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Original Article

PHENOLIC CONTENTS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF FLOWER, LEAF AND STEM EXTRACTS OF *FERULAGO ANGULATA* (SCHLECHT) BOISS

FARIDEH AZARBANI[1*], ZEINAB SAKI[2], ALI ZAREEI[1], ABDOLNASSER MOHAMMADI[1]

[1]Department of Biology, Lorestan University, Khorramabad, Iran, [2]Department of Chemistry, Lorestan University, Khorramabad, Iran. Email: frazarban@gmail.com

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ABSTRACT

Objective: *Ferulago angulata* known as Chavir is an Iranian endemic plant that has been used as the food preservative and natural flavor since long times. In the present study, antibacterial and antioxidant activities of aqueous and methanol extracts from flower, leaf and stem of *F. angulata* (Schlecht.) Boiss from Lorestan Province of Iran were evaluated. Total phenol, flavonoid and anthocyanin contents were also determined.

Methods: Antibacterial activity was studied by disc diffusion method. Diphenylpicrylhydrazyl (DPPH) assay was used to determine the antioxidant property. The total phenol, flavonoid and anthocyanin contents were measured by Folin Ciocalteu, $AlCl_3$ and pH differential methods, respectively.

Results: None of the sample extracts showed significant antibacterial activity against tested pathogens. All extracts had inhibitory activity on DPPH radicals with concentration-dependent manner. IC_{50} values ranged from 214±8.2 to 1606±94.4 µg/ml. The total phenol, flavonoid and anthocyanin contents were in the range of 59±0.017 to 483.5±0.098 mg of Gallic acid equivalents per g of dried extract, 0.5±0.004 to 539±0.29 mg of Quercetin equivalents per g of dried extract and 0.43±0.02 to 4.2±0.82 mg Cyanidin-3-glucoside, respectively.

Conclusion: The Flower aqueous extract exhibited the maximum phenolic compounds and antioxidant activity but, the stem methanol extract was the weakest one.

Keywords: *Ferulago angulata*, Antibacterial, Antioxidant, Phenol, Flavonoid, Anthocyanin.

INTRODUCTION

Free radicals are chemical species that have unpaired electrons in their external orbital [1]. They are highly reactive species capable of wide spread oxidation and peroxidation of proteins, lipids and DNA which are associated with chronic degenerative diseases including cancer, coronary artery disease, diabetes, etc [2]. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [3]. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects [4]. Hence, there is a need for investigation of safer antioxidants from natural sources. Many plants in the world show strong antioxidant activities due to their phenolic contents [5]. The genus *Ferulago* from Apiaceae family consists of 35–40 species worldwide, 8 species exist in Iran and 3 of them grow natively or are endemic [6]. Traditionally this plant was added to dairy and oil ghee to prevent decay besides adding a pleasant taste to them. The extracted essence is also used in perfume and cosmetic industries. *Ferulago* has a long history of use in folk medicine for its sedative, tonic, digestive and anti-parasitic effects [7]. There have been some published reports on its significant antibacterial, antioxidant and antidiabetes properties [8-10]. The *Ferulago angulata* known in Iran as Chavir has two subspecies; subsp. *angulata* (Schlecht.) and subsp. *carduchorum* [11]. This study represents the first report of antioxidant capacity, antibacterial activity, total phenol, total flavonoid and anthocyanin contents of the aqueous and methanol extracts obtained from flower, leaf and stem of *F. angulata* (Schlecht.) Boiss from the Ronje mount in Lorestan Province, Iran.

MATERIALS AND METHODS

Plant materials

The aerial parts (flower, leaf and stem) of *F. angulata* were collected from Ronje mountain in Lorestan Province of Iran during May to June 2013. The plant was identified and authenticated by the Dr. Hamed Khodayari, Department of Biology, Lorestan University, Iran.

