



Investigation of Antioxidant Activities of Different Plants Grown in Baku

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ABSTRACT: This work aims to evaluate the antioxidant activities of various plants (*Eupatorium maculatum*, *Pinus eldarica* and *Ficus benghalensis*) grown in Baku. Antioxidants are bioactive compounds that prevent cell damage by combating oxidative stress and play an important role in the prevention of many chronic diseases including cardiovascular diseases, cancer and neurodegenerative disorders. The antioxidant potential of plants is closely related to the amount of phenolic compounds and flavonoids they contain. Within the scope of the study, three different plant species (*Eupatorium maculatum*, *Pinus eldarica* and *Ficus benghalensis*) widely distributed in Baku were selected and their extracts were prepared using ethanol. Then, in order to evaluate the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay and cupric reducing antioxidant capacity (CUPRAC) assay were applied. At the same time, total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminum nitrate spectrophotometric methods. As a result of the analyzes, it was determined that some plants have high antioxidant potential, therefore, they have high potential for use in the pharmacological and functional food industry. It was revealed that plant extracts with high phenolic content in particular exhibited stronger antioxidant effects. The research results indicate that Baku's local plant resources can be an important source of raw materials for the health and food industries. This study may provide the basis for more detailed biochemical studies of local plant species in the future and for a broader evaluation of their therapeutic potential. At the same time, the research results provide a useful scientific basis for further investigation of the application possibilities of new natural antioxidant resources in various industrial sectors.

KEYWORDS: Antioxidant activity, *Eupatorium maculatum*, *Ficus benghalensis*, flavonoid, phenolic, *Pinus eldarica*.

INTRODUCTION

Oxidative stress is a process that occurs as a result of excessive accumulation of free radicals in the body and the inability of the antioxidant defense system to neutralise these radicals. This leads to damage to cell membranes, proteins and DNA, increasing the risk of developing various chronic and degenerative diseases such as cardiovascular pathologies, cancer, diabetes, neurodegenerative diseases and premature ageing [1]. Modern scientific research shows that dietary and lifestyle changes, especially the consumption of foods rich in natural antioxidants, can reduce the harmful effects of oxidative stress. Therefore, the effects of natural antioxidants on human health are widely investigated and it is important to find new natural plant antioxidants [2]. Plants are of particular importance as a source of natural antioxidants because they contain phenolic compounds, flavonoids, carotenoids, vitamins (vitamin C and E) and other bioactive components. These substances help to neutralize free radicals, have an anti-inflammatory effect and strengthen the immune system. Therefore, it is of great scientific and practical importance to investigate the antioxidant activities of various plants and to evaluate their biomedical potential. In particular, the study of local flora species growing in certain geographical regions provides a scientific basis for the widespread use of these plants in the food and pharmaceutical industry as well as revealing their health benefits [3, 4].

Eupatorium maculatum, a member of the Asteraceae family, is a perennial herb that grows mostly in wetlands, river banks, swamps and moist meadows, especially in the eastern and southeastern regions of North America. This plant is of great importance both ecologically and pharmacologically. It has been shown that the roots and flowers of some plants are rich in flavonoids, terpenoids, alkaloids and other beneficial substances. It is used by Native American people to relieve ailments such as swelling and fever. It has been also found that *Eupatorium maculatum* extracts are effective in the prevention and treatment of bacterial infections, especially shows the good antibiotic effect. *Eupatorium maculatum* also has anti-inflammatory and analgesic effects. These effects favourably support the use of the plant in folk medicine since ancient times [5, 6].

Pinus eldarica is a species of pine belonging to the *Pinus* species and naturally grows widely in the mountainous regions of the Southern Caucasus, especially in the Eldar Mountains of Azerbaijan. This species has great importance both ecologically and



economically [6]. *Pinus eldarica* is popularly known as a medicinal tree used in traditional herbal medicine for the treatment of bronchial asthma and various skin diseases [8]. Previous studies have also been demonstrated that several *Pinaceae* members have high antioxidant activity due to their rich polyphenolic profiles [9].

