Journal of Medicinal Plants and By-products (2024) 1: 51-56
https://doi.org/10.22092/jmpb.2022.358593.1472

Original Article

Phytochemical Profiles and Antioxidant Activity of Iranian *Melia azedarach* L.

Marzie Kamali[1,2*], Jafar Valizadeh[3], Hamid Reza Shaterian[1] and Javad Mottaghipisheh[4]

[1]*Department of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran*
[2]*Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
[3]*Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran*
[4]*Center for Molecular Biosciences (CMBI), Institute of Pharmacy/Pharmacognosy, University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria*

Article History

Received: 06 May 2022
Accepted: 11 November 2022

Keywords
Antioxidant activity
Essential oil
Melia azedarach
Trans-nerolidol
Phytochemical profile

***Corresponding author**
mkamali@sbmu.ac.ir

ABSTRACT

Melia azedarach L. (Meliaceae family) is traditionally consumed to treat rheumatic pains, astringent, skin diseases, and diuretics. The present study aims to investigate the chemical composition of the flowers essential oil, as well as *in vitro* antioxidant activities of the leaves and fruits extracts of *M. azedarach* by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays. The GC-MS analysis of the essential oil identified oxygenated sesquiterpenes (43.7%) as the major chemical class. The volatile oil was rich in *trans*-nerolidol (39.5%), 1,4-dimethoxybenzene (11.6%), 2-phenylacetaldehyde (9.1%), and phenyl ethyl alcohol (7%). The leaves ethanolic extracts (LEE) indicating the IC_{50} values 149 ± 0.05 µg/mL and 20.31 ± 0.03 mmol of the $FeSO_4$/g of the extract exhibited the strongest radical scavenging activity assessed *via* DPPH and FRAP assays, respectively. The higher antioxidant activity of LEE was correlated with the higher total phenolic contents (42.86 ± 0.02 µg gallic acid/mg extract) compared to the fruits (26.8 ± 0.05 µg gallic acid/mg extract). To the best of our knowledge, this is the first report on the essential oil compositions and the antioxidant activity of this species collected from the southeast of Iran. This study highlights that the *M. azedarach* essential oil can be a rich natural source of *trans*-nerolidol with diverse biological activities. Additionally, the ethanolic extract of leaves has significant antioxidant activities. Therefore, these findings might direct further complementary phytochemical and biological investigations of this species.

INTRODUCTION

Essential oils (EOs) are volatile classes of secondary metabolites produced in various parts of many higher plants [1]. These plant products are responsible for the plant's odor characteristic, while they are reported to possess diverse medicinal properties, such as antioxidant, antimicrobial, anti-anxiety, antidepression, and pain-relieving properties [2,3]. Various ailments, such as inflammation, cancer, diabetes, atherosclerosis, arthritis, Parkinson's and Alzheimer's diseases, and AIDS, are induced by free radicals damaging several biofactors [4]. Over half of the plant species have medicinal effects and considerable antioxidant potential owing to their valuable combined enzymatic and non-enzymatic antioxidant defense systems against free radicals [5].

Melia azedarach L. (Figure 1) belonging to the Meliaceae family is known as Chinaberry, bead tree, Margosa tree, and Persian lilac, and is native to Iran, China, and India, while it is grown in some parts of the world [6].

The plant has been traditionally consumed to treat rheumatic pains, astringent, skin diseases, and diuretics [7]. Leaf, bark, root, and seed extracts revealed several biological properties, such as anticancer, antimalarial, antioxidant, antifertility, antifungal, antiviral, and antibacterial activities [8]. Previous studies identified tetranortriterpenoids, terpenoids, limonoids, steroids, flavonoids, fatty

acids, and essential oil in this species [9]. The present study aimed to identify the essential oil of *M. azedarach* harvested from the southeast of Iran, to evaluate the antioxidant activities of the leaf and fruit extracts *via* DPPH and FRAP assays, and to analyze their total phenolic contents.

MATERIALS AND METHODS

Plant Material

The leaves, flowers, and fruits of *M. azedarach* L. were gathered from the Botanical Garden of Sistan and Baluchestan University, Zahedan Province, southeastern Iran, in March and May 2012. A botanist named Dr. Jafar Valizadeh carried out the identification of the plant samples. Furthermore, the L, Fl, and Fr's voucher specimens (no. 6735, 6736, and 6737, respectively) were deposited at the herbarium of the Department of Biology, Faculty of Science, Sistan and Baluchestan University.

