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Isolation and identification of Gallic acid from the Elaeagnus angustifolia leaves and determination of total phenolic, flavonoids contents and investigation of antioxidant activity

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Abstract

Elaeagnus angustifolia has many potential applications in drugs, detergents, perfumes, herbal teas and show various biological and pharmacological activities, such as anti-inflammatory, antipyretic and other effective treatment of disease. Gallic acid (GA) and its derivative methyl gallate (MG) are well studied plant phenolics. They have exhibited anticancer effects in several cancer cell lines. In the present work, The *E. angustifolia* leaves were collected from the enclosure of Azerbaijan Shahid Madani University trees and were dried in the shade condition; they were milled and extracted successively with n-hexane, ethyl acetate and methanol in a Soxhlet apparatus for 24 h. Further study on the methanol extract of this plant has resulted in the isolation of Gallic acid. The structure of the compound was established by spectroscopic methods. Antioxidant activities of flavonoid and phenolic compounds were also measured.

Keywords: Elaeagnus angustifolia; Gallic acid; isolation; soxhlet extractor.

Introduction

Elaeagnus angustifolia is a deciduous small tree or shrub that can reach a height of 5-10 m. The plants have shiny brownish red spin. Stems, leaves, flowers, and fruits are covered with silver-white scales. Leaves are inconspicuously veined, lance-shaped or linear lanceolate, 3-7 cm long and 1-1.3 cm

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wide, obtuse at the apex, and cuneate. Appearing from May to June, the fragrant flowers are erect or nearly erect, and have bell-shaped calyx tubes and a conspicuous, glabrous, conical floral disc, which surround the base of the style. Fruits are pink, elliptic, 9-12 mm long and 6-10 mm wide, and mature in September.

E. angustifolia (Figure 1) has many potential applications in drug manufacturing and thus is an excellent candidate for an investigation into its delivery in nanocapsule form. *E. angustifolia* is a calcium-rich herb of *Elaeagnaceae* family that has been found to have a variety of therapeutic effects. Anti-inflammatory, antipyretic and other effective treatment of diseases have the subject of study of the plant [1-6].

According to the present knowledge, *E. angustifolia* leaves comprise a mixture of different bioactive compounds belonging to various chemical types, such as phenolic compounds [7-11], they have been reported to show a wide range of physiological properties including anti-allergenic, anti-artherogenic, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effect [12-15]. Gallic acid which is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, has various medicinal uses, it is cytotoxic against certain cancer cells without harming normal cells, and possess anticarcinogenic properties and scavenger activity against several types of harmful free radicals. Gallic acid and its structurally related compounds are found widely distributed in fruits and plants. Gallic acid and its catechin derivatives are also present as one of the main phenolic components of both black and green tea. Esters of Gallic acid have a diverse range of industrial uses, as antioxidants in food, in cosmetics and in the pharmaceutical industry. In addition, Gallic acid is employed as a source material for inks, paints and color developers. Studies utilizing these compounds have found them to possess many potential therapeutic properties including anti-cancer and antimicrobial properties [16-20].

Experimental

All reagents were purchased from Fluka, Merk and Aldrich chemical companies. All solvent were distilled before use and stored over a drying agent. Column chromatography was conducted with silica gel 230-400 mesh, Merck and Silica TLC analysis was performed on Merck F254 silica gel plates (20×20 cm). IR spectra were recorded with a Shimadzu FTIR-408 spectrophotometer as KBr pills. ^{1}H NMR and ^{13}C NMR spectra

were recorded on a Brucker 400 AC spectrometer in $CDCl_3$ as a solvent at room temperature.

