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Polyphenol composition and antioxidant activity from the vegetable plant *Artemisia absinthium* L.

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Abstract

The aim of this work was to establish the antioxidant capacity and the polyphenolic profile of *Artemisia absinthium* that could potentially be used in the human diet. An ultra-high performance liquid chromatographic method was used to identify and quantify individual phenolic compounds of the *A. absinthium* leaves. A total of 20 polyphenolic compounds were identified and quantified in *A. absinthium* leaves, including hydroxybenzoic acids, hydroxycinnamic acids, flavonols and other groups of phenolic compounds. The ultra-high performance liquid chromatography (UHPLC) analysis of the phenolic compounds profile revealed that salicylic acid was the dominant phenolic compound present in the leaves extract followed by myricetin, caffeic acid, gallic acid and ferulic acid. The *A. absinthium* leaves was extracted with 3 different solvents (ethyl acetate, methanol and water) and screened for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. The antioxidant activity of *A. absinthium* was assessed by evaluating the 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power and phosphomolybdenum assay. Water extract exhibited the highest phenolic content (134.47 mg gallic acid equivalent 100g DW^{-1}) whereas, ethyl acetate extract showed highest flavonoid content (87.04 mg quercetin equivalent 100g DW^{-1}). The water extract possesses highest antioxidant activity towards DPPH, reducing power and phosphomolybdenum assay. The antioxidant activity among the *A. absinthium* leaves extracts assayed through all the three methods was found to be water extract > methanol extract > ethyl acetate extract. Compared with those of major commercial leafy vegetables, leaves of *Artemisia* contain high content of phenolic acids and flavonoids, which provide significant health benefits.

Keywords: *Artemisia absinthium;* antioxidant activity; 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity; ultra-high performance liquid chromatography (UHPLC).
Abbreviations: DPPH: 1,1-diphenyl-2-picrylhydrazyl; UHPLC: Ultra-high performance liquid chromatography.

Introduction

Artemisia absinthium L., commonly known as wormwood belongs to the Asteraceae family and grows as a perennial herb with fibrous roots. It grows naturally on non-cultivated, arid ground, on rocky slopes and at the edge of footpaths and fields. The plant is recognized by its characteristic odor which makes it useful for making a plant spray against pests. It is used in companion planting to suppress weeds as its roots secrete substances that inhibit the growth of surrounding plants. The plant has repellant activity against insect larvae. The plant is used as an ingredient in the spirit absinthe, and is used for flavoring in some other spirits and wines, including bitters, vermouth and pelinkovac. It is also used to spice mead (Grieves, 1931; Tariku et al., 2011). In Korea, wormwood is traditionally used as colour and flavor agent for the preparation of green *songpyeon*, a type of *dduk/tteok* (Korean rice cake), eaten during the Korean thanksgiving festival of Chuseok in the autumn. The young leaves picked in the spring when it is still young is used to prepare the juice which is used as an ingredient in the dough preparation to make green *songpyeon*. The chemical constituents of leaves and flowers include silica, two bitter substances (absinthin and anabsinthine), thujone, tannic and resinous substances like malic and succinic acid (Tariku et al., 2011). Wormwood is used as stomachic, antiseptic, antispasmodic, carminative, cholagogue, febrifuge and anthelmintic. The extracts of the plant have shown to inhibit strong antimicrobial activity (Fiamegos et al., 2011). The pure wormwood oil is very poisonous, but with proper dosage poses little or no danger. The oil is a potential source of novel agents for the treatment of leishmaniasis (Tariku et al., 2011). In recent years, there has been a trend towards the use of natural phytochemicals present in natural resources like vegetables, fruits, oilseeds and herbs which serve as potential antioxidants and functional ingredients (Kaur and Kapoor, 2001; Elliott, 1999). Polyphenolic compounds are a group of low and medium molecular weight secondary metabolites that are widely distributed in plants, which can be divided into two major subgroups: flavonoids and phenolic acids. Phenolic acids include mainly hydroxybenzoic acids (e.g. benzoic, gentisic or *p*-anisic acids) and hydroxycinnamic acids (e.g. caffeic or ferulic acid conjugates, sinapic acid). There is a much higher quantity and diversity of hydroxycinnamates than hydroxybenzoates and they consist of *p*-coumaric, caffeic, ferulic and sinapic acids either glycosylated or esterified with quinic, shikimic or tartaric acids. In fruits and leaves, the main

