

Quantitative analysis and evaluation of antioxidant activity of medicinal plant *Moringa Oleifera* L.

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ABSTRACT

The medicinal plant *Moringa Oleifera* L. leaf methanol extract was analyzed for its total polyphenol content, total saponin content, total phlobatannin content and total flavonoid content. Four *in-vitro* antioxidant assays [2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), metal chelating activity, and total antioxidant capacity (TAC). were done to measure the reducing power and the scavenging ability of the extract. The antioxidant analysis indicated that IC₅₀ and percentage (%) inhibition were dose-dependent and showed the highest antioxidant activity ($7.05 \pm 0.2 \mu\text{g/mL}$) for metal chelating ferrous ion activity followed by $2.54 \pm 0.01 \mu\text{g/mL}$ for FRAP, $3.90 \pm 0.02 \mu\text{g/mL}$ for DPPH, and $1.50 \pm 0.02 \mu\text{g/mL}$ for TAC. Thus the methanol extract of *Moringa oleifera* leaf has great potential as natural natural therapy, anti-oxidant source, and for the conservation of raw and processed foods.

Key words: Antioxidant activity, Evaluation, *Moringa oleifera*, Phytochemicals, Quantitative estimation.

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1. INTRODUCTION

Many antioxidants of variable molecular structures are significant in dietary systems. Their efficiency is affected by redox potential, processing, activation energy of antioxidants, stability, pH, and consistency. Free radicals are produced during the oxidative reaction, if the chain reaction continues these can cause cellular harm. Antioxidants prevent these interactions by eliminating free radicals and inhibiting further oxidative reactions (32). Antioxidants are responsible for the defense mechanism of organisms against disease-associated free radical (10), thus, consumption of plant-derived antioxidants prevents degenerative illnesses, such as cancer and Parkinson's (25,35). Antioxidants guard the cells by transforming ROS to non-radical species, disrupting the auto-oxidant chain reaction caused by ROS and decreasing the regional concentrations of oxygen (9,21).

The human body has very robust and complex antioxidant defence mechanism to shield the cells and organ systems to neutralize free radicals beside ROS, which includes endogenous and exogenous components. It include antioxidant phytonutrients present in plant foods, antioxidant enzymes, nutrient-derived antioxidants, and metal-binding proteins (34). Strong antioxidant capacity to scavenge harmful ROS has been linked to plant's tolerance to diverse environmental stresses. Enhancing plant's tolerance to diverse environmental stresses. Enhancing plants tolerance to various environment stress depends on the strong antioxidant ability to scavenge harmful ROS. Therefore, high antioxidant ROS levels are maintained in both stressed and unstressed cells in the endoplasmic reticulum, mitochondria, cell walls, plasma membranes, chloroplasts, peroxisomes and apoplast (38).

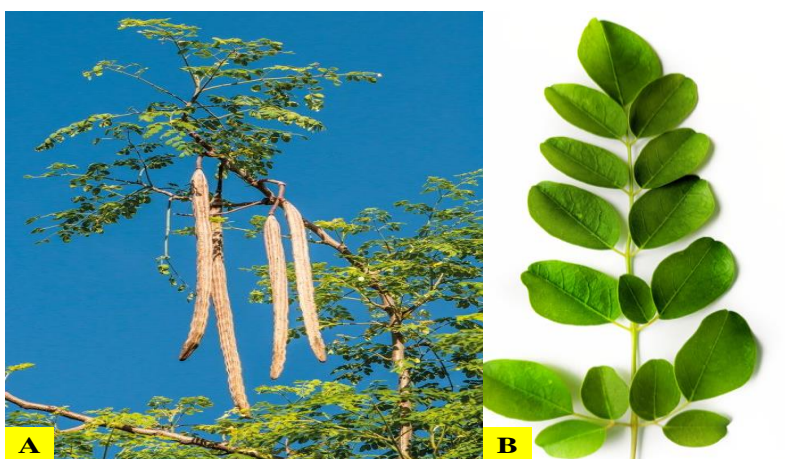


Figure 1. Experimental plant *Moringa oleifera*. (A) full plant. (B). leaves

Moringa oleifera (family: Moringaceae) is indigenous to the hills of Himalayas, India. It is widely grown in Bangladesh, Afghanistan, Thailand, Cambodia, Nepal, Sri Lanka, Pakistan, Indonesia, the Philippines, South and Central America and West Indies. It

has biological activities [anti-diabetic, anthelmintic, anti-oxidant, wound healing, anticancer, reducing hyperglycemia, anti-inflammatory, hepatoprotective, antimicrobial, and immunomodulatory activities (1,23,16,28, 29,15,11)]. Its pharmacological activities are due to various phytochemicals that act as effective free radical scavengers and thus is key factor in the medicinal plant antioxidant activity (3).