Figure 1. *E. Angustifolia*

Mass spectrum was attained by double-focusing mass spectrometer. The λ max and color intensity of lawsone were determined on a Philips PU 8620 UV spectrophotometer in DMSO solvent using a 1-cm quartz cell. Gallic acid was isolated from *E. angustifolia* leaves and identified by HPLC apparatus. The chromatography apparatus consisted of a Jasco (Tokyo, Japan) PU-1580 isocratic pump and a Jasco UV-1575 spectrophotometric detector, a Rheodyne 7725i manual injector equipped with a 20 µl loop (Rheodyne, Cotati, CA, USA). The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface. Melting points were determined on an Electrothermal 9100 melting point instrument.

Isolation and characterization of Gallic acid from E. *angustifolia* leaves powder

Sample of *E. angustifolia* leaves were collected from the enclosure of Azarbaijan Shahid Madani University trees in the month of April 2014. Drying was carried out at room temperature and shade. A laboratory mill was used to grind the leaves. The air-dried, powdered leaves of *E. angustifolia* (100 g) were extracted with 250 mL hexane in a Soxhlet apparatus to remove unwanted fat. After drying, the residue was extracted with ethyl acetate and methanol respectively. The extracted methanol was used for the isolation of Gallic acid. The methanol is removed completely in vacuum to leave a white solid (100 mg) as crude product. The crude Gallic acid is purified by thin layer chromatography. The product was chromatographed over silica gel by Chloroform: methanol: acetic acid in a ratio of 13.3: 2.7: 1 v/v. The melting point of the isolated, pure Gallic acid was in the range of 257-260 ^{o}C which is same as the literature value, 260 ^{o}C.

UV-Vis spectrum of Gallic acid in methanol solvent is recorded. The absorption is observed at 310 nm. Standard Gallic acid and isolated Gallic acid from methanolic extract of *E. angustifolia* leaves identified by HPLC apparatus. The peaks at retention time of 2.30 and 3.25 are in methanol solvent and Gallia acid, respectively (Figures 2 and 3).

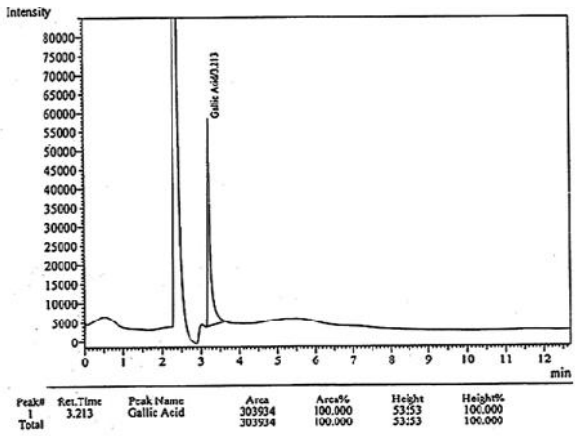

Figure 2. HPLC chromatogram of standard Gallic acid

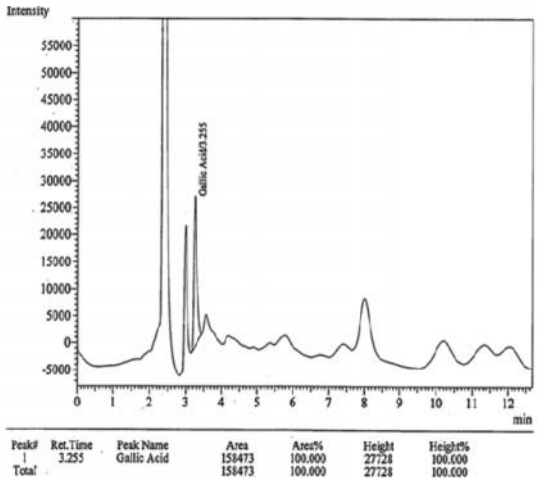

Figure 3. HPLC chromatogram of Gallic acid extracted of *E. angustifolia*

The IR spectrum revealed the presence of carboxylic group (2700-3600 cm^{-1}), hydroxyl phenolic groups (3284, 3382), carbonyl group (1705) and an aromatic moiety (1541, 1618) (Figure 4).