hydroxycinnamates result from the esterification of caffeic acid groups(s) with quinic acid, the most frequent and abundant caffeoylquinic acid isomer being 5-*0*-caffeoylquinic acid (chlorogenic acid). In cereal grains, ferulic acid esters are the most common hydroxycinnamates. Flavonoids, perhaps the most important single group of phenolics in foods, comprise a group of over 4000 aromatic plant compounds; they include anthocyanins, proanthocyanidins, flavonols and catechins (Carvalho et al., 2010). Epidemiological studies have provided evidence of beneficial health effects of dietary fruits and vegetables, and the beneficial effects have been attributed at least in part to secondary metabolites, including flavonoids and hydroxycinnamic acids (Nijveldt et al., 2001). Phenolic compounds are associated with a high number of biological activities and one with special interest is the antioxidant capacity. The consumption of antioxidant compounds or foods with high levels of these compounds is associated in prevention and reduction of the risk of diseases associated to free radical reactions. The increase of degenerative diseases such as coronary heart disease, diabetes, cancer and age related diseases has required the urgency to find new natural sources of non-toxic antioxidant compounds (Katalinic et al., 2010). The aim of our study was to investigate the phenolic composition and antioxidant activity of extracts from different solvents from leaves of *A. absinthium*. According to the recommendations, the antioxidant effects in three different bioassays were studied, besides determination of total phenolics and flavonoids. The study of polyphenolic composition is an important scientific agenda for food and nutritional sciences, which may contribute to the improvement of conventional foods with added health benefits being very useful to determine these chemicals in plants, in the field of nutrition, pharmacology and agronomy. To the best of our knowledge, this is the first report on the phenolic composition and antioxidant activity from the leaves of *A. absinthium.*

Results and discussion

Evaluation of total phenolic and flavonoid content

The total phenolic content of the extracts from leaves of *A. absinthium* was determined by Folin-Ciocalteu (FC) method and the results are expressed as equivalents of gallic acid (Table 1). Among the three extracts, water extract had the highest (134.47 mg 100g DW^{-1}) amount of phenolic compounds followed by methanol (131.18 mg 100g DW^{-1}), and ethyl acetate (51.49 mg 100g DW^{-1}). The total flavonoid content varied from 18.85 to 87.04 mg quercetin 100g DW^{-1} (Table 1). Similar results were observed with other species of *Artemisia*. For instance, the alcoholic extract of *Artemisia argentea* contained total phenolic content of 152.8 mg 100g DW^{-1} and flavonoid content of 109.20 mg 100g DW^{-1} plant material (Gouveia and Castilho, 2011). Similar results were also reported from other *Artemisia* species (Carvalho et al., 2011). The levels of total phenolics determined in this way are not absolute measurements of the amounts of phenolic compounds, but are in fact based on their chemical reducing capacity relative to gallic acid (Elliot, 1999). The phenolic compounds are the dominant antioxidants that exhibit scavenging efficiency on free radicals, and reactive oxygen species are numerous and widely distributed in the plant kingdom (Prior and Cao, 2000).

