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Research Article

Preliminary Screening of *Artemisia argyi* for Antioxidant Potentials

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ABSTRACT

Introduction *Artemisia argyi* is an aromatic herb traditionally used to help treat menstrual disorders, infertility, epistaxis, uterine bleeding in pregnancy, excessive menstrual flow and acts as an antipruritic. The present investigation was undertaken to appraise the antioxidant profile and antioxidant capacity of *Artemisia argyi* by using polar solvent extraction. Methods The aqueous, ethanol and methanol leaf extracts of *Artemisia argyi* were screened for the presence of phytochemicals. Total phenolic content and total flavonoids content were evaluated using the Folin-Ciocalteu method and Aluminium chloride colorimetric method. Antioxidant profile for flavonoids, hydroxybenzoic acids, hydroxycinnamic acids were quantified using reverse phase high performance liquid chromatography (RP- HPLC). Radical attenuating abilities of the different extracts were investigated using ABTS radical scavenging ability, DPPH radical scavenging activity, ferric reducing power (FRAP), iron chelating activity and nitric oxide (NO) scavenging activity. Results The methanol extract showed the strongest extractability for hydroxybenzoic acid, hydroxycinnamic acids and flavonoids. The methanol extract of *Artemisia argyi* possessed highest total phenolic and flavonoid contents. The lowest EC_{50} values for ABTS, DPPH and NO were recorded in methanol extract at 0.11 ± 0.01 mg/ mL, 63.34 ± 1.10 µg/mL and 0.96 ± 0.01 mg/ mL respectively. In FRAP assay, methanol extract exhibited the highest frap value, 2.38 ± 0.06 mmole Fe^{2+} equivalents /g extract. The aqueous extract showed the highest iron chelating activity with the EC_{50} value of 3.05 ± 0.07 mg/ mL. EC_{50} values of ABTS, DPPH and NO assay showed negative correlation with the total phenolic and total flavonoid content while iron chelating and FRAP assays expressed positive correlation. Conclusions This study revealed that *Artemisia argyi* possess greater antioxidant potential in methanol and addresses the health promoting effects of *Artemisia argyi* to become a lucrative nutraceutical source for antioxidants.

Keywords *Artemisia argyi*, HPLC, phenolic, flavonoid, antioxidant activities.

INTRODUCTION

Free radicals are group of atoms with unpaired electrons and they are highly reactive species. They can be generated either in the normal body metabolism as endogenous or from the exogenous sources such as ingestion of foreign chemicals or pollutants. However, they could be beneficial in terms of signaling, regulatory molecules and destroy viruses and bacteria at physiologic levels. If excess, free radicals in our bodies could cause oxidative stress which adversely alter the cell structures as interacting with lipids, proteins, and DNA which would trigger diseases such as Parkinson's disease, cancer, atherosclerosis, stroke, rheumatoid arthritis, neuro degeneration, and diabetes. Hence, the antioxidants are present as reducing agents to neutralize free radicals. They can be classified into endogenous (enzymatic or non-enzymatic compounds in the body) and exogenous (externally supplied from the foods)[1-3]. Spices and herbs have been used to improve flavors and antioxidant capacity since ancient time. Plant foods are rich in phytochemicals which are the non-nutritive plant chemicals that have protective or disease preventive action. They are natural bioactive compounds that interplay with nutrients and dietary fiber in protective function and possess many properties such as antioxidants, anti-microbial and physiological activities[4]. These bioactive compounds in the human diet are largely contributed by plants. They are secondary metabolites in plants such as the phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen containing compounds (alkaloid, amino acids, peptides and amines) and carotenoids are well known sources of antioxidants[5]. Successful determination of biologically active compounds from plant is largely dependent on the type of solvent used in extraction procedure. The water, ethanol, methanol, chloroform, hexane and ether are often the solvent to extract bioactive compounds[6]. Solvent extraction is frequently used for isolation of the antioxidants and all extraction yield, phenolic content and antioxidant activity of the extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity. The water and methanol (polar solvents) showed the high total phenolic contents and antioxidant activities[7,8]. Polar solvents are playing the important role for obtaining high fractions of high antioxidant activity and total phenolic contents.The genus, *Artemisia* belongs to the *Asteraceae* family and is the largest of the flowering plants. *Artemisia* species are aromatic or fragrant plants which contain essential oil.

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Table 1: Phytochemical constituents of leaf crude extracts of *Artemisia argyi*.

Secondary metabolites	Aqueous extract	Ethanolic extract	Methanolic extract
Alkaloid	-	-	-
Antraquinone	-	-	-
Flavonoid	+	+	+
Phlobatannin	-	-	-
Saponin	+	+	+
Steroid	-	+	+
Tannin	-	+	+
Terpenoid	-	-	-
Essential oil	-	+	+

(+) Presence of phytochemical compounds. (-) Absence of phytochemical compounds.

