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Comprehensive Evaluation of Madhuca longifolia: Morphological, Phytochemical, and Pharmacological Perspectives: A Research

Santosh Shukla*[1], Prof. (Dr.) Shikhar Verma[2], Prof. (Dr.) Pranay Wal[3]

*[1]Ph.D Research Scholar, School of Pharmacy, Maharishi University of Information Technology, Lucknow, 226008, Uttar Pradesh, India.
[2]Dean, School of Pharmacy, Maharishi University of Information Technology, Lucknow, 226008, Uttar Pradesh, India.
[3]Dean, Pranveer Singh Institute of Pharmacy, Kanpur, Uttar Pradesh, India.

Corresponding Author:

Santosh Shukla

Ph. D Research Scholar, School of Pharmacy, Maharishi University of Information Technology, Lucknow, 226008, Uttar Pradesh, Bharat.

Email address: sshukla364@gmail.com

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ABSTRACT

Background with Aim: Madhuca longifolia (MDL) is a plant known for its various medicinal properties. This study aimed to comprehensively evaluate the morphological characteristics, extractive values, phytochemical constituents, and bioactive compounds of different parts of MDL.

Methods: Morphological characteristics of MDL flower, bark, and root sections were examined, followed by an assessment of extractive values using ethanol solutions. Phytochemical screening tests were performed on extracts, and various parameters such as ash values, water-soluble extractive values, and loss on drying were determined. Thin layer chromatography (TLC) was employed to identify bioactive compounds, and isolation techniques were utilized for further validation.

Results: MDL exhibited distinct morphological characteristics across its flower, bark, and root sections. Extractive values varied, with the highest yield observed in the bark extract. Phytochemical screening revealed the presence of steroids, triterpenoids, saponins, alkaloids, glycosides, tannins, phenolic compounds, and flavonoids. TLC analysis confirmed the presence of alkaloids, tannins, and phenols/flavonoids in MDL samples, with quercetin successfully isolated from bark extracts.

Conclusion: This comprehensive analysis highlights the diverse pharmacological potential of Madhuca longifolia, underscored by its rich phytochemical composition. The presence of bioactive compounds suggests promising therapeutic applications, warranting further investigation and development of MDL-based formulations for pharmaceutical and healthcare purposes.

KEYWORD: Piper Betel, Phytoconstituents, Methanolic Extract, Ethanolic extract, Aqueous Extract

INTRODUCTION

Herbal Medicine

Herbal medicine, formally known as botanical or phytotherapy, constitutes an enduring therapeutic practice deeply rooted in historical traditions, characterized by its utilization of plant-derived remedies to address diverse health maladies. This comprehensive examination shall explore the domain of herbal medicine, encompassing its historical antecedents, fundamental principles, contemporary applications, and potential advantages and constraints.

Herbal medicine is a discipline within the realm of scientific inquiry wherein botanical formulations are employed for the mitigation of various pathological conditions. Notably, botanical substances persist in assuming a pivotal role within primary healthcare paradigms across many developing nations, as corroborated by Sukanya et al. in 2009. Indigenous systems of medicine such as Ayurveda, Siddha, Unani, and Folk medicine represent key repositories of traditional knowledge and therapeutic practices. The empirical validation of the efficacy and safety of traditional wisdom in healthcare, as expounded by Fabricant et al. in 2001, remains a paramount research endeavor in this domain. Remarkably [1], even in the contemporary milieu, herbal medicine continues to harbor vast reservoirs of untapped potential, with nearly 80% of the global populace residing in developing nations reliant upon botanical resources for their healthcare needs (1-3).