UHPLC separation and determination of phenolic compounds in wormwood leaf extract

Flavonols, hydroxybenzoic acid and hydroxycinnamic acid compounds present in vegetables have been considered a therapeutic agent due to their beneficial health effects, such as their supposed protection against certain cancers, cardiovascular diseases and aging (Ross and Kasum, 2002). Moreover, these compounds have also been used as a source of colors for food products, mainly anthocyanins, in alternative to synthetic dyes whose harmful effects upon human health have often been assumed and, in some cases demonstrated. Therefore qualitative and quantitative analysis of the wormwood leaf extract was made using UHPLC as described in the experimental part and the results are presented in Table 2. The phenolic compounds in the wormwood leaves extract were identified by comparisons to the retention time and UV spectra of authentic standards while the quantitative data were calculated from the calibration curves. Salicylic acid was the dominant phenolic compound in wormwood leaf extract; it constituted about 14399.45 µg g^{-1}, followed by myricetin (1086.55 µg g^{-1}), caffeic acid (80.60 µg g^{-1}), gallic acid (63.99 µg g^{-1}) and ferulic acid (53.55 µg g^{-1}). Similar variations in the phenolic compounds were reported in different *Artemisia* species (Carvalho et al., 2011). The three flavonols identified in the analysis were myricetin, quercetin and kaempferol. Myricetin was the most dominant flavonols in the wormwood leaves studied as it accounted for the largest proportion of the total flavonols content (Table 2). In the hydroxycinnamic acid group, caffeic acid was the most dominant hydroxycinnamic acid followed by ferulic acid and *o*-coumaric acid. Salicylic acid was the dominant compound in the hydroxybenzoic acid group followed by gallic acid, vanillic acid, β-resorcylic acid and protocatechuic acid. Previous work has 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 (Wang et al., 1999). The results suggest that flavonols like myricetin, quercetin, together with hydroxybenzoic acid, hydroxycinnamic acid and other group of phenolic acids play a predominant role in the leaves of wormwood. In humans, the presence of flavonoids may contribute to the neutralization of cell-damaging free radicals and the maintenance of heart health (Ross and Kasum, 2002). The presence of hydroxycinnamic and hydroxybenzoic acids in our diets may also contribute to bolster cellular antioxidant defenses and to maintain a healthy vision. Although flavonoids are increasingly recognized as playing important roles as antioxidant, further work is necessary to uncover the full potential of these compounds in the improvement of human health.

Evaluation of antioxidant capacity by DPPH- radical scavenging activity

The free radical scavenging activity of the extracts was tested through DPPH method (Katerere and Eloff, 2005) and the results were compared with BHT (Figure 1). 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. The wormwood leaf extracts were able to reduce the stable radical DPPH- to the yellow colored diphenylpicrylhydrazine. The IC_{50} values of the

Table 1. Total phenolic (expressed as gallic acid equivalents) and flavonoid content (expressed as mg quercetin g^{-1}) from leaves extracts of *A. absinthium.*

Extract	Total phenolic content (mg 100g DW^{-1})	Total flavonoid content (mg 100g DW^{-1})
Ethyl acetate	51.49 ± 0.015^{c}	$87.04 \pm 0.11^{*a**}$
Methanol	131.18 ± 0.011^{b}	41.21 ± 0.04^{b}
Water	134.47 ± 0.016^{a}	18.85 ± 0.01^{c}

*Data represents mean values ± SD of three replicates. **Mean values with common letters are not significantly different at $P \leq 0.5$ according to Duncan's multiple range test (DMRT).

