ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed(Group –I) Journal Volume 10, Iss 12, December 2021

PHYTOCHEMICAL EVALUATION AND DETERMINATION OF ANTIOXIDANT ACTIVITIES OF MORINGA OLEIFERA

Keshamma E[1*] and Shobha Rani K[2]

[1]Associate Professor, Department of Biochemistry, Maharani's Science College for Women (Maharani Cluster University), Palace Road, Bengaluru, Karnataka, India.

[2]Assistant Professor, Department of Botany, Government First Grade College, Chikkaballapur, Karnataka, India.

***Corresponding Author Dr. Keshamma E**

keshamma.blr76@gmail.com

ABSTRACT

Dietary phytochemicals are considered as an effective tool to cure various human physiological disorders. The leaves of *Moringa oleifera* are rich in bioactive compounds, and reported to possess various pharmacological activities. Therefore, we aimed for quantitative estimation of phytochemicals present in *M. oleifera* leaves and determination antioxidant activities of leaf parts of *M. oleifera*. Leaves of *M. oleifera* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 50% methanol. The free radical scavenging activity of the methanolic leaf extract of *M. oleifera* and of standard ascorbic acid were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. Results portrayed that methanolic leaf extract of *M. oleifera* exhibited inhibition percentage of 4.56%, 7.89%, 15.82%, and 31.58% at a concentration range of 25µg/mL, 50µg/mL, 100µg/mL, and 200µg/mL respectively with an IC_{50} value of 38.48 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 53.54 µg/mL. In conclusion, the presence of secondary metabolites especially polyphenols in the methanolic leaf extract of M. oleifera led to the demonstration of its antioxidant properties. Therefore, methanolic leaf extract of *M. oleifera* could be exploited in the development of polyherbal formulations with antioxidant claims.

Keywords: Moring oleifera, Leaves, Methanol, Extract, Antioxidant agents, Polyphenols

INTRODUCTION

Multi-purpose medicinal plants have continued to elicit research interest and commercial attention globally as natural resources considered rich in nutritional and pharmacological properties to treat and cure several diseases.[1,2] Several medicinal products as multitherapeutic agents have been derived from such herbs and plants and considered safe for consumption.[3,4]

Moringa oleifera is a plant commonly termed as horseradish tree, the ben oil tree, or the drumstick tree. This plant of Indian origin with several medicinal properties has been used for several hundred years by various countries.[5] The leaves of this wonder tree are rich in phytochemicals and nutrients such as beta carotene, essential minerals, and proteins, and hence the tree is often known as a "miracle tree" and "mother's best friend".[6] The leaves of this miracle tree can be consumed in several methods that will aid in the long-term maintenance and enhancement of nutritional status. Some countries of the African subcontinent have emphasized the development of these leaves as therapeutic nutritional supplements.[7] In traditional medicine,

these leaves have been explored for their use in the management of obesity, some cancers, hysteria, diabetes mellitus, and vitamin C deficiency.[8,9]

M. oleifera grows up to 5-12 meters belongs to the family Moringaceae (Figure 1A and Figure 1B), and all the part of this plant is culturally very important for its nutritional and pharmacological values.[8,10] It is reported that the *M. oleifera* contains many phytoconstituents such as flavonoids, alkaloids, saponins, saccharides, glucosinolates, tannins, phenolic acids, and nitrile glycosides etc. These complex natural phytochemicals contribute to its various pharmacological activities. Previous studies reported about the antioxidant and anti-inflammatory activities of *M. oleifera* leaves.[11]

Figure 1: Showing *M. oleifera* plant (A) and leaf parts of *M. oleifera* (B)

With these viewpoints, in the current study we aimed for quantitative estimation of phytochemicals present in *M. oleifera* leaves and determination antioxidant activities of leaf parts of *M. oleifera*.