Ficus benghalensis is a tree species belonging to the *Ficus* genus and native to the Bengal region of India. This species thrives in tropical and subtropical climates and likes high humidity environments. Bengal ficus is also known for its drought tolerance; This feature makes it adaptable to various ecosystems. This species has great ecological and cultural importance. A considerable number of *Ficus* species have been utilised within the domain of folk medicine for an extensive period of time. These species have been employed in the treatment of a variety of ailments, including flatulence, gastritis, vomiting, hypotension, diabetes, and dysentery [10]. It has been demonstrated by preceding studies that all extracts of *Ficus benghalensis* possess antioxidant and significant immunomodulatory activities [11].

Baku and its surroundings have a rich plant diversity and some plants growing in the region are used in traditional medicine by local people. However, information on the antioxidant properties of these plants is limited and their bioactive compounds have not yet been fully investigated. Therefore, this study aims to determine the antioxidant capacity of ethanol extracts obtained from the leaves of three different plants (*Eupatorium maculatum*, *Pinus eldarica* and *Ficus benghalensis*) growing in Baku using various antioxidant methods.

MATERIALS AND METHODS

1. Preparation of plant extracts

The leaves of *Eupatorium maculatum*, *Pinus Eldarica* and *Ficus benghalensis* plants were collected from Baku, Azerbaijan and the leaves were washed, dried in an oven at an average temperature of 50°C and pulverised. The dried plant samples were extracted with ethanol solvent at room temperature for 24 hours in a shaker. After extraction, filtration was carried out using filter paper and the solvent was removed by collecting the filtrate. The solids obtained were weighed and the stock solution was prepared by dissolving in ethanol at the desired concentrations.

2. Antioxidant activity

The antioxidant properties of the ethanol extracts of *E. maculatum*, *P. Eldarica* and *F. benghalensis* plant leaves were investigated by using DPPH and CUPRAC methods. Furthermore, the total phenolic and flavonoid contents of the extracts were also determined in order to provide support for the antioxidant properties of the extracts.

A. DPPH Radical Scavenging Activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical activities of the ethanol extracts of the plant leaves and ascorbic acid as a standard were determined according to the method used by Brandt-Williams et al. [12] A solution of DPPH was prepared by dissolving 25 mg of DPPH in 10 mL of ethanol and the solution kept in the dark at 4°C. Plant leaf extracts at various concentrations (between 100-500 µg/mL) were added to this solution and kept for 30 minutes at room conditions. The absorbance values were then measured at a wavelength of 517 nm in a spectrophotometer. Ascorbic acid was used as standard. The experiments were repeated three times. The decreasing absorbance value indicates the free radical scavenging activity, which is the measure of the DPPH solution. The capacity of DPPH free radical was calculated using the following equation,

$$\text{DPPH Radical Scavenging capacity (\% Inhibition)} = [(A_0 - A_1) / A_0] \times 100$$

A_0 = Control absorbance

A_1 = Sample absorbance

B. Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The antioxidant test of the CUPRAC method was applied to plant extracts using the procedure used by Apak et al. [14]. To a test tube, the following solutions were added: 1 ml of each copper(II) solution, neocuprin solution and ammonium acetate buffer and 30, 50, 80, 100 and 150 ml of the extract and mixed with vortex. Then the solutions were kept at room temperature for 30 min. The completed reaction mixtures were read at a wavelength of 450 nm. Trolox was used as the positive standard. The experiments were performed in 3 replicates for all concentrations.

C. Total Phenolic Content

Total phenolic compounds were determined by Folin-Ciocalteus reagent. 0.5 ml extract, 1 ml Folin-Ciocalteu reagent and 3 ml sodium carbonate solution in 20% water were added to the test tube and mixed with the help of vortex. The mixture was kept at room temperature for 2 hours and 30 minutes and the results were measured at 750 nm and calculated as gallic acid equivalent (GAE) [15].