Madhuca longifolia

Madhuca longifolia, commonly known as Mahua, is a versatile tree native to the Indian subcontinent. Belonging to the family Sapotaceae, this species is highly valued for its various uses in traditional medicine, food, and industry. Its natural habitat spans across the Indian peninsula, from the foothills of the Himalayas to the southern tip of India. The Mahua tree is known for its striking appearance, with glossy, elliptical leaves and fragrant, creamy-white flowers that bloom during the spring season. These flowers are a significant cultural symbol in many regions of India and are used to make various traditional delicacies and beverages. One of the most remarkable features of Madhuca longifolia is its multiple applications. Almost every part of the tree holds some utility. The flowers are used to produce Mahua liquor, a popular alcoholic beverage in tribal communities. The seeds yield valuable oil, known for its medicinal properties and use in cooking. Additionally, various parts of the tree have been utilized in Ayurvedic and traditional medicine for treating ailments ranging from skin disorders to respiratory issues [2]. Furthermore, Mahua plays a crucial role in the ecosystem, providing habitat and sustenance for a diverse array of wildlife, including birds, insects, and mammals. Despite its rich cultural and ecological significance, Mahua faces threats from deforestation, habitat loss, and overexploitation. Efforts are underway to conserve and sustainably manage this invaluable species to ensure its continued presence for future generations.

Taxonomical Classification of *Madhuca longifolia*

Madhuca longifolia belongs to the *Sapotaceae* family, which includes various species of trees and shrubs commonly known as sapotes. Within the genus Madhuca, M. longifolia is one of the prominent species, distinguished by its elongated leaves and fragrant flowers.

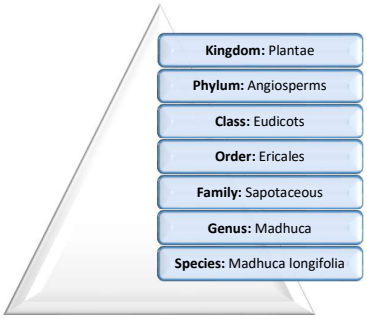

Figure 1: Taxonomical Classification of Madhuca Longifolia

Chemical Constitution

Madhuca longifolia, commonly known as Mahua, contains various chemical constituents across its different parts, each contributing to its diverse uses in traditional medicine, food, and industry. Here are some of the main chemical constituents found in different parts of the Mahua tree [3]:

Plant Part	Chemical Constituents	Properties/Uses
Seed Oil	Oleic acid, linoleic acid, palmitic acid, tocopherols (vitamin E), sterols, triterpenoids	Rich in unsaturated fatty acids, minor constituents contribute to antioxidant properties and health benefits.
Flowers	Saponins, flavonoids, tannins, glycosides	Medicinal properties, characteristic aroma, and flavor
Leaves	Alkaloids, flavonoids, phenolics, tannins	Antioxidant, antimicrobial, anti-inflammatory properties
Bark	Alkaloids, tannins, flavonoids, glycosides	Pharmacological activities including antidiabetic, antimicrobial, and wound-healing properties
Roots	Alkaloids, flavonoids, terpenoids	Believed to possess bioactive compounds, less studied compared to other parts of the plant

Table 1: Chemical Constituent reported in Madhuca Longifolia

Pharmacological Value of Leaf extract

Madhuca Longifolia has been studied for its potential medicinal and pharmacological benefits. In the alternating heptagons that are provided below, the list of numerous pharmacological benefits has been compiled.

- **Antioxidant activity:** Mahua contains phenolic compounds and flavonoids that scavenge free radicals.
- **Anti-inflammatory properties:** Compounds in Mahua exhibit anti-inflammatory activity, potentially useful for conditions like arthritis.
- **Antimicrobial and antifungal activity:** Mahua parts contain bioactive compounds with antimicrobial properties.

- **Antidiabetic effects:** Mahua extracts may help regulate blood glucose levels and improve insulin sensitivity.
- **Wound healing:** Mahua bark and leaves promote wound healing, attributed to antibacterial and anti-inflammatory properties.
- **Hepatoprotective activity:** Mahua extracts protect the liver from damage caused by toxins and oxidative stress.
- **Anticancer potential:** Some compounds in Mahua exhibit cytotoxic effects against cancer cells, suggesting potential in cancer treatment [5].