MATERIALS AND METHODS

Collection Leaves of *M. oleifera*

The leaves of *M. oleifera* were collected from agricultural places in and around Chikkaballapur, Karnataka, India. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[12]

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *M. oleifera* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of 50% methanol. The extract was concentrated by distilling the solvent in a rotary flash evaporator and

dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.[12]

Quantitative Estimation of Phytochemicals

Estimation of total phenolic content

The total phenolic content of the methanolic leaf extract of *M. oleifera* was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth and Gillespie.[13] Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid, and expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Estimation of total flavonoid content

The total flavonoid content of the methanolic leaf extract of *M. oleifera* was determined using aluminum chloride ($AlCl_3$) method.[14,15] The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C. This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. 2 mL of 1M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Assay of Antioxidant Activity

DPPH free radical scavenging assay

The free radical scavenging activity of the methanolic leaf extract of *M. oleifera* and of standard solution (ascorbic acid) were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as described by Karadag et al.[16] The assay mixture contained 2 mL of 1.0 mmol/L DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (10-500 µg/mL). The solution was rapidly mixed and incubated in dark at 37°C for 20 min. The decrease in absorbance of each solution was measured at 517 nm. Ascorbic acid is a well-known antioxidant was used as positive control while DPPH radical solution with 1 mL ethanol was taken as blank. The radical scavenging (%) was calculated by the following formula:

Free radical scavenging activity (%):

Absorbance of control – Absorbance of sample/Absorbance of control X 100

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

RESULTS

The results of quantitative estimation of phytochemicals in methanolic leaf extract of *M. oleifera* was represented in Table 1 and plotted in Figure 2. Results implied that total phenolic quantity was found to be highest (18.54 mg GAE/g extract) in methanolic leaf extract of *M. oleifera* when

compared with total flavonoid quantities (4.41 mg QE/g extract).

Table 1: Quantitative analysis of phytochemicals of methanolic leaf extract of *M. oleifera*

Phytochemicals	**Methanolic leaf extract of *M. oleifera***
Total Phenolics	18.54 mg GAE/g extract
Total flavonoids	4.41 mg QE/g extract

Values are expressed mean; n=3

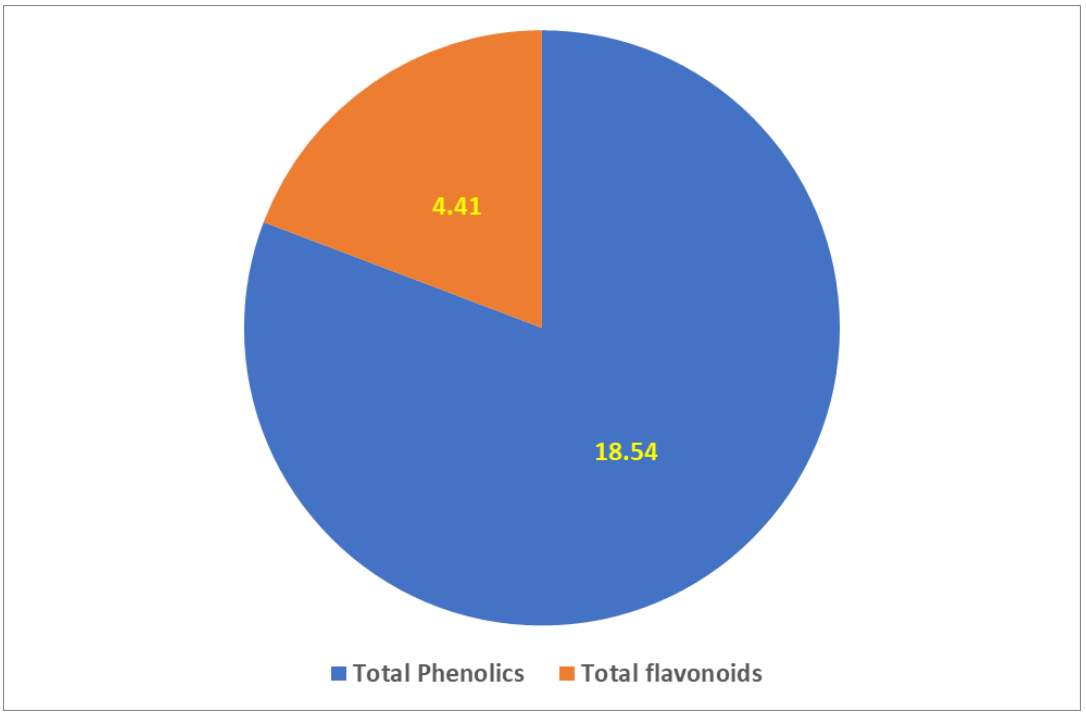

Figure 2: Quantitative analysis of phytochemicals of methanolic leaf extract of *M. oleifera*

The results of effect of methanolic leaf extract of *M. oleifera* on DPPH radical-scavenging activity was represented in Table 2. Results depicted that methanolic leaf extract of *M. oleifera* exhibited inhibition percentage of 4.56%, 7.89%, 15.82%, and 31.58% at a concentration range of 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL respectively with an IC_{50} value of 38.48 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 53.54 µg/mL.