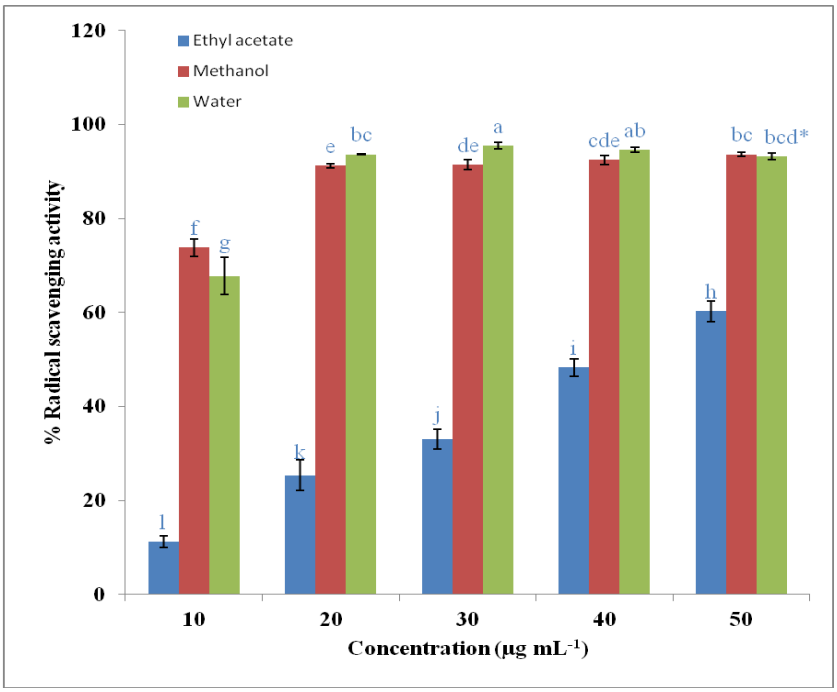

Fig 1. Free radical-scavenging activity of the leaves extracts from *A. absinthium* at different concentrations by DPPH method. Each sample was assayed in triplicate for each concentration. Experimental results were means ± SD of three parallel measurements. Mean values with common letters are not significantly different at $P \leq 0.5$ according to Duncan's multiple range test (DMRT).

extracts were methanol (6.77 µg mL^{-1}), water (7.37 µg mL^{-1}), and ethyl acetate (41.45 µg mL^{-1}) respectively. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid) and aromatic amines (e.g., *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois, 1958). The positive correlation between polyphenolic content of the extracts and its antioxidant activity is well documented (Huang and Mau, 2006). Therefore, the content of total phenolic compounds in the extracts might explain their high antioxidant activities. In this study, the extracts exhibited a concentration dependent antiradical activity by inhibiting DPPH- radical (Figure 1). Of the different extracts, water extract exhibited the highest antioxidant activity of 95.56% at 30 µg mL^{-1} concentration, followed by methanol (91.49%) and ethyl acetate (33.08%) respectively at the same concentration and it indicates that compounds with strong radical-scavenging capacity are of high polarity (Figure 1). One of the possible mechanisms is polyphenolic associated compounds. Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radical, quenching singlet and triple oxygen or decomposing peroxide (Li et al., 2007). Butylated hydroxytoluene (BHT) showed similar degree of free radical scavenging activity with that of the extracts at low concentration points. The DPPH activity of BHT exhibited 92.04% at 30 µg mL^{-1} concentration.

Evaluation of reductive potential

Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been claimed to explain the antioxidant activities (Re et al., 1999; Diplock, 1997). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. 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 (Figure 2). The reducing power of the extracts followed the order of water > methanol > ethyl acetate extract. The reducing properties are generally associated with the presence of reductones (Pin-Der Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Li et al., 2007). Our data on the reducing capacity of extracts suggested that reductone-associated and hydroxide groups of compounds can act as electron donors and can react with free

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

Compounds	Concentration (µg g^{-1})
Flavonols	
Myricetin	1086.55 ± 40.043*[b**]
Quercetin	30.90 ± 0.565[cd]
Kaempferol	12.95 ± 0.303[cd]
Hydroxycinnamic acid	
Caffeic acid	80.60 ± 0.848[c]
p-Coumaric acid	9.10 ± 0.141[d]
Ferulic acid	53.55 ± 2.61[cd]
m-Coumaric acid	0.24 ± 0.007[d]
o-Coumaric acid	32.90 ± 0.005[cd]
Hydroxybenzoic acid	
Gallic acid	63.99 ± 0.827[cd]
Protocatechuic acid	29.20 ± 0.001[cd]
β-Resorcylic acid	35.55 ± 0.070[cd]
Vanillic acid	47.00 ± 0.282[cd]
Salicylic acid	14399.45 ± 30.90[a]
Other Phenolic compounds	
Rutin	44.00 ± 0.282[cd]
Vanillin	12.55 ± 0.494[cd]
Hesperidin	36.00 ± 0.007[cd]
Resveratrol	21.40 ± 0.007[cd]
Naringenin	8.15 ± 0.070[d]
Formononetin	11.10 ± 0.707[cd]
Biochanin A	4.55 ± 0.070[d]