Extraction procedure

The shade dried flowers, leaves and stems of the plant ware crushed into powder with blender. The powdered material was extracted three times with methanol/water at room temperature in the cycle of 24h each. The filtrates of extracts were combined and dried under a vacuum in a rotary evaporator at 40°C to pursue further analysis.

Chemicals

Gallic acid, butylated hydroxy toluene (BHT), Folin–Ciocalteu, Diphenylpicrylhydrazyl (DPPH) and Quercetin were procured from Sigma. The rest of the chemicals and solvents used were of analytical grade and were procured from either Sigma or Merck.

Four pathogenic bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* were procured from Iranian Research Organization for Science and Technology. Nutrient agar was used as bacterial media.

Antibacterial activity

Antibacterial activity of samples was evaluated against *Bacillus cereus* (PTCC 1556), *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330) and *Klebsiella pneumonia* (PTCC 1053) by disc diffusion method [12]. Nutrient agar plates were inoculated with the overnight bacterial culture. The compounds at different concentrations were placed on the surface of the inoculated plates. Amikacin was used as standard. After incubation at 37°C for 24h, the diameter of inhibition zone was measured.

Antioxidant activity

Antioxidant activity of extracts was measured according to DPPH method [13]. The DPPH method is based on the reduction of DPPH when mixed with an antioxidant [14]. Briefly, 50 µl of different concentrations (10–500 µg/ml) of samples was added to 5 ml of a freshly prepared 0.004% methanol solution of DPPH as the free radical source.

The mixture was shaken vigorously and allowed to stand at room temperature in the dark. After 30 minutes, absorbance was measured at 517 nm. The DPPH scavenging activity was expressed as the inhibition percentage and was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Where $A_{Control}$ is the absorbance of DPPH without sample and A_{Sample} is the absorbance of DPPH in the presence of sample. The results were compared to BHT as standard. Ability of sample to scavenge 50% of DPPH radicals was measured as IC_{50}.

Total Phenolic Content (TPC)

Total soluble phenols were determined using Folin-Ciocalteu reagent [25]. Briefly, 100 µl of sample (1 mg/ml) and different dilution of Gallic acid solution were added to 6 ml of distilled water. Then, Folin-Ciocalteu reagent (1 ml) was added and the contents of the tube were mixed thoroughly. After 3 min, 1.5 ml of 20% Na_2Co_3 solution were added. The reaction mixture was vortexed and the test tubes were placed in dark at room temperature for 2h with intermittent shaking. The absorbance was read against the blank at 765 nm. Phenol contents of the samples were calculated on the basis of the standard curve for Gallic acid. Total phenols were expressed as mg of Gallic acid equivalents (GAE) per g of dried extract.

Total Flavonoid Content (TFC)

Total flavonoid content was measured using the aluminum chloride colorimetric assay [16]. 500 µl of extract (1 mg/ml) and different dilution of Quercetin solution were added to 10 ml volumetric flask containing 4 ml of water. To the above mixture 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added, allowed to stand for 6 minutes, 2 ml of 1M NaOH was added and then distill water immediately added to a final 10 ml volume.

Then the solution was thoroughly mixed and the absorbance was measured against the blank at 510 nm. Total flavonoids were expressed as mg of Quercetin equivalents (QE) per g of dry weight of extract.

Total Monomeric Anthocyanin content (TMA)

The TMA content was determined by the pH differential method described by Giusti and Wrolstad [17]. This method is based on the fact that anthocyanins absorb light at 520 nm at pH1.0 but not at pH 4.5. Thus, the difference in absorbance at 520 nm is directly proportional to anthocyanin concentration [18].