Essential Oil Extraction

Hundred-fifty g of the fresh flower was subjected to the steam distillation apparatus. The essential oil (EO) was subsequently extracted thrice, each within 4 h. The extracted EO was dehydrated with anhydrous sodium sulfate (Na_2SO_4) and stored in a sealed vial at 4 °C until further experiments.

Plant Extracts Preparation

The fruits and leaves samples were dried under shade and then powdered. Fifty g of each material was individually macerated three times with 150 mL EtOH at room temperature for 24 h. Afterward, the solvents were removed under reduced pressure conditions. The ethanolic extracts were kept at 4 °C for phytochemical and antioxidant investigations.

Essential Oil Composition

The gas chromatography (GC) coupled to flame ionization detector (FID) analysis of the EO was performed on an Agilent Technologies 7890A GC system under the following conditions: HP 5MS column (30.0 m × 0.25 mm, 0.25 µm particle size), oven temperature program: holding initially at 50 °C for 1 min, then increased to 250 °C with 2.5 °C/min for 15 min. The injector and detector (FID) temperature were set at 250 and 275°C, respectively. N_2 was the carrier gas, and the column flow rate was 1.5 mL/min with a split ratio of 1:10.

The GC-MS analysis was conducted by an Agilent Technologies 7890A GC system supplied with an HP 5MS column (30.0 m × 0.25 mm, 0.25 µm) combined with a GC/MS/MS QQQ 7000B mass-selective detector. The operating conditions were the same as described for the GC-FID analysis; however, the carrier gas was He. The MS conditions were adjusted at 70 eV ionization voltages, and the mass range was from 35 to 374 atomic mass units. The chemical constituents were identified by comparing their mass spectra to Wiley libraries and their retention index, which was calculated using the retention times of the *n*-alkanes (C8 - C18) series and previously reported in the literature [10, 11].

Antioxidant Assessments

DPPH Radical Scavenging Activity

The ability of the extracts in scavenging free radicals was evaluated through the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay [12]. In brief, 1 mL of the DPPH solution (1.5×10^{-4} M) was mixed into 2.5 mL of the plant ethanolic extracts and BHT (butylated hydroxytoluene) as the utilized positive control. Moreover, 30 min after the incubation, the absorbance of the sample solutions was recorded at 517 nm using a UNICO UV-2100 Spectrophotometer (China). The inhibition of DPPH was computed using the following formula:

$$I_{DPPH}\ (\%) = 100 \times [A_{control} - (A_{sample} - A_{blank})/A_{control}]$$

Where A_{sample}, A_{blank}, and $A_{control}$ are expressed as absorbance of sample, blank, and control, respectively.

Ferric-reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the leaf and fruit ethanolic extracts was further experimented. In the FRAP assay, an analyzed sample at different concentrations is mixed with a measured volume of freshly prepared FRAP reagent including the Fe^{3+}-TPTZ salt. Antioxidants can reduce this salt to colored Fe^{2+}-TPTZ form [13]. Initially, (a) 300 mM of acetate buffer (pH 3.6), (b) 10 mM of TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and (c) 20 mM $FeCl_3.6H_2O$ were prepared. A FRAP reagent solution was prepared by adding a, b, and c in a ratio of 10:1:1 v just before the test. Afterward, 3.6 mL of the FRAP reagent was added to 0.4 mL distilled water and 80 mL of each sample: the extracts or ascorbic acid as the positive control. After 10 min incubation at 37 °C, the absorbance of the colored solution was read

at 595 nm. The calibration curve of aqueous solutions of Fe^{2+} (concentrations ranges of 0.1~1 mM/L) was determined, and its results were expressed as mmol of $FeSO_4.7H_2O$/g of the extract.

Total Phenolic Contents

Phenolic compounds of higher plants, including flavonoids, phenolic acids, tannins, and phenolic diterpenes, play crucial roles in possessing scavenging free radicals and antioxidants [14]. Total phenolic contents (TPCs) from the extracts were determined with the Folin-Ciocalteu reagent [15]. In brief, 1 mL of the extracts (400 µg/mL) in EtOH was mixed with 5 mL Folin-Ciocalteu reagent diluted 1/10 with distilled water. After 10 min, 4 mL of the sodium carbonate solution (75 mg/mL) was added to the mixture, and 30 min later, the absorbance was recorded at 765 nm. The phenolic contents were represented as µg of the gallic acid/mg of the extract.

