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Original Article

Antioxidant Activities of Traditional Plants in Qaen by DPPH Free Radical- Scavenging Assay

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Abstract

Background and Aim: In humans, many diseases are associated with the accumulation of free radicals. Antioxidants can scavenge free radicals and minimize their impact. Therefore, the search for naturally occurring antioxidants of plant origin is imperative.

Methods: Here, we aimed to investigate the antioxidant and free radical scavenging properties of methanolic extracts from *Plantago major*, *Asperugo procumbens*, *Fumaria paviflora*. Methanolic extract of *P. major*, *A. procumbens*, *F. paviflora* leaf is a potential source of natural antioxidants and serves as an effective free radical scavenger and/or inhibitor. Hence, of *P. major*, *A. procumbens*, *F. paviflora* might be a good plant-based pharmaceutical product for several diseases caused by free radicals.

Results: In this experiment, we examined different parts (leaf) of *A. procumbens* and found that methanolic extract of *A. procumbens* leaf, which contains large amounts of phenolic and flavonoid compounds, exhibited the highest antioxidant and free radical scavenging. A positive correlation (P-value < 0.005) was observed between phenolic content and free radical (DPPH) scavenging efficiencies.

Conclusion: Methanolic extract of *A. procumbens* leaf is a potential source of natural antioxidants and serves as an effective free radical scavenger and/or inhibitor. Hence, *A. procumbens* might be a good plant-based pharmaceutical product for several diseases caused by free radicals.

Keywords: Anti-radical properties; Extraction; DPPH; Medicinal plants.

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Introduction

There is strong evidence that many dangerous pathophysiological processes, such as cancer, diabetes, and cardiovascular and neurodegenerative diseases, are associated with the accumulation of free radicals (19, 23, 15). A free radical is an atom or molecule that has an unpaired electron and is therefore unstable. This unstable radical tends to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells, thus causing protein and DNA damage (19). Such radical- caused cell damage can become more widespread due to weakened cellular antioxidant defense systems. All biological systems have innate antioxidant defense mechanisms that remove damaged molecules, but these mechanisms can be inefficient. Therefore, dietary intake of antioxidants is imperative to protect cells from damage caused by free radicals.

Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons from antioxidants to these damaged cells. Antioxidants also turn free radicals into waste by- products, which are eliminated from the body (26). Consumption of antioxidant-enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals (10). Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C (22). Although phenolic compounds are commonly found in both edible and in non-edible

herbs, cereals, fruits, vegetables, oils, spices, and other plant materials (3), scientific information on the antioxidant properties of endemic plants is scarce because the availability of endemic plants is limited to certain regions and only known by local populations. Therefore, the assessment of such properties remains an interesting and useful task, particularly to find promising sources of natural antioxidants for functional foods and/or nutraceuticals (30, 4).

The results of ethnobotanical and ethnopharmacological studies of different species of *P. major, A. procumbens, F. paviflora* indicate the potential use of these plants for the treatment of a large variety of diseases. Due to the increasing interest in the relationship between antioxidants and diseases, it is important to measure the overall antioxidant activity of *P. major, A. procumbens, F. paviflora*. Therefore, the objective of this study was to evaluate the antioxidant and free radical scavenging activity as well as polyphenol contents of methanolic extractives from different parts of *P. major, A. procumbens, F. paviflora*.

Methods

Chemicals and reagents

DPPH, methanol, ethanol, acetone, and hexane were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

Plant material

The plants *P. major, A. procumbens, and F. paviflora* were collected from Qaen (a city in South Khorasan Province, Iran) local farms in spring and summer.

Plants Material Extraction

The aerial parts plants were collected, shade dried for seven days, and ground. The dried powder of plants (1g) was soaked in 10 ml methanol- water (80:20, v/v), ethanol- water (50:50), acetone, and hot hexane. Extraction was carried out at ambient temperature for 24 h. The ratio of methanol and ethanol with water used yielded the highest yield of phenolic compounds and flavonoids during preliminary trials. Each extract was filtered with Whatman No. 1 filter paper. The solvents evaporated at 40°C in a rotary evaporator (Buchi Laboratory) (2).

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin- Ciocalteu method described by Wolfe et al. (29), An aliquot of the extract was mixed with a 2 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 20 min at 25°C for color development. Absorbance was then measured at a 760 nm UV-spectrophotometer (Shimadzu, USA). Samples of extract were evaluated at a final concentration of 0.1 and 0.15 mg/mL. Total phenolic contents were expressed in terms of gallic acid equivalent, GAE (standard curve equation: $y= 0.091X+ 0.167$, $R^2 = 0.994$), and mg of GA/g of dry extract. The experiment was repeated three times at each concentration.