Artemisia argyi also known as Chinese mugwort, it has been used as traditional Chinese medicine to help treat lower abdomen pain, menstrual disorders, infertility, spitting of blood, epistaxis, uterine bleeding in pregnancy, excessive menstrual flow and external as an antipruritic. Besides, it wisely used for moxibustion and even in flavoring or as vegetable in our diet. The nutritional values of the plant have been reported to high contents of essential amino acids, high amount of polyunsaturated fatty acids, good DPPH scavenging activity, high vitamin C, total phenolic compound contents, and volatile compounds[9]. The essential oil of this plant has been reported have antioxidant and insecticides activities[10-12]. However, there is no much antioxidant profile that has been studied by using polar solvents in this plant. In recent years, many studies are more on the plant extracts and essential oils for their potential antioxidant activity. The availability of the natural plant extracts can meld with the pleasant taste and smell with preservation as to avoid lipid deterioration, oxidation and spoilage by microorganisms[13].The current study appraised the antioxidant profile and antioxidant capacity of *Artemisia argyi* by using polar solvent extraction (aqueous, ethanol and methanol) and compared their activity to evaluate the outcome for their application as a functional food and nutraceutical source for antioxidants.

MATERIALS AND METHODS

Preparation of plant extract

Plant sample (*Artemisia argyi*) was purchased from local market, Kampar in January of 2015. The plant sample was identified and authenticated by Prof. H.C Ong. The leaves of *Artemisia argyi* were washed under running tap water and air dried under shade. The dried leaves were ground to find powder using an electric blender and 50 g of the powder was soaked with 500 mL of three selected solvent (deionized water, 95 % ethanol and methanol) at room temperature for three days. Each sample was filtered through filter paper and the solutions were stored in 4 °C. The procedure was repeated for another two cycles of extraction by using the previous filtrates to ensure complete extraction of phytochemical compounds. The ethanol and methanol filtered solutions were sent to rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) for the removal of solvents. Aqueous filtered solution was centrifuged (8000 g, 15 min) and the supernatant was freeze dried (LaboGene, Lynge, Denmark) until a constant weight was observed. The aqueous, ethanol and methanol crude extracts were stored in 4 °C for the following analysis.

Phytochemical screening

Aqueous, ethanol and methanol leaf extract of *Artemisia argyi* were subjected to preliminary phytochemical screening for the detection of various plant secondary metabolites[14].

Test for alkaloid

Wagner test: 2 mL of each filtrate added with 1 % HCl + steam. Then add 1 mL of the solution with 6 drops of Wagner's reagent. The brownish-red precipitate indicates the presence of alkaloid.

Test for antraquinone

Borntrager's test: 1mL of 10 % ammonia added into 2 mL of chloroform extract extract. The pink red color in the ammoniacal (lower) layer indicates the presence of antraquinone.

Test for flavonoid

Shinoda's test: 1 mL of concentrated HCl and magnesium turnings were added into 2 mL of methanolic extract. The pink red or green blue coloration indicates the presence of flavonoid.

Test for phlobatannin

2 mL extract was boiled with 2 mL of 1 % HCl. The formation of red precipitate indicates the presence of phlobatannin.

Test for saponin

Foam test: 2 mL of filtrate added with 5 mL of distilled water and shaken well. The persistence of foaming indicates the presence of saponin.

Test for steroid

Liebermann-Burchardt test: 1 mL of methanol extract added with 1mL of chloroform, 2 mL of acetic anhydride and 2 drops of concentrated H_2SO_4. The dark green coloration indicates the presence of steroid.

Test for tannin

Braemer's test: 2 mL of methanol extract added with 10 % alcoholic ferric chloride (1:1) and the dark blue colouration indicates the presence of tannin.

Test for terpenoid

Liebermann-Burchardt test: 1 mL of methanol extract added with 1 mL of chloroform, 2 mL of acetic anhydride and 2 drops of concentrated H_2SO_4. The reddish pink coloration indicates the presence of terpenoid.

