Research Article

# Analysis of Phenolic, Flavonoid and Antioxidant Activity of *Moringa oleifera* (Lam.) Root

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**ABSTRACT**

*Moringa oleifera* (Lam.) (Moringaceae) is commonly known as drumstick and it's the best source of nutrition and used as a traditional medicine for various ailments. Antioxidant activity *Moringa oleifera* (Lam.) roots was identified as an active principle against DPPH, superoxide, $ABTS^{\cdot+}$, nitric oxide, hydrogen peroxide radicals and also showed highest inhibition scavenging effect on reducing power, hydroxyl radical and also have potent $Fe^{2+}$ metal chelating activity. The total phenolic and flavonoids content were also evaluated and found to be 55.26 ± 1.04 mg/g expressed in Gallic acid equivalents (GAE) and 200.62 ± 3.30 mg/g quercetin equivalent respectively. The present work revealed the active principle against free radicals in the methanol extract of *Moringa oleifera* root.

**Keywords:** *Moringa oleifera*; Traditional Medicine; Antioxidant activity; Bioactive components.

## INTRODUCTION

The reactive oxygen species produced in cells including hydrogen peroxide ($H_2O_2$), superoxide anion and hydroxyl radicals that are highly reactive results in cell death and tissue damage. Oxidative stress contributes to a wide range of diseases such as Alzheimer's, Parkinson's, diabetes, rheumatoid arthritis and neurodegenerative diseases that affect motor neurons[1]. The plant is a rich source of antioxidants, hence appropriate utilization of it lowers risk of nervous disorders, heart disease and also protects against some cancers[2]. Natural antioxidant from plants mainly exists due to phenolic compounds such as flavonoids, phenolic acids and tocopherols. Synthetic antioxidants like butylated hydroxytoluene and butylated hydroxy anisole (BHA) have adverse effects. Hence, there is a need for natural antioxidants with high nutritional values and fewer or no side effects[3].

*Moringa oleifera* Lam. belongs to Moringaceae family which is distributed in India, Srilanka, Thailand, Pakistan, Singapore, Africa and Burma. *M. oleifera* leaves contain high protein content with significant quantities of all the essential amino acids. It is utilized mainly in Africa, India and other parts of the world as it is a good source of food supplement[4]. *M. oleifera* has long been widely used for many phyto-therapeutics as it possesses various activities such as antimicrobial, antitumor, antioxidant, antiurolithiatic, antiatherosclerotic and central nervous system activity, etc. There have been numerous reports about the antimicrobial, antioxidant and other activities of *M. oleifera* leaves, but only a few referred to other parts of *M. oleifera*, especially root[5]. Therefore, this study was aimed to evaluate the phenolic constituents, flavonoid content and antioxidant activities of the methanolic extract of *M. oleifera* root to explore their therapeutic efficiency at *in vitro* level.

## MATERIALS AND METHODS

*M. oleifera root sample* was collected from in and around the surrounding areas of Annur, Coimbatore and Tamil Nadu. The species was identified and authenticated by the Botanical Survey of India (BSI), Coimbatore wide No.BSI/SRC/5/23/2010-11/Tech-1849 and the voucher specimen was deposited at BSI for future reference. The roots were cut into small pieces and shade dried for nine-ten weeks. The dried roots were powered and extracted with methanol in the ratio of 1:5. 100 g of the sample was soaked in 500 ml of methanol for 24 hours in an orbital shaker and filtered through a Whatman No. 1. Filter paper and evaporated to dry using vacuum desiccator. The total phenolic content and total flavonoid content in the methanolic extract of *M. oleifera* root were determined according to the methods described by Singleton *et al*. (1999)[6] and Marinova *et al*. (2005)[7] respectively.

Various concentrations (200-1000 µg/ml) of methanol extract of *M. oleifera* root were used to measure the antioxidant activity by various assays. DPPH radical scavenging activity was determined following the method described by Sreejayan and Rao (1996)[8]**.** Reducing power of the extract was measured according to the method illustrated by Oyaizu (1986)[9]. The $Fe^{2+}$ chelating activity of the extract was evaluated following Dinis *et al.* (1994)[10]. The $ABTS^{\cdot+}$ radical cation decolorization assay was examined based on the description of Re *et al.* (1999)[11].

The hydrogen peroxide radical ($H_2O_2$) scavenging activity was assessed by the method described by Ruch *et al.* (1989)[12]. Superoxide anion ($O_2^{\cdot-}$) scavenging activity was assessed according to the method elucidated by Winterbourn *et al*. (1975)[13]**.** The effect of hydroxyl radical ($^{\cdot}OH$) scavenging was assessed according to the method reported by Nagai *et al.* (2006)[14]. Nitric oxide radical ($NO^{\cdot}$) scavenging activity was measured according to Griess reaction by Green *et al.* (1982)[15]. Ascorbic acid was taken as a positive control (reference standard antioxidant) for all the tested assays. The absorbance of the test solution and the percentage inhibition was calculated in all the assays. All experiments were repeated at least thrice. The results were expressed as Mean ± Standard deviations.

