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COMPREHENSIVE ANALYSIS OF BIOACTIVE COMPOUNDS AND THERAPEUTIC POTENTIAL OF *MORINGA OLEIFERA* LAM

Vetal Shraddha[1*], Kakad Subhash[1], Tambe Harshad[2], Naik Ravishankar[2], Landge Aditya[2]

*[1,2]Department of Biotechnology, P. V. P. College, Pravaranagar, Ahilyanagar, MS, India.

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***Corresponding Author**
Vetal Shraddha
Department of Biotechnology, P. V. P. College, Pravaranagar, Ahilyanagar, MS, India.

ABSTRACT

Moringa oleifera Lam. plant leaves have gained significant attention in recent years, due to its impressive array of essential minerals and antioxidants. M. oleifera leaves are rich in essential minerals, bioactive compounds and anti-nutritional factor, rendering them significant for both nutritional and economic applications. The current investigation examined the presence of phytochemicals including alkaloids, flavonoids, glycosides, phenols, terpenoids, saponins, tannins, steroids, carbohydrate, and minerals such as calcium, magnesium, sodium, iron, along with zinc through Atomic Absorption Spectrophotometry (AAS), in addition their antimicrobial and antioxidant capabilities. This evaluation serves to validate the medicinal properties of *Moringa oleifera* Lam. in preventing disorders related to oxidative stress, thereby endorsing its application in pharmaceuticals, functional foods, and nutraceutical formulations.

KEYWORDS: *Moringa oleifera* Lam, Mineral Content, Phytochemicals, Antioxidant and Antimicrobial Activity.

INTRODUCTION

Moringa oleifera Lam, popularly known as ‘Drumstick’ which is found in tropical India to Africa. Moringa oleifera which belongs to family Moringaceae. *Moringa oleifera* Lam. a plant well-known for its nutritional and medicinal properties. Additionally, moringa leaves are utilized to regulate blood pressure and blood glucose level. *Moringa oleifera* Lam. is rich in nutrients including vitamins, minerals & antioxidant. It has been reported to have various health benefits such as reducing inflammation, improving cardiovascular health and

supporting immune function. *Moringa oleifera* Lam. contains high protein content, making it for malnutrition prevention. It has the potential benefits in managing neurodegenerative diseases like Alzheimer's.[1,2] Minerals are vital nutrients that are necessary for sustaining a number of bodily processes. They improve immunological function, control blood pressure and promote bone health. As a significant source of beta-carotene, vitamin C, protein, calcium, magnesium, zinc and potassium, *Moringa oleifera* Lam. leaves is a good source of both macro and micronutrients that the body requires. Among the several minerals found in *Moringa oleifera* Lam. calcium which is necessary for bone health, potassium which regulates the blood pressure, magnesium which support the regulatory activity of immune cells and reduce the risk of neurodegenerative diseases, zinc which has antioxidant activity & involved in hormone regulation particularly in the regulation of insulin and thyroid hormones. Its incorporation into daily diets can help address nutritional deficiencies and promote overall wellness.[3,4] *Moringa oleifera* Lam. leaves are an appreciable source of alkaloids, carotenoids, saponins, isothiocyanates, phenolic acid, terpanoids & flavonoids. This high bioactive profile is famous to boost immune system and defeat reactive oxygen species. By enhancing fecal excretion, the saponins that were isolated from the leaves have anti-cancer properties, lower plasma cholesterol and decrease enterohepatic circulation of bile acids.[5] *Moringa oleifera* Lam. contain free radical scavenging molecules such as phenol, alkaloids, amines with a great tendency of antioxidant activity. The tannin extracted from the leaves helps in treating inflammations related conditions such as arthritis and also used in organic farming to protect crops from pests and diseases. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. Preliminary phytochemical screening of plant is primarily an important aspect in finding the chemical constituents in plant material. Hence, the present investigation is carried out to find the phytochemicals present in leaves of *Moringa oleifera* Lam. With its rich composition of bioactive compounds, *Moringa oleifera* Lam. continues to be a valuable plant for scientific research including development of natural drugs for treating inflammation, infections and oxidative stress-related diseases. Analyzing phytochemicals helps in validating traditional medicine, developing pharmaceutical and nutraceutical products and ensuring the safety & efficacy of plant-based remedies.[6,7]

