mm) column and the absorbance was measured at 280 nm. The mobile phases were 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The injection volume was 4 μl and the linear gradient of UHPLC solvents was as follows: 0 min, 92% A: 8% B; 0–2.2 min, 90% A: 10% B; 2.2–5 min, 85% A: 15% B; 5–7.5 min, 84.5% A: 15.5% B; 7.5–8.5 min, 82.2% A: 17.8% B; 8.5–13 min, 55% A: 45% B; 13–14 min, 0% A: 100% B; and 14–15 min, 92% A: 8% B. The run time was 15 min and the flow rate was 500 μl/min.

Solutions of available pure known compounds, gallic acid, homogentisic acid, protocatechuic acid, gentisic acid, β-resorcylic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, vanillin, ρ-coumaric acid, salicylic acid, ferulic acid, m-coumaric acid, rutin, o-coumaric acid, myricetin, resveratrol, quercetin, naringenin, kaempferol, formononetin and biochanin A were chromatographed as external standards. All standards were dissolved in methanol before injections in the analytical UHPLC system. Their ranges of concentration used were 25, 50, 100, 150 μg/ml. Phenolic compounds of leaf extract were identified by comparing their retention times with those of pure compounds. The results were expressed as μg/g of each compound from the total phenolic compounds.

DPPH radical scavenging assay

The antioxidant activity of the extracts from *A. scaber* leaves, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by [14]. Different concentrations (25 to 100 μg/ml) of the extracts were taken in different test tubes with 4 ml of a 0.006% MeOH solution of DPPH-. Water/methanol in place of the extracts was used as control. Absorbance at 517 nm was determined after 40 min of incubation at room temperature. Radical scavenging activity was expressed as the inhibition percentage, and was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control at 40 min reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the sample at 40 min.

Assay of reductive potential

The reducing power of the extracts was determined according to the method of [18]. Different extracts of concentration (100 to 500 µg/ml) in 1 ml of distilled water was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$], and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 650 *g* for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of *A. scaber* leaf extracts was evaluated by the method of [21]. An aliquot of 0.1 ml of sample solution (1 mg/ml) was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under same conditions as the rest of the sample. For samples of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (mg/g of extract).

RESULTS

Total phenolic and flavonoid contents

A. scaber leaf extracts were analyzed for total phenolic and flavonoid contents. The total phenolic content of the extracts from *A. scaber* leaf was determined by FC method and the results are expressed as equivalents of gallic acid (Table 1). Among the five extracts, ethyl acetate extract had the highest (322.43 mg/g) amount of phenolic compounds and lowest was observed in water extract (23.67 mg/g). The total flavonoid content varied from 0.34 to 6.51 mg QE/g (Table 1).

UHPLC analysis of phenolic compounds in A. scaber *leaf extract*

The qualitative and quantitative analyses of the *A. scaber* leaf extracts were made using UHPLC and the results are presented in Table 2. The phenolic compounds in the *A. scaber* leaf extracts were identified by comparisons to the retention time and UV

Table 1
Total phenolic (expressed as gallic acid equivalents) and flavonoid content (expressed as mg quercetin/g) from leaf extracts of *A. scaber*

Extract	Total phenolic content (mg/g)	Total flavonoid content (mg/g)
Ethyl acetate	322.43 ± 4.66[a]	6.51 ± 0.28[a]
Petroleum ether	35.08 ± 1.12[d]	2.23 ± 0.06[d]
Butanol	202.34 ± 5.13[b]	4.43 ± 0.10[b]
Methanol	183.39 ± 5.59[c]	3.12 ± 0.09[c]
Water	23.67 ± 0.39[e]	0.34 ± 0.02[e]

Data represents mean values±SD of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

spectra of authentic standards while the quantitative data were calculated from the calibration curves. *A. scaber* leaf extracts contained total flavonols (4908.40 µg/g), hydroxybenzoic acid (261.60 µg/g), hydroxycinnamic acid (156.15 µg/g) and other phenolic compounds (164.50 µg/g). The three flavonols identified in the analysis were myricetin, quercetin and kaempferol. Myricetin (4850.45 µg/g) was the most