*Data represents mean values ± SD of three replicates. **Mean values with common letters are not significantly different at $P \leq 0.5$ according to Duncan's multiple range test (DMRT).

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

Extract	Antioxidant capacity [as equivalent to α-tocopherol (mg g^{-1})]
Ethyl acetate	187.48 ± 0.094*[a**]
Methanol	168.28 ± 0.013[b]
Water	161.75 ± 0.054[b]

*Data represents mean values ± SD of three replicates. **Mean values with common letters are not significantly different at $P \leq 0.5$ according to Duncan's multiple range test (DMRT).

radicals to convert them to more stable products, and thereby terminate radical chain reactions.

Evaluation of antioxidant capacity by phosphomolybdenum method

The antioxidant capacity of the wormwood leaf extracts was measured spectrophotometrically through phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte, and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the extracts was found to decrease in the order: water > methanol > ethyl acetate extract (Table 3).

Materials and methods

Chemicals

Methanol, acetonitrile, glacial acetic acid and distilled water (HPLC grade) were purchased from J.T. Baker (USA), and hydrochloric acid was purchased from Daejung Co. (Daejung chemicals & Materials Co. Ltd, Siheung, Gyeonggi-Do, Korea). Dimethyl sulfoxide (DMSO), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, protocatechuic acid, β-resorcylic acid, vanillic acid, caffeic acid, vanillin, ρ-coumaric acid, salicylic acid, ferulic acid, m-coumaric acid, rutin, o-coumaric acid, hesperedin, myricetin, resveratrol, quercetin, naringenin, kaempferol, formononetin and biochanin A were purchased from Sigma-Aldrich (USA).

Plant material

The *Artemisia absinthium* L. leaves (500 g) were procured from local market in April 2012 at Gwangjin-gu, Seoul, South Korea. A voucher specimen is deposited in the Department of Applied Biosciences, Konkuk University, Seoul, South Korea, and identified by Dr. Ill-Min Chung.

Preparation of extracts

A. absinthium 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 40 ± 2°C for 2 days. The dried leaves were finely powdered. The dried powder was extracted with 5 x 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 ethyl acetate until the extracts were nearly colorless. Solvents were removed in vacuo, and extracts were obtained respectively.

Determination of total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965). 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 colour was determined at 765 nm, using a Macasys Optizen 2120UV plus UV-spectrophotometer (Macasys, Korea). The concentration of the total phenolic content was determined as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The estimation of phenolic compounds from the extracts was carried out in triplicate and the results were averaged.

Determination of total flavonoid content

Total flavonoid content of the extracts was determined by using the aluminium chloride colorimetric method as described by Willet (2002), with some modifications. Extracts (0.5 mL), 10% aluminium chloride (0.1 mL), 1M 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 Macasys Optizen 2120UV plus UV-spectrophotometer (Macasys, 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 with 10 mL of acetonitrile and 2 mL of 0.1 N hydrochloric acid. The