RESULTS AND DISCUSSION

Essential Oil Composition

The EO extracted from *M. azedarach* fresh flowers was characterized as the pale-yellow color possessing a pleasant odor (oil yield: 0.01 % v/w based on fresh weights). The GC-MS analysis identified nine fragrant constituents representing 87.2% of the total compounds (Table 1). The volatile oil contained primarily oxygenated sesquiterpenes (43.7%), and the major compounds were identified as *trans*-nerolidol (39.5%), 1,4-dimethoxybenzene (11.6%), 2-phenylacetaldehyde (9.1%), and phenyl ethyl alcohol (7%).

According to *Ghomi et al*., *trans*-nerolidol (38.9%) was rich in the *M. azedarach* EO collected from the central part of Iran. Although, viridiflorol (8.1%), bicyclogermacrene (8.2%), *β*-caryophyllene (7.2%), *cis*-caryophyllene (7.1%), 1,8-cineole (6.1%), and camphor (5.7%) were identified from the flower EO, none of them was characterized in the present EO [16]. Consistent with the present study, *trans*-nerolidol (70%) has been identified as the main Indian flower's volatile oil constituent; however, due to its richness in *n*-nonanal (4.78%) and *cis*-nerolidol (3.99%), the chemical profile was not the same [17]. Many investigations have confirmed parameters affecting EO contents and compositions of various plant populations [18]. According to another report by *Lau et al*., benzaldehyde (68.50%) and phenylacetaldehyde (22.26%) were characterized in the plant flowers extracted *via* the solid-phase micro-extraction (SPME) method. Nevertheless, using the current steam distillation method, the percentage of benzaldehyde and phenylacetaldehyde was 4.5% and 9.1%, respectively [19]. Various factors affect different volatile oil compositions, while in this case, the difference might correlate with the EO's extraction method and the plant's growth location.

Fig. 1 The aerial parts of *M. Azedarach* L.

Antioxidant Activity

As Table 2 shows, among the leaf and fruit ethanolic extracts assessed through the DPPH assay, the leaf exhibited potent antioxidantl effects compared to the fruit extracts with the IC_{50} values of 149 ± 0.05 µg/mL and >200 µg/mL, respectively.

To confirm the DPPH results, the ferric reducing power of the leaf and fruit ethanolic extract was further elaborated, showing the reduction values 20.31 ± 0.03 and 12.51 ± 0.02 mmol of the $FeSO_4$/g extract, respectively. Reduction power values of the samples and positive control were determined according to the linear regression equation ($y = 0.2481x - 0.0055$, $R^2 > 0.99$) (Table 2). According to the DPPH and FRAP results, the leaf ethanolic extract exhibited higher antioxidant activity than fruits did.

In one previous study, the IC_{50} values of the leaf aqueous, methanolic, and ethanolic extracts were calculated as 62 µg/mL, 66 µg/mL, and 58 µg/mL by the DPPH assay [20]; however, comparably these samples indicated higher scavenging activity than our plant materials did, which is possibly caused by different phytochemical profiles. Orhan *et al*. also in

one comparative study reported that the leaf methanolic extract possessed higher antioxidant activities than the fruits evaluated *via* DPPH and FRAP assays [7]. Furthermore, in another study, the defatted ethanolic extract of the leaf demonstrated significant DPPH activity compared to ascorbic acid as the positive control with the IC_{50} values of 30.55 µg/mL and 50.11 µg/mL, respectively [21]. No previous study compared the antioxidant effects of defatted and non-defatted extracts of this plant. However, studies investigating other plants indicate that defatting does not cause a significant difference in DPPH results. While defatting has affected the results of the FRAP method; this improvement has been attributed to the removal of fats [22].

Table 1 Essential oil components of the fresh flowers of *Melica Azedarach* L.

