

Phytochemical Constituents of Leaves of *Moringa oleifera* Grow in Cuddalore District, Tamil Nadu, India

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ABSTRACT

Aim: The plant products are used to treat various diseases instead of synthetic drugs owing to their least side effects. One of the important plants is *Moringa oleifera*. The micronutrients of *Moringa oleifera* leaves (MOL) have anti-inflammatory, antidiabetic, antihypertensive, antiepileptic, and antitumor properties. Literature explored the contents of MOL from various countries and thus, the present study aims to evaluate the existence of phytochemicals present in the MOL grow in the Cuddalore District, Tamil Nadu, India.

Materials and methods: The maceration technique was employed to extract the active contents of the powdered leaves with 70% ethanol. The acquired crude extract was subjected to detailed phytochemical analysis. We have tested MOL for the presence of alkaloids (Mayer's test), proteins and amino acids (xanthoproteic test and ninhydrin test), flavonoids (alkaline reagent test), glycosides (Legal's test), saponins (foam test), and tannin (gelatin test). The presence of reducing sugars, carbohydrates, phytosterols, and triterpenoids was tested by Fehling's test, Molisch's test, Liebermann-Burchard's test, and Salkowski's test, respectively. Standard chemical tests were used for testing steroids, anthraquinones, and fats and fixed oils.

Results: Stronger presence of some chemical compounds like proteins and amino acids, flavonoids, alkaloids, steroids, and saponins as well as other ingredients were detected. The extract showed weak positivity for phytosterols, reducing sugars, and fats and fixed oils. But carbohydrates, anthraquinones, tannin, and triterpenoids were not identified in the extract of MOL.

Conclusion: The presence of alkaloids, flavonoids, and saponins can exhibit stronger antioxidant activity against the free radicals, which are of great medicinal value. The active components extracted from MOL may be useful as a drug in various diseases induced by the reactive oxygen species.

Keywords: Drumstick leaves, Maceration, Phytochemical, Saponins.

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INTRODUCTION

The plant products are used to treat various diseases instead of synthetic drugs owing to their least side effects. Hence, the field of ancient medicine reached peak growth in the last few years. In India, the drugs derived from plants form the major component of traditional medicine like Ayurveda and Siddha. The investigation of herbal substances was conducted due to their efficient medicinal value, which laid the platform for the discovery of newer medicines to cure various ailments.¹ One of the important plants with its great medicinal value is *Moringa oleifera* leaves (MOL), which belongs to the monogeric genus *Moringaceae* family.

The *Moringaceae* family contains 13 species among which one of them is *Moringa oleifera*. It is widely cultivated in countries like India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, other parts of Asia, the Arabian Peninsula, Africa, southern Florida, West Indies, Paraguay, Peru Mexico, and Brazil.² It is a small, fast-growing deciduous tree with soft white wood and gummy bark that usually grows up to 9-m height. The feathery foliage of tripinnate leaves originates from the main axis which may have a length of 30–75 cm.^{1,2} In India, the leaves and pulp form an integral part of the food for many centuries. All the parts of *M. oleifera* like leaves, pods, and pulps of drumstick, bark and root are traditionally used for various diseases, but leaves are most commonly used and thus, a Tamil proverb mention the great value of the tree as "*Murungaiyai nattavan verungaiyodu povan*" which means a man who cultivates and eats all parts of the tree will become strong physically and never uses a walking stick in his geriatric period.

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The MOL is a significant source for protein, amino acids, calcium, potassium, iron, zinc, as well as vitamins like A, B, C, and E.³ It also has polyphenol, phenolic acids, flavonoids, alkaloids, a simple sugar, tannins, vitamins, rhamnose, carotenoids, phytates, isothiocyanates, saponins, oxalates, and triterpenoid glucosinolates.² Many investigations explored that the micronutrients of MOL have anti-inflammatory, antidiabetic, antihypertensive, antiepileptic, and antitumor properties.³ It shows high antioxidant property against free radicals induced tissue damage because it contains phenolic compounds.³ Literature explored the contents of MOL from various countries and thus, the present study aims to evaluate the existence

of phytochemicals present in the MOL grow in the Cuddalore district, Tamil Nadu, India.