Numerous phytochemicals from various chemical classes have been reported to exhibit inhibitory effects against a wide range of microorganisms in vitro. *Moringa oleifera* Lam. synthesized active secondary metabolites such as alkaloid and flavonoids which has been

shown antibacterial activity against various bacterial strains. Pterygospermin is a flavonoid compound present in *Moringa oleifera* Lam. which interact with bacterial cell membrane, leads to disruption of membranes integrity, ultimately resulting in the death bacterial cell. The growing concern over antibiotic resistance has further highlighted the importance of *Moringa oleifera* Lam. as a source of natural antimicrobial agents for use in medicine, food preservation and in agriculture.[8,9] Gallic acid, a plant- derived phenolic compound, is well known for its strong antioxidant, anti-inflammatory, antimicrobial and anticancer properties. It is abundant in plants such as *Moringa oleifera* Lam. where it contributes significantly to medicinal and therapeutic effects. In *Moringa oleifera* Lam. gallic acid reduces intracellular ROS accumulation caused by toxic metals (arsenite, cadmium, nickel and lead) through its hydroxyl and carboxyl groups, which play key role in metal chelation. It also helps to regulate triglyceride levels and adipose cell size. Knowledge of such phytochemicals is important for synthesis of complex compounds and the development of health-promoting products.[10,11] The antioxidant activity of *Moringa oleifera* Lam. is crucial for its therapeutic potential, as it protects against oxidative damage linked to aging and diseases. One of the most frequently applied techniques for evaluating this activity is the Hydrogen Peroxide Scavenging Assay, where ascorbic acid serves as a standard. Strong scavenging activity validates the antioxidant potency of *Moringa oleifera* Lam. and supports its application in pharmaceuticals, nutraceuticals and functional food.[12,13]

MATERIALS AND METHODS

1. Collection of plant materials

The plant material was collected from Wadzari Bk, Sangmner (Located 19.7051°N, 74.3239°E) Ahmednagar district, Maharashtra in March 2024. A voucher specimen of plant has been deposited in the herbarium of Department of Botany P.V.P. College Pravaranagar. The plant was identified with the help of assemble literature.

2. Plant extracts preparation

The plant leaves were washed thoroughly with running tap water, then air dried under shade, and then the plant leaves was grinded in mixer. The powder was kept in plastic bags with labeling. The plant extract was prepared by magnetic stirrer method. About 5 gm of powdered plant leaves was mixed with 250 ml of solvent (distilled water) containing flask and place it on orbital rotary shaker for overnight at 120 rpm. Place the magnetic pins into the conical & set the magnetic stirrer to moderate speed (200 rpm) at 40°C for 1 hrs. After

stirring, filter the mixture using Whatman filter paper. Collect the filtrate and further used for preliminary phytochemical analysis.

3. Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using the following standard methods.[14-16]

Test for alkaloids

2-3 ml of plant extract was mixed with 1-2 drops of conc. HCL. 2-3 ml of Mayer's reagent was then added. The presence of alkaloids is revealed by formation of white ppt.

Test for flavonoid

2 ml of 2N NaOH solution was added to 3 ml of plant extract. The presence of flavonoids is indicated by the yellow colour.

Test for glycoside

1-2 ml of plant extract was mixed with 3 ml of chloroform and 10% NH_4OH solution. The presence of glycosides is indicated by the pink colour.

Test for phenolic

1-2 ml of plant extract, 2 ml of distill water was added, followed by a few drops of 10% $FeCl_3$. Phenols are indicated by the presence of green or blue colour.

Test for terpenoid

1-2 ml of chloroform and 2 ml of conc. H_2SO_4 was added to 0.5 ml of plant extract. Terpanoids indicated by the presence of red or brown colour at the interface.

Test for saponin

2 ml of extract was mixed with 2 ml of distilled water and shaken for 15 min. The presence of saponins is revealed by the formation of a 1-2 cm layer of foam.

Test for tannin

Tannins were tested by adding 2 ml of 5% ferric chloride to the 1 ml of plant extract. The presence of tannins showed by the dark blue or greenish-black colour.

Test for ninhydrin

1-2 drops of ninhydrin reagent added to 2 ml of the plant extract and heated for few minutes. The presence of amino acids is indicated by blue or violet colour.

Test for steroid

1-2 ml of plant extract and 1-2 ml of chloroform ($CHCl_3$) was added; along with 1-2 drops of conc.H_2SO_4. The formation of a bluish brown ring indicates the presence of phyto-steroids.

Test for carbohydrates

2-3 ml of the plant extract was treated with 2 ml of Molish's reagent and 1-2 drops of conc.H_2SO_4, resulting in the formation of a purple colour, that confirms the presence of carbohydrates.