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to Dasgupta and De (6) method. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (at concentrations of 50, 100, 200, 500, and 1000 ppm). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as the percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{The absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The quality of the radical scavenging property of plants was determined by calculating the IC_{50}. The IC_{50} value is the concentration of each plant extract required

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The effect of different solvents on the antioxidant activity of plants was determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at a 5% significant level using the SAS software (2001) program.

Results

Figure 1, shows the amount of total phenolic compounds of the methanolic extracts of *P. major, A. procumbens, and F. paviflora*. Among the extracts, *F. paviflora* had a higher amount of total phenolic compounds than the other extracts. At a concentration of 100 µg / mL, *F. paviflora*, *P. major* and *A. procumbens* were 19, 11, and 8 mg of GA / g, respectively (Fig 1).

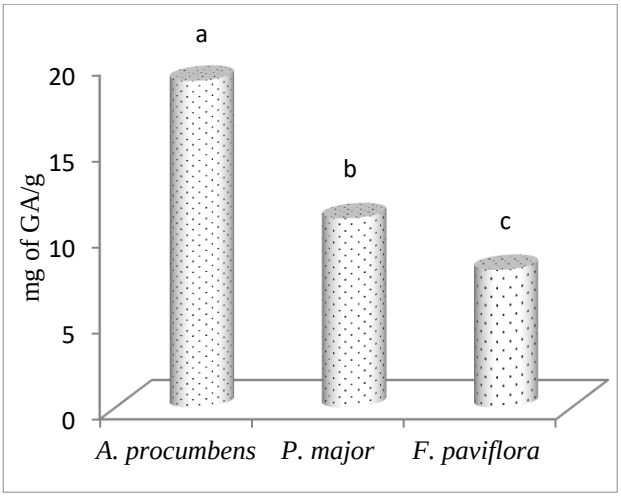

Figure 1. Determination of amount of total phenolic compounds with methanolic extractives of *P. major*, *A. procumbens*, *F. paviflora*. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, p < 0.05) for all tested dosages.

Among the medical plants, *A. procumbens* showed the highest total antioxidant capacity followed by *P. major* and *F. paviflora* (Fig 2). A positive correlation (P-value < 0.005) was observed between DPPH, amount of total phenolic compounds and IC_{50} efficiencies inhibition activity.

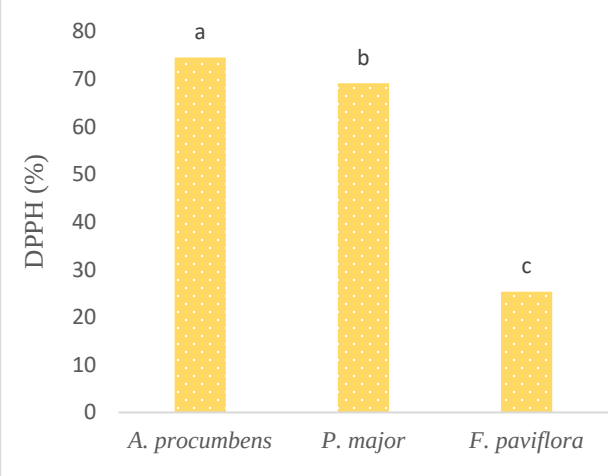

Figure 2. Determination of DPPH radical scavenging activity of methanolic extractives of *P. major*, *A. procumbens*, *and F. paviflora*. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, p < 0.05) for all tested dosages.

Results showed that solvent significantly (p < 0.05) influenced IC_{50} scavenging activity (Fig 3). The IC_{50} the index was used to express the inhibition effects of free radicals in the DPPH method. In this way, the lower the amount of IC_{50} value, the higher the inhibition potency. The IC_{50} the index was used to express the inhibition effects of free radicals in the DPPH method. In this way, the lower the amount of IC_{50} value, the higher the inhibition potency. According to Fig (3) data, the minimum amount of IC_{50} by *A. procumbens* plant in extraction with (80 %), and the maximum IC_{50} is related to the *F. paviflora* (Fig. 3).