Test for volatile oil

2 mL extract added with 0.1 mL of dilute NaOH followed by addition of small quantity of dilute HCl and the white precipitates formation indicates the presence of volatile oil

Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis

RP-HPLC analysis using the Shimadzu HPLC system (Shidmadzu Co., Kyoto, Japan) equipped with a SPD-20A UV/Vis Detector was used to quantify the antioxidant compounds. The Restek Pinnacle™ II C18 column (150 mm x 4.6 mm x 5.0 µm) (Restek, Bellefonte, PA, USA)

Table 2: Contents of selected hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids in aqueous, ethanolic and methanolic leaf extracts

Leaf extract	Hydroxybenzoic acids (mM/g dry matter)			
	Protocatechuic acid	*p*-hydroxybenzoic acid	Gallic acid	Vanillic acid
Aqueous	n.d.	0.11 ±0.00	0.78 ± 0.04	n.d.
Ethanolic	n.d.	n.d.	0.86 ± 0.02	n.d.
Methanolic	0.12 ± 0.00	0.21 ± 0.00	0.33 ± 0.01	0.37 ± 0.04
Leaf extract	Hydroxycinnamic acid (mM/g dry matter)			
	Caffeic acid	Ferulic acid	Neochlorogenic acid	*p*-coumaric acid
Aqueous	n.d.	n.d.	0.34 ± 0.02	n.d.
Ethanolic	n.d.	n.d.	0.01 ± 0.00	n.d.
Methanolic	0.02 ± 0.00	n.d.	0.37 ± 0.00	0.05 ± 0.00
Leaf extract	Flavonoids (mM/g dry matter)			
	D-(+) catechin		Quercetin hydrate	
Aqueous	0.09 ± 0.02		0.32 ± 0.02	
Ethanolic	n.d.		0.07 ± 0.01	
Methanolic	9.88 ± 0.08		0.02 ± 0.00	

n.d., not detected. Data are presented as mean ± standard errors (n = 3)

Table 3: Total phenolic and total flavonoid contents of the leaf extracts of *Artemisia argyi*.

Bioactive compounds	*Artemisia argyi* extracts		
	Aqueous	Ethanolic	Methanolic
Total phenolics (mg GAE/g dry matter)	68.87 ± 0.70	80.53 ± 2.66	234.52 ± 0.99
Total flavonoids (mg QE/ g dry matter)	83.37 ± 31.88	449.81 ± 5.59	737.72 ± 25.55

GAE, gallic acid equivalents; QE, quercetin equivalents, values reports as mean ± standard errors (n=3)

was used to perform chromatographic separations. The binary gradient described by Kaisoon et al[15] was used with a slight modification. The solvents comprising water was adjusted to pH 2.74 by pH meter (Mettler-Toledo Inc., Greifensee, Switzerland) with acetic acid as solvent A and acetonitrile as solvent B. The running conditions were column fixed at 38 °C and injection volume of 20 µl, 0.5 mg/mL of each extract. The analysis was carried out at a flow rate of 0.8 mL/min and the UV detection wavelengths, 280 nm for hydroxybenzoic acid, 320 nm for hydroxycinnamic acid and 320 nm for flavonoids. Identification and quantification of phenolic compounds and flavonoids were carried out by using the external standard method by comparing their retention time and peak areas with the pure standards.

Determination of Total Phenolic Contents

Total phenolic content in each of the crude extract was determined by using Folin-Ciocalteu method as described by Azlim Almey et al[16]. Gallic acid was used as standard and 1 mg/mL standard stock solution of gallic acid was prepared. The working concentration between 0.02 mg/mL to 0.14 mg/mL were prepared by the diluting the stock with deionized water. 100 µL of extract was added with 750 µL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes at room temperature. 750 µL 6 % sodium carbonate was then added and mixed gently. After 90 minutes, the absorbance was read at 725 nm using Vis spectrophotometer (Biochrom, Berlin, Germany). The total phenolic contents were expressed in mg gallic acid equivalents (GAE)/g of dry matter using the standard calibration curve of gallic acid.

Determination of Total Flavonoid Contents

The total flavonoid content of each crude extract was measured using an assay modified from Wong et al[3]. Quercetin hydrate was used as standard and 1 mg/mL standard stock solution of quercetin hydrate was used. The working concentration between 0.2 mg/mL to 1mg/ml were prepared by the diluting the stock with methanol. 200 µL of extract was added to 150 µL of 5 % sodium nitrite ($NaNO_2$) and then incubated at room temperature for 6 minutes. After that, 150 µL of aluminum chloride hexahydrate ($AlCl_3.6H_2O$) was added to the mixture and incubated at room temperature for 6 minutes. Next, 800 µL of 10 % NaOH was added and the absorbance was measured at 510 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The total flavonoid contents were expressed in mg quercetin equivalents/g dry matter using the standard calibration curve of quercetin.

