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PHYTOCHEMICAL ANALYSIS AND ISOLATION OF FLAVONOIDS FROM MORINGA OLEIFERA

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ABSTRACT

Moringa oleifera, commonly known as the drumstick tree, is widely recognized for its nutritional and medicinal properties. Flavonoids, a diverse group of plant secondary metabolites, contribute significantly to its therapeutic potential. This study explores the phytochemical profile of Moringa oleifera, focusing on the extraction, isolation, and characterization of flavonoids. Employing advanced analytical techniques, we identified key flavonoid compounds and evaluated their potential biological activities. The findings reinforce the value of Moringa oleifera in pharmacological applications and provide insights for further research.

Keywords: Moringa Oleifera,, Flavonoids, Phytochemical.

I. INTRODUCTION

The genus Moringa encompasses 13 species, with Moringa oleifera being the most widely cultivated due to its adaptability and multifaceted uses. Known as a "miracle tree," it is rich in bioactive compounds, including vitamins, minerals, and secondary metabolites. Among these, flavonoids have garnered attention for their antioxidant, anti-inflammatory, and anticancer properties.

II. MATERIALS AND METHODS

2.1 Plant Material Collection

Fresh leaves of Moringa oleifera were collected from a cultivated farm in SAM university campus . The plant material was authenticated by a botanist Dr. Jagrati Tripathi Asst. Prof of Botany Govt. College Khimlasha and assigned a voucher specimen number for future reference.

2.2 Preparation of Extracts

The leaves were shade-dried and ground into a fine powder. Approximately 100 g of powdered leaves were subjected to Soxhlet extraction using ethanol as the solvent. The crude extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further analysis.

2.3 Preliminary Phytochemical Screening

Standard qualitative tests were conducted to detect flavonoids, alkaloids, saponins, tannins, and phenolic compounds. For flavonoid detection, the aluminum chloride test and Shinoda test were performed.

Tests for Alkaloids

Mayer's Reagent Test: 1.36 gm of mercuric chloride and 3 gm of potassium iodide were dissolved in water to make 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream coloured precipitate shows the presence of alkaloid.

Hager's Reagent Test: It is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

Wagner's Reagent Test: It is a solution of potassium triiodide in water which was prepared by dissolving 1.3 gm iodine in a solution of potassium iodide (2 gm) in water to make 100 ml. Formation of brown precipitate after addition of this reagent in extract indicates the presence of alkaloids.

Test for Flavonoids

Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbons and conc. hydrochloric acid was added drop wise. Pink scarlet color appears after few minutes, indicated the presence of flavonoids.

Zinc Hydrochloride Test: To the test solution add a mixture of Zinc dust and conc. hydrochloric acid. It gives red color after few minutes confirms flavonoids.

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Tests for Carbohydrates

Molish test: About 0.1 gm of the sample was dissolved in 2 ml of water and added 2-3 drops of 1% ethanolic solution of alpha napthol and then carefully poured 2 ml of concentrated sulphuric acid down the side of the test tube so that it forms a heavy layer at the bottom. A deep violet colour is produced if carbohydrates are present.

Fehling's Test: 1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and boiled for 1 minute. Equal volume of sample was then added, heated in boiling water bath for 5-10 minutes. First a yellow then brick red color shows the presence of carbohydrates.

Test for Saponins

Foam Test: About 2 gm of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins.

Heamolytic Test: Sample was added to one drop of blood placed on glass slide. Hemolytic zone indicated the presence of saponins.

Test for Tannins: Sample was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents;

$FeCl_3$ Solution Test: A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue color shows the presence of tannins.

Lead acetate Test: A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate indicates the presence of tannins.

Test for Amino acids

Ninhydrin test: To the 3 ml of crude sample 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish colour indicated presence of amino acids.

Test for anthraquinone glycosides

Borntrager's Test: To the 3ml of the sample, dilute sulphuric acid was added, boiled and flittered. To the filtrate equal volume of chloroform was added and shaken. After separating the organic layer, ammonia was added. Turning pink of ammonical layer indicates the presence of said glycosides.

Tests for sterol/steroids

Salkowaski Test: Few mg of the sample was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid and shaken. The development of red colour in the chloroform layer indicates the presence of sterols/steroids.

Test for Terpenoids

Liebermann–Burchard Test: Few mg of the sample was dissolved in 1ml of chloroform and few drops of acetic anhydride. Concentrated sulphuric acid was added by the side of the test tube. Production of purple color indicates the presence of triterpenoids and blue–green color indicates the presence of sterols.

Test for Proteins

Biuret Test: To the sample 4% NaOH and few drops of 1% $CuSO_4$ were added. Violet or pink colour indicates the presence of proteins.

Xanthoproteic Test: Sample was mixed with 1ml of concentrated sulphuric acid, formation of precipitate shows positive test.

Millon's Test: 3ml of sample was mixed with Millon's reagent, formation of precipitate indicates the presence of proteins.