Sample extracts were diluted in a 1:12 ratio (v: v) with potassium chloride and sodium acetate buffers (pH 1.0 and 4.5, respectively) in separate vessels. After 15 min incubation at room temperature, the absorbance of each solution was read at 520 and 700 nm (to correct the haze) versus a blank cell filled with distilled water. Results were expressed as equivalents of Cyanidin-3-glucoside (C3G), which is the most common anthocyanin pigment found in nature [19].

$$\text{Anthocyanin (mg/l)} = (A \times MW \times DF \times 10^3) / (\varepsilon \times l)$$

Where, $A=(A_{520}-A_{700})_{pH=1}-(A_{520}-A_{700})_{pH=4.5;}$ MW=449.2 g/mol for C3G; DF=dilution factor; l=pathlength in cm; ε=26900 L mol^{-1} cm^{-1} for C3G; and 10^3=factor for conversion from g to mg.

Statistical analysis

All the experiments were performed in triplicate and results expressed as means ± SD. Statistical analyses were performed using one-way ANOVA by SPPS version 15.0 and Excel 2007. The P-values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Antibacterial activity

In the current study, four different bacteria, namely *B. cereus*, *S. aureus*, *E. coli* and *K. pneumonia* were used to screen the possible antibacterial activity of extracts. The results show that none of the extracts displayed significant activity against microorganisms tested. However, the flower methanol extract at a concentration 120 µg/disc exhibited a weak activity (13 mm) against *B. cereus.* In a study by Taran et al. [20]. the essential oil of aerial parts of *F. angulata subsp. carduchorum* showed activity against *S. aureus* (MIC: 15 µg/ml).

However, Hosseini et al. [21]. reported that essential oils and extracts of *F. angulata ssp. angulata* exhibited no considerable activity against *Shigella boidii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus and Enterococcusfaecalis* even in the high concentration (10%).

DPPH radical scavenging activity

DPPH is a stable free radical which has been commonly used to determine the antioxidant activity of natural compounds [22]. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen atom or electron donating antioxidant. The reduction capability of DPPH radical determines by the decrease in its absorbance at 517 nm induced by antioxidants [23]. Figure 1 shows the concentration-dependent free radical scavenging activity of the methanol extracts of the flower, leaf and stem of *F. angulata* compared with BHT. The results indicated that all methanol extracts of *F. angulata* have different levels of inhibitory activity on DPPH radicals.

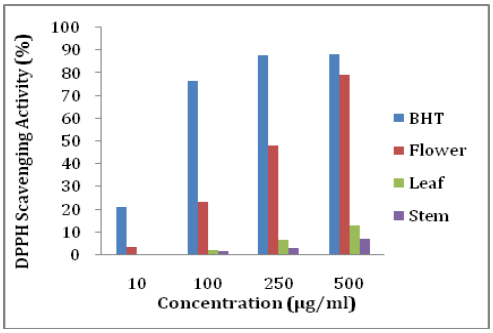

Fig. 1: DPPH scavenging activity of methanol extracts of *F. angulata* aerial parts and BHT.

At a concentration 500 µg/ml, the scavenging activities of methanol extracts of the leaf and stem were 13% and 7.2%, respectively, while at the same concentration, that of the flower reached 79%. The DPPH scavenging activities of aqueous extracts are presented in Figure 2. It was observed that the activity increased in a concentration dependent manner. At a concentration 500 µg/ml, the scavenging activities of aqueous extracts of the flower, leaf and stem were 89%, 84.6% and 67.2%, respectively. As are shown in Table 1, the flower aqueous extract with an IC_{50} value of 214±8.2 µg/ml was superior to the other extracts studied, but the stem methanol extract (IC_{50}: 1606±94.4 µg/ml was the weakest one. The aqueous extracts of the flower, stem and leaf exhibited a significantly higher inhibitory effect compared to their methanol extracts. In a study by Ghasemi Pirbalouti et al. [24] the IC_{50} value 28 µg/ml was found for hydrodistilled essential oil prepared from the stem and leaves of *F. angulata* (Schlecht.) Boiss.

