Asian Journal of Chemistry; Vol. 27, No. 12 (2015), 4587-4590

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.19244

Investigation on Total Phenolic Contents of *Elaeagnus multiflora*

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Received: 14 April 2015; *Accepted*: 22 May 2015; *Published online*: 29 August 2015; AJC-17513

Phenolics as metabolites possess antioxidant activity and can protect the body from damage caused by free radical induced oxidative stress. Investigation of total phenolics from selected fractions of *Elaeagnus multiflora* was carried out by Folin-Ciocalteau method. The phenolic contents of fruit extract in methanol, *n*-hexane, chloroform and ethyl acetate fractions of *E. multiflora* were 70 ± 23.1, 15 ± 2.3, 20 ± 2.3 and 25 ± 2.3, respectively. In leaves methanol, *n*-hexane, chloroform and ethyl acetate fractions showed 55 ± 11.5, 40 ± 4.0, 46 ± 3.1 and 205 ± 3.1, respectively while in young branches total phenolics were 67 ± 22.5 (methanol), 40 ± 2.3 (*n*-hexane), 30 ± 5.6 (chloroform) and 36 ± 3.0 (ethyl acetate). These results suggested that *E. multiflora* is a good candidate as a natural anti-oxidant.

Keywords: *Elaeagnus multiflora* Thunb, Folin-Ciocalteau reagent, Gallic acid, Total phenolics.

INTRODUCTION

Plant secondary metabolites have broad and diverse group of chemical compounds. The major classes of these secondary metabolites are phenolics, terpenes, flavoniods, flavones and alkaloids. Metabolites work together having amazing impact on plants physiological mechanism such as signalling, interaction with environment and defence against biotic and abiotic factors[1,2]. They play significant role in the metabolism of plant for instance in development of phenylpropanoid metabolism. Phytochemicals including flavonoids, stilbenes and lignans are the most abundant phenolic compounds in these plants[3]. These phenolic antioxidants show the ability to protect body from damages caused by free radicals include oxidative stress[4]. The capability of hindrance, inhibiting, or to put the oxidation of oxidizable supplies by scavenging free radicals and diminishing oxidative stress caused through the extreme quantities of reactive oxygen and nitrogen species (ROS/RNS *e.g.* superoxide anion, hydrogen peroxide, hydroxyl radical, peroxinitrite) beat endogenous antioxidant capability, leading to oxidation of various biomolecules such as enzyme, proteins, DNA and lipids[5]. The opposite associations between fruits and vegetables ingestion and the danger of oxidative stress associated disease[6], such as cardiovascular disease, cancer or osteoporosis have been partially recognized to phenolics[7]. It has been planned that the antioxidant properties of phenolic compounds can be mediated by the subsequent mechanism firstly scavenging fundamental species such as ROS/RNS, secondly suppressing by inhibiting some enzymes or chelating sketch metals concerned in free radical construction and thirdly up regulating or shielding the antioxidant security[8]. For the purpose of defence against harsh action of free radicals, nature has been created an antioxidant defence system composed of group of compounds and enzymes strong enough to remove free radicals before they can cause injure to the cells and new borne tissues. Several phenolic antioxidants can produce an auto-oxidation process and behave pro-oxidation below certain situation. Instead of terminating a free radical chain reaction by reacting with a second radical, the phenoxy radical may also cooperate with oxygen and manufacture from quinines (P=O) and superoxide anion (O_2^-) as exposed below[9]:

$$PO^\bullet + O_2 \rightarrow P{=}O + O_2^-$$

However, transition metal ions could also encourage pro oxidant activity of phenolic antioxidants as confirmed by the subsequent reactions[10].