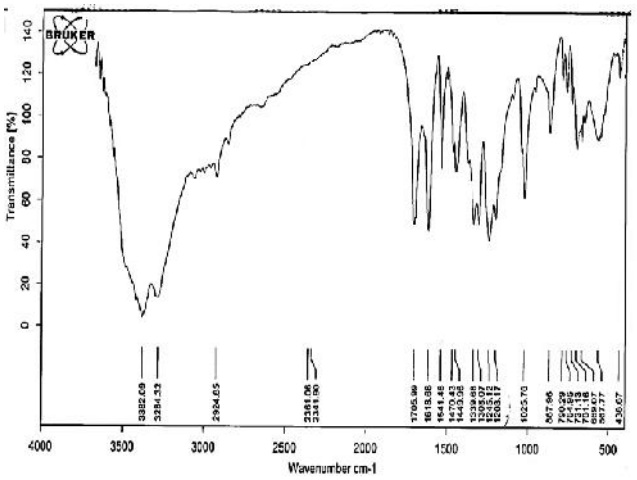

Figure 4. FT-IR spectrum of Gallic acid

Signals at ∂_H = 12.2, 8.8, 9.2 and 6.9 ppm and their related coupling constant in ^{1}HNMR spectrum is related to Carboxylic acid, number 1, number 2 and number 3 protones respectively (Figure 5).

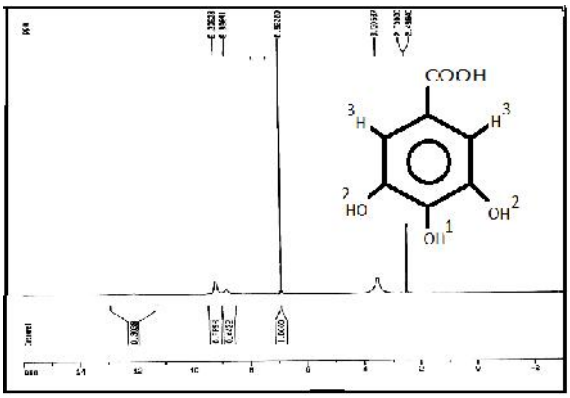

Figure 5. ^{1}HNMR spectrum of Gallic acid

Five signals were observed in the ^{13}CNMR spectrum, which corresponds to 5 carbon atoms in this compound [167.6 (C=O), 138.1 (C-1), 145.5 (C-2), 108.8 (C-3), 120.5 (C-4)] (Figure 6).

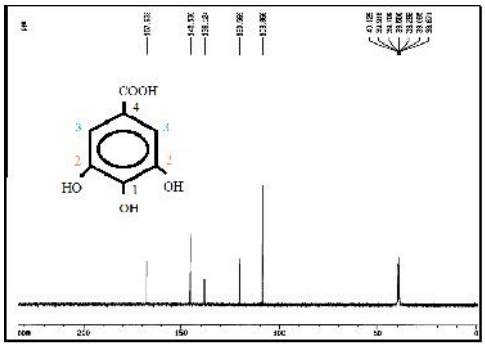

Figure 6. ^{1}HNMR spectrum of Gallic acid

Endothermic peaks in 100 and 267 ^{o}C in DSC thermograms are related to water solvent and extracted material from *E. angustifolia* leaves, this amount is consistent with the melting point mentioned in the literature for Gallic acid (Figure 7).

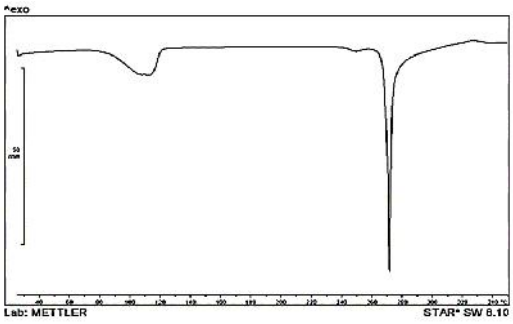

Figure 7. DSC thermograms of Gallic acid

Information of TGA thermogram for extracting material shows that the demolition started at 130 °C and 270 °C at the ends (Figure 8).