MATERIALS AND METHODS

Sample Collection and Preparation of the Extract

The leaves of horseradish were harvested from a village in Cuddalore district, Tamil Nadu, India and authenticated by a senior Botanist. The dust particles in the leaves were washed with water and then subjected to air and oven-drying method at 44°C for 4 hours and ground into a fine powder using a domestic electric blender. The maceration technique was employed to extract the active contents of the powdered leaves with 70% ethanol at a ratio of 1:40, w/v for 72 hours at room temperature with occasional shaking. The resultant extract was drained with Whatman no 1 filter paper and the remaining substance was re-extracted by the above process and solvent up to the exhaustion of the marc.⁴ Then, the rotary evaporator model was used to remove the solvent and finally, the phytochemical analysis was performed in the crude extract.

Phytochemical Screening

Phytochemical examinations were carried out for the extracts as per the standard tests.

Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid (HCl) and filtered.

Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow-colored precipitate indicates the presence of alkaloids.

Detection of Proteins and Amino Acids

Xanthoproteic test: The extracts were treated with a few drops of conc. nitric acid. The formation of the yellow color indicates the presence of proteins.

Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. The formation of blue color indicates the presence of amino acid.

Detection of Flavonoids

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. The formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

Detection of Glycosides

Extracts were hydrolyzed with diluted HCl and then subjected to test for glycosides.

Legal's test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. The formation of pink to blood-red color indicates the presence of cardiac glycosides.

Detection of Steroids

One milliliter of the crude plant extracts was dissolved in 10 mL of chloroform and to it was added an equal volume of concentrated sulfuric acid from sides of the test tube. The upper layer turns into a red and the sulfuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Detection of Saponins

Foam test: 0.5 g of the extract was shaken with 2 mL of water. If foam produced persists for 10 minutes it indicates the presence of saponins.

Detection of Tannin

Gelatin test: To the extract, a 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.

Detection of Reducing Sugars

Fehling's test: About 0.5 g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5 mL of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Detection of Carbohydrates

Extracts were dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test: Filtrates were treated with two drops of alcoholic α -naphthol solution in a test tube. The formation of the violet ring at the junction indicates the presence of carbohydrates.

Detection of Anthraquinones

Five milliliters of extract were boiled with 10 mL of sulfuric acid and filtered while hot. The filtrate was shaken with 5 mL of chloroform, the chloroform layer was pipette out into another test tube then 1 mL of dilute ammonia is added. The resulting solution was observed for color changes. The change in color indicates the presence of anthraquinones.

Detection of Phytosterols

Liebermann-Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled, and cooled. Conc. sulfuric acid was added. The formation of a brown ring at the junction indicates the presence of phytosterols.

Detection of Triterpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of conc. sulfuric acid, shaken and allowed to stand. The appearance of golden yellow color indicates the presence of triterpenes.

Fats and Fixed Oils Test

It was done by filter paper press test in which extracts were pressed in filter paper and results were observed.

RESULTS

The presence of phytochemicals in the ethanolic extract of MOL extract is summarized in the Table 1.

Stronger presence of some chemical compounds like proteins and amino acids, flavonoids, alkaloids, steroids, and saponins were detected. The extract showed weak positivity for phytosterols, reducing sugars, and fats and fixed oils. But carbohydrates,

Table 1: The phytochemical screening of the ethanolic extract of MOL collected from Cuddalore District, Tamil Nadu