Test for gallic acid

Take 2 ml of plant extract and add 1-2 drops of 1% ferric chloride solution to the extract. A greenish-black colour indicates the presence of gallic acid.

4. Quantitative analysis of gallic acid

Dissolve the dried plant leaf extract in aqueous solution. Prepare the standard gallic acid solution (100µg/ml). 1 ml of plant sample is transferred to a test tube. Then 0.5 ml of Folin-Ciocalteau reagent was added. Incubate the tube for 3 min. Then add 0.5 ml of sodium carbonate solution. Incubate the tube for 2 hrs. After incubation, measure the absorbance at 670nm. Prepare standard curve of gallic acid and use them to estimate the concentration of unknown sample.[17]

Test for Micro-organisms

The bacterial isolates were obtained from the laboratory unit of Department of Microbiology, Pravara Medical Trust Loni, and authenticated using standard biochemical tests as described by Cheesbrough. The isolates were maintained on a freshly prepared nutrient agar slant.

5. Antimicrobial assay

The freshly prepared nutrient agar plates were placed in dryer for approximately 15 minutes to eliminate surface moisture. The test organism were then aseptically inoculated onto the plates using the spread plate method. Tetracyclin discs served as the positive control for bacterial cultures, while DMSO extract discs were used as the negative control. The

inoculated plates were incubated at 37°C for 24-38 hours. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zones, which were recorded in millimeters.[18]

6. Antioxidant assay

Hydrogen Peroxide Scavenging Assay

Stock solution of ascorbic acid (100 µg/ml) was prepared in distilled water. Prepare a 40mM hydrogen peroxide solution in the standard phosphate buffer (pH 7.4). Mix 1ml of hydrogen peroxide with 1ml of *Moringa oleifera* Lam. leaf extract. Incubate the mixture at room temperature for 10-15 min & measure the absorbance of mixture at 695 nm using spectrophotometer. Calculate the percentage of scavenging of hydrogen peroxide using the following formula.[19,20]

H_2O_2 Scavenging Activity (%) = [(Absorbance of control – Absorbance of sample) / (Absorbance of control)] x 100

RESULTS AND DISCUSSION

The present study explored the mineral potential, phytochemical composition and pharmacological activities of *Moringa oleifera* Lam. The results obtained from the phytochemical screening of *Moringa oleifera* Lam. leaves are systematically summarized in Table 1. The phytochemical analysis revealed the presence of various bioactive compounds in *Moringa oleifera* Lam. leaves. The phytochemicals activities observed in the *Moringa oleifera* Lam. are the indicatives of these plants could be a possible source to obtain new and effective herbal drug to real treatments. Table 2. shows the mineral profiling of *Moringa oleifera* Lam. which indicates notable concentrations of calcium, magnesium, sodium, iron and zinc. These essential minerals play a vital role in bone strength, immune system support & regulation of numerous physiological functions. The sample were examined using an Atomic Absorption Spectrophotometer (AAS). Table 3. shows the effect of plant extract on antibacterial activity. Using the disc diffusion technique, the sample was tested for its antibacterial activity against gram-negative bacteria. As shown in Table 4. *Moringa oleifera* Lam. shows a significant antioxidant activity comparable to that of ascorbic acid. These results indicate that *Moringa oleifera* Lam. could play a potential role in protecting against diseases associated with oxidative stress. Current research indicates that the *Moringa oleifera* Lam. extract exhibits antimicrobial activity, suggesting their potential use as antimicrobial

agents in the development of new therapeutic drugs. The antibacterial and antioxidant effects of these bioactive compounds play crucial role in pharmacological studies aimed at producing more effective medications. The presence of minerals in significant amount underscores the plant traditional use as a treatment of various health conditions.

Table 1: Qualitative phytochemical screening of *Moringa oleifera* Lam. leaf extract.

Obs. No.	Test	Aqueous Extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	-
4.	Phenols	+
5.	Terpenoids	+
6.	Saponins	+
7.	Tannins	+
8.	Ninhydrin	+
9.	Steroids	+
10.	Carbohydrate	+
11.	Gallic acid	+

'+' indicates presence and '-' indicates absence of activity

Table 2: Mineral composition of *Moringa oleifera* Lam. (Percentage).