$$Cu^{2+} \text{ or } Fe^{3+} + POH \longrightarrow Cu^{+} + \text{ or } Fe^{2+} + PO^{\bullet} + H^{+}$$

$$PO^\bullet + RH \longrightarrow POH + R^\bullet$$

$$R^\bullet + O_2 \longrightarrow ROO^\bullet$$

$$ROO^\bullet + RH \longrightarrow ROOH + R^\bullet$$

$$ROOH + Cu^{+} \text{ or } Fe^{2+} \longrightarrow Cu^{2+} \text{ or } Fe^{3+} + RO^\bullet + OH^-$$

Phenolic compounds constitute one of the most frequent and omnipresent groups of metabolites and are an integral component of the human diet. It was found that in addition to primary antioxidant activity, this group of compound displays

a broad diversity of biological functions which are mainly connected to modulation of carcinogenesis. Various *in vitro* and *in vivo* systems have been in employment to determine the anticarcinogenic and anticancer potential of these natural phenolic compounds or extracts[11]. *Elaeagnus multiflora* Thunb is a deciduous or semi-evergreen shrub or small tree growing to 2-8 m tall, having up to 30 cm diameter with dark brown bark. The shoots are covered with red-brown scales. The leaves are ovate to elliptic, 3-10 cm long and 2-5 cm broad. The flowers are solitary or in pairs in the leaf axils, fragrant, with a four-lobed pale yellowish-white corolla 1.5 cm long. The flowering season starts in mid-spring. The fruit is round to oval drupe 1 cm long, ripe in mid- to late summer. The *E. multiflora* is native to P.R. China and Japan and has for centuries been cultured as a decorative as well as for its touchy fruit. The fruit is juicy and edible, with an acidic taste. It has also been conventionally used in China as a handling for cough, diarrhoea and foul sores and yet for the cancer[12].

The *Elaeagnus* family consists of everlasting plants as food stuff additives contain constituents of terpenes, phenols, aliphatic alcohols, flavonoids, saponins and tannins. A number of identified bioactive chemicals particularly terpenes such as thymol and carvacraol are also isolated from plants of this family[13]. Antibacterial source such as epigallocatechin from *Elaeagnus galabra* have been recognized[14]. Leaves and fruits of *E. angustifolia* member of this Genera and the *E. multiflora* have an antipyretic consequence[15], muscle relaxant activity[16], and anti-nociceptive and anti-inflammatory possessions[17]. Taking into the consideration that scarcity of obtainable data concerning the medicinal properties in fruit of *E. multiflora* has been investigated like the antioxidant, antiplatelet aggregation, anti-inflammatory and tyrosinase inhibitory activities[18]. Other species of the genus *E. multiflora* plants are actinorhizal, growing in symbiosis with the *Actinobacterium Frankia* in the soil. These bacteria fix atmospheric nitrogen, making it available in usable form for the host plant and indirectly for other nearby plants. The plant family of *E. multiflora* also possesses antioxidant activity which can be used for the treatment of cardiovascular heart diseases as well as it was reported to put forth antioxidative, antiproliferative and anti-inflammatory effects. The extract of seed and flash of *E. multiflora* may supply to suppress cancer growth through its anti-inflammatory and antiprolifrative effects and have likelihood to control tumour cell growth[19].

The present study was conducted to analyze the phenolic components in different parts of native species of *E. multiflora* from Gilgit-Baltistan considering that it has the nutritional and functional material in fruit and leaves and the young branches as well as having the development and capacity to repair the damage cells caused by reactive oxygen species. The results were compared for the phenolic contents in young branches, leaves and fruits of *E. multiflora*. This study revealed that the young branches of *E. multiflora* contain more phenolics than fruits and leaves.

EXPERIMENTAL

The chemicals used in this study were obtained from local suppliers of different companies. Folin-Ciocalteaue phenol reagent was purchased from MP Biomedical Industries, France. Organic solvents like *n*-hexane and ethyl acetate were purchased from Tedia, United States of America. Chloroform from the Merck, Germany. Gallic acid and sodium carbonate from Scharlu and acetone from the BDA, AnalaR.