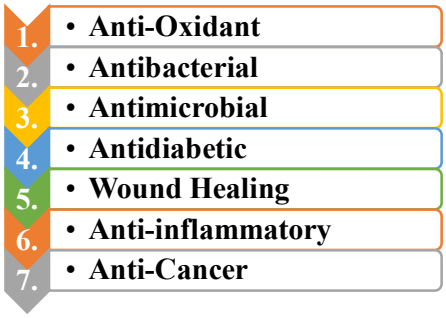

Figure 2: Pharmacological activities found in *Madhuca Longifolia* [10-11]

MATERIAL AND METHOD

Procurement of Plant material

Madhuca Longifolia specimens, including the flower, bark, and root, were gathered from a village near Gorakhpur, Lucknow. Dr. Avadhesh Mishra, Director of the Integrated Biotechnological Research Institute in Lucknow, verified the authenticity of the plant components (Authentication Reference No. IBRI/23/C031). The leaves were rinsed with tap water to eliminate dirt and impurities, and then let dry in a well-ventilated area for 10 days. Afterward, they were dried for 10 minutes in a tray dryer, ground to reduce their size using a grinder, and finally separated into fine powder using a No. 40 sieve. The fine powder obtained was preserved for subsequent analysis by sealing it in zipper bags and placing them in desiccators [6-7].

Organoleptic Evaluation

During conducting an organoleptic examination, some sensory factors were noted, including odor, color, taste, shape, apex, and leaf texture.

Preparation of Extract

The dried wood, bark, and leaf were pulverized using a grinder. 60 grams of each powder were uniformly packed into the soxlet extractor and treated to extraction with 210 milliliters of ethanol. Following the extraction process, the solvent was evaporated and the resulting extracts were concentrated using a water bath until a solid residue formed [8]. The residue was then stored in a desiccator. The raw extract was utilized for pharmacognostic analysis, phytochemical screening, in-vitro assessment of antioxidant activity, and the extraction of a bioactive component.

Qualitative Phytochemical screening

The extract was subjected to various phytochemical tests to identify specific compounds:

1. Alkaloid Tests:

Hager's Test: A few drops of Hager's reagent (picric acid solution) were added to the extract. The formation of a yellow precipitate indicated the presence of alkaloids.

Mayer's Test: Mayer's reagent (potassium mercuric iodide solution) was mixed with the extract, producing a cream precipitate, confirming alkaloids.

Dragendroff's Test: Adding Dragendroff's reagent (potassium bismuth iodide solution) to the extract resulted in an orange precipitate, indicating alkaloids.

Wagner's Test: Treating the extract with Wagner's reagent (iodine-potassium iodide solution) produced a reddish-brown precipitate, confirming the presence of alkaloids.

2. Steroids Tests:

Liebermann-Burchard Test: The extract was dissolved in chloroform, followed by the addition of acetic anhydride and sulfuric acid. A reddish-violet precipitate at the junction confirmed the presence of steroids.

Salkowski Test: The extract was mixed with sulfuric acid in chloroform, leading to a reddish-brown layer in chloroform and green fluorescence in the acid layer, indicating steroids.

3. Flavonoid Tests:

Ferric Chloride Test: A neutral ferric chloride solution was added to the extract, forming a blackish-red color, indicating flavonoids.

Lead Acetate Test: Adding lead acetate solution resulted in a yellow precipitate, confirming flavonoids.

4. Saponins Test:

Foam Test: The extract was diluted with distilled water and shaken. A 1 cm foam layer formed, confirming the presence of saponins.

5. Protein and Amino Acid Tests:

Millon's Test: Millon's reagent was added to the extract, producing a red color, indicating proteins and amino acids.

Biuret Test: The extract was mixed with sodium hydroxide and copper sulfate solution, resulting in a violet color, confirming proteins and amino acids.

6. Glycosides and Sugars Tests:

Molisch's Test: Concentrated sulfuric acid and Molisch's reagent were added to the extract, forming a reddish-violet ring, indicating glycosides and sugars.

Fehling's Test: Boiling the extract with Fehling's solution resulted in a brick-red precipitate, confirming glycosides and sugars.

7. Phenolic Compounds and Tannins Tests:

Ferric Chloride Test: The addition of ferric chloride to the extract caused a bluish-black color to appear, which indicates the presence of phenolic compounds and tannins.

Gelatin Test: When the extract was combined with a gelatin solution, a white precipitate formed, confirming the phenolic compounds and tannins in the extract.

These tests established the presence of a range of phytochemicals, including alkaloids, steroids, flavonoids, saponins, proteins, amino acids, glycosides, sugars, as well as phenolic compounds and tannins [9-11].