2. MATERIALS AND METHODS

2.1 Preparation of *M. oleifera* extract

The *M. oleifera* leaves were collected from Baraut, Baghpat district, Uttar Pradesh, India (Fig. 1). The leaves were shade dried for 7 days and were powdered in Mixer / Grinder. The powdered leaves at 50 g / 250 ml were extracted in methanol solvent for 72 h with slight shaking at rocker shaker. The extract was filtered through muslin cloth and collected, evaporated using a rotary evaporator, and chilled at room temperature in a petri dish and stored at 4°C in air-tight containers (13,14,26). Methanol solvent was used due to its high polarity in extract polar compounds from the test sample.

2.2 QUANTITATIVE ANALYSIS

2.2.1 Polyphenols

Quantitative estimation of polyphenols was done by the Folin Ciocalteu (FC) method with few modifications (2). Different concentration of methanol extract 100-100 µl were taken in individual test tubes and added 2 ml FC reagent. After 5 min, each tube added 2.5 ml of sodium carbonate, and mixture reaction solution was incubated at room temperature (RT) for 90 min. The absorbance was recorded at 760 nm by using gallic acid as standard and expressed in (mg GAE/g) of extract.

2.2.2 Flavonoids

Different concentrations of the extract 100-100 µl were taken in individual test tubes and 500 µl of AlCl₃ was added. The mixture was incubated for 40 min. The absorbance was recorded at 430 nm wavelength using a UV spectrophotometer by using quercetin as standard. Total flavonoid content was expressed as (mg QE/g of extract) of extract (20).

2.2.3 Phlobatannins

Phlobatannin was determined by the Folin-Denis (FD) method (18) with minor modifications. One mL of different concentrations of sample was taken in labeled test tubes and to each tube added 10 mL MQ water, 10 mL sodium carbonate and 2.5 mL FD reagent which resulted in the development of a bluish-green colour after 20 min incubation. The absorbance was recorded at 550 nm wavelength using tannic acid as standard. Total phlobatannin content was expressed as tannic acid equivalent per (mg TAE/g of extract) of extract.

2.2.4 Saponins

Different concentrations of samples and standards were prepared. The test sample (100 μ l) was added with 400 μ l of VAA, 1.6 ml of PCA, and placed in water bath a (70-75°C) for 15 min. After 2 min chilling on ice, 3 ml of GAA was added, vortexed and mixed well. The absorbance was recorded at 550 nm (17). The values were expressed as saponin equivalents (mg SQE/g) of extract.

2.3. ANTIOXIDANT ACTIVITY

2.3.1 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay

Different concentrations of methanol extract 100-100 μ l from *M. oleifera* leaves were taken in individual test tubes. To each test tube 1000 μ l of DPPH (0.3 mM) was added. The test tubes were incubated in dark for 25 min. Ascorbic acid (AA) was used as a standard and absorbance was recorded at 517 nm wavelength (16). The extract concentration providing 50 % inhibition (IC₅₀) was obtained by linear regression. The given equation was used to evaluate the scavenging activity (SA).

$$SA (\%) = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100 \quad (1)$$

Where, A_{control} : Absorbance of control, A_{sample} : Absorbance of tested sample

2.3.2 Ferric reducing antioxidant potential (FRAP)

Different concentrations of methanol extract 100-1000 μ l were taken in test tubes. To each test tube, 2 ml FRAP reagent (acetate buffer-50ml + TPTZ-5ml + FeCl₃- 5 ml) was added. The test tubes were incubated in dark for 25-30 min (37). Using BHA as a reference and expressed as μ M Fe (II)/g., the absorbance was recorded at 593 nm after incubation. The linear graph equation, $y = mx + c$ was obtained from the standard curve and the value of 'y' was evaluated.