Peak	Compounds	RI* [a]	Area (%)	Identification methods [b]
1	Benzaldehyde	960	4.5	MS, RI
2	Phenylacetaldehyde	1036	9.1	MS, RI
3	2-phenylethanol	1106	7.0	MS, RI
4	1,4- dimethoxybenzene	1165	11.6	MS, RI
5	2,2-dimethyl-1-phenylpropan-1-one	1272	3.6	MS
6	1a,2,5,5,6- pentamethyl-*cis*- 4a,1a,5,6,7,8 -hexahydro-*4H*-chromene	1448	5.1	MS
7	*trans*-Nerolidol	1563	39.5	MS, RI
8	*α-epi*-Cadinol	1639	4.2	MS, RI
9	Ethyl hexadecanoate	1925	2.6	MS, RI
	Total		87.2	

[a] Retention Index

[b] MS (Mass spectra) compared to Wiley, RI retention indices in literature published

Table 2 Antioxidant activities of the leaf and fruit ethanolic extracts of *M. azedarach* L. using DPPH and FRAP assays

Samples	DPPH assay (IC_{50}: µg/mL)	FRAP assay (mmol of $FeSO_4$/g extract)
Leaf ethanolic extract	149±0.05	20.31±0.03
Fruit ethanolic extract	>200	12.51±0.02
BHT (Butylated hydroxytoluene)	11.9±0.05	-
Ascorbic acid	-	121.54±0.05

Total Phenolic Contents

Total phenolic contents (TPCs) of the leaf and fruit ethanolic extracts of *M. azedarach* were determined by the Folin-Ciocalteu assay based on the linear calibration curve of gallic acid ($y = 0.0262x + 0.0075$, 5–150 µg/mL, $R^2 > 0.98$). Consequently, the leaf ethanolic extract demonstrated higher TPC with 42.86 ± 0.02 µg gallic acid/mg extract followed by the fruit ethanolic extract with 26.8 ± 0.05 µg gallic acid/mg extract. According to our results, comparison of the TPCs of the leaf ethanolic, hexane, ethyl acetate, and aqueous extracts of *M. azedarach* were collected from Indonesia, it was shown that ethanolic (102.18 µg gallic acid/mg) and ethyl acetate (109.43 µg gallic acid/mg) extracts were the richest in the compounds [23]. Similarly, the Indian leaf ethanolic extract of *M. azedarach* with the highest amount of phenolic compounds exhibited the most significant antioxidant activity compared to aqueous and methanolic extracts [20]. However, an insignificant TPC was previously reported from the fruit ethanolic extract of the collected plant from the center of Iran (16 µg gallic acid/mg extract) [24]. The differences observed between the results can be owing to different geographical regions, collection seasons, or other environmental factors. Many researchers demonstrated TPCs as the main responsible marker compounds for considerable biological potency, including anti-radical effects [25]. Although environmental effects on phenol levels may influence the antioxidant activity, it is necessary to detect the presence of other antioxidant compounds such as flavonoid and tannin in the plant [22].

CONCLUSION

Identification of the *M. azedarach* flower volatile oil indicated the presence of several vital secondary

metabolites, specifically *trans*-nerolidol, an acyclic sesquiterpene alcohol, as the main compound. Besides its essential role in the plant defense system, also exhibited several biological activities, such as antiparasitic, antimicrobial, antioxidant, antibiofilm, anti-inflammatory, antinociceptive, skin penetration enhancer, antiulcer, insect repellent, and anticancer properties. Additionally, the present study revealed that the leaf ethanolic extract possessed more potent antioxidant activity than the fruit extract assessed through both DPPH and FRAP assays, which were correlated with higher phenolic components. The present experiment demonstrated that this species could be considered a valuable natural source. Further complementary phytochemical and biological investigations of *M. azedarach* are required, in particular the isolation and identification of the dominant secondary metabolites that are responsible for the bioactivities.

ACKNOWLEDGEMENT

The authors would like to thank Sistan and Baluchestan University for supporting this project. We are also grateful to Zahra Sarmadi for providing GC-MS.