Phytochemical Parameter

Extractive Values

- **Loss on Drying:** The moisture content is measured by drying 5-6 grams of sample at 110°C until a constant weight is achieved.

Ash Values:

- **Total Ash:** Two grams of sample are incinerated at 600°C, and the ash is weighed.
- **Acid Insoluble Ash:** The ash is treated with hydrochloric acid, filtered, and the insoluble residue is weighed [12].
- **Water Soluble Ash:** The ash is treated with water, and the weight difference between total ash and insoluble material gives the water-soluble ash percentage [13].

Extractive Values:

- **Alcohol Extractive Value:** Five grams of sample are macerated in ethanol, filtered, evaporated, and dried to determine the extractive percentage [14].
- **Water Extractive Value:** Five grams of sample are soaked in water, filtered, evaporated, and dried to calculate the water-soluble extractive percentage.

Total Flavonoid Content

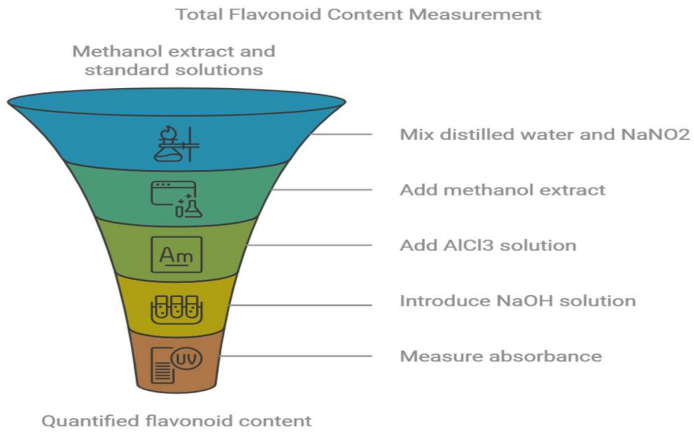

Figure 3: Determination of Total Flavonoid Content

Total Phenolic Content

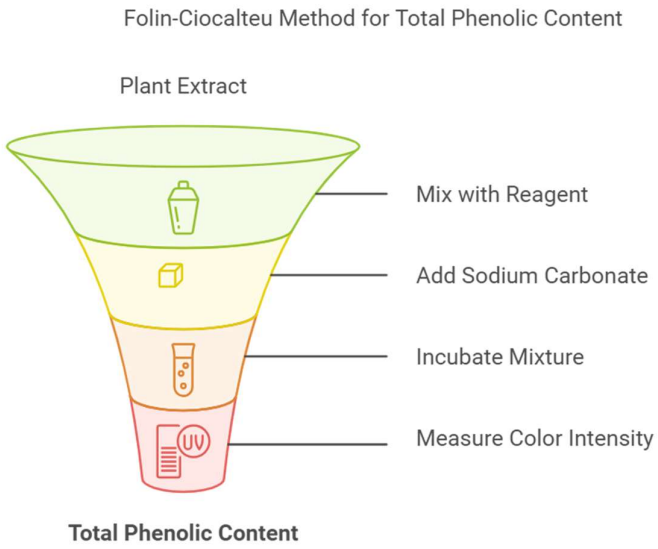

Figure 4: Determination of Total Phenolic Content

Thin Layer Chromatograph (TLC)

Estimation of phytoconstituents by thin-layer chromatography (TLC)

Thin-layer chromatography was performed using standard methods. Small quantities of sample root extract (2 mg/ml) were dissolved in their respective solvents. A solvent system developed by varying the concentration of mobile phases was employed. The plates were visualized directly after drying and with the help of a UV-TLC viewer [15]. The R_f value of the different spots was calculated and the values obtained were matched with the reference values [16].

The R_f value was calculated as follows.

$$R_f = \frac{\text{Distance traveled by the sample}}{\text{Distance traveled by the solvent}}$$

Isolation of Bioactive Compounds

Column Chromatography:

Ten grams of the bark extracts of *Madhuca longifolia* were used to make the admixture, which was then placed in a silica gel column (100-200 mesh, 100 g). Elution was then performed using 100% hexane, 50% acetate, 25% methanol, and 100% methanol in the respective ratios. Elutants were combined into five fractions based on the profile obtained from a thin layer chromatogram (TLC) [17].