Determination of 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging ability

The ABTS radical cation scavenging activity of each extract was determined according to the method described by Wong et al[3]. The 8 mg/mL of ABTS stock solution and 1.32 mg/mL of potassium persulfate ($K_2S_2O_8$) were mixed and kept in dark for 16 hours at room temperature. Next, 80.2 mL of 1 M potassium hydrogen phosphate (K_2HPO_4) was mixed with 19.8 mL of 1M potassium dihydrogen phosphate (KH_2PO_4) to obtain 100 mL of 100 mM, pH 7.4 potassium phosphate buffer. Then, the potassium phosphate buffer was used to dilute the ABTS stock solution as working solution until the absorbance reached

Table 4: The antioxidant capacity of aqueous, ethanolic and methanolic leaf extracts of *Artemisia argyi* based on ABTS , DPPH and NO, FRAP and iron chelating assays.

Leaf extract of *Artemisia argyi* or Standard	Iron chelating activity assay $\mathbf{EC_{50}}^{**}$	DPPH radical scavenging assay $\mathbf{EC_{50}}^{*}$	NO scavenging assay $\mathbf{EC_{50}}^{**}$	ABTS radical cation scavenging assay		FRAP assay
				$EC_{50}{}^{**}$	TEAC***	FRAP values****
Aqueous extract	3.05 ± 0.07	105.73 ± 6.72	2.34 ± 0.06	0.20 ± 0.00	1.07 ± 0.04	0.39 ± 0.01
Ethanolic extract	4.21 ± 0.15	107.95 ± 1.32	1.05 ± 0.01	0.20 ± 0.01	1.07 ± 0.06	0.68 ± 0.00
Methanolic extract	4.20 ± 0.15	63.34 ± 1.10	0.96 ± 0.01	0.11 ± 0.01	1.95 ± 0.24	2.38 ± 0.06
Ascorbic acid	-	6.55 ± 0.10	0.27 ± 0.00	-	-	-
EDTA	0.023 ± 0.001	-	-	-	-	-

* µg/mL; ** mg/mL; *** mmole Trolox equivalents/ g dry matter; **** mmole Fe2+ equivalents / g dry matter

Table 5: Correlation analysis between phenolics, flavonoids and different parameters.

Assays	Correlation coefficient (r)	
	Phenolics	Flavonoids
$EC_{50}$of ABTS	-0.992*	-0.818NS
$EC_{50}$of DPPH	-0.966NS	-0.757NS
E C_{50} of Metal chelating	0.544NS	0.890NS
$EC_{50}$of NO	-0.601NS	-0.918NS
FRAP values	0.998*	0.886NS

NS not significant; *significant at P<0.05.

0.700 ± 0.005 at 734 nm. For the determination, the 0.1 mL of extract was added to 1 mL of ABTS working solution and kept in dark for 10 minutes. Then, the absorbance was measured at 734 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The ABTS cation radical scavenging ability (%) was calculated as below:

ABTS cation radical scavenging ability

(%) = 1- $\frac{A_{sample}}{A_{control}}$ x 100 %

$A_{control}$ = absorbance of control reaction without plant extract

A_{sample}= absorbance of reaction with plant extract

The Trolox (0 to 0.25 mM) was used as standard and the relative antioxidant capacities were expressed in EC_{50} and TEAC values (mmole Trolox equivalents/100 g dry matter).

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical-scavenging activity of each crude extract was determined by method modified from Settharaksa et al[17]. The 0.1 mM of DPPH reagent was prepared. 500 µL of crude extract was added to 500 µL DPPH reagent and the mixture was shaken kept in dark for 30 minutes at room temperature. Next, the absorbance was measured at 517 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The blank was prepared by replacing the DPPH reagent with methanol. The DPPH radical scavenging ability (%) was calculated according to the following formula:

DPPH free radical scavenging activity

(%) = 1- $\frac{A_{sample}}{A_{control}}$ x 100 %

$A_{control}$ = absorbance of control reaction without plant extract

A_{sample}= absorbance of reaction with plant extract

The ascorbic acid (0 to12 µg/mL) was used as reference and the antioxidant activity was expressed in EC_{50} which the concentrations of each extract required to scavenge 50% of DPPH radicals.

Determination of ferric reducing power (FRAP)

The FRAP of each extract was determined by method modified from Settharaksa et al[17]. The sodium acetate buffer (300 mM, pH 3.6) was prepared and it was adjusted to pH 3.6 by adding of 16 mL glacial acetic acid, and the final volume was made up to 1 L and stored at 4°C. The FRAP reagent was made by mixing the sodium acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) and $FeCl_3.6H_2O$ (20 mM) in the ratio of 10:1:1 and pre-warmed to 37 °C before used. For the determinations, 0.2 mL of extract was added to FRAP reagent and left in 37 °C water bath for 5 minutes. Next, the absorbance was measured at 593 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The ferrous sulfate heptahydrate (0 to 0.40 mM) was used as standard and the results were expressed in mmole Fe^{2+}equivalent.