Collection and preparation of sample: The authentic plant species of *Elaeagnus multiflora* Thumb. (800 g) were collected from Oshikhandass valley of Gilgit-Baltistan during July 2014 and placed under shade to make them dry for 2 weeks at chemistry laboratory, Karakorum International University, Gilgit. The plant was identified by resident taxonomist Mr. Qamar Abbas, Department of Biological Sciences, Karakorum International University, Gilgit. The dried plant sample was then crushed in separate parts in to small pieces and transferred to separate glass bottles after weighing (fruits, 71 g; leaves, 57 g and young branches, 43 g). The dry crushed samples were soaked in methanol solvent (95 %) for 24 h. The methanolic extract was obtained through rotary evaporator and further fractions were prepared in different solvents by solvent-solvent extraction method. Folin-Ciocalteaue method was used to determine the total phenolics in each extract.

Preparation of solutions

Standard solution: Standard solution of gallic acid was prepared by dissolving 0.2 g of gallic acid in 1 L of 80 % methanol.

Percent solution: For liquid substances percent solution is geared up by dissolving amount of solute in 100 mL of solution. For example 80 % methanol solution was prepared by 80 mL methanol in 20 mL distilled water.

Molar solution: The solution which is made by dissolving amount of substance equal to its molecular weight in a solvent to make 1 L solution is known as molar solution. *e.g.* molar solution of sodium carbonate is prepared by dissolving 75 g in water to make 1 L aqueous solution of sodium carbonate.

Gallic acid: Gallic acid has capacity to retain the phenolic contents. Different type of concentrations of gallic acid solution were prepared to get the standard curve and absorbance for example, 50, 100, 200, 300, 400 and 500 and then prepared saturated solution of sodium carbonate. Sodium carbonate has the capacity to react with water to make buffer.

General procedure

Folin-Ciocalteau phenol method: Total phenolic contents of fruits, leaves and young branches of *Elaeagnus multiflora* was determined by Folin-Ciocalteau method based on Singleton and Rossi, using gallic acid as standard phenolic compound. The absorbance of different solutions of gallic acid having different concentrations like 50, 100, 200, 300, 400 and 500 were used to obtain a standard curve. After the preparation of standard solution of gallic acid 50 µL of solution was taken in test tube with the help of micropipette and 450 mL distilled water was added in the test tube in addition to 2 mL sodium carbonate solution in each test tube after the addition of 2.5 mL of Folin-Ciocalteau reagent and put each test tube in the water bath for incubation for 1.5 h at 30 °C with continuous shaking after every 5 min. Absorbance was determined against each sample by using UV-visible spectrometer when solutions achieved room temperature. The methanolic extract

(2-5 mg) of the berries (fruit), leaves and young branches of *E. multiflora*. were taken in different vials and dissolved in 80 % methanol followed by addition of 450 mL distilled water and 2.5 mL Folin reagent. After an interval of 5 min, 2 mL of sodium carbonate solution was also added and incubated on water bath for 0.5 h at 35 °C. The solutions were kept at room temperature for 1.5 h with regular shaking then absorbance was determined by using UV-visible spectrometer.

RESULTS AND DISCUSSION

The fruits, leaves and young branches of *Elaeagnus multiflora* Thunb. were taken for the estimation of total phenolic contents. All these parts showed the positive results for the phenolic content. The Folin-Ciocalteau phenol method was used for determination of phenolic content by using gallic acid as a standard phenolic compound. The Folin-Ciocalteau method involves the transport of electron from phenolic complexes to phosphomolybdic acid or phosphotungestic acid complexes, which are examined spectrometrically at 765 nm. Throughout the procedure of extraction 85 % of active interfering compounds are unconcerned. The absorbance of gallic acid increases with increase in absorption and a straight line is obtained[20]. The absorbance readings of gallic acid are shown in Table-1. The fruits, leaves and young branches of a medicinal plant *E. multiflora* were tested for phenolic contents using Folin-Ciocalteaue phenol method. First the whole fruit extract was used for the estimation of phenolic contents. Every part of plant contained considerable amount of phenolics but in compression among different parts of *E. multiflora*, leaves showed highest amount of phenolic contents (205 ± 3.1) than young branches (67 ± 2.5) and in turn young branches exhibits more phenolics than fruits (70 ± 3.1) in all of solvent extracts except methanol where reverse trend has observed.