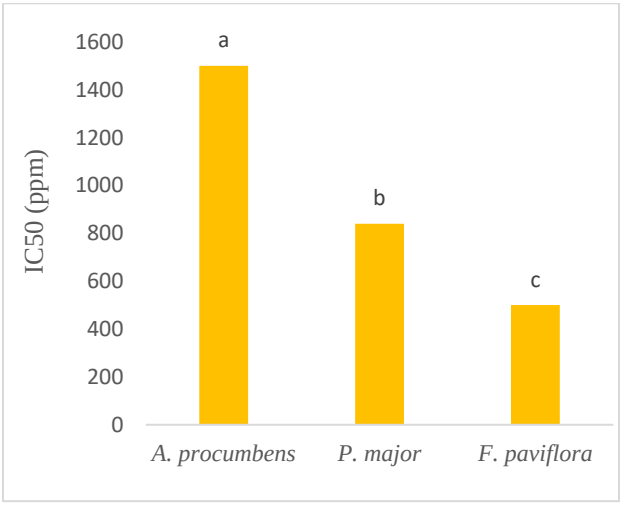

Figure 3. Determination of IC_{50} of methanolic extractives of *P. major*, *A. procumbens*, *F. paviflora*. All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3, p < 0.05) for all tested dosages.

Discussion

Total phenolic content of the extractives showed a significant and strong positive correlation (P-value < 0.005) with free radical (DPPH) scavenging efficiencies inhibition. Our results are consistent with the data published previously (20, 17). Thus, the polyphenolic constituents of the extracts may be the major contributors to the antioxidant activity in free radical neutralization and lipid peroxidation inhibition. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (11). Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to a yellow-colored product, diphenylpicryl hydrazine, by the addition of the extract in a concentration-dependent manner. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Polyphenol contents and

tocopherols scavenge the DPPH radicals by their hydrogen donating ability (11, 14). The results obtained in this study suggest that all the extracts from *A. procumbens* showed radical scavenging activity by their electron transfer or hydrogen donating ability. Total polyphenols content and radical scavenging antioxidant activity are highly correlated (11).

Free radicals that participate in the fats peroxidation process play an important role in causing diseases like cancer and cardiovascular disease (5). DPPH radicals are widely used to determine the ability to quench free radicals of various natural products and are adopted as a model compound for the free radicals that will be established by lipids (28). *A. procumbens* extracts with all solvents have the highest inhibition percentage of DPPH radicals in comparison to the other extracts. *F. paviflora* had the lower DPPH free radical scavenging activity. Also, observed methanol extracts had the highest amount of phenolic compounds (25). Yu et al. (32), reported that phenolic compounds in methanol (80%) of peanut shells were 1.90 mg / g, respectively. These researchers introduced ethanol, as the most effective solvent in the extraction of phenolic compounds. The results obtained in this study were consistent with the present research. The results showed that the plant type significantly affected the extraction of phenolic compounds and the *A. procumbens* with 19 mg Gallic acid/g had the highest extraction, respectively (24). In the study of Mazarei et al. (21) 50 microliters/ml of methanol solvents in thyme had DPPH free radicals scavenging activity of 82.2%. The proper use of medicinal plants requires the recognition of existing chemical compounds because it is the presence of chemical compounds that leads to the therapeutic effect of the plant (18). Fazeli et al. (8) obtained IC_{50} hydroalcoholic essence of yarrow and thyme were respectively 42.65 and 2.35 µg / ml. The investigation of (12) showed that the concentration of 5 mg/mL of peppermint extract has a range of 52/93% of the ability to absorb DPPH free radical. Abdi et al. (1) reported the amount of $IC_{50}$of peppermint essence as 4/60 ppm. In the study of Hosseini et al. (2012), the antioxidant effect of blue essence by (IC_{50}= 84/94 µg/ml) and Ethyl acetate by (IC_{50}= 725µg/ml) of the rosemary was high. In the study (8), the amount of IC_{50}in hydro alcoholic essence of rosemary was 92/80 mg per mL. The anti-radical activity of rosemary essential oils is related to the presence of polar compounds such as Rosmarinic acid and other phenolic acids. The study done on phenolic compounds of rosemary essence showed this activity that consists of Carnosine, carnosol, and Rosmarinic acid (7). Rosmarinic acid reacts with DPPH during a period and is in a constant state for 10 minutes while the reaction rate of Carnosine acid is lower and requires around 30 minutes to reach a steady state (31). Kamkar et al. (16) reported IC_{50} level of savory extracts in methanol, respectively, 76/30 mg / mL with less than the results reported in this study (9) reported the IC_{50} of methanol extract in savory as 67/23 ppm. Shafiee Dastjerdi and Mazoji (27) obtained IC_{50} of methanol, (70%) in *Ziziphora clinopodiodes* by Alborz province Chalus region and Tehran province respectively as 234.94 ppm. Different results have been reported in different studies to determine the anti-radical properties of studied plants. The reason for the difference in the stated results can be due to the number, conditions of the region, the conditions of cultivation, the conditions of preservation and drying, and different experimental conditions, such as the solvent type, and the extraction time.

