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Nutritional Analysis and Anti-Oxidant Study ofMoringa oleifera from Marathwada Region

Shashank Dahe[1], Lahu Hingne[1]
Aditya Pharmacy College, Department of Pharmacognosy Beed, Maharashtra, India[1]

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ABSTRACT:
Moringa oleifera, also known as the drumstick tree, is a nutrient-rich plant with significant antioxidant properties. Its leaves and pods contain essential nutrients such as proteins, carbohydrates, fiber, vitamins (A, C, and E), and minerals. These components contribute to overall health by improving immunity, reducing cholesterol, and supporting digestion. The plant also contains secondary metabolites like flavonoids, alkaloids, and tannins, which offer pharmacological and nutritional benefits.Moringa is commonly used in Southeast Asia in soups and lentil dishes, but its potential as a food supplement and for food fortification remains underexplored. Its high dietary fiber content aids in cholesterol reduction and protection against cancer and digestive disorders. Proximate analysis confirms Moringa's nutritional value, but its composition varies based on cultivation and environmental factors.Regular testing ensures safety for consumption. Additionally, Moringa's antioxidant activity, due to polyphenols, helps combat oxidative stress and related diseases.In conclusion, Moringa oleifera is a valuable, nutrient-dense plant with great potential to address malnutrition and improve human health, warranting further research and exploration.

I. INTRODUCTION:

Moringa oleifera, also known as the drumstick tree or "Tree of Life," is widely grown in India and tropical regions worldwide. India ranks second in global fruit and vegetable production, with horticultural crops covering 21.83 million acres (1). Despite this, many local and wild vegetables remain underutilized due to limited scientific research on their nutritional value (2).

Moringa is highly nutritious, with its leaves, flowers, and pods rich in protein, calcium, magnesium, phosphorus, iron, and vitamins A, C, and E. It is used for food, animal feed, and herbal medicine. Studies highlight its bioactive compounds—flavonoids, alkaloids, and phenolics—which contribute to its antioxidant, anti-inflammatory, liver-protective, anti-diabetic, and anticancer properties (3).

Research shows that soil and climate impact Moringa's nutrient composition (4). Dried leaves contain high protein, fat, fiber, and minerals, but cooking reduces some of these nutrients (5).Moringa is gaining global recognition as a nutraceutical, incorporated into teas, baked goods, and beverages (6). Clinical studies suggest benefits for iron levels and weight maintenance in women, but more research is needed to confirm its effectiveness as a dietary supplement (7).

With its exceptional nutritional and medicinal properties, Moringa oleifera has the potential to combat malnutrition and improve health (8). However, further research is necessary to optimize its dietary and medicinal applications while addressing concerns about anti-nutrient factors (9).

II. MATERIALS AND METHODS:

1. Preparation of Leaves and Pods Powder:

- The fresh plant leaves were washed thoroughly and carefully with distilled water andair dried for 7 days.
- Leaves was crushed and blended in Mixer for size reduced to mesh size #40 and used for physicochemical analysis.
- The powdered stored in closedcontainer and utilized for extraction.
- Approximately 5 kg of moringa pods were cooked in a pressure cooker for 2 hours, after which the pulp was scraped off and collected in a stainless-steel plate to be driedfor 2 days at room temperature.

2. Vitamin Analysis:

A. Vitamin A: The analysis was done as described previously (10).The test substance added with 1ml of chloroform & 5ml of antimony trichloride.Shaken well & observed for the color development. Vitamin A is

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present intest substance if it shows transient blue color.

B. Tocopherol (Vitamin E): The analysis was done as described previously (11). The test substance was mixed with 2ml of ethanol, 0.2ml of HNO3 followed by boiling for 5min. Tocopherol gives yellow to red color.

C. Vitamin C (Ascorbic Acid): The analysis was done as described previously (12). Test substance when added with 5ml distilled water, 5%w/v solution of sodium nitroprusside & 2ml dilute sodium hydroxide & mixed it with0.6ml HCl drop wise & stirred it well. Yellow color turns blue indicates presence ofascorbic acid.

3. DPPH Activity:

- The analysis was done as described previously (13).
- Dilute ascorbic acid in methanol to obtain concentrations of 20, 40, 60, 80, and 100 µg/ml.
- Dissolve the plant extract in methanol at the desired concentration.
- Prepare a 0.1 mM solution of DPPH in methanol.
- Mix 2 ml of the prepared DPPH solution with 2 ml of the plant extract solution in a test tube.
- For the control, combine 2 ml of DPPH solution with 2 ml of methanol (without the plant extract).
- Shake the mixtures thoroughly and leave them at room temperature for 30 minutes, protected from light.
- Use a UV-Vis spectrophotometer to measure absorbance at 517 nm.
- Determine the antioxidant activity using the formula:

$$\%\text{Scavenging Activity} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Where:

- A_{control} = Absorbance of the control (DPPH + methanol)
- A_{sample} = Absorbance of the test sample (DPPH + plant extract)