Determination of iron chelating activity

The chelation of ferrous (Fe^{2+}) ions of each extract was determined by the method described by Hasson and Jamaludin[18]. 950 µL of extract was mixed with 50 µL of $FeCl_2$ (2 mM), the mixture was added to 200 µL ferrozine (5 mM) and shaken and left at room temperature for 10 minutes. Next, the absorbance was measured at 562 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The percentage inhibition of ferrozine-Fe^{2+} complex was calculated as follows:

Inhibition of ferrozine-Fe^{2+} complex

(%) = $\frac{A_{contol-}\ A_{sample}}{A_{control}}$ x 100 %

$A_{control}$ = absorbance of control reaction without plant extract

A_{sample}= absorbance of reaction with plant extract

The ethylenediaminetetraacetic acid (EDTA) (0 to 35 mg/mL) was used as reference and the results were expressed in EC_{50}which the concentration of each extract is required to inhibit 50% of ferrozine-Fe2 + complex.

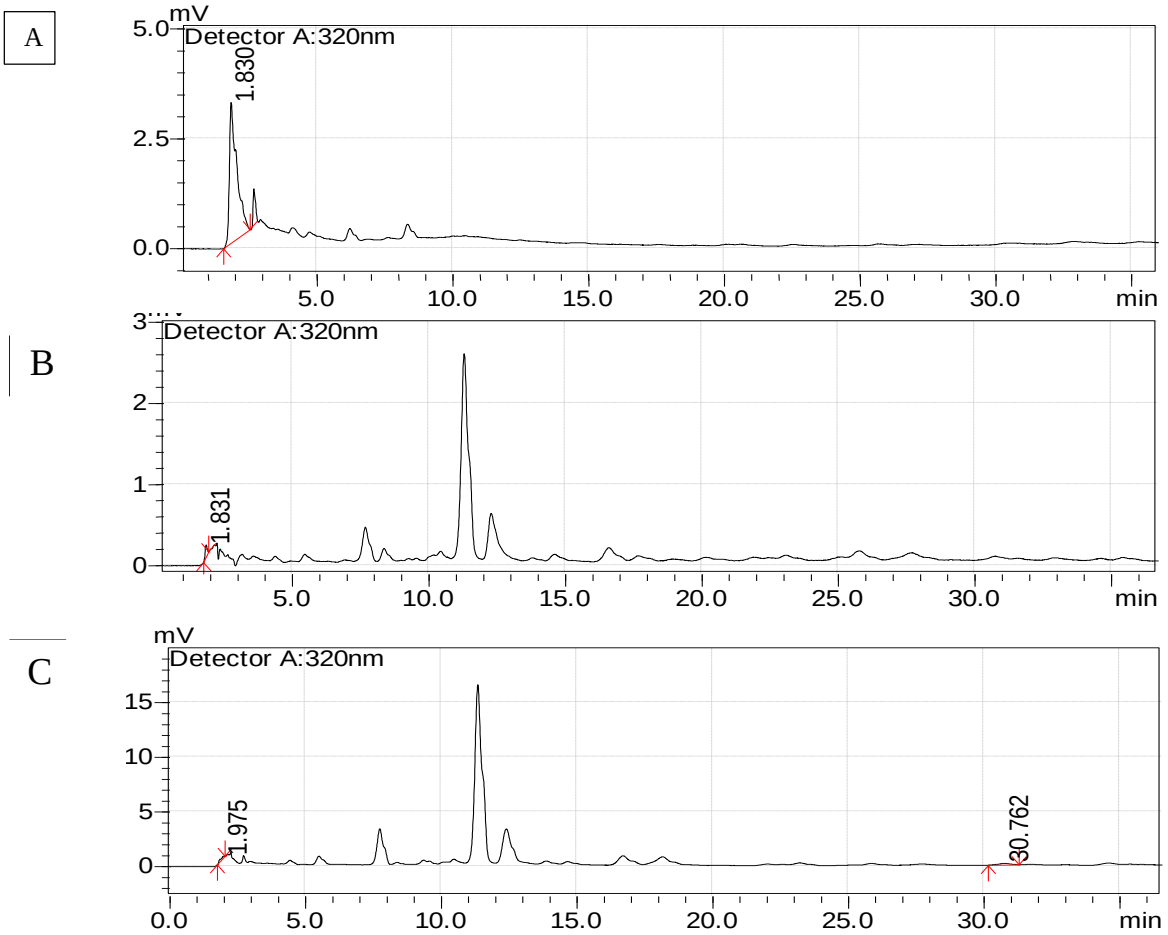

Figure 1: The HPLC chromatogram at 320 nm. A, aqueous leaf extract; B, ethanolic leaf extract; C, methanolic leaf extract.