Table 2: Effect of methanolic leaf extract of *M. oleifera* and ascorbic acid on DPPH radical-scavenging activity

Conc. of Methanolic Leaf extract of *M. oleifera* (µg/mL)	**Inhibition (%)**	**Conc. of Ascorbic Acid (µg/mL)**	**Inhibition (%)**

25	4.56 ± 0.08	25	85.45 ± 0.18
50	7.89 ± 0.16	50	88.48 ± 0.21
100	15.82 ± 0.21	100	91.28 ± 0.19
200	31.58 ± 0.34	200	93.42 ± 0.26
IC_{50} (ug/mL) = 38.48		IC_{50} (ug/mL) = 53.54	

Values were expressed as Mean ± SD; n=3

DISCUSSION

Among the ancient civilizations, India is well known for possessing an extensive array of medicinal plants.[17] Moreover, plant-derived substances have recently become of great interest owing to their versatile applications.[18] Many of the aromatic and medicinal plants found in India's forests are harvested to be used as raw materials for the manufacturing of medications and scented products. Medicinal plants have been valued for their long history due to their diverse pharmacological effects, which are believed to be attributed to secondary plant metabolites such as tannins, alkaloids, flavonoids, glycosides, and steroids.[17] *M. Oleifera* is a widely recognized herb with a wide range of therapeutic uses. It is frequently used as an ingredient in polyherbal formulations.[19]

DPPH free radical scavenging activity is an easy and widely used method for testing *in-vitro* antioxidant activity of natural compounds or plant extracts.[20] DPPH is a stable free radical at room temperature, purple in color. Its reduction capability to accept an electron or a hydrogen radical from antioxidants is determined by measuring decrease in its absorbance values at 517 nm.[17] Therefore, in this study we aimed for quantitative estimation of phytochemicals present in *M. oleifera* leaves and determination antioxidant activities of methanolic leaf extract of M. oleifera using DPPH radical scavenging method.

Results of our study revealed that methanolic leaf extract of *M. oleifera* exhibited inhibition percentage of 4.56%, 7.89%, 15.82%, and 31.58% at a concentration range of 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL respectively with an IC_{50} value of 38.48 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 53.54 µg/mL. In this study DPPH radical scavenging activity of methanolic leaf extract of *M. oleifera* was compared with standard ascorbic acid, Findings inferred that although ascorbic acid had higher scavenging activity at all tested concentrations than the extract, the extract still showed good free radical scavenging activity. The free radical scavenging property of *M. oleifera* may be one of the mechanisms by which this plant is effective as a traditional medicine.[17]

Phenolics and flavonoids have at least one hydroxyl ion substituted with aromatic ring and can form chelate complexes with the metal ions thereby getting easily oxidized and are the means for donating electrons to scavenge free radicals.[21,22] Literature reports evidenced that higher phenolic content in *M. oleifera* is correlated with increased antioxidant activity.[23] Furthermore, linear correlation between phenolic content and antioxidant activity was also been reported by various

research investigators in the literature.[24,25] In our study the total phenolic quantity was found to be highest (18.54 mg GAE/g extract) in methanolic leaf extract of *M. oleifera* when compared with total flavonoid quantities (4.41 mg QE/g extract). These findings implied that the antioxidant activities exhibited by methanolic leaf extract of *M. oleifera* in our study could be accredited to its phenolic constituents. Concurrently Keshamma reported antioxidant activities of methanolic extract of *Elephantopus scaber* which contains considerable amounts of polyphenols and flavonoids.[12]

CONCLUSION

In conclusion, this study evidenced the antioxidant properties of methanolic leaf extract of *M. oleifera*. The secondary metabolites, primarily polyphenols, that are present in methanolic leaf extract of *M. oleifera* could be responsible for its antioxidant properties. Consequently, methanolic leaf extract *M. oleifera* may be used in the development of formulations of natural antioxidant agents. Nevertheless, to confirm the safety, effectiveness, and potential mode of action of antioxidant activities of *M. oleifera*, more *in-vivo* research is advised.