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Table 1: Showing Phytochemical screening of various crude extracts of Moringa oleifera Lam

S. No.	Tests	Plant type	Observation for extracts	
			Petroleum Ether	Methanol
1	**Carbohydrates**			
1.1	Molish test	M. oleifera	Negative	Positive
1.2	Fehling	M. oleifera	Negative	Negative
1.3	Barfoed's Test	M. oleifera	Negative	Positive
2.	**Proteins and amino acids**			
2.1	Biuret's test	M. oleifera	Negative	Negative
2.2	Ninhydrin test	M. oleifera	Negative	Negative
3	**Glycosides**			
3.1	Legal's test	M. oleifera	Positive	Positive
3.2	Keller-Killani test	M. oleifera		Positive
4	**Saponins**			
4.1	Froth test	M. oleifera	Negative	Positive
5	**Alkaloids**			
5.1	Mayer's test	M. oleifera	Negative	Positive
5.2	Hager's test	M. oleifera	Negative	Positive
5.3	Wagner's test	M. oleifera		Positive
6	**Flavonoids**			
6.1	Lead acetate test	M. oleifera	Positive	Positive
6.2	Alkaline reagent test	M. oleifera	Positive	Positive
7	**Triterpenoids and Steroids**			
7.1	Libermann-burchard,s test	M. oleifera	Positive	Negative
7.2	Salkowski,s test	M. oleifera		Positive
8	**Tannins and Phenols**			
8.1	Ferric chloride test	M. oleifera	Positive	Positive
8.2	Lead acetate test	M. oleifera	Positive	Positive
8.3	Gelatin test	M. oleifera		Positive

2.4 Isolation of Flavonoids The concentrated ethanol extract was fractionated using liquid-liquid partitioning with solvents of increasing polarity (Toulene : Aceteic acid : Formic acid5 : 3.5 : 0.5)). The ethyl acetate fraction, showing the highest flavonoid content, was subjected to column chromatography using silica gel as the stationary phase and gradient elution with ethyl acetate and methanol.

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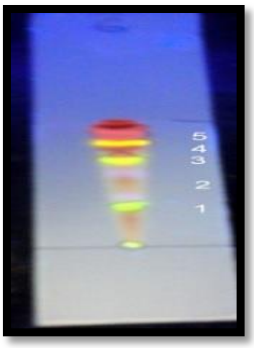
Table 2 Showing TLC of crude extracts of Moringa oleifera L.

Methanolic extract	Solvent system	Detecting reagent	Rf Value
Moringa oleifera L.	Toulene : Aceteic acid : Formic acid (5 : 3.5 : 0.5)	Sprayed with vanillin-sulphuric acid and heated at 100^{0}C for 5 minutes.	0.08 0.39 0.44 0.7 0.73

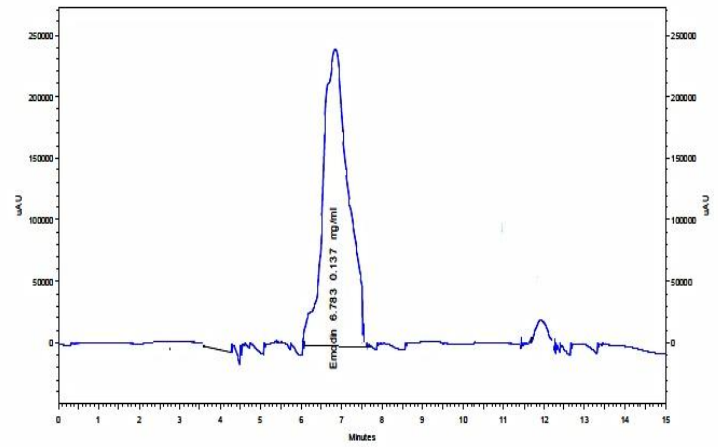


III. RESULTS AND DISCUSSION

3.1 Preliminary screening revealed the presence of flavonoids, alkaloids, tannins, and phenolic compounds in the ethanolic extract. The ethyl acetate fraction demonstrated the highest flavonoid concentration, corroborating previous studies.

Table3: HPLC showing presence of flavonoids in isolated fraction of Moringa oleifera L.

S. No.	Lit. Ret. Time	Exp. Ret. Time	Compound
1.	3.620	4.416	Unknown
2.	**6.220**	**7.659**	**Purified fraction**
3.	10.581	11.312	Unknown



Isolated CompoundsTwo major flavonoids were isolated and identified:

Quercetin, Kaempferol ,These compounds were identified based on their spectral data and comparison with literature values.The antioxidant activity of the isolated flavonoids was evaluated using the DPPH radical scavenging assay. Both compounds exhibited significant antioxidant potential, with quercetin showing higher activity. The results suggest that *Moringa oleifera* flavonoids could serve as natural antioxidants in pharmaceutical and nutraceutical applications.

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IV. CONCLUSION

This study highlights the rich phytochemical diversity of Moringa oleifera, focusing on the isolation and characterization of flavonoids. The identified compounds, quercetin and kaempferol, demonstrate promising antioxidant activity. These findings support the therapeutic potential of Moringa oleifera and encourage further research into its bioactive compounds.

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