Conclusion

The biologically active pure compound is better than crude extract. However, to get the overall view regarding the phytochemical composition and biological activities of any plant, it is important to pick the most potential part by investigating several parts (leaf) of that plant. Here, we examined different parts (leaf) of *P. major*, *A. procumbens*, *F. paviflora* and found that the methanolic extract of *A. procumbens* leaf, contains large amounts of phenolic and flavonoid compounds, exhibited the highest antioxidant and free radical scavenging. A positive correlation (P-value < 0.005) was observed between phenolic content and free radical (DPPH) scavenging efficiencies. These investigation assays indicate that *P. major*, *A. procumbens*, *F. paviflora* leaves are significant sources of natural antioxidants, which could help to prevent the progression of various diseases caused by free radicals, such as certain cancers. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the plant extract.

Acknowledgments

Note declared.

Conflict of Interest

The authors declare no conflict of interests.

Funding/Support

This study was conducted without financial support.

Ethics

This research was done in the laboratory on non- human materials and no need for Ethics Committee approval.

Authors' Contribution

All authors had equal roles in the design of the study, interpretation of results, and writing of the manuscript.

References

1. Abdi GR, Shakarpour M, Sallami SA, Bertay SM. Effect of water stress on functional properties, antioxidant capacity and peppermint peppermint metabolites. Journal of Horticulture. 2018;49(3):715-727.

2. Arabshahi-Delouee S, Urooj A. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chemistry. 2006;102(3):1233–1240.

3. Atere TG, Akinloye OA, Ugbaja, RN, Ojo, DA, Dealtryd G. *In vitro* antioxidant capacity and free radical scavenging evaluation of standardized extract of *Costus afer* leaf. Food Science and Human Wellness. 2018;7(4):266-272

4. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by products: antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99: 191–203.

5. Chohra D, Ferchichi L, SelimCakmak Y, Zengin G, Mohammed Alsheikh S. Phenolic profiles, antioxidant activities and enzyme inhibitory effects of an Algerian medicinal plant (*Clematis cirrhosa* L.). South African Journal of Botany. 2020;132:164-170

6. Dasgupta N, De B. Antioxidant activity of someleafy vegetables of India: a comprative study. Food chemistry. 2007;101(2):471-474.

7. Elmastas M, Dermirtas I, Isildak O, Aboul-Enein HY. Antioxidant Activity of S-Carvone Isolated from Spearmint (*Mentha Spicata* L. Fam Lamiaceae). J. Liquid Chromatography & Related Technol. 2006;2(10):1465–1475.

8. Fazeli Nasab B, Rahnama M, Mazaraei A. Evaluation of the relationship between antioxidant properties and antimicrobial properties of extracts of 9 medicinal plants. Journal of Mazandaran University of Medical Sciences. 2017;149(27): 78-63.

9. Guo H, Saravanakumar K, Wang MH. Total phenolic, flavonoid contents and free radical scavenging capacity of extracts from tubers of *Stachys affinis*. Biocatalysis and Agricultural Biotechnology. 2018;15:235-239.

10. Hamid K, Saha MR, Urmi KF, Habib MR, Rahman MM. Screening of different parts of the plant Pandanus odorus for its antioxidant activity. Int J Appl Biol Pharm. 2010;1:1364–8.

11. Hara K, Takao Someya T, Sano K, Sagane, Y, Watanabe T, Wijesekara RGS. Antioxidant activities of traditional plants in Sri Lanka by DPPH free radical-scavenging assay. Data in Brief. 2018;17:870-875.

12. Hashemi M, Zare MA, Naghebi S, Raisey M, Hassanzadeh Azar H. Evaluation of Chemical Composition, Antibacterial, Antifungal and Antioxidant Properties of Sage, Peppermint and Oregano. Laboratory Journal. 2015;9(2): 56-47.

13. Hoseyni N, Maleki Rad AS, Genghis Ashtiani S, Nazemi M. Investigating the Essential Oil Capacity and Different Processes of Methanolic Extract of T. Shirazi, Maryam Goli, Rosemary, Khalavash and Cinnamon in the Control of Free Radicals. Journal of Shaheed Sadoughi University of Medical Sciences. 2012;2(1):28-38.