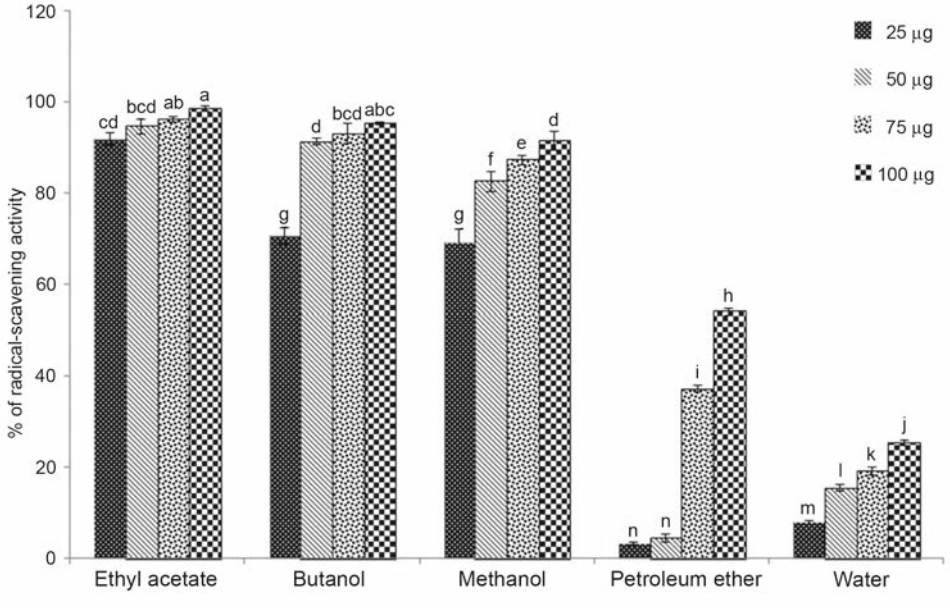

Fig. 1. Free radical scavenging activity of the extracts from *A. scaber* leaf at different concentrations by DPPH method. Data represents mean values ± SD of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

Table 2
Major phenolic compounds identified in the *A. scaber* leaf extract by UHPLC analysis

Compounds	Concentration (µg/g)
Flavonols	
Myricetin	4850.45 ± 23.40[a]
Quercetin	25.30 ± 1.00[fg]
Kaempferol	32.65 ± 0.35[ef]
Total	4908.40
Hydroxycinnamic acid	
Caffeic acid	104.20 ± 1.22[b]
p-Coumaric acid	8.95 ± 0.57[fg]
Ferulic acid	29.10 ± 1.00[ef]
m-Coumaric acid	0.05 ± 0.01[h]
o-Coumaric acid	12.55 ± 3.18[g]
Chlorogenic acid	1.30 ± 0.70[h]
Total	156.15
Hydroxybenzoic acid	
Gallic acid	61.05 ± 1.63[d]
Protocatechuic acid	29.10 ± 1.00[ef]
β-Resorcylic acid	27.70 ± 1.10[ef]
Vanillic acid	26.00 ± 0.55[ef]
Syringic acid	12.25 ± 0.27[fg]
Gentisic acid	84.50 ± 1.25[c]
Salicylic acid	21.00 ± 10.88[ef]
Total	261.60
Other phenolic compounds	
Rutin	39.90 ± 5.09[ef]
Vanillin	12.65 ± 0.57[efg]
Homogentisic acid	55.65 ± 0.63[d]
Resveratrol	29.70 ± 1.00[fg]
Naringenin	10.30 ± 0.90[g]
Formononetin	11.70 ± 0.75[g]
Biochanin A	4.60 ± 0.14[gh]
Total	164.50

Data represents mean values±SD of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