## RESULTS AND DISCUSSION

Total phenol contents of methanol extract of *M. oleifera root* were found to be 55.26 ± 1.04 mg/g expressed in Gallic acid equivalents (GAE) and the linear curve value was obtained with y = 0.003, x = 0.0166 ($R^2$=0. 9963). The results implied that the high content of the total phenolic content was present in the *M. oleifera* root extracts (Figure 1).

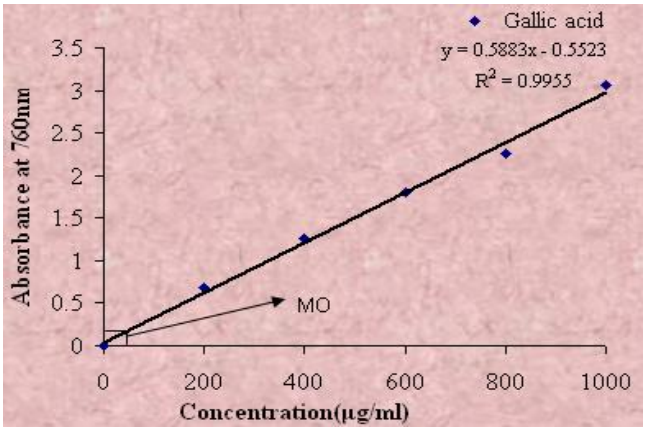


**Figure 1:** Quantification of Total phenolic content (TPC). The content was calculated as Gallic acid equivalent (mg QE/g).

The regression equation of the calibration curve of quercetin is y=1. 062, x-0.1003, $R^2$ = 0. 9785 (Figure 2).

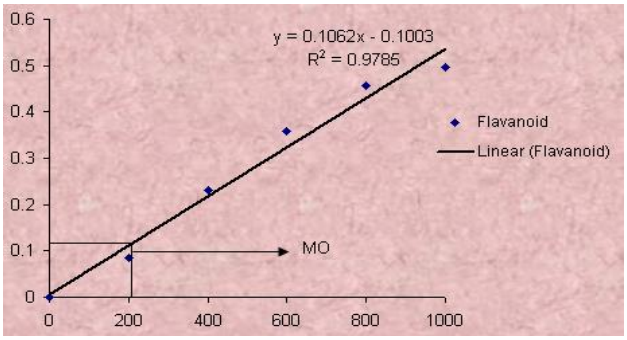


**Figure 2:** Quantification of Total flavonoid content. The content was calculated as quercetin equivalent (mg GE/g).

The extracts of *M. oleifera* root contained 200.62 ± 3.30 mg/g quercetin equivalent which is an expression of a high content of flavonoids in *M. oleifera root extract*. The total phenol and flavonoid results were fluctuating with other reported findings of different parts of *M. oleifera* obtained from a different place such as Kenya, Nigeria and Pakistan, which may be due to the variances in soil composition and weather conditions of those regions[16, 17].

Generally, the practice of a single method to measure the overall antioxidant potential is not suggested due to the different modes of action and the complexity of natural phytochemicals present in the sample that might lead to inaccurate findings. Hence, in this work different scavenging and reduction assays (DPPH, Reducing Power, $Fe^{2+}$, $ABTS^{\cdot+}$, $H_2O_2$,$O_2^{\cdot-}$,$^{\cdot}OH$ and $NO^{\cdot}$) were tested to assess the antioxidant potentials of *M. oleifera* root methanolic extracts (Figure 3). DPPH radical scavenging activity was increased from 77.38 ± 0. 01% to 96.40 ± 0. 08% at a concentration of 200 to 1000 µg/ml and standard ascorbic acid was 96.87 ± 0.47% at 1000 µg/ml. DPPH radical scavenging activity showed significantly lower $IC_{50}$ value in methanol extract (133.33 ± 5.77 µg/ml) compared to ascorbic acid (115.00 ± 8.66 µg/ml). The lowest $IC_{50}$ value indicates a high DPPH radical scavenging activity. Methanol extract exhibited the lowest $IC_{50}$ value hence it has high DPPH radical scavenging activity. This is similar in the context of the report that expressed potent DPPH radical scavenging activity of *M. oleifera* leaf extract[18].

The reducing power of *M. oleifera* root extracts was increased with increased concentrations (Figure 3). For instance, the reducing power of methanol root extract and ascorbic acid were 2.201 at a concentration of 1000 µg/ml. The reducing ability of methanol extracts (0.402 - 1.837 / 200 – 1000 µg/ml) was due to the reduction of $Fe^{3++}$ to $Fe^{2++}$ form. The reducing power of *M. oleifera* is due to components in the extract that donated electron which reacts with the free radicals and breaks the free radical chain[19].