REFERENCES

1. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother Res. 2007;21(1):17-25.
2. Popoola JO, Adebayo AH, Taiwo OS, Ayepola OO, Okosodo EF. Studies on local knowledge and in vitro cytotoxicity of *Moringa oleifera* L., *Andrographis paniculata* N. and *Asystasia vogeliana* B. extracts. Res J Appl Sci. 2017;12(2):180-90.
3. Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. Biotech. 2016;6(2):203.
4. Sheba LA, Anuradha V. An updated review on *Couroupita guianensis* Aubl: a sacred plant of India with myriad medicinal properties. J Herbmed Pharmacol. 2020;9(1):1-11.
5. Paliwal R, Sharma V, Pracheta. A review on horse radish tree (*Moringa oleifera*): a multipurpose tree with high economic and commercial importance. Asian J Biotechnol 2011;3(4):317–328.
6. Leone A, Spada A, Battezzati A, et al. Cultivation, genetic, ethnopharmacology, phytochemistry, and pharmacology of *Moringa oleifera* leaves: an overview. Int J Mol Sci 2015;16(6):12791–12835.
7. Rodríguez-Pérez C, Quirantes-Piné R, Fernández-Gutiérrez A, et al. Optimization of extraction method to obtain a phenolic compound rich extract from *Moringa oleifera* Lam leaves. Ind Crops Prod 2015; 66:246–254.
8. Kasolo JN, Bimenya GS, Ojok L, et al. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J Med Plants Res2010;4(9):753–757.
9. Gupta S, Jain R, Kachhwaha S, et al. Nutritional and medicinal applications of *Moringa oleifera* Lam. review of current status and future possibilities. J Herb Med 2018; 11:1–11.
10. Abdulkarim SM, Long K, Lai OM, Muhammad SK, Ghazali HM. Frying quality and stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils. Food chemistry. 2007;105(4):1382-9.
11. Xu YB, Chen GL, Guo MQ. Antioxidant and anti-inflammatory activities of the crude extracts of *Moringa oleifera* from Kenya and their correlations with flavonoids. Antioxidants. 2019 Aug 9;8(8):296.

12. Keshamma E. A Study on Quantitative Analysis of Phytochemicals and Evaluation of In-vitro Antioxidant Activity of Leaf Extracts of *Elephantopus scaber*. International Journal of Recent Developments in Science and Technology. 2011;1(6):80-91.
13. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2007; 2(4): 875-877.
14. Kariyone T, Hashimoto Y, Kimura M. Microbial studies of plant components. IX. Distribution of flavonoids in plants by paper chromatography. J Pharm Soc. 1953; 73:253–6.
15. Naghski JS, Fenske CS, Jr, Couch IF. Use of paper chromatography estimation of quercetin in rutin. J Am Pharm Assoc. 1951; 40:613.
16. Karadag A, Ozeelik B, Saner S. Review of methods to determine antioxidant capacities. Food Anal Method 2009; 2: 41-60.
17. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in vitro antioxidant and anti–inflammatory activity of flowers of *Moringa oleifera*. Asian Pacific journal of tropical biomedicine. 2013;3(8):623-7.
18. Keshamma E. Phytochemical Screening and Determination of Antioxidant Activity of Rhizome of Acorus Calamus. International Journal of Food and Nutritional Sciences. 2022;11(7):3508-3515.
19. Rao KV, Gopalakrishnan V, Loganathan V, Nathan SS. Anti-inflammatory activity of *Moringa oleifera*. Lam. Ancient Science of Life. 1999;18(3&4):195-8.
20. Philips A, Philips S, Arul V, Padmakeerthiga B, Renju V, Santha S, et al. Free radical scavenging activity of leaf extracts of *Indigofera aspalathoides*: An in vitro analysis. J Pharm Sci Res 2010; 2: 322-328.
21. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem 2003; 51:2144-55.
22. Sreedam CD, Kaiser H, Jahan BI, Sultana S, Md. Islam S. In vitro antioxidant activity of different parts of the plant diospyros discolor. Res J Agric Biol Sci 2010; 6:472-5.
23. Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG, Afanas'ev IB. Influence of metal ions on flavonoid protection against asbestos-induced cell injury. Arch Biochem Biophys 2001; 385:129-37.
24. Borneo R, Leon EA, Aguirre A, Ribotta, P, Cantero JJ. Antioxidant capacity of medicinal plants from the province of cordoba (Argentina) and there in vitro testing in model food system. Food Chem 2008; 112:664-70.
25. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 2004; 94:550-7.