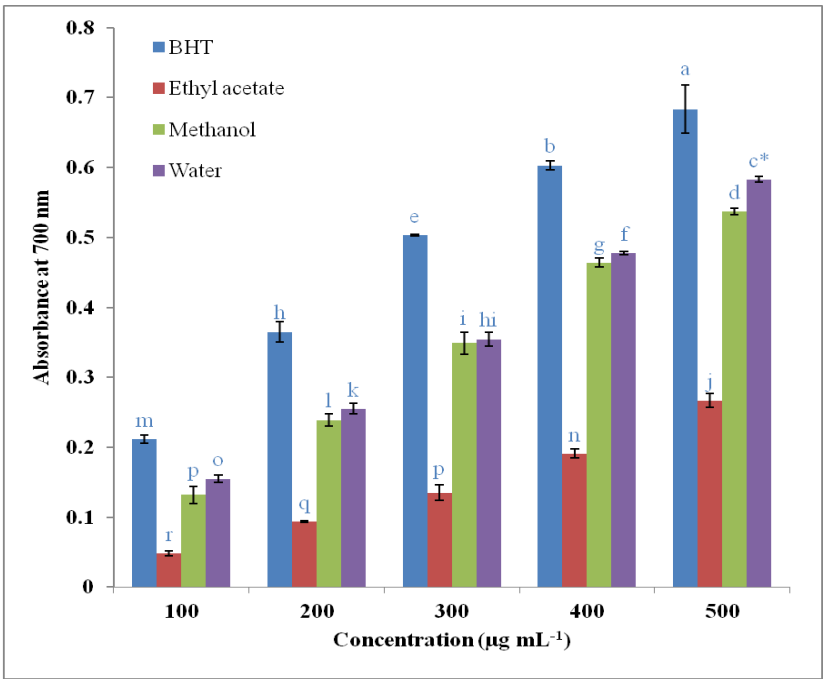

Fig 2. Reducing power of the extracts from *A. absinthium* leaves at different concentrations. Each sample was assayed in triplicate for each concentration. Experimental results were means ± SD of three parallel measurements. Mean values with common letters are not significantly different at $P \leq 0.5$ according to Duncan's multiple range test (DMRT).

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 (Model; ACCELA PDA DETECTOR, CA, USA) system. Separation was primarily achieved using a HALO C_{18} (2.7 µm, 2.1 x 100 mm) column and the absorbance were 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^{-1}. Solutions of available pure known compounds, gallic acid, protocatechuic acid, *β*-resorcylic acid, vanillic acid, caffeic acid, vanillin, *ρ*-coumaric acid, salicylic acid, ferulic acid, *m*-coumaric acid, rutin, *o*-coumaric acid, hesperedin, 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^{-1}. Phenolic compounds of leaf extract were identified by comparing their retention times with those of pure compounds. The results were expressed as µg g^{-1} of each compound from the total phenolic compounds.

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the extracts from wormwood leaves, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH-) free radical was determined by the method described by Katerere and Eloff (2005) with some modifications. Briefly, different concentrations (10 to 50 µg mL^{-1}) 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:

Radical scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}]$ x 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 Oyaizu (1986) with some modifications. Briefly, different extracts of concentration (100 to 500 µg mL^{-1}) 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 indicates increased reducing power. All analysis were run in triplicate and averaged.

Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of wormwood leaf extracts was evaluated by the method of Prieto et al. (1999) and the results are expressed as equivalents of α-tocopherol (mg g^{-1} DW of extract).

Expression of data and statistical analysis

The data was presented as mean ± standard deviation (SD) for the three determinations and the statistical significance between groups was analyzed by one way ANOVA followed by Duncan's test using SPSS software package version 12.0 (SPSS Inc., Chicago). P-values less than 0.5 were considered significant.

Conclusions

In our present study, the decreasing order of antioxidant activity among the *A. absinthium* leaves extracts assayed through all the three methods was found to be water extract > methanol extract > ethyl acetate extract. This order is similar to the phenolic contents of the extracts that showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract. The UHPLC analysis of the phenolic compounds profile revealed that salicylic acid was the dominant phenolic compound present in the wormwood leaves extract followed by myricetin, caffeic acid, gallic acid and ferulic acid. The knowledge of the phenolic profile, occurring in the wormwood holds great significance from both dietary and nutritional point of view.

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Ye-Ji Lee and Ill-Min Chung contributed equally to this work.

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