2.3.3 Total antioxidant capacity (TAC)

TAC was estimated by the phosphomolybdenum method of Prieto *et al.* (24). Different concentrations of methanol extract 100-1000 μ l were taken in test tubes and to each test tube, 1000 μ l ammonium molybdate, 1000 μ l sodium phosphate and 1 ml sulphuric acid was added. All tubes were incubated in a water bath for 90 min at 95 °C. The absorbance was taken at 695 nm and AA was used as standard, sodium phosphate buffer as blank and a standard curve was prepared. The linear graph equation, $y = mx + c$ was obtained from the standard curve, and the value of 'y' was evaluated. The TAC was expressed as ascorbic acid equivalent (mg ACE/g) of extract.

2.3.4 Metal chelating capacity

Different concentrations of test samples and standard (EDTA) were prepared. The sample (500 μ l) was stirred with 500 μ l of FeSO₄, 1 ml tris HCl buffer, 0.5 ml of bi-pyridyl solution, 0.4 ml of hydroxylamine HCl and 2 ml of ethanol. At 522 nm absorbance was measured (36). Equation 1 was used to calculate the content of the extract to chelate ferrous ions.

2.4. Statistical analysis

The results were expressed as mean \pm standard error (SE) in triplicates. Microsoft Office Excel 2011 was used for the data analysis. The IC₅₀ value for above mention all parameters was calculated by regression equation. The IC₅₀ value is inversely proportional to the antioxidant activity.

3. RESULTS AND DISCUSSION

Phytochemicals of *M. olifera* leaves (polyphenols, flavonoids, tannins and saponins) in methanol extract were analyzed and summarized in Fig 2, 3 and Table 1. Polyphenolics content was 43.48 ± 0.05 mg GAE/g of extract.

Table 1. Total polyphenol, flavonoid, phlobatannin and saponin contents in *M. olifera* leaves.

Test Samples		Total Polyphenols	Total Flavonoids	Total Phlobatannin	Total Saponins
Leaf extract		43.78 \pm 0.04	59.61 \pm 0.08	39.18 \pm 0.12	40.31 \pm 0.03
Standard	Gallic Acid	45.67 \pm 0.04	-	-	-
	Quercetin	-	60.03 \pm 0.11	-	-
	Tannic Acid	-	-	38.63 \pm 0.09	-
	Saponin Quillaja.	-	-	-	38.84 \pm 0.04

The values are means of the triplicates \pm SE.

(i). **Polyphenols:** These are the major group of plant secondary metabolites and consists of numerous hydroxyl groups on aromatic rings. The polyphenolic content is an indicator of its antioxidant capacity (7). They also have various biological activities (antioxidant, anticancer, neuropharmacological, detoxifying, and immune-enhancing agents), hence used widely in pharma and cosmetic industries (5). They are used to treat various ailments viz., diabetes, heart disease, osteoporosis, cancer and respiratory disorders (27).

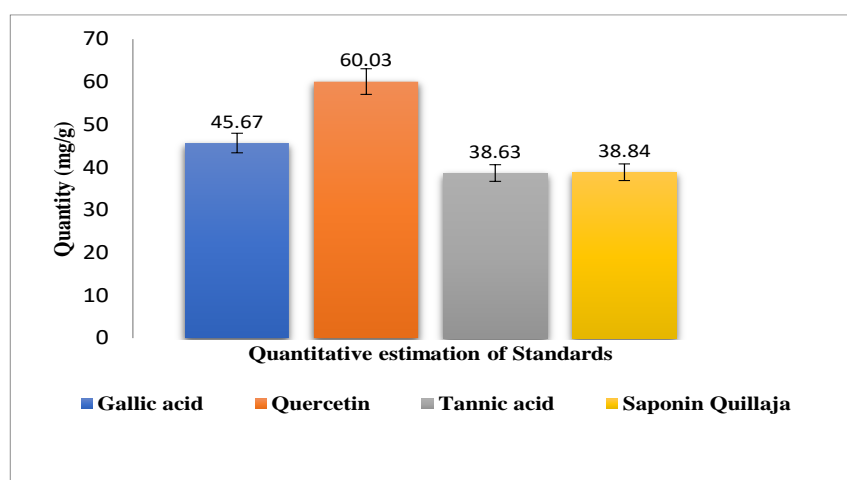


Figure 2. Quantitative estimation of various standards

(ii). Flavonoids: Their content in the sample was 59.61 ± 0.08 mg QE/g of extract. Flavonoids are sub-classified into flavones, flavanols, flavans, chalcones, anthocyanidins and isoflavonoids. Various functional groups in flavonoids have biological properties anticancer, anti-inflammatory, antioxidant, and antimicrobial (22).