Isolation of bioactive compound:

Standard procedures were followed while performing thin-layer chromatography [18]. Samples in small amounts (2 mg/ml) were dissolved in the appropriate solvents. A typical dose of quercetin (1 mg) was dissolved in methanol. Throughout the screening process, several mobile phases with various concentrations were used, and the one in which the separation of flavonoids was evident was chosen: Acetate ethyl: Acid formic: Water (100:11:11: 26) in GAA. Immediately upon drying, all plates were seen using a UV TLC viewer at 254 and 366 nm. The visualizing agent anisaldehyde-sulphuric acid was employed. It was determined what the Rf value of each of the several spots that were seen was. After the plates were kept in the iodine chamber, dark brown bands were

formed. These bands were then scratched and suspended in the mobile phase separately for three to four days. Following the removal of the mobile phase, the vacuum evaporated, and the residue was gathered until there was enough for dosing and spectrophotometric analysis [19]. The plates developed under UV light showed fluorescent spots in 100% ethyl acetate fraction similar to the standard quercetin compound. The selected purified material was subjected to its HPTLC and IR spectra. The eluant was subjected to TLC, the sample and standard are shown in the figure, which confirms the detection and isolation of quercetin from the bark extracts of *Madhuca longifolia* [20].

RESULT AND DISCUSSION

1. **Morphological characters of plant material**

The three plant elements of *Madhuca longifolia* (MDLE) the flower, bark, and root sections—have all had their numerous morphological characteristics, such as color, scent, taste, size, shape, etc., examined. Every component of the plant has a distinct smell and is green in color. The flavor of leaves is similar to that of mint. The table below displays the combined findings of the macroscopical investigation.

S. No	Parameter	Flower Components	Bark Components	Root Components
1	Color	Brownish Hue	Light Brown Shade	Greenish Tint
2	Odor	Unique Aroma	Characteristic Smell	Distinct Aroma
3	Taste	Typical Flavor	Distinct Flavor	Typical Taste
4	Leaf Dimensions	5-10.1 cm	4 mm	8-17 cm
5	Shape	Broadly Spread	Broadly Spread	Broadly Spread

Table 3: Morphological Characters of Madhuca Longifolia

2. **Extractive Value**

S. No	Plant Part	Yield (% w/w)
1	Flower extract	13.41
2	Bark extract	14.70
3	Root extract	11.82

Table 3: Extractive Values of different extracts of Madhuca Longifolia

3. **Phytochemical screening OR Phytoconstituent screening**

Phytochemical Screening tests were performed by using different parts of MDLE extract such as flower, bark, and root, and was found that be roots of plants had good phytocompounds. Concentrates of plant material have demonstrated the presence of sugars, glycosides, tannins, saponins, and phenol. It is not completely certain that all proteins are absent from the concentrates. This analysis shows that the ethanolic separate is composed of more components. According to a preliminary investigation, the leaf extract included a significant amount of bioactive secondary compounds, including flavonoids, phenols, alkaloids, tannins, glycosides, and carbohydrates. This species may have some therapeutic promise based on the presence of these components. Furthermore, the presence of a few phytoconstituents may be the reason for the healing properties of the two distinct concentrates.

S. No	Chemical Tests	Flower	Bark	Root
1	Steroids and Triterpenoids	+	+	-
2	Saponins	+	++	+++
3	Alkaloids	+++	+	+
4	Glycosides	+	+	+
5	Tannins and Phenolic compounds	++	+	+++
6	Flavonoids	++	++	+++
7	Proteins	-	-	-
8	Carbohydrates	-	-	-