Sr. No.	Sample	Calcium	Magnesium	Zinc	Sodium	Iron
1.	*Moringa oleifera* leaf extract	01.22	00.40	0.011	1.41	0.61

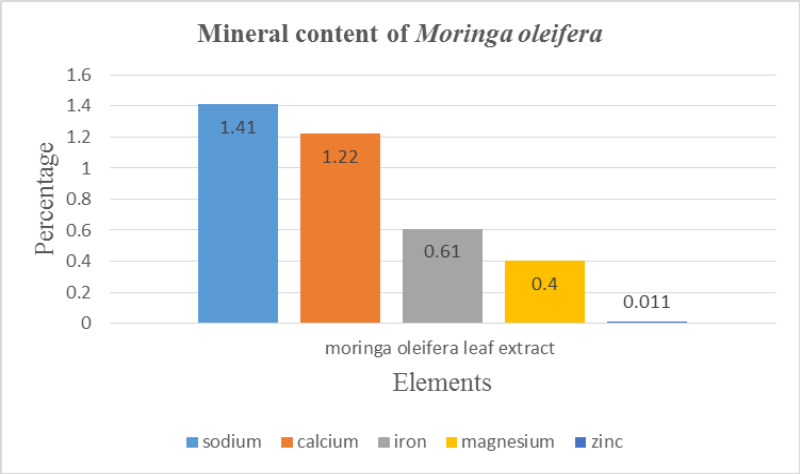

Fig. 1: Mineral composition of *Moringa oleifera* Lam.

The above study demonstrates that 1gm of *Moringa oleifera* leaf powder provides approximately 12.2 mg of calcium, 4 mg of magnesium, 0.11 mg of zinc, 1.41 mg of sodium and 0.61 mg of iron.

Table 3: Anti-microbial activity of *Moringa oleifera* Lam.

Sr. No.	Bacterial isolates	Tetracyclin (Gram positive)	
		Sample in µg/ml	Zone of inhibition (mm)
1.	*Escherichia coli*	250 µg/ml	7 mm
		500 µg/ml	9 mm
		750 µg/ml	10 mm
		1000 µg/ml	13 mm

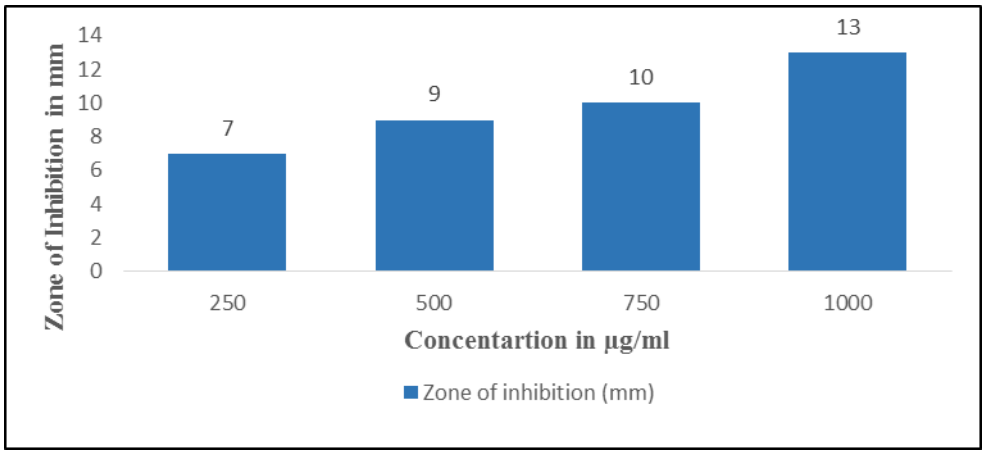

Fig. 2: Anti-microbial activity against *Escherichia coli.*

Table 4: Antioxidant activity.

Sr. No.	Bacterial isolates	Tetracyclin (Gram positive)	
		Sample in µg/ml	Zone of inhibition (mm)
1.	*Pseudomonas aeroginosa*	250 µg/ml	6 mm
		500 µg/ml	8 mm
		750 µg/ml	10 mm
		1000 µg/ml	11 mm