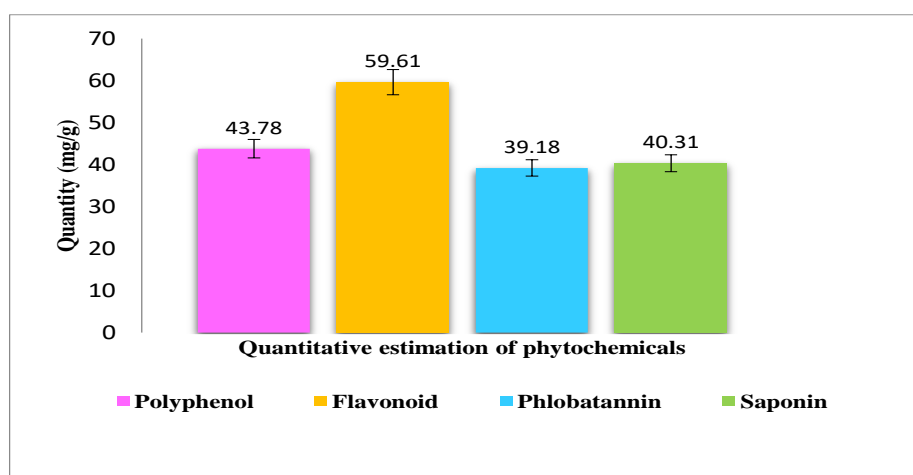


Figure 3. Quantitative estimation of phytochemicals in *M. olifera* leaves.

(iii). Phlobatannin: Its content in the sample was 39.18 ± 0.12 mg TAE/g of extract. They are produced under biotic and abiotic stresses in plants. They have antioxidant property owing to their phenolic rings and hydroxyl groups. These compounds are potent anti-carcinogenic, anti-mutagenic agents and have antioxidant activity (8).

(iv). Saponins: Their content in the sample was 40.31 ± 0.03 mg SQE/g of extract. Saponins binds to cholesterol and forms an insoluble complex that is excreted through the bile, hence prevents the re-absorption of cholesterol. Thus saponins are useful to cure hypercholesterolemia (19). These findings suggested that *M. olifera* is an important medicinal plant for the treatment of various ailments.

The extract very effectively scavenge the free radicals due to extract's ability or transfer of an electron or hydrogen to neutralize the DPPH free radical (33). It reduced DPPH to diphenyl picrylhydrazine giving a yellow color which indicated successful free radical scavenging (Fig 4). The IC_{50} of DPPH of methanol extract from the leaf was 3.90 ± 0.02 .

The methanol extract reduced the ferric-tripyridyltriazine (Fe^{III} -TPTZ) to ferrous-tripyridyltriazine (Fe^{II} -TPTZ), indicating high antioxidant activity. The antioxidant activity of extract of 1 mg/ml concentration was 2.54 ± 0.01 . The higher value of FRAP indicated the greater antioxidant activity of the *M. olifera*.