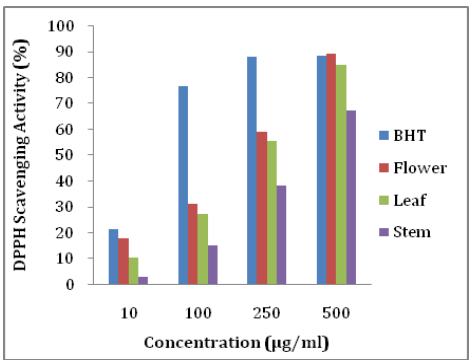

Fig. 2: DPPH scavenging activity of aqueous extracts of *F. angulata* aerial parts and BHT.

Total phenolic contents

Phenolic compounds have been reported to be associated with antioxidant activity due to the presence of hydroxyl groups in their structures [25,26]. Therefore in the current study, levels of TP, TF and

anthocyanin contents of the test extracts were determined. Table 1 reports the results of TP, TF and anthocyanin contents analyses. TPC values of the extracts were expressed as mg Gallic acid equivalents/g dried extract and ranged from 59±0.017 to 483.5±0.098 mg GAE/g dw. The TFC values of the extracts were expressed as mg Quercetin equivalents/g dried extract and varied from 0.5±0.004 to 539±0.29 mg QE/g dw. The highest TPC and TFC were observed in aqueous extract of the flower and stem respectively, whereas those of the methanol stem extract were the lowest.

The highest and lowest anthocyanin contents were found in aqueous extract of flower (4.2±0.82 mg C3G/g. dw) and methanol extract of leaf (0.43±0.02 mg C3G/g. dw), respectively. Antioxidant activity, TPC and TFC of aqueous extracts from flower, leaf and stem were higher than that of the methanol extracts. The differences could possibly be due to the solvents polarities. In a study by Hosseini et al. [13] phenolic content values 229.2 and 202.9 mg GAE/g were obtained for ethyl acetate fraction and methanol extract of this plant.

Table 1: Phenol, flavonoid and anthocyanin contents and IC_{50} values of *F. angulata* extracts.

Extracts	Plant parts	IC_{50} (µg/ml)	Phenols (mg GAE/g dw)	Flavonoids (mg QE/g dw)	Anthocyanins (mg C3G/g dw)
Aqueous extracts	Flower	214±8.2	483.5±0.098	299±0.16	4.2±0.82
	Leaf	238±4.1	291.7±0.057	108.5±0.13	1.26±0.51
	Stem	278±7.6	387.2±0.011	539±0.29	1.88±0.73
Methanol extracts	Flower	226±5.3	258.1±0.074	254±0.009	1.15±0.72
	Leaf	864±29.5	75.4±0.016	3.5±0.003	0.43±0.02
	Stem	1606±94.4	59.0±0.017	0.5±0.004	1.67±0.77

Data are expressed as mean ± SD (n=3). GAE mg/g dw, QE mg/g dw, and C3G mg/g dw represent mg of Gallic acid equivalents, mg of Quercetin equivalents and mg of Cyanidin-3-glucoside equivalents per g of dried extract, respectively.

CONCLUSION

The obtained results showed that all of tested extracts have different levels of phenolic compounds and antioxidant activity. The highest TP, TF, anthocyanin and antioxidant activity occurred in the aqueous extracts. As a result, aqueous extract of *F. angulata* flowers having the highest phenolic compounds and antioxidant activity could be used as a good natural source of antioxidants in different industries.