Determination of nitric oxide scavenging activity

The nitric oxide scavenging activity of each extract was measured using method modified from Chai and Wong[19]. 800 µL of extract was added to 200 µL sodium nitroprusside (5.68 mM, pH 7.4) and the mixture was stand at room temperature under light source for 30 minutes. Next, the 50 µL Griess reagent was added and the mixture was incubated under dark at room temperature for 10 minutes. The absorbance was measured at 546 nm by using Vis spectrophotometer (Biochrom, Berlin, Germany). The nitric oxide scavenging activity (%) was calculated as follows:

Nitric oxide scavenging activity

(%) = 1- $\frac{A_{sample}}{A_{control}}$ x 100 %

$A_{control}$ = absorbance of control reaction without plant extract

A_{sample}= absorbance of reaction with plant extract

The ascorbic acid (0 to 0.6 mg/mL) was used as standard and the results were expressed in EC_{50} which the concentrations of each extract required to scavenge 50% of nitric oxide.

Data Analysis

All experiments were carried out in triplicate and data are presented as mean ± standard error. Data were analysed using Microsoft Excel 2010 with add-ins Analysis ToolPak.

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary screening tests are useful in the detection of bioactive principles and subsequently may lead to drugs discovery and development[20]. In the present study, the aqueous, ethanol and methanol leaf extract of *Artemisia argyi* were screened for various constituents which are given in Table 1. The result suggests the presence of flavonoid, saponin, steroid, tannin and essential oil in both ethanol and methanol leaf extract. Only flavonoid and saponin were detected in aqueous leaf extract. Among the solvents, methanol and ethanol revealed the presence of more phytochemicals than aqueous extract. The yield and compositions of phytochemicals were mainly affected by different types of solvents applied with varying polarities[21]. As the methanol has the polarity index 5.1, which can easily extract various polar compounds and certain group of non-polar compounds compared to water and ethanol. Regarding its toxicity and a potential domestic therapeutic application, the ethanol seems to be quite satisfactory alternatives to extract the phytochemical compounds. In plant, the secondary metabolites were produced for the adaption to the environment to against several environmental stresses and for the process of co-evolution with others interacting organism[22]. The presence of wide range of phytochemicals as well as the secondary