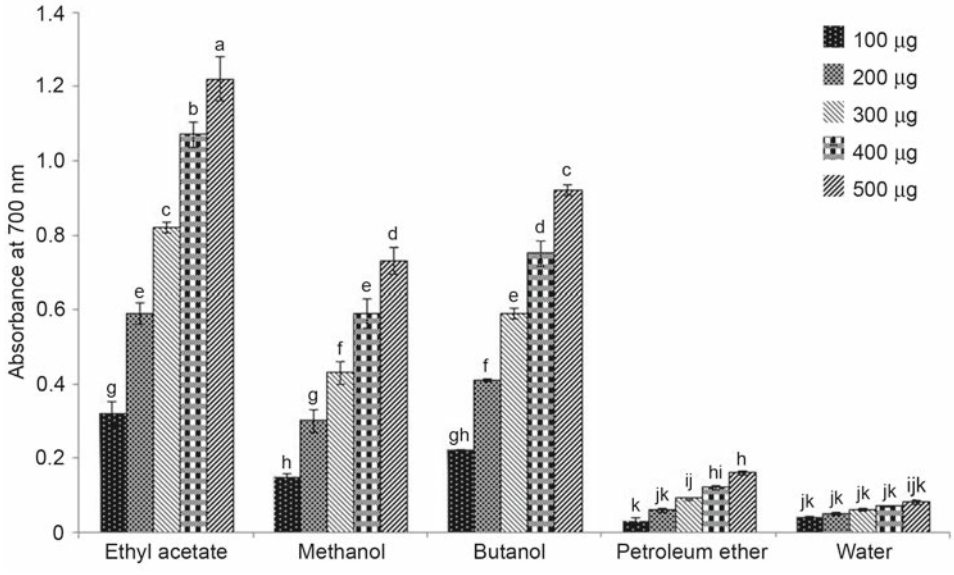

Fig. 2. Reducing power of the extracts from *A. scaber* leaf at different concentrations. Data represents mean values ± SD of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

dominant flavonols it accounted for the largest proportion of the total flavonols content, whereas kaempferol (32.65 µg/g) and quercetin (25.30 µg/g) were detected in lower amounts in *A. scaber* leaf extracts. In the hydroxycinnamic acid group, caffeic acid (104.20 µg/g) was the most dominant hydroxycinnamic acid, followed by ferulic acid (29.10 µg/g), o-coumaric acid (12.55 µg/g) and p-coumaric acid (8.95 µg/g) present in *A. scaber* leaf extracts. Gentisic acid (84.50 µg/g) was the dominant compound in the hydroxybenzoic acid group followed by gallic acid (61.05 µg/g), protocatechuic acid (29.10 µg/g) and β-resorcylic acid (27.70 µg g/l). The other phenolic compounds like homogentisic acid (55.65 µg g/l), rutin (39.90 µg g/l) and resveratrol (29.70 µg g/l) were also found in *A. scaber* leaves (Table 2).

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was tested through DPPH method and the results were compared with BHT (Fig. 1). The *A. scaber* leaf extracts were able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine. The IC_{50} values of the extracts were ethyl acetate (13.60 µg/ml), butanol (17.66 µg/ml), methanol (18.05 µg/ml), water (158.42 µg/ml) and petroleum ether (376.50 µg/ml), respectively. In this study, the extracts exhibited a concentration dependent antiradical activity by inhibiting DPPH radical (Fig. 1). Of the different

extracts, ethyl acetate extract exhibited the highest antioxidant activity of 98.61% at 100 µg/ml concentration, followed by butanol (95.42%), methanol (91.55%), petroleum ether (54.15%) and water (25.34%), respectively, at the same concentration and it indicates that compounds with strong radical scavenging capacity are of medium polarity (Fig. 1). The DPPH activity of BHT exhibited 92.04% at 50 µg/ml concentration.

Assay of reductive potential

In the present study, the extracts exhibited effective reducing capacity at all concentration points. The reducing capacity of the extracts increased with increase in the concentration (Fig. 2). The reducing power of the extracts followed the order of ethyl acetate > butanol > methanol > petroleum ether > water extract.

Evaluation of antioxidant capacity by phosphomolybdenum method

The antioxidant capacity of the *A. scaber* leaf extracts was measured spectrophotometrically through the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte, and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. Results indicated that ethyl acetate extract has higher antioxidant capacity (110.96 mg α-tocopherol equivalent/g dw) than other four extracts which showed antioxidant capacity in the following order: butanol (103.78 mg α-tocopherol equivalent/g dw) > methanol (98.46 mg α-tocopherol equivalent/g dw) > petroleum ether (87.53 mg α-tocopherol equivalent/g dw) > water (26.76 mg α-tocopherol equivalent/g dw) (Table 3).