4. Total Antioxidant Capacity:

- The analysis was done as described previously (14).
- Dilute gallic acid in distilled water to obtain concentrations of 20, 40, 60, 80, and 100 µg/mL
- Measure 0.1 mL of 100 µg/mL and 200 µg/mL Moringa oleifera extract separately
- Combine 1 mL of TAC reagent with each standard and sample solution
- Prepare a blank by mixing 0.1 mL of distilled water with 1 mL of TAC reagent
- Securely cap all tubes and incubate in a boiling water bath at 95°C for 90 minutes
- Allow the tubes to cool to room temperature before taking measurements
- Use a UV-Vis spectrophotometer to measure absorbance at 695 nm, using the blank as the reference
- Construct a standard curve from gallic acid absorbance values
- Express total antioxidant capacity as mg gallic acid equivalents (GAE) per gram of the sample

5. Cytotoxicity activity:

- The analysis was done as described previously (15).
- The Sulforhodamine B (SRB) assay is a fast, sensitive, cost-effective, and high-throughput method used to quantify cellular proteins.
- It plays a crucial role in in vitro drug screening within the Developmental Therapeutic Program of the National Cancer Institute (NCI), USA.
- In this assay, cells were seeded at a density of 5000 cells per well in 100 µL of medium in 96-well flat-bottom microtiter plates (Eppendorf Inc, USA).
- The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow cell adherence.
- Herbal extracts and the standard drug were dissolved in dimethyl sulfoxide (DMSO) and further diluted with plain medium.
- The test samples were added in triplicates at final concentrations of 0.1, 1, 10, and 100 µM, followed by incubation for 48 hours at 37°C.

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- Wells containing only cells served as the negative control, while those treated with Adriamycin (ADR) functioned as the positive control. Each experiment was conducted in triplicate.
- After 48 hours, morphological changes in the cells were observed, and images of cells treated with the highest drug concentration were captured using a Phase Contrast Inverted Microscope (Model Eclipse Ti-S, NIKON Co., Japan) equipped with a digital camera.
- The cellular proteins were then fixed using 10% trichloroacetic acid, stained with SRB dye, and subsequently eluted with 10 mM Trizma base.
- Absorbance was measured at 540 nm, with a reference wavelength of 690 nm, using a plate reader (Model Sunrise, Tecan Inc., USA).
- The percentage of cell growth was calculated based on the ratio of the average absorbance (O.D.) of the test wells to that of the control wells.

III. RESULTS AND DISCUSSION:

1. Determination of Vitamins:

Vitamins	Results (mg/100g)	
	Leaves	**Pods**
Vitamin A	501	1.2
Vitamin E	51.3	0
Vitamin C	400	201

The vitamin content is summarized in Table 1. MOL demonstrated significantly higher levels of vitamin A (501 mg/100 g) and vitamin C (400 mg/100 g) compared to vitamin E (51.3 mg/100 g). On the other hand, MOP contained a greater amount of vitamin C (201 mg/100 g) but had a much lower vitamin A content (1.2 mg/100 g). The vitamin A levels in Moringa pods were found to be minimal. Among the vitamins analyzed, vitamin C was the most abundant, with higher concentrations in MOL (501 mg/100 g) than in MOP (201 mg/100 g). Additionally, the results indicate that Moringa pods lack vitamin E.

2. Determination of DPPH Radical Scavenging Activity:

The findings of the DPPH radical scavenging activity are illustrated in Figure 1 comparing the results with the well-known antioxidant, Vitamin C. Based on the analysis of Figure 1, it is evident that Moringa exhibits strong DPPH radical scavenging effects. The antioxidant properties of Moringa leaves and pods were remarkable, with significantly lower IC50 values of 2.0280 µg/mL and 4.0697 µg/mL, respectively.

The IC50 value represents the concentration required to reduce 50% of DPPH radicals and was calculated based on the percentage reduction of DPPH. Since IC50 values are inversely related to antioxidant activity, lower values indicate a higher antioxidant capacity. The results are presented as the mean of triplicate measurements at varying concentrations, with error bars indicating standard deviation. The chart also highlights significant variations in DPPH content among the samples from different locations. Additionally, Vitamin C, used as a standard, exhibited higher scavenging activity than all the tested samples.