D. Total Flavonoid Compounds

For the determination of total flavonoid content, 0.5 ml of each plant extract was taken, 0.1 ml of sodium acetate was added and after 1 minute 0.1 ml of 10% $\text{Al}(\text{NO}_3)_3$ was added and shaken. The volumes were then made up to 5 ml with ethanol. The absorbance values of the mixtures kept at room temperature for 50 min were measured at 450 nm wavelength. The results obtained were calculated as quersetin equivalent (QE) [16].

RESULT AND DISCUSSION

A. DPPH Radical Scavenging Activity

DPPH radical scavenging activities of *Eupatorium maculatum*, *Pinus Eldarica* and *Ficus benghalensis* plants grown in Baku region were used to determine their antioxidant activities. The plant leaves were extracted with ethanol and used at different concentrations (100-500 mg/L) to determine DPPH free radical scavenging activity. Ascorbic acid was used as a standard for comparison. The results obtained are shown in Figure 1. When the results obtained were evaluated, it was found that the DPPH radical scavenging capacity of the ethanol extracts of the three plant leaves ranged from 13% to 74%. It was observed that the radical scavenging activity increased with increasing concentration of the plant extracts. Among the plant extracts, *Pinus eldarica* leaf extract showed the highest DPPH scavenging activity with 74% inhibition. However, when the DPPH free radical scavenging activity of all the ethanol plant extracts was compared with standard ascorbic acid, it was found that all of them exhibited lower activity than the standard.

Khater et al. investigated the DPPH radical scavenging activity of the methanol extract of *Pinus eldarica* growing in Cairo Governorate, Egypt and determined the DPPH scavenging capacity as 37.60 % [17]. In the study undertaken by Singh et al., a methanol extract of *Ficus benghalensis* was prepared and collected from Gorakhpur, India. It was found that the DPPH radical scavenging activity of the extract increased with increasing extract concentration, reaching approximately 74% at a concentration of 150 $\mu\text{g/ml}$ [18]. The results of the ethanol extract of the plants collected from Baku, are consistent with these findings.

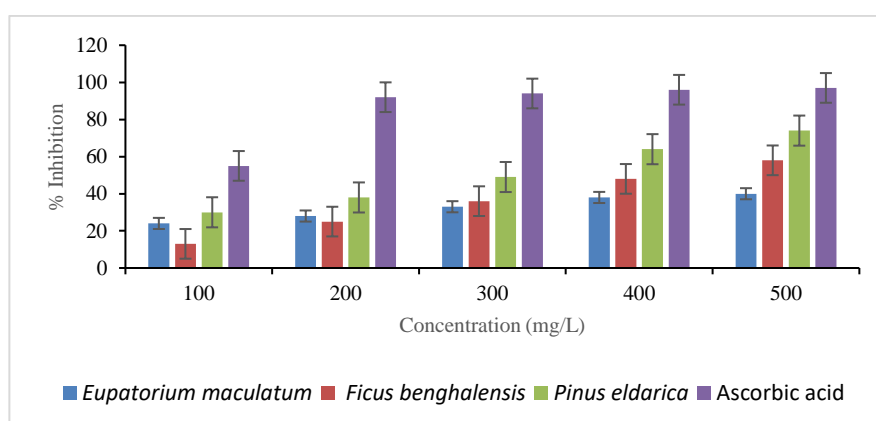


Figure I. DPPH Free Radical Scavenging Capacity of the Ethanol Extract of Plant Leaves

B. Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The leaves of three different plants (*Eupatorium maculatum*, *Pinus Eldarica* and *Ficus benghalensis*) growing in Baku region were extracted with ethanol and the CUPRAC copper reducing capacity method was used to determine the antioxidant activity of these extracts at concentrations of 30-150 mg/L. Trolox was used as standard. The results obtained are shown in Figure II. When

the results were evaluated, it was determined that the copper ion reduction capacities of all ethanol plant extracts were lower than the standard trolox. Among the plants, the ethanol extract of *Eupatorium maculatum* leaves showed the highest reduction capacity.