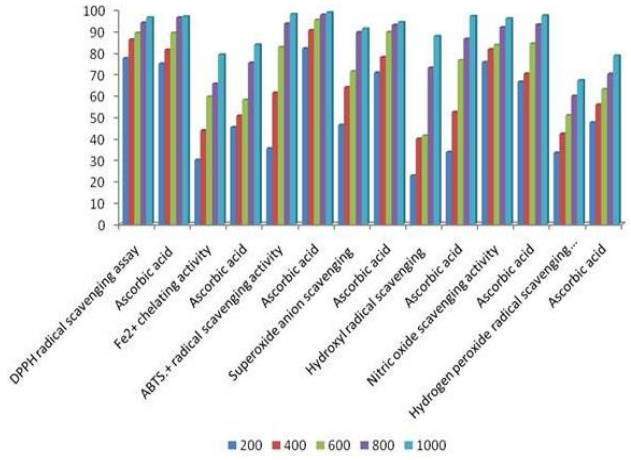


**Figure 3:** *In vitro* antioxidant activity of *Moringa oleifera* (Lam.) root

The $Fe^{2+}$ chelating activity of *M. oleifera* root showed potent chelating power as 79.11 ± 0.52% and standard L-Ascorbic acid was 83.70 ± 0.12%. The higher chelating power was due to the compounds present in the extracts that chelate metal ions in Fenton and Haber-Weiss

reactions where the iron generates free radicals through and prevents oxidative damage. The $IC_{50}$ value of methanol extract was 458 µg/ml, and the standard was found to be 420 µg/ml, which were lesser than *M. oleifera* root extracts. From the result, it was evident that the root extract possessed $Fe^{2+}$ chelating activity and might play a protective role against oxidative damage induced by metal-catalyzed decomposition reactions. Single electrons of $Fe^{2+}$ possess the ability to move by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals[20].

*M. oleifera* root extract exhibited good $ABTS^{\cdot+}$ radical scavenging activity and the percentage of inhibition (Figure 3) was found to be 97.97 ± 0.07% which is comparable with standard ascorbic acid 98.78 ± 0.06%. $IC_{50}$ values in scavenging abilities on $ABTS^{\cdot+}$ radicals of root extract were 305 ± 5.00 µg/ml and ascorbic acid 121.67 ± 2.89 µg/ml. The results obtained indicate that the extract possesses good $ABTS^{\cdot+}$ radical scavenging activity. $ABTS^{\cdot+}$ radical cation is reactive towards most antioxidants including phenolics, thiols and vitamin C[21].

The methanol extract of *M. oleifera* root extract exhibited good superoxide scavenging activity of 91.22 ± 0.07% at a concentration of 1000 µg/ml which is almost close to ascorbic acid 94.18 ± 0.06% (Figure 3). $IC_{50}$ values of root sample (243.33 ± 5.77 µg/ml) remained to be higher than ascorbic acid (155 ± 8.66 µg/ml). The result suggests that *M. oleifera* root possesses potent superoxide radical scavenging activity.

The scavenging activity increases in a dose-dependent manner 87.73 ± 0.07% and 98.07 ± 0.52% at a concentration of 1000 µg/ml for methanol root extract and ascorbic acid respectively (Figure 3). $IC_{50}$ value obtained for the root extract 645.12 ± 5.00 µg/ml were higher than the ascorbic acid 378.33 ± 2.89 µg /ml. *M. oleifera* root extracts showed better scavenging abilities on hydroxyl radical which is an extremely reactive free radical that induces severe damage[22].

The nitric oxide scavenging activity in *M. oleifera* root extract increases in a dose-dependent manner in methanol (96.01 ± 0.12%) and ascorbic acid (97.33 ± 0.08 %) (Figure 3). $IC_{50}$ value of methanol extract and ascorbic acid were found to be 158.33 ± 7.64 µg/ml and 95.00 ± 8.66µg/ml respectively. Results revealed that the extract showed radical scavenging activity against Nitric oxide, which is a diffusible free radical that plays many roles as an affecter molecule in diverse biological systems[23].

Antioxidants scavenge dangerous oxidative products by donating electrons to $H_2O_2$ and neutralize it to water. The extracts scavenge hydrogen peroxide in a dose-dependent manner (33.34 ± 0.13, 42.21± 0.05, 50.77± 0.09, 57.99 ± 0.03, 67.12 ± 0.06) (Figure 3). $IC_{50}$ values of methanol and L-ascorbic acid were found to be 610 ± 5.00 µg/ml and 228.33 ± 7.64 µg/ml. The results of all tested assays indicated that the methanolic extract of *M. oleifera* root had the premier antioxidant activity. The results of this study are coordinate with those reported by Atawodi *et al*. (2010)[24] and Xu *et al*. (2019)[5].

## CONCLUSION

The present study revealed that *M. oleifera* root possesses antioxidant activity against DPPH, $O_2^{\cdot-}$, $ABTS^{\cdot+}$, $NO^{\cdot}$ and $H_2O_2$ which exhibited the highest inhibition scavenging effect on reducing power assays. In addition, they have persuasive. OH activity and $Fe^{2+}$ metal chelating activity. This report summarizes the natural antioxidant efficiency of *M. oleifera* root extract and opens up the possibilities to explore further. In future, the bioactive compounds present in the root of *M. oleifera* will be isolated and *in vivo* antioxidant activity will be tested for their use as a natural therapeutic agent.

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