Table 3
Antioxidant capacity of *A. scaber* leaf extracts
by phosphomolybdenum method

Extract	Antioxidant capacity [as equivalent to α-tocopherol (mg/g)]
Ethyl acetate	110.96 ± 3.18[a]
Methanol	98.46 ± 2.71[c]
Petroleum ether	87.53 ± 4.04[d]
Butanol	103.78 ± 1.50[b]
Water	26.76 ± 0.29[e]

Data represents mean values±SD of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

DISCUSSION

In the present study, significant differences were observed in the amount of total phenolic and flavonoid content found in the extracts obtained by using different solvents (Table 1). Among the various solvents used, ethyl acetate showed higher levels of phenolics and flavonoids. In contrast, methanol extract exhibited higher levels of phenolics and flavonoids in some leafy vegetables [2]. The phenolic compounds like flavonols, hydroxybenzoic acids and hydroxycinnamic acids which are present in most of the vegetables, herbs and spices are considered as an important therapeutic agent because of their beneficial effects on human health, such as protection of certain types of cancers, aging and cardiovascular diseases [22]. Quercetin, kaempferol, myricetin, and their glycosides were the predominant flavonols, occurring in many medicinal herbs associated with anticancer activity [3]. In a previous work it has been established that the antioxidant properties of some plants are partly due to low molecular mass phenolic compounds, particularly flavonoids, which are known to be potent antioxidants [28]. The results suggest that flavonols like myricetin, kaempferol, together with hydroxybenzoic acid, hydroxycinnamic acid and other group of phenolic acids play a predominant role in the leaves of *A. scaber*. In our study, the presence of phenolic compounds implies that the consumption of *A. scaber* leaves is beneficial for human health.

DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH-solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. One of the possible factor of mechanisms is the role of polyphenolic associated compounds. In our present investigation the ethyl acetate fraction exhibited the highest antioxidant activity when compared with other solvent extracts. Similar results were reported in the case of *Coriandrum sativum* [29] and *Synurus deltoids* [11]. In contrast, methanol extracts showed the highest antioxidant activity in four leafy vegetables [25]. The positive correlation between polyphenolic content of the extracts and its antioxidant activity is well documented [10]. Therefore, the content of total phenolic compounds in the extracts might explain their high antioxidant activities. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones [19], which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom [8]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing capacity of extracts suggested that ethyl acetate fraction exhibited highest activity when compared with other solvent extracts, which indicates that ethyl acetate fraction contains large amount of reductone-associated and hydroxide groups of compounds which can act as electron donors and react with free radicals to convert them to more stable products, and thereby terminate radical chain reactions. All the extracts obtained from *A. scaber* leaves were able to reduce Mo (VI) to Mo (V). Ethyl acetate extract exhibited highest

phosphomolybdenum activity compared to other extracts. In contrast to our results, ethanol extract exhibited highest phosphomolybdenum activity compared to other extracts of *Abrus precatorius* leaves [9].

CONCLUSION

Our results showed that *A. scaber* were rich in phenolic constituents and demonstrated good antioxidant activity measured by different methods. This species, rich in flavonoids and phenolic acids, could be a good source of natural antioxidants. Therefore, qualitative and quantitative analysis of major individual phenolics in the *A. scaber* could be helpful for explaining the relationships between total antioxidant capacity and total phenolic contents. The results obtained can be important in understanding the role played by this green leafy vegetables in the prevention and treatment of several diseases.

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REFERENCES

1. Ames, B. N., Shigenaga, M. K., Hagen, T. M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA 90,* 7915–7922.
2. Bhat, R., Liong, M. T., Abdorreza, M. N., Karim, A. A. (2013) Evaluation of free radical scavenging activity and antioxidant potential of a few popular green leafy vegetables of Malaysia. *International J. Food Properties 16,* 1371–1379.
3. Cai, Y., Luo, O., Sun, M., Corke, H. (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci. 74,* 2157–2184.
4. Cao, G., Sofic, E., Prior, R. L. (1997) Antioxidant and pro-oxidant behavior of flavonoids: Structure activity relationships. *Free Radic. Biol. Med. 22,* 749–760.
5. Cook, N. C., Samman, S. (1996) Flavonoids–chemistry, metabolism, cardio protective effects, and dietary sources. *Nutr. Biochem. 7,* 66–76.
6. Di Matteo, V., Esposito, E. (2003) Biochemical and therapeutic effects of antioxidants in the treatment of Alzeimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Curr. Drug Targets CNS Neurol. Disord. 2,* 95–107.
7. Gerber, M., Boutron-Ruault, M. C., Hercberg, S., Riboli, E., Scalbert, A., Siess, M. H. (2002) Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bulletin du Cancer 89,* 293–312.
8. Gordon, M. H. (1990) The mechanism of the antioxidant action *in vitro*. In: Hudson, B. J. F. (ed.) *Food Antioxidants*. Elsevier, London, pp. 1–18.
9. Gul, M. Z., Ahmad, F., Kondapi, A. K., Qureshi, I. A., Ghazi, I. A. (2013) Antioxidant and antiproliferative activities of *Abrus precatorius* leaf extracts – an *in vitro* study. *BMC Complement Altern. Med. 13,* 53–64.
10. Huang, S. J., Mau, J. L. (2006) Antioxidant properties of methanolic extracts from *Agaricus blazei* with various doses of γ-irradiation. *LWT-Food Sci. Technol. 39,* 707–716.