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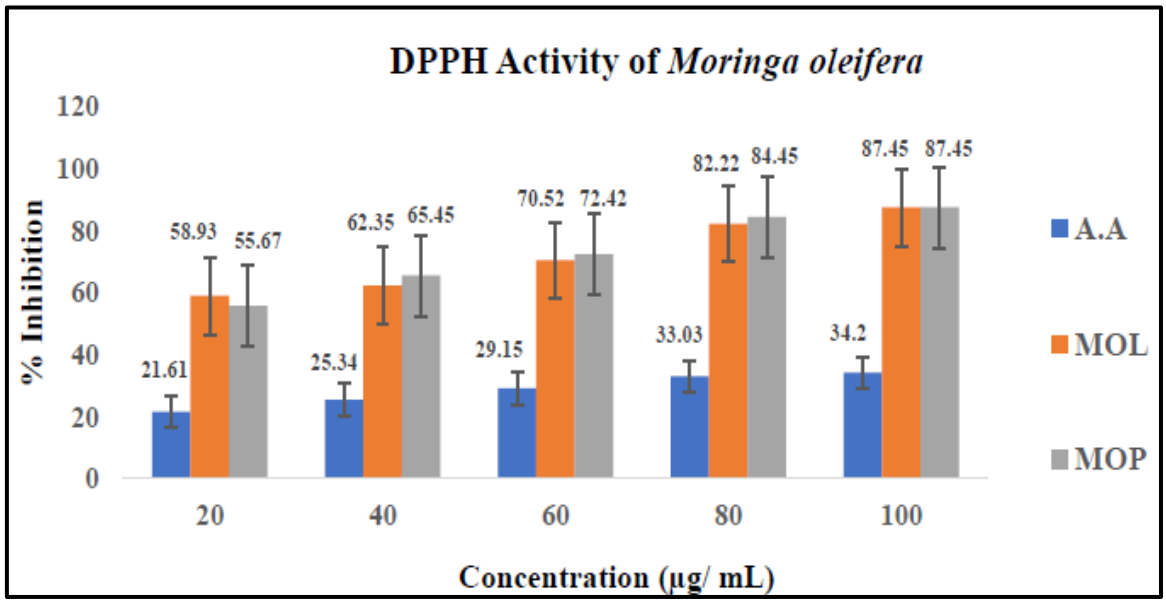

Fig.1 DPPH activity of Moginga oleifera

3. Cytotoxic effect of Moringa oleifera in cancer cells:

The anticancer screening data Table. 20 revealed that MOP extract has moderate antitumor agent with GI50 values >80 µg mL−1 , towards all cell lines. The cytotoxicity of MOP extract and Adriamycin, a standard cytotoxic drug against Human Pancreatic Cancer Cell Line (MIA-PA-CA-2), Human Prostate Cancer cell line (PC-3) and Treatment of Three cell lines with MOP extract and Adriamycin resulted in a considerable inhibition of cell growth, where MOP showed a stronger inhibitory effectagainst all selected four cell lines.

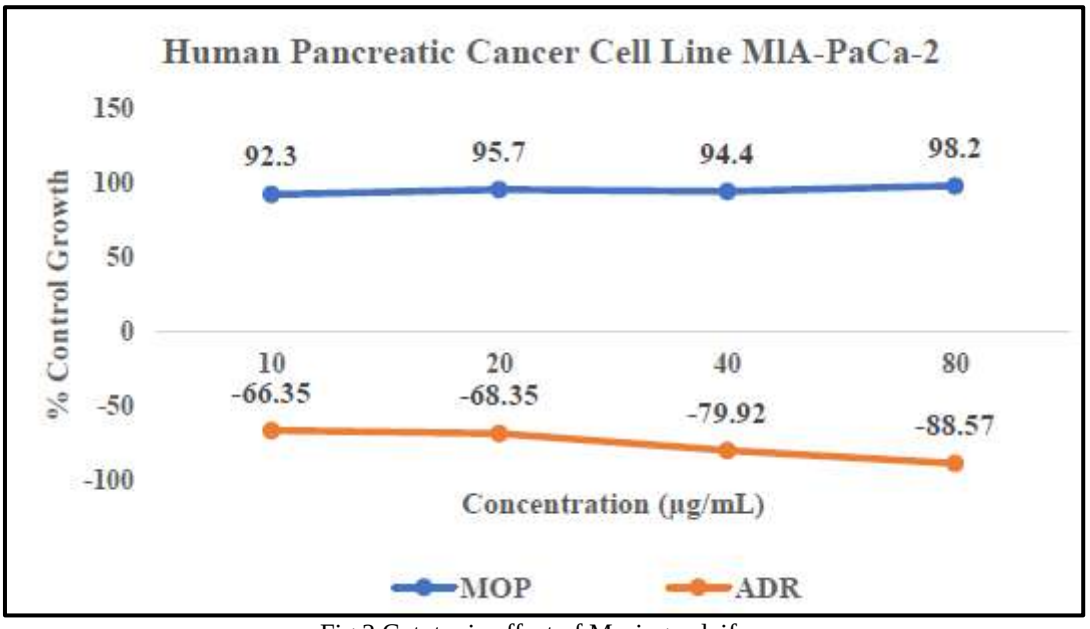

Fig.2 Cytotoxic effect of Moringa oleifera

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IV. CONCLUSION:

In conclusion, presence of essential vitamins like vitamin A, ascorbic acid (vitamin C), and alphatocopherol (vitamin E) in Moringa leaves and pods highlights their potential in maintaining a healthy body and preventing various health conditions. These vitamins contribute to normal vision, protect against oxidative stress-related damage, regulate the immune response, facilitate wound healing, and provide relief from coughs and colds. Moringa's rich vitamin content makes it a valuable resource in combating malnutrition.

Moringa oleifera is a highly nutritious plant with significant antioxidantactivity. Its leaves and pods contain essential nutrients, vitamins, and phytochemicalsthat can contribute to human health and nutrition. Further research and explorationof this plant's properties can provide valuable insights into its role in addressingmalnutrition and promoting well-being.

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