TABLE-1
ABSORBANCE OF STANDARD SOLUTION OF GALLIC ACID AT DIFFERENT CONCENTRATIONS

S. No.	Concentration of solutions (mg/L)	Absorbance of standard solution of gallic acid
1	50	164 ± 2.4
2	100	210 ± 2.9
3	200	290 ± 5.0
4	300	335 ± 6.4
5	400	415 ± 4.6
6	500	460 ± 3.4

Estimated phenolics in different solvent fractions like *n*-hexane, chloroform, ethyl acetate, methanol and water of three parts of *E. multiflora,* ethyl acetate, water and methanol and showed higher amount of phenolic contents (205, 171, 70, respectively) whereas chloroform and *n*-hexane contained least amount of phenolic contents (46 and 40, respectively) as shown in Table-2. The chloroform fraction of *E. multiflora* showed higher amount of phenolics in leaves (46 ± 3.1 mg equivalent of gallic acid) followed by young branches (31 ± 5.0) as compared to fruits (20 ± 2.3) and similar trend in hexane, ethyl acetate and water fractions was observed, whereas in methanolic extract fruits exhibited higher phenolics (70 ± 3.1) followed by young branches (67 ± 2.5) and leaves (55 ± 1.5). These results indicated that the concentration of phenolics in medicinal plant species are not only depends on different parts but also the nature of solvents.

TABLE-2
PHENOLIC CONTENTS OF FRUITS, LEAVES AND YOUNG BRANCHES OF *Elaeagnus multiflora*

S. No.	Extract type	Phenol content (mg equivalent of gallic acid)		
		Fruits	Leaves	Young branches
1	Methanolic	70 ± 3.1	55 ± 1.5	67 ± 2.5
2	*n*-Hexane	15 ± 2.3	40 ± 4.0	40 ± 2.3
3	Chloroform	20 ± 2.3	46 ± 3.1	31 ± 5.0
4	Ethyl acetate	25 ± 2.3	205 ± 3.1	36 ± 3.0
5	Water	28 ± 4.8	171 ± 3.8	45 ± 2.3

Conclusion

For present studies the authentic sample was collected from Oshikhandass valley of Gilgit-Baltistan and identified as *Elaeagnus multiflora* Thunb. The plant material was subjected for extraction process to evaluate the phenolic contents. Different solvent extractives of the fruits, leaves and young branches of a medicinal plant *E. multiflora* were tested for phenolic contents by using Folin-Ciocalteaue phenol method by taking gallic acid as a standard phenolic compound. The whole plant extract was used for estimation of phenolic contents. Every part of plant contains phenolic contents but the fruits contains higher amount of phenolics as compared to leaves and young branches in the methanolic extract. In *n*-hexane fraction the phenolic content is greater in leaves and young branches than fruits, but relatively low than methanolic extract. In chloroform fraction the phenolic content is much higher in leaves than *n*-hexane but lower concentration of phenolics than that of methanolic extract. The amount of phenolic content in ethyl acetate fraction was highest than methanolic fraction and all other fractions. This study showed that the ethyl acetate extract of leaves of *E. multiflora* contains highest amount of phenolic contents and exhibited the greatest reducing potential and radical scavenging activity. On the basis of these results it can be concluded that *E. multiflora* is rich in bioactive phenolic compounds which should be isolated for further investigations. The leaves, fruits and young branches of *E. multiflora* could be exploited as phenolic, antioxidant additives and as nutritional supplements for prolong existence.

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