11. Jiang, Y., Hu, W., Lee, K. C., Wang, M. H. (2013) Antioxidant and anti-inflammatory activities of different solvent fractions from ethanol extract of *Synurus deltoides* (Aiton) Nakai leaves. *Food Sci. Biotechnol. 22,* 215–223.
12. Jung, C. M., Kwon, H. C., Seo, J. J., Ohizumi, Y., Matsunaga, K., Saito, S., Lee, K. R. (2001) Two new monoterpene peroxide glycosides from *Aster scaber*. *Chem. Pharm. Bull. 49,* 912–914.
13. Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., Heinonen, M. (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem. 47,* 3954–3962.
14. Katerere, D. R., Eloff, J. N. (2005) Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed Anti-HIV/AIDS phytomedicine. *Phyto. Res. 19,* 779–781.
15. Kim, C. M., Sin, M. K., An, T. K., Lee, K. S. (1997) *Dictionary of Chinese Herbs*. Jung Dam Publisher, Seoul, Korea, p. 1431.
16. Kumpulainen, J. T., Salonen, J. T. (1999) *Natural Antioxidants and Anti-carcinogens in Nutrition*, *Health and Disease*. The Royal Society of Chemistry, UK, pp. 178–187.
17. Kwon, H. C., Jung, C. M., Shin, C. G., Lee, J. K., Choi, S. U., Kim, S. Y., Lee, K. R. (2000) A new caffeoyl quinic acid from *Aster scaber* and its inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase. *Chem. Pharm. Bull. (Tokyo) 48,* 1796–1798.
18. Oyaizu, M. (1986) Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J. Nutr. 44,* 307–315.
19. Pin-Der-Duh, X. (1998) Antioxidant activity of burdock (*Arctium lappa* L): its scavenging effect on free radical and active oxygen. *J. Am. Oil Chem. Soc. 75,* 455–461.
20. Pourmorad, F., Hosseinimehr, S. J., Shahabimajd, N. (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol. 5,* 1142–1145.
21. Prieto, P., Pineda, M., Aguilar, M. (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analyt. Biochem. 269,* 337–341.
22. Ross, J., Kasum, C. (2002) Dietary flavonoids: Bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr. 22,* 19–34.
23. Serafini, M., Bellocco, R., Wolk, A., Ekstrom, A. M. (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology 123,* 985–991.
24. Singleton, V. L., Rossi, J. A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult. 37,* 144–158.
25. Subhasree, B., Baskar, R., Keerthana, R. L., Susan, R. L., Rajasekaran, P. (2009) Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chem. 115,* 1213–1220.
26. Tiwari, A. K. (2001) Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy. *Curr. Sci. 81,* 1179–1187.
27. Waladkhani, A., Clemens, M. R. (1998) Effect of dietary phytochemicals on cancer development. *Int. J. Mol. Med. 1,* 747–753.
28. Wang, H., Nair, M. G., Strasburg, G. M., Booren, A. M., Gray, J. I. (1999) Antioxidant polyphenols from tart cherries *(Prunus cerasus)*. *J. Agric. Food Chem. 47,* 840–844.
29. Wangensteen, H., Samuelsen, A. B., Malterud, K. E. (2004) Antioxidant activity in extracts from coriander. *Food Chem. 88,* 293–297.
30. Willet, W. C. (2002) Balancing life-style and genomics research for disease prevention. *Science 296,* 695–698.