14. Huang D, Ou B, Prior RI. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005;53:1841–56.

15. Islam S, Nasrin N, Khan MA, Hossain ASMS, Islam F, Khandokhar P. Evaluation of antioxidant and anticancer properties of the seed extracts of Syzygium fruticosum Roxb. Growing in Rajshahi, Bangladesh. BMC Complement Altern Med. 2013;13:142.

16. Kamkar A, Thorian F, Akhondzadeh Basti A, Mathaqi A, Shariati Far N. Evaluation of Chemical Composition of Savory Essences and Comparison Antioxidant Activity with Aquatic and Alcoholic Extract. Journal of Veterinary Research. 2013;68(2):183-190.

17. Kedare BS, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011;48:412–22.

18. Kolahi M, Mokhtari B, Mirzaee N. A phytochemical study and comparison of the effect of pomegranate extracts spongy tissue on colon cancer cells caco$_2$. Jundishapur Sci Med J. 2016;15:201-15. [In Persian]

19. Kumar Dubey, R, Upadhyay G, Singh V, Pandey S. Antioxidant potential and free radical scavenging activity of *Parkia roxburghii*, G. Don, a lesser known leguminous tree from North East India. South African Journal of Botany. 2020;131:454-461

20. Liu SC, Lin JT, Wang CK, Chen HY, Yang DJ. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. 2009;144: 577–81.

21. Mazarei A, Mousavi Nick SM, Fahmeda L. Analysis of antioxidant, flavonoid and phenolic activity of ethanolic, hydro, estonian and methanolic extracts of thirteen medicinal plants. Nova Biologica Reperta. 2018;4(4):309-299.

22. Mwamatope B, Tembo D. Chikowe I, Kampira E, Nyirenda C. Total phenolic contents and antioxidant activity of *Senna singueana, Melia azedarach, Moringa oleifera* and *Lannea discolor* herbal plants. Scientific African. 2020:9: e00481

23. Naskar S, Mazumder UK, Pramanik G, Bala A, Haldar PK, Islam A. Comparative in vitro antioxidant activity of different parts of Cocos nucifera (Linn.) on reactive oxygen and nitrogen species. Int. J Pharm Pharm Sci. 2011;3:104–7.

24. Oliveira I, Coelho V, Baltasar R, Pereira JA, Baptista P. Scavenging capacity of strawberry tree (*Arbutus unedo* L.) leaves on free radicals. Food and Chemical Toxicology. 2009;47:1507-1511.

25. Peschel W, Sanchez-Rabaneda F, Dn W, Plescher A, Gartzia I, Jimenez D, Lamuela-Raventos R, Buxaderas S, Condina C. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem. 2006;97:137-150.

26. Shahdadi F, Payandeh M, Salehi Sardoei A. Comparison of Antioxidant Activity of Dracocephalum polychaetum Bornm and Nepeta cataria L. and Their Effect on Probiotic Bacteria in a Simulated Gastrointestinal Environment. Journal of Medical Microbiology and Infectious Diseases. 2021;9(1):5-11.

27. Shafiee Dastjerdi L, Mazoji A. Antioxidant Activity and Total Phenols Content of Different Solvent Extracts of Ziziphora clinopodioides from Three Geographical Locations in Iran. Journal of Chemical and Pharmaceutical Research. 2016;8(11): 243-248.

28. Sridevi G, Srividya S, Sembulingam K, Sembulingam P. An evaluation of *in vitro* and *in vivo* free radical scavenging and antioxidant potential of ethanolic extract of Pergularia daemia. Biocatalysis and Agricultural Biotechnology. 2018;15:131-137.

29. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51:609–14.

30. Wu SQ, Li R, Jiang ZT, Wang Y, Tan J, Tanga SH. Evaluation of antioxidant active ingredients of spikenard essential oil by ultra-fast gas chromatography electronic nose and radical scavenging mechanism. Industrial Crops and Products. 2020:112489

31. Yesil-Celiktas O, Girgin G, Orhan H, Wichers HJ, Bedir E, Vardar-Sukan F. Screening of free radical scavenging capacity and antioxidant activities of Rosmarinus officinalis extracts with focus on location and harvesting times. European Food Research and Technol. 2007;224(4):443-451.

32. Yu J, Ahmedna M, Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry. 2005;90:199-206.