S. no.	Phytochemical	Reagents used	Observation	Presence of phytochemical
1	Alkaloids	Mayer's test	Formation of yellow precipitation	++
2	Proteins and amino acids	Xanthoproteic test	Formation of yellow color	++
		Ninhydrin test	Formation of blue color	+
3	Flavonoids	Alkaline reagent test	Formation of yellow to colorless	++
4	Glycosides	Legal's test	Formation of pink to red color	+
5	Steroids	Sulfuric acid test	Formation of yellow green fluorescence	++
6	Saponins	Foam test	Formation of foam	+++
7	Tannin	Gelatin test	No formation of precipitation	Absent
8	Reducing sugars	Fehling's test	Formation of precipitation	+
9	Carbohydrates	Molisch's test	No formation of violet ring	Absent
10	Anthraquinones	Sulfuric acid test	No color change	Absent
11	Phytosterols	Liebermann-Burchard's test	Formation of brown ring	+
12	Triterpenoids	Salkowski's test	No formation of yellow color	Absent
13	Fats and fixed oil	Filter paper press test	Observe the oil content	+

+++ , Present in high concentration; ++, Present in moderate concentration; +, Present in low concentration

anthraquinones, tannin, and triterpenoids were not identified in the extract of MOL.

DISCUSSION

There are few research studies are available regarding the potential nutritional value of the plant grown in India. However, in the last decade, the medicinal properties associated with MOL has earned much focus among the people because the dietary supplements are natural substances. Different extraction techniques and solvents decide the amount of phytochemicals present in the plant extract. On the one hand, El Sohaimy et al. demonstrated that 70% of methanol extracted the maximum level of flavonoids but not with the same amount of ethanol and water.⁵ On the other hand, Vongsak et al. showed maceration with 70% ethanol is the excellent extraction method and solvent to drag the potential antioxidant compounds from an extract of MOL for medicinal usage.⁴

The present study showed the stronger presence of some compounds like protein and amino acids, flavonoids, alkaloids, steroids, and saponin, which is in agreement with Okiki et al. The phytochemical analysis in MOL from Nigeria revealed high concentrations of alkaloids, flavonoids, and saponins; 446.67 ± 12.11 , 846.67 ± 11.07 , and 844.17 ± 27.82 mg/100 g, respectively. The concentrations of phytates and tannin are more but with low-oxalate content.⁶ Valdez-Solana et al. reported a higher amount of chlorogenic acid, rutin and luteolin, apigenin quercetin, and kaempferol.⁷ The findings of the present study are supported by Shanmugavel et al., who revealed that MOL collected from Puducherry, which is rich in phenolic compounds, flavonoids, and vitamin C possessing strong antioxidant activity.⁸ Vats and Gupta reported phytochemicals and antioxidant potential of hydroethanolic extract of leaves of *M. oleifera* from India revealed that leaves possessed the highest content of total phenolics.⁹ Similarly, Abdulkadir et al. showed the total flavonoid content was found to be higher in both methanolic and hexane extract of MOL than any other part of the plant.¹⁰ Furthermore, Abbas et al. showed the presence of phytochemicals in the crude ethanolic extract of *M. oleifera* harvested from Sudan and explored the compounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins, and anthraquinone.¹¹

There are various mechanisms by which phenolics may act as antioxidants: *via* scavenging free radicals, donating hydrogen atom, quenching singlet oxygen, chelating metal-ion, or acting as substrates to be assaulted by superoxide. Other mechanisms may be involved *in vivo* where phenolics may prevent oxidation of α -tocopherol or themselves being oxidized; or by donating a hydrogen atom to the α -tocopherol radical, they may reproduce α -tocopherol. Thereby, prolonging the period of low-density lipoprotein accumulated with lipid hydroperoxides.¹² Thus, the presence of alkaloids, flavonoids, and saponins can exhibit stronger antioxidant activity against the free radicals, which are of great medicinal value. Hence, the MOL can be a potential source for the nutrients to improve the health status of the animals and humans.

CONCLUSION

The present study suggests that MOL cultivated in Cuddalore District contains stronger presence of some phytochemicals, such as, alkaloids, flavonoids, saponins, steroids, glycosides, and proteins. These active components of MOL can be used as a drug in various diseases induced by the reactive oxygen species.

FUTURE PERSPECTIVES

Further investigations must be conducted to isolate the phytochemicals and their mechanism of action when the compounds applied as drugs.

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