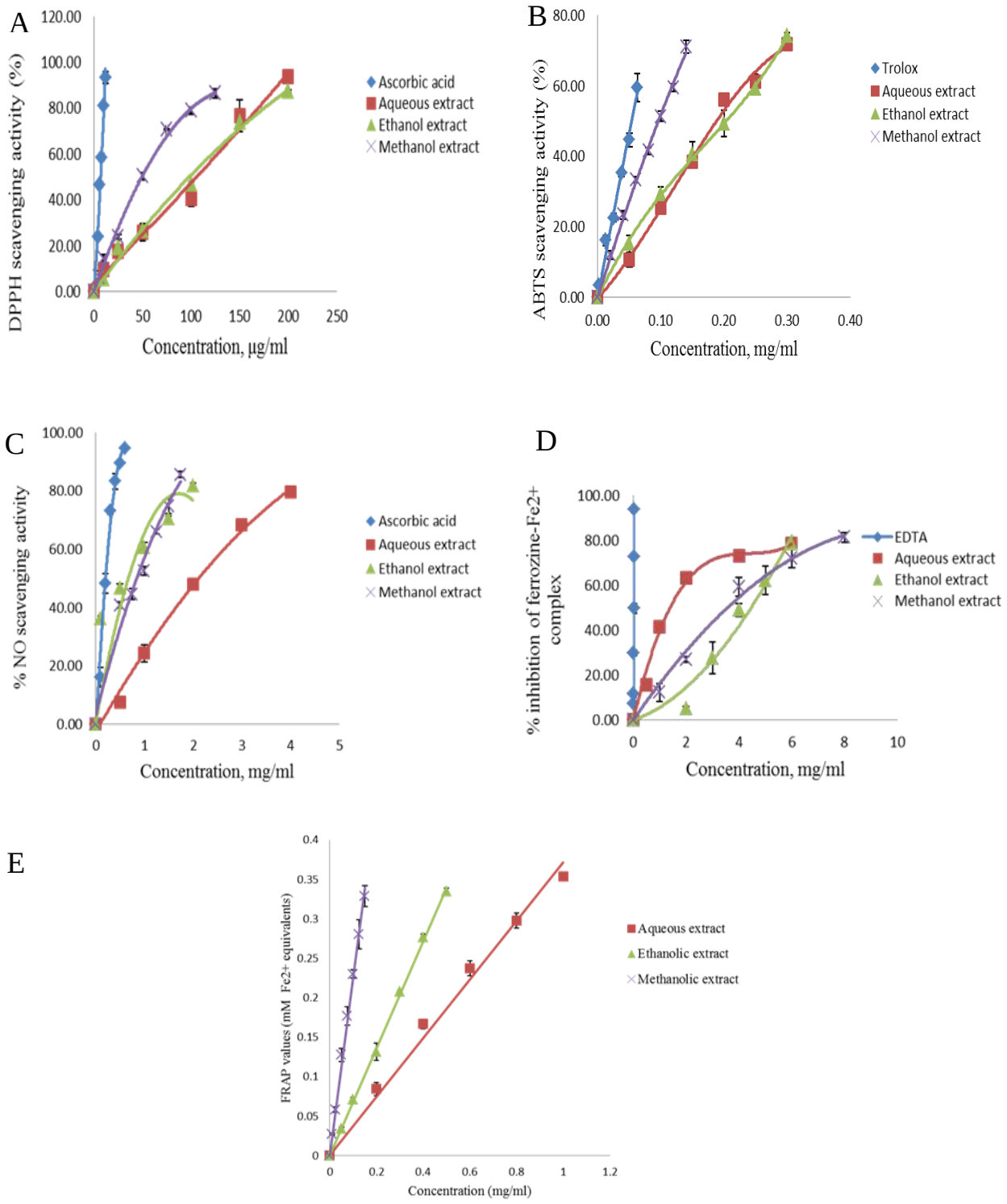

Figure 2: The antioxidant capacity of aqueous, ethanolic and methanolic leaf extracts of Artemisia argyi. A, DPPH radical scavenging activity; B, ABTS radical cation scavenging ability; C, NO scavenging activity; D, iron chelating activity; E, ferric reducing power (FRAP). Data are mean ± standard errors (n=3).

metabolites could be beneficiary to the population in a multitude of ways. The flavonoids and phenolic compounds have been reported to free radical scavenging abilities, anti-carcinogenic and anti-inflammatory. Tannins have the antifungal properties and steroids as well as saponins exhibited the effects of anticancer, cholesterol reduction, anti-inflammatory and antioxidant. Essential oils have been reported have antibacterial, antifungal, antiviral, insecticidal and antioxidant properties[23,28]. The presence of flavonoid, saponin, steroid, tannin and essential oil in the leaf extracts of *Artemisia argyi* leading the plant potentially has the nutritionally or pharmacological values.

HPLC profiles of hydroxybenzoic acids, hydroxycinnamic acids and flavonoids

HPLC is one of the techniques to separate and quantify the phenolic compounds. In general, phenolic extracts are applied to an HPLC instrument utilizing a reversed phase C18 column with UV-Vis detector and polar acidified solvents in order to suppress the ionization of phenolic acids[29,30]. In this study, four selected hydroxybenzoic acids and hydroxycinnamic acids, and two selected flavonoids in the aqueous, ethanol and methanol leaf extracts of *Artemisia argyi* were assessed. The contents were identified by comparing the retention times of their peaks with their pure standards. In hydroxybenzoic acids, the proto catechuic acid, *p*-hydroxybenzoic, gallic acid and vanillic acid were detected in methanol extract. By contrast, the *p*-hydroxybenzoic and gallic acid were detected in aqueous extract and only gallic acid was detected in ethanol extract. As the methanol extracted them evenly, the water extracted the *p*-hydroxybenzoic and gallic acid and ethanol was fully extracted the gallic acid in the highest amounts (0.86 ± 0.02 mM/g dry matter) compared to water and methanol. Among the four hydroxycinnamic acids, caffeic acids, neochlorogenic acid *p*-coumaric acid were detected in methanol extract. Only the neochlorogenic acid was detected in both aqueous and ethanolic extract. However, the methanol possessed the highest amount of neochlorogenic acid, 0.37 ± 0.00 mM/g dry matter. Consistently, the Figure 1, chromatogram at 320 nm showed the high extractability of methanol, as the methanol extract exhibited more peaks and higher intensity in the same peaks compared to aqueous and ethanol extract. Between the flavonoids, quercetin was detected in all extract while the aqueous extract posed the highest amount, 0.32 ± 0.02 mM/g dry matter. The D-(+) catechin was not detected in ethanol extract only. By contrast, the methanol extract contained the significant highest amount, 9.88 ± 0.08 mM/g dry matter. Methanol produced the best results to extraction of catechin and epicatechin in tea leaves and grape seeds compared to water, ethanol, and ethyl acetate in the technique of extraction with pressurized liquids as described in Zulema et al[31]. Table 2 summarizes the tentative characterization of these selected phenolic compounds.