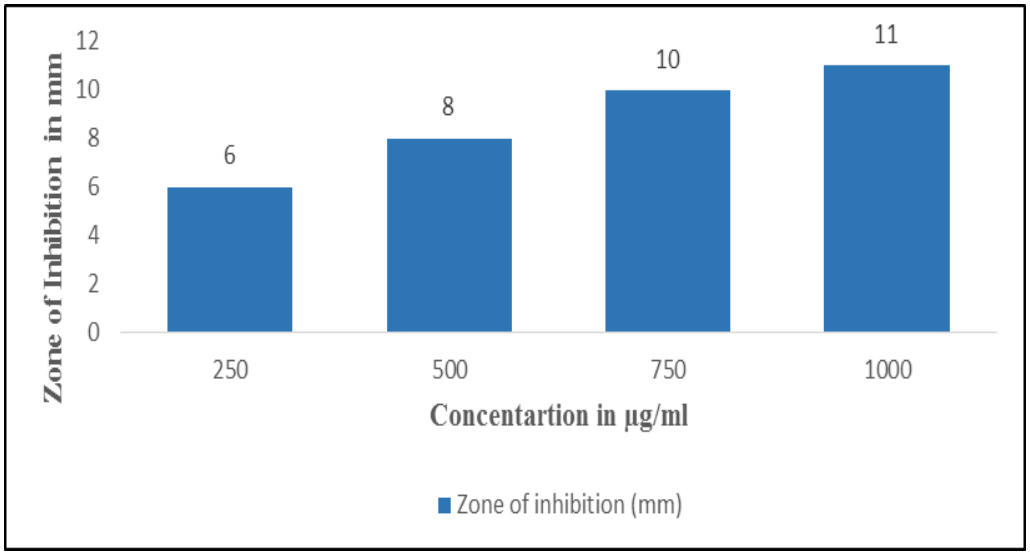

Fig. 3: Anti-microbial activity against *Pseudomonas aeroginosa.*

Hydrogen peroxide scavenging assay

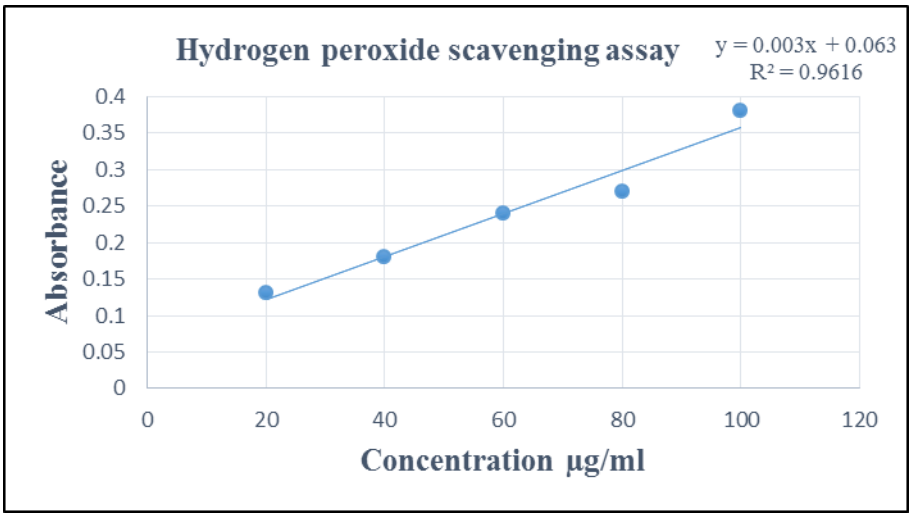

Fig. 4: Graphical representation of Hydrogen peroxide scavenging assay.

Hydrogen peroxide scavenging activity (%) = [(Absorbance of control – Absorbance of sample) / (Absorbance of control)] x 100

The above result interprets that the plant sample (*Moringa oleifera* Lam.) has 63.41 % antioxidant activity compared to the standard ascorbic acid, indicating that sample has higher antioxidant activity.

CONCLUSION

Currently, significant investigations are underway regarding plants because of their varied chemical components, rendering them essential for numerous scientific explorations. This research explored the mineral composition, phytochemical characteristics, and therapeutic properties of *Moringa oleifera* Lam. Analysis confirms that *Moringa oleifera* Lam. serves as a valuable source of key minerals, including calcium, magnesium, sodium, iron and zinc. Phytochemical screening confirmed the presence of several bioactive constituents including alkaloids, flavonoids, terpenoids, saponins, tannins and phenolic. Antioxidant potential was assessed through the hydrogen peroxide scavenging assay, which demonstrated strong free-radical scavenging activity. Furthermore, antimicrobial evaluation showed notable inhibitory effects against different microorganisms, particularly *Escherichia coli* & *pseudomonas aeroginosa.* Collectively, the study underscores the significance of *Moringa oleifera* Lam. as a promising natural source for developing therapeutic agents with health-promoting benefits. These findings further suggest its potential in the discovery and formulation of novel, more effective antimicrobial drugs derived from natural origins.

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