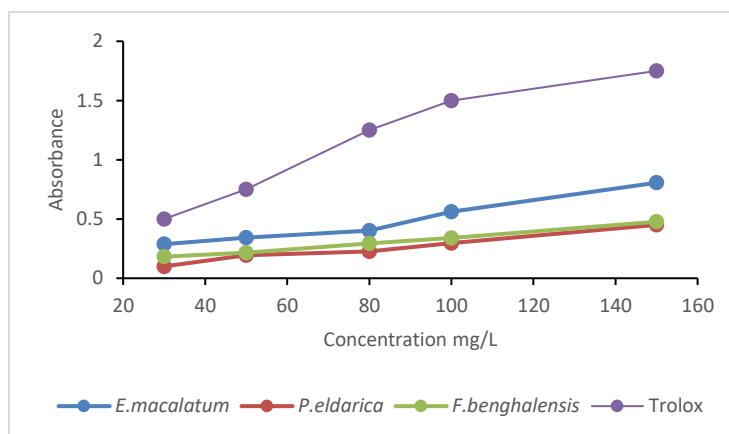


Figure II. Copper Ion Reducing Antioxidant Capacity (CUPRAC) of the Ethanol Extract of the Plant Leaves

C. Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid contents of ethanol extracts of three different plant (*Eupatorium maculatum*, *Pinus Eldarica* and *Ficus benghalensis*) leaves collected from Baku were determined and the results are given in Table I. Tulasi et al. investigated the total amount of phenolic and flavonoid substances by extracting Ficus in different solvents and found 18, 64 (mg/GAE/g) and 8, 21 (mg/QE/g) in ethanol extracts, respectively [19]. In another study, Khater et al. investigated the total phenolic and flavonoid content of *Pinus eldarica* plant obtained from Cairo Governorate of Egypt by extracting them with ethanol and water mixture [17]. According to their results, they determined the total phenol and total flavonoid content of white pine as 67.60 mg/g GAE and 49.80 mg/g as catchin. Our findings are similar to these results.

Table I: Total Phenolic and Flavonoid Content in the Ethanol Extract of Plant Leaves

Example	Total Phenol (mg/GAE/g)	Total Flavonoid (mg/QE/g)
<i>Eupatorium maculatum</i>	8,34	21,65
<i>Ficus benghalensis</i>	8,01	7,70
<i>Pinus eldarica</i>	13,18	13,20

(GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent.)

CONCLUSIONS

In the present study, the antioxidant properties and total phenol and flavonoid content of the leaves of three different plants (*Eupatorium maculatum*, *Pinus eldarica* and *Ficus benghalensis*) grown in Baku were determined. The results obtained were in accordance with the existing literature, and *P. eldarica* demonstrated the highest activity in terms of DPPH radical scavenging capacity among the plants studied. However, the CUPRAC values revealed that *E. maculatum* plant extract exhibited superior antioxidant activity in comparison to other plant extracts that were tested, attributable to its high total flavonoid content. The results of this study showed that some plant species grown in Baku have good antioxidant activity and there is a strong relationship between their phenolic and flavonoid contents and their antioxidant potential. The results of this study showed that some plant species grown in Baku have good antioxidant activity and there is a strong relationship between their phenolic and flavonoid contents and their antioxidant potential. It is thought that the results of this study may provide insights into the determination of the potential of plant species from Baku to be used in functional foods and medical preparations, as well as suggest new directions for future studies.