Legend: +++: Excellent, ++: Good, +: Present, -: Absent

Table 4: Phytochemical screening of Piper betel leaves

4. Phytochemical parameters

Extractive, Ash, Water-Soluble Extractive, and Loss on Drying Values of MDLE

S. No	Parameter	Part of Plant	Yield (% w/w)
1	**Ethanol Extractive Values**	Flower extract	13.41
		Bark extract	14.70
		Root extract	11.82
2	**Ash Values**	Flower extract	4.28
		Bark extract	5.81
		Root extract	1.17
3	**Water-Soluble Extractive**	Flower extract	14.01
		Bark extract	13.20
		Root extract	11.07
4	**Loss on Drying**	Flower extract	3.02
		Bark extract	5.36
		Root extract	1.70
5	**Total Ash**	Flower extract	4.7
		Bark extract	4.9
		Root extract	3.1
6	**Water-Soluble Ash**	Flower extract	11.14
		Bark extract	12.72
		Root extract	10.01
7	**Acid-Insoluble Ash**	Flower extract	7.32
		Bark extract	6.71
		Root extract	4.83

Table 5: Extractive, Ash, Water-Soluble Extractive, and Loss on Drying Values of MDLE

Total Flavonoid Content

Rutin was used as a standard in the spectrophotometer assay for the quantitative assessment of flavonoid content, with a few minor adjustments. In summary, 0.25 mL

of standard solutions and 1.25 mL of distilled water were combined with 75 µL of 5% NaNO2. 75 µL of 10% AlCl3 was added after 6 minutes. 0.5 mL of 1 M NaOH was added to the mixture after an additional 5 minutes. The mixture's absorbance was measured right away at 510 nm in comparison to a prepared water blank. The amount of total flavonoids was reported in milligrams of rutin equivalents (RE). Table and Fig display the standard drug's total phenolic content, respectively.

Sr. No.	conc.(µg/ml)	Absorbance (at 510 nm)
1	10	0.005
2	20	0.008
3	40	0.011
4	60	0.017
5	80	0.02
6	100	0.026

Table 18: Total flavonoid Content of MDLE

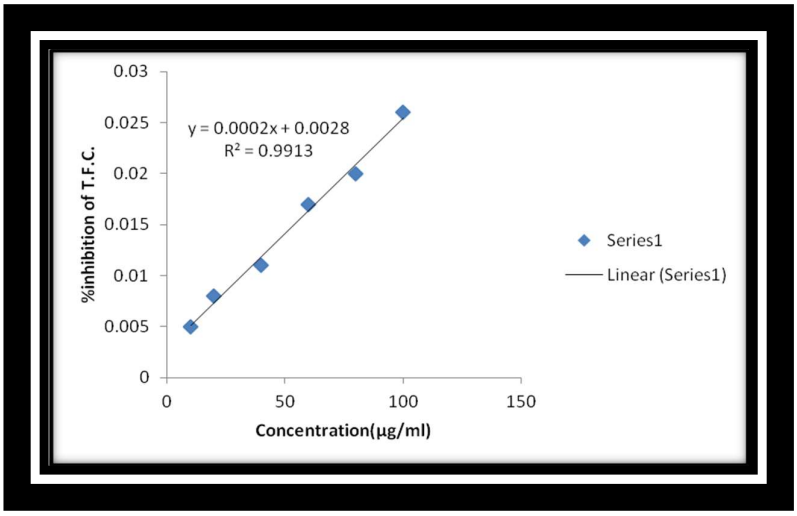

Figure 2: Standard curve of Rutin for Total Flavonoid content

Total Phenolic Content

A solution of gallic acid was prepared and varying volumes were diluted with distilled water. After adding Folin-Ciocalteu reagent and sodium carbonate, the mixtures were left to react for 2 hours with occasional shaking. The absorbance of the blue color formed was then measured at 760 nm. A standard curve for gallic acid was created based on these measurements.

Sr. No.	Conc.(µg/ml)	Absorbance (at 760 nm)
1	1	0.05
2	5	0.053
3	10	0.061
4	50	0.132
5	100	0.283
6	150	0.412