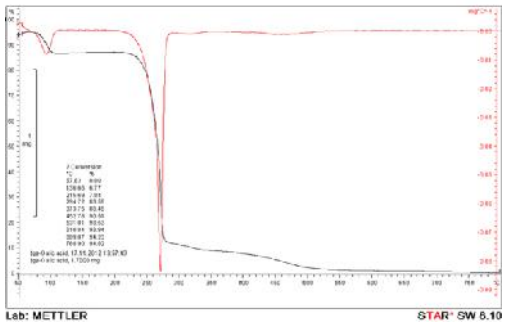

Figure 8. TGA thermograms of Gallic acid

Determination of antioxidant activity of the extracted material

The antioxidant activity of the extracted methanol was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method [21-23] with slight modification.

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) is a well-known radical and a trap ("scavenger") for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 517 nm or in the EPR signal of the DPPH (Figure 9).

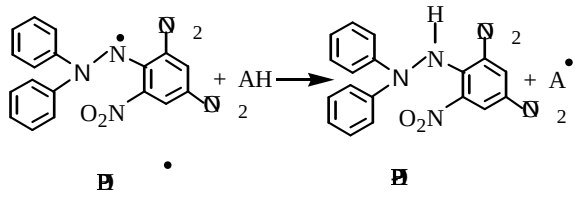

Figure 9. The reaction of DPPH Free radical (2,2'-diphenyl-1-picrylhydrazyl) with antioxidant where AH is donor molecule and A• is free radical produced.

In the classical procedure, fresh DPPH in methanol is used; at first, different concentrations of methanol extract is prepared as follows: 30, 50, 70, and 100 µl of sample added to 970, 950, 930, and 900 µl fresh DPPH solution respectively, So that the final volume becomes 1 mL. After 5 minutes the observance of solutions at 517 nm is determined (Figure 10 and 11). By increasing the concentrations of extract methanol, the absorbance of samples is reduced. This means that the extract methanol has antioxidant properties. The results are expressed as radical scavenging activity (RSA%) using the following equation:

RSA% = [absorbance at 517 nm (t = 0) – Absorbance at 517 nm (t = t) × 100/absorbance at 517 nm (t = 0)], where (t = 0) is the absorbance of the control at t = 0 min; and t = t is the absorbance of antioxidant at t = 5, or 30 min.

The antioxidant concentration necessary to decrease the initial DPPH• Concentration by 50% inhibition (named as inhibition concentration IC_{50} or efficiency concentration EC_{50}) is also often used for the comparison of the antioxidant capacities of different compounds or extracts of nature samples. In this study, IC_{50} values are 0.33 g/l. A lower value of IC_{50} indicates higher antioxidant activity. The IC_{50} value is calculated using the graph obtained by plotting the inhibition percentage against the extract or compound concentration.

However, the values of IC_{50} parameter were expressed in different units: in grams of antioxidant per kg of DPPH• [24] , the molar or the mass ratio of antioxidant to DPPH• [25-29], as µmoles of an antioxidant [30-32], or in a concentration unit as mg mL^{-1}[33].

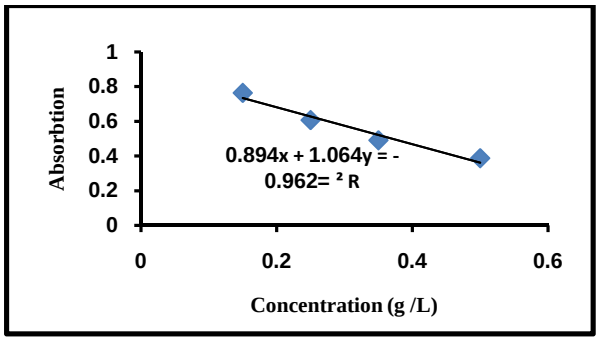

Figure 10. The absorbance curve of DPPH at different concentrations of methanolic extract at 517nm