REFERENCES

1. Akinmoladun, F. O., Akinrinlola, B. L., & Komolafe, O. T. (2020). Antioxidants and their roles in human health. *Biomedicine & Pharmacotherapy*, 129, 110467.
2. Al-Owais, M., & Khan, R. A. (2019). Phytochemical constituents and antioxidant potential of medicinal plants. *Journal of Natural Products Research*, 33(5), 678-690.
3. Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agro-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(4), 191-203.
4. Ferreira, I. C. F. R., Martins, N., & Barros, L. (2017). Phenolic compounds and its bioavailability: In vitro bioactive compounds or health promoters?. *Bioactive Natural Products*, 32(3), 67-85.
5. S.Q. Wang, S.L. He, M.Z. Zhang, Y.X. Zhang, Q.Y. Wang, C.Y. Zhang, T.Y. Liu, B. Liu, J.Y. Han, J.C. Qin ve Diego A. Sampietro (2020) - Chemical composition and allelopathic potential of essential oils from *Eupatorium maculatum* on *Lolium perenne* L. and *Echinochloa crusgalli* L. *Allelopathy Journal*, 49 (1): 51-62.
6. Bayramov, A. M., & Həsənova, G. Q. (2021). Azərbaycan florasında rast gəlinən bəzi dərman bitkilərinin antioksidant xüsusiyyətləri. *Azərbaycan Milli Elmlər Akademiyası (AMEA) Biologiya və Tibb Jurnalı***, 73(2), 45-52.
7. Kaundun S.S., Fady B., Lebreton P. 1997. Genetic differences between *Pinus halepensis*, *Pinus brutia* and *Pinus eldarica* based on needle flavonoids. *Biochemical Systematics and Ecology* 25: 553–562.
8. Mamedov N, Craker LE. (2001) Medicinal plants used for the treatment of bronchial asthma in Russia and Central Asia. *Journal of herbs, species and medicinal plants*, 8(2-3): 91-117.
9. Iravani S, Zolfaghari B. (2014). Phytochemical analysis of *Pinus eldarica* bark. *Research in pharmacological science*, 9(4): 243-250.
10. Joshi, M.C., P.M., M. P.J., 1980. Some folk medicines of Dangs, Gujarat state. *Bull. Medico Ethnobot. Res.*, 1, 8–24.
11. Karmakar, S., Paul, S., Biswas, N.M., Khanam, J., Kar, S.K., Mukherjee, H., Poddar, S., 2020. A pharmacological audit and advancement on the recent trend of research on *Ficus benghalensis* L. including its In Vitro hepatoprotective activity. *Clin. Phytosci.* 6 (1), 1–3.
12. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology***, 28(1), 25-30.
13. Gülçin, İ. (2020). *Antioxidants and antioxidant methods: An updated overview*. ***Archives of Toxicology***, 94(3), 651-715.
14. Apak R, Guclu, K, Demirata B, Ozyurek M, Celik SE, Betasoglu B, et al. (2007). Comparative evaluation of various total antioxidant capacity assay applied to phenolic compounds with the CUPRAC assay. *Molecules*. 12(7):1496-547
15. Kim DO, Jeong SW, Lee CY. (2003). Antioxidant capacity of phenolics phytochemicals from various cultivars of plums. *Food Chem*. 81:321–6.
16. Park Y-S, Jung S-T, Kang S-G, Heo BK, Arancibia-Avila P, Toledo F, et al. (2008). Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chem* 2008;107:640–8.
17. Khater, O. R., El-Kholie, E. M., & Sherif, A. A. (2020). Nutritional Characterizations Of White Pine (*Pinus eldarica*) Nuts. *Journal of Home Economics*, 30(4).
18. Singh, S. P., & Verma, N. K. (2019). Evaluation of the Antioxidant Activity of *Ficus benghalensis* Plant Extracts of Barks. *International Journal of Pharmacy and Biological Sciences-IJPBSTM*, 9(2), 487-491.
19. Tulasi, C. D. S. L. N., Rani, A. S., & Manjula, B. (2015). Screening of phyto-chemicals, TLC profiling, total flavonoid and phenolics content, anti-oxidant activity and anti-microbial activity of *Ficus benghalensis* Linn and *Ficus religiosa* Linn latex. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(9), 22-29.