Total phenolic and total flavonoid contents

Most of the phenolic and flavonoid compounds were often linked with antioxidant activities. As shown in Table 3, the highest levels of total phenolic and total flavonoid were detected in methanol extract, followed by ethanol and aqueous extract. These results were strongly linked with the results of phytochemical screening and HPLC analysis in this study. As reported, the ethanol and methanol extracted the five constituents out of nine, but only two constituents were detected in aqueous extract. The HPLC analysis above also revealed the best extractability in methanol compared to ethanol and water.

Antioxidant activities

Natural antioxidants are multifunctional and high interest as alternative source to artificial or synthetic antioxidants to reduce oxidation in complex food systems. There are several factors that may influenced the antioxidant activity, such as the multiplicity and heterogeneity of plant matrix, soil and climatic conditions of the plantation region and even the experimental conditions used for product achievement. The antioxidant properties of a plant extract cannot just evaluated by a single method due to the complexity of nature phytochemicals. Hence, several antioxidant assays should be conducted and compared to generate a more complete antioxidant properties or profile[20]. In the present study, five methods were used to assess the antioxidant capacity of aqueous, ethanol and methanol extracts (ABTS, DPPH, NO, FRAP and iron chelating assays). Results are showed in Figure 2, the methanol extract showed a superior antioxidant capacity for all assays except the iron chelating assay, in which aqueous extract showed the best result. The chelating activity was mainly based on the number and position of phenolic hydroxyl groups. Previous studies also claimed that the non-phenolic compounds (such as proteins, polysaccharides, oleoresins and saponins) are main responsible for the chelating activity. However, the flavonoids such as naringin, pelargonidin, phloridzin, and hesperitin had reported no chelating activity[32,33]. In this study, the water extracted the more chelating-related compounds compared to ethanol and methanol. The results of ABTS, DPPH, NO and iron chelating assays also reported in term of $EC_{50.}$ Consistently, the ABTS expressed in mmole Trolox equivalents/ g dry matter and FRAP in mmole Fe^{2+} equivalents / g dry matter (FRAP values) in Table 4. Although the *Artemisia argyi* is traditionally edible, the present of antioxidant capacity that measured in vitro cannot be extrapolated simply to the in vivo situation, because bioavailability, metabolism and biotransformation as well as chemical reactivity are important in the determination of the in vivo capacity[34]

Correlations between Phenols and Flavonoids

According to the results discussed previously, the different solvents to extract *Artemisa argyi* exhibited different antioxidant activities. Obviously, the composition of bioactive phytochemical contents also differed between different extraction whereby the methanol can extract most of these compounds evenly, as supported in phytochemical screening, HPLC analysis and total phenolic and flavonoid contents in this study. Phenolic acid and flavonoids might contributed in antioxidant activity, hence the correlations between analyzed parameters were assessed which detailed in Table 5. The EC_{50} of ABTS, DPPH, NO showed negative correlation with total phenol (r = -0.992, -0.966, 0.601 respectively) and flavonoids (r = -0.818, -0.757, -0.918 respectively) thereby suggesting a pronounced influence of the phenolic and flavonoids in the antioxidant activity. Our findings are similar with results of various workers suggesting these assays' EC_{50} were negatively correlated with phenolic and flavonoids[19,20,35,36]. The FRAP values positive correlate to total phenolics (r = 0.998) and flavonoids (r = 0.886). A study conducted by Akinola et al[37], a strong correlation was found between FRAP and flavonoid (r = 0.83), FRAP and polyphenols (r = 0.80) and FRAP and phenolic acid (r = 0.86) in the ten selected *Zingiberaceae* species (Gingers) Rhizomes. Contradictory, the EC50 metal chelating even showed positive correlation

to phenolic and flavonoid content, as described previously[32,33]. Some studies revealed that no such correlation between antioxidant activity and phenol and flavonoid content. The phenol and flavonoids are not the only metabolites affecting the antioxidant activities of the plant extracts. Other phytochemicals such polysaccharides and peptides may also have an influence on the antioxidant potentials of different plants[38.]

CONCLUSIONS

The methanol extract of *Artemisia argyi* possessed the highest total phenolic contents and total flavonoid contents compared to ethanol and aqueous extracts. It also exhibited the highest antioxidant capacity in ABTS, DPPH, NO and FRAP assays except iron chelating assay in which the water is best solvent to extract chelating-related compounds. The methanol potentially can be the solvent to extract *Artemisia argyi* and might further access other profiles such as antibacterial, anticancer and antifungal. The good anti-oxidative capacity, total phenolic & flavonoid compounds address the potential health promoting effects of *Artemisia argyi* to become a potential nutraceutical source for antioxidants.

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