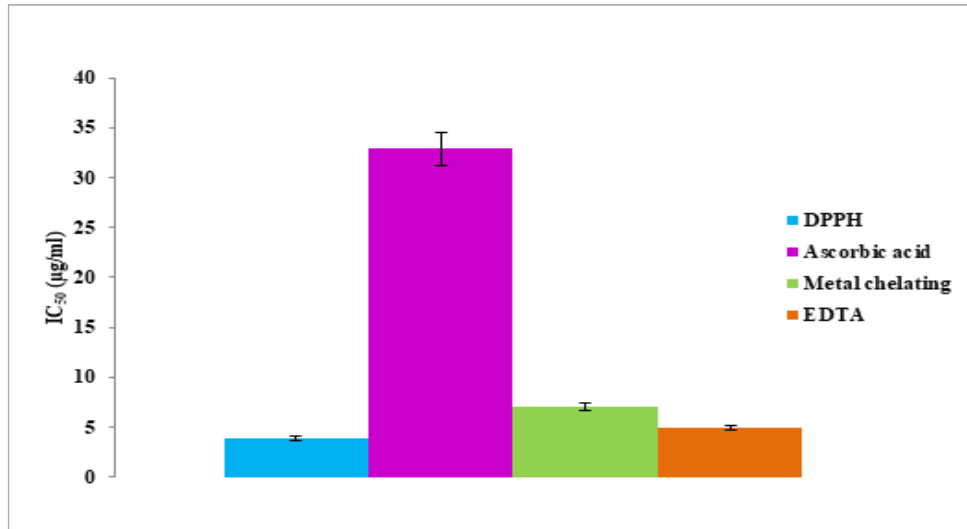


Figure 5. IC₅₀ values of *M. olifera* leaves extract and standards through antioxidant scavenging activity.

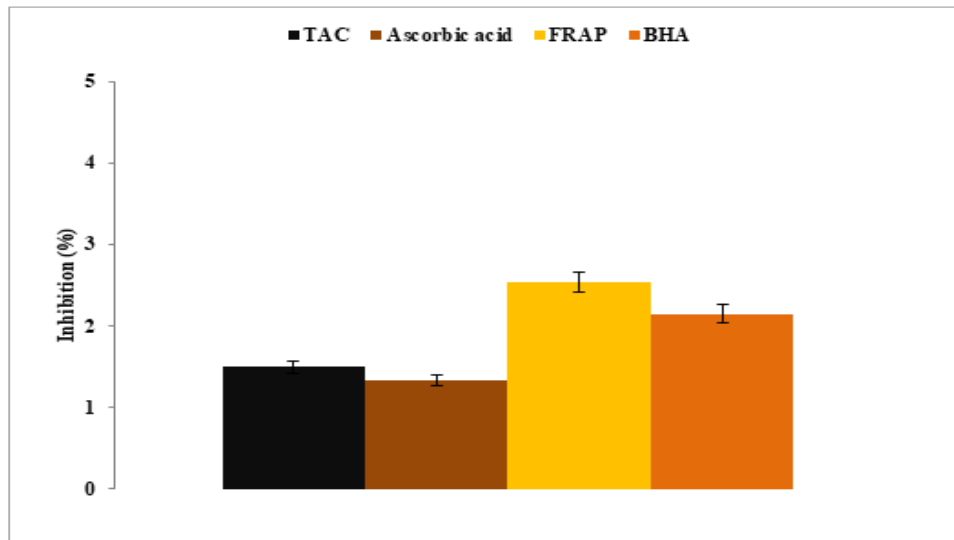


Figure 6. Inhibition (%) of *M. olifera* leaves extract and standards through antioxidant scavenging activity.

4. FUTURE PERSPECTIVES

The increased use of pesticides, chemicals, smoking, pollutants and some synthetic drugs increases the risk of diseases induced by free radicals. The antioxidant properties of medicinal plants to prevent and delay the oxidative damage in target molecule caused by ROS. The herbal extract cure diseases without any side effects.

5. CONCLUSIONS

Antioxidant properties of a plant-derived bioactive compound showed the pathway to treat various human ailments as they block oxidative chain reactions. The antioxidant analysis indicated that IC₅₀ and percentage (%) inhibition were dose-dependent and showed the highest antioxidant activity ($7.05 \pm 0.2 \mu\text{g/mL}$) for metal chelating ferrous ion activity. It might due to presence of numerous bioactive compounds. Even at relatively small concentrations, they inhibited the oxidation process, and thus play various physiological roles in the body. Free radicals are implicated in various diseases viz. degenerative disorders such as cancer and Alzheimer.

Abbreviations used: GA: Gallic acid; Q: Quercetin; TA: Tannic acid; SQ: Saponin quillaja; AA: Ascorbic acid; BHA: Butylated hydroxyanisole; EDTA: Ethylenediamine tetraacetic acid; GAA: Glacial acetic acid; PCA: Perchloric acid; VAA: Vanillin-acetic acid.

AUTHORS CONTRIBUTION

RA and YS conceived of the present idea for the article. HB, VK and HV performed the literature search, data analysis and wrote the manuscript. RA and YS provided critical feedback and helped to shape the final draft.

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DECLARATION

We declare that all authors of this Ms. Have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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