Table 19: Quantitative estimation of Gallic acid

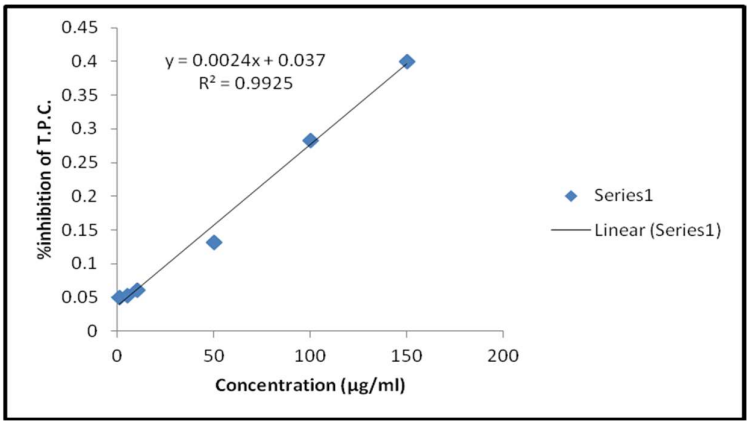

Figure 3: Standard curve for Gallic acid

5. Thin Layer Chromatography

The Thin Layer Chromatography (TLC) test of *Moringa oleifera* methanol extract (MDLE) was performed using standard methods. The extract was dissolved in methanol, and solvent systems were adjusted to optimize mobile phase concentrations. After development, the TLC plates were examined both visually and under UV light, and the Rf values were calculated and compared with reference standards for alkaloids, tannins, phenols, and flavonoids.

Thin Layer Chromatography (TLC) Analysis of MDLE

Test	Solvent System	Number of Spots	Rf Value	Inference
Alkaloids	Cyclohexane: Chloroform: Diethyl amine (5:4:1)	2	0.375	Quinone
			0.236	Naphthoquinone Derivatives
Tannins	Chloroform: Ethyl acetate: Acetic acid (50:50:10)	1	0.605	Cinnamic Acid Derivatives
Phenol/Flavonoids	Toluene: Ethyl acetate: Formic acid (36:12:5)	2	0.676	6-Hydroxy Flavones
			0.706	6-Hydroxy Flavone Derivatives

Table 20: Determination of R_f values

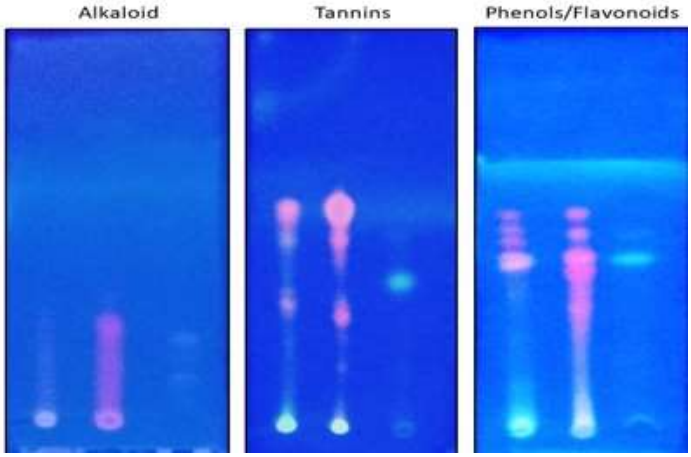

Figure 4: TLC Images of selected extracts

CONCLUSION

The study on *Madhuca longifolia* (MDLE) covered various aspects such as morphological analysis, extractive values, phytochemical screening, thin layer chromatography (TLC), and the isolation of bioactive compounds. The morphological assessment highlighted distinct features of the plant's flower, bark, and root, including their color, odor, taste, size, and shape. Extractive value analysis showed that bark extracts had the highest yield of bioactive compounds. Phytochemical screening confirmed the presence of secondary metabolites like alkaloids, tannins, flavonoids, glycosides, and phenolic compounds, indicating the plant's medicinal potential. Additionally, phytochemical parameters like ash values, water-soluble extractives, loss on drying, and total ash content provided quantitative insights into the plant's composition. Assays for total flavonoid and phenolic content further verified the abundance of these compounds. TLC analysis identified alkaloids, tannins, phenols, and flavonoids with distinct Rf values, showcasing the chemical diversity of MDLE. Quercetin was also isolated from the bark extract, reinforcing the plant's richness in bioactive constituents. Overall, the study highlights the pharmacological potential of *Madhuca longifolia* and its suitability for developing novel therapeutic agents, laying the groundwork for further research into its medicinal applications.

Conflict of interest: The authors declare no conflict of interest.

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