Determination of total phenolic content

The total phenolic content of the *E. angustifolia* leaves extracts was determined using the Folin-Ciocalteu reagent [34]. The reaction mixture contained: 200 µL of diluted methanolic extract, 800 µLof freshly

prepared diluted Folin-Ciocalteu and 2 mL of 7.5% sodium carbonate. The final mixture was diluted with 7 mL deionized water. Mixtures were kept in dark at ambient condition for 2 h to complete the reaction. The absorbance at 517 nM was measured. Gallic acid was used as standard (Figure 12) and the results were expressed as mM Gallic acid/mg *E. angustifolia* leaves. This value was 0.038.

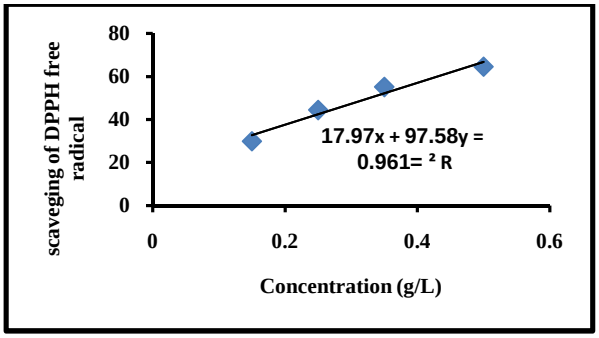

Figure 11.

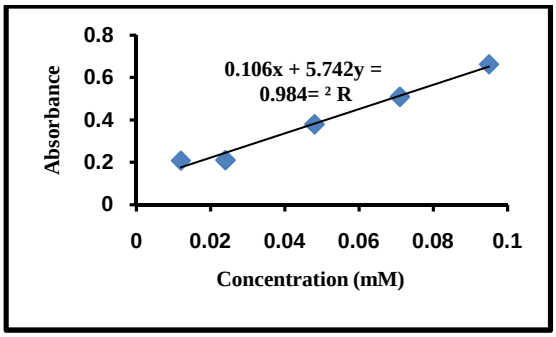

Figure 12.

Determination of total flavonid content

Total flavonid content was determined using aluminium chloride ($AlCl_3$) according to mentioned method [35], using quercetin as a standard (Figure 13). The plant extract (0.1 mL) was added to 0.3 mL distilled water, followed by 5% $NaNO_2$ (0.03 mL). After 5 min at 25°C, $AlCl_3$ (0.03 mL, 10%) was added. Then after further 5 min, the reaction mixture was treated with 0.2 mL of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and absorbance was measured at 517 nm. The results were expressed as mm quercetin/mg *E. angustifolia* leaves extracts. This value was 0.1736.

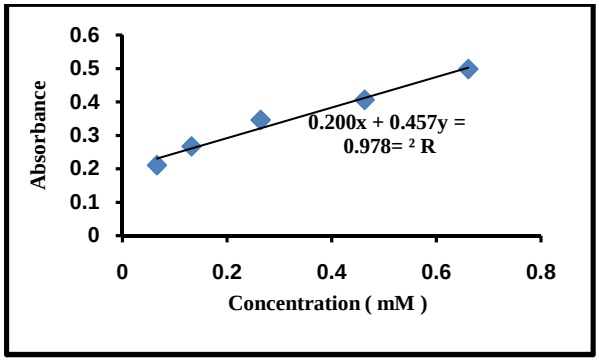

Figure 13.

Conclusion

The results of the current study showed that the extract methanol of *E. angustifolia* leaves had the highest scavenging activity. The amounts of phenol compounds of *E. angustifolia* correlates to their scavenging effect. These results demonstrate that the antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effect of different Phyto compounds.

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