CONFLICT OF INTERESTS

Declared None

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REFERENCES

1. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266:37–56.
2. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation and cancer: how are they linked? Free Radic Biol Med 2010;49:1603-16.
3. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of Smilax excelsa L. leaf extracts. Food Chemistry 2008;110:571–83.
4. Ku CS, Mun SP. Antioxidant activities of ethanol extracts from seeds in fresh Bokbunja (Rubus coreanus Miq.) and wine processing waste. Bioresour Technol 2007;99:2852–6.
5. Katalynic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 2006;94:550-7.
6. Khalighi Sigaroodi F, Hadjiakhoondi A, Shafiee A, Mozaffarian VA, Shahverdi AR, Alavi SHR. Phytochemical analysis of ferulago burnardii tomk and m pimen. J Daru 2006;14:214-21.
7. Yesilbag D, Eren M, Agel H, Kovanlikaya A, Kovanlikaya F. Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum sod activity. Br Poult Sci 2011;52:472-82.
8. Khalighi Sigaroodi F, Hadjiakhoondi A, Shahverdi A, Mozaffarian V, Shafiee A. Chemical composition and antimicrobial activity of the essential oil of ferulago bernarda tomk and m. Pimen Daru 2005;13(3):100-4.
9. Khanahmadi M, Janfeshan K. Study on antioxidation property of Ferulago angulata plant. Asian J Plant Sci 2006;5:521-6.
10. Demetzos C, Perdetzoglou D, Gazouli M, Tan K, Economakis C. Chemical analysis and antimicrobial studies on three species of Ferulago from Greece. Planta Med 2000;66:560-3.
11. Rechinger KH, Hedge IC, Lamond JM. Flora Iranica. Graz: akademische Druck-und Verlagsanstalt 1987;428-30.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45(4):493–6.
13. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytochem Res 2000;14:323-8.
14. Molyneux P. The Use of DPPH to estimate antioxidant activity. Songklanakarin J Sci Technol 2004;26:211-9.
15. Yu L, Perret J, Harris M, Wilson J, Haley S. Antioxidant properties of bran extracts from Akron wheat grown at different locations. J Agricultural Food Chem 2003;51:1566–70.
16. Kumar S, Kumar D, Manjusha KS, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of Citrullus colocynthis (L.) Schrad. Methanolic fruit extract. Acta Pharm 2008;58:215-20.
17. Giusti MM, Wrolstad RE. Characterization and measurement of anthocyanins by uv-visible spectroscopy. In: Wrolstad RE, Schwartz SJ, editors. Handbook of food analytical chemistry. New York: Wiley; 2005. p. 19–31.
18. Wrolstad RE, Durst RW, Lee J. Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 2005;16:423–8.
19. Francis FJ, Markakis PC. Food colorants: anthocyanins. Crit Rev Food Sci Nutri 1989;28:273–314.
20. Taran M, Ghasempour HR, Shirinpour E. Antimicrobial activity of essential oils of Ferulago angulata subsp. Carduchorum. Jundishapur J Mic 2010;3(1):10-4.
21. Hosseini N, Akbari M, Ghafarzadegan R, Changizi Ashtiyani S, Shahmohammadi R. Total Phenol, antioxidant and antibacterial activity of the essential oil and extracts of ferulago angulata ssp. angulata. J Medic Plants 2012;11(43):80-5.
22. Ozturk M, Ozturk FA, Duru ME, Topcu G. Antioxidant activity of stem and root extracts of Rhubarb (Rheum ribes): an edible med plant. Food Chem 2007;103:623-30.
23. Yuan JP, Li X, Xu SP, Wang JH, Liu X. Hydrolysis kinetics of secoisolariciresinol diglucoside oligomers from flaxseed. J Agric Food Chem 2008;56:10041-7.
24. Ghasemi Pirbalouti A, Sedaghat L, Hamedi B, Tirgir F. Chemical composition and antioxidant activity of essential oils of three endemic medicinal plants of Iran. Bangladesh J Bot 2013;42(2):327-32.
25. Villano D, Fernandez Pachon MS, Moya ML, Troncoso AM, Garcia-Parilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH Free radical. Talanta 2007;71:230-5.
26. Pahar B, Chakraborty S, Chaudhuri S, Sengupta B, Sengupta PK. Binding and antioxidant properties of therapeutically important plant flavonoids in biomembranes: insights from spectroscopic and quantum chemical studies. Chem Physics Lipids 2012;165:488-96.