REFERENCES

1. Dehsheikh A.B., Sourestani M.M., Dehsheikh P.B., Mottaghipisheh J., Vitalini S., Iriti M. Monoterpenes: Essential oil components with valuable features. Mini Rev Med Chem. 2020;20:958-974.
2. Mottaghipisheh J., Maghsoudlou M.T., Valizadeh J., Arjomandi R. Antioxidant activity and chemical composition of the essential oil of Ducrosia anethifolia (DC.) Boiss. from Neyriz. J Medicinal Plants by- Products. 2014;3:215-218.
3. Mottaghipisheh J., Kiss T., Tóth B., Csupor D. The Prangos genus: a comprehensive review on traditional use, phytochemistry, and pharmacological activities. Phytochem Rev. 2020;19:1449-1470.
4. Moon J-K., Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem. 2009;57:1655-1666.
5. Krishnaiah D., Sarbatly R., Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Chem Eng Res Des. 2011;89:217-233.
6. Kasote D.M., Katyare S.S., Hegde M.V., Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015;11:982-991.
7. Orhan I.E., Guner E., Ozturk N., Senol F., Erdem S.A., Kartal M., Sener B. Enzyme inhibitory and antioxidant activity of *Melia azedarach* L. naturalized in Anatolia and its phenolic acid and fatty acid composition. Ind Crops Prod. 2012;37:213-218.
8. Oelrichs P.B., Hill M.W., Vallely P.J., MacLeod J.K., Molinski T.F. Toxic tetranortriterpenes of the fruit of Melia azedarach. Phytochemistry. 1983;22:531-534.
9. Khan M.F., Rawat A.K., Pawar B., Gautam S., Srivastava A.K., Negi D.S. Bioactivity-guided chemical analysis of Melia azedarach L.(Meliaceae), displaying antidiabetic activity. Fitoterapia. 2014;98:98-103.
10. Adams R.P. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation. 2007.
11. Van Den Dool H., Kratz P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A. 1963;11:463-471.
12. Blois M.S. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-1200.
13. Benzie I.F., Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-6.
14. Kahkonen M., Hopia A., Vuorela H., Rauha J., Pihlaja K. Kujala TSl, Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem.1999;47:3954–3962.
15. Nickavar B., Esbati N. Evaluation of the antioxidant capacity and phenolic content of three Thymus species. J Acupunct Meridian Stud. 2012;5:119-125.
16. Safaei-Ghomi J., Ahmadi T., Batooli H. GC-MS identification of essential oil components and in vitro investigation of antioxidant activity of methanol extracts from flower and fruit fractions of *Melia azedarach* cultivated in central Iran. Chem Nat Compd. 2010;46:816-818.
17. Kharkwal G., Pande C., Tewari G., Panwar A., Pande V. Volatile terpenoid composition and antimicrobial activity of flowers of *Melia azedarach* Linn. from north west Himalayas, India. J Indian Chem Soc. 2015;92:141-145.
18. Piri E., Mahmoodi Sourestani M., Khaleghi E., Mottaghipisheh J., Zomborszki Z.P., Hohmann J., Csupor D. Chemo-diversity and antiradical potential of twelve Matricaria chamomilla L. populations from Iran: proof of ecological effects. Molecules. 2019;24:1315.
19. Lau K-M., Su W-S., Chien S-C., Wang S-Y., Senthil Kumar K. *Melia azedarach* flowers and their volatile components improved human physiological and psychological functions. J Essent Oil-Bear Plants. 2021;24:1200-1211.
20. Nahak G., Sahu R.K. In vitro antioxidative acitivity of Azadirachta indica and *Melia azedarach* Leaves by DPPH scavenging assay. Nat Sci. 2010;8:22-28.
21. Marimuthu S., Balakrishnan P., Nair S. Phytochemical investigation and radical scavenging activities of *Melia azedarach* and its DNA protective effect in cultured lymphocytes. Pharm Biol. 2013;51:1331-1340.

22. Njanje I., Bagla V.P., Beseni B.K., Mbazima V., Lebogo K.W., Mampuru L., Mokgotho M.P. Defatting of acetone leaf extract of Acacia karroo (Hayne) enhances its hypoglycaemic potential. BMC Complement Altern Med. 2017;17:482.
23. Ervina M., Poerwono H., Widyowati R., Matsunami K. Sukardiman. Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of *Melia azedarach* L. wild type leaves. Biotechnol Rep. 2020;25:e00437.
24. Afkhami-Ardekani O., Esmailidehaj M., Afkhami-Ardekani M., Esmaeili H., Afkhami-Ardekani A. *Melia azedarach* L. fruit extract effect on plasma lipid profile and cardiac and hepatic functions of diabetic rats. Iran j diabetes obes. 2017;9:155-163.
25. Laughton M.J., Halliwell B., Evans P.J., Robin J., Hoult S. Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin: effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. Biochem Pharmacol. 1989;38:2859-2865.