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Research Article

Pharmacognostic Standardization, Phytochemical Profiling, and Chromatographic Isolation of Bioactive Constituents from *Mallotus philippensis* Leaves

Arjun Kashnia*, Dr. Pankaj Sharma, Dr. Jaya Sharma, Dr. Monika Jain

Department of Pharmaceutical Sciences, APEX University, Jaipur, Rajasthan

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ABSTRACT

Background: Mallotus philippensis is recognized for its diverse therapeutic properties, heavily attributed to its secondary metabolites. This study aimed to standardize the crude leaf drug, evaluate its phytochemical profile, and isolate major bioactive constituents. Methods: Physicochemical parameters were evaluated following WHO guidelines. The leaves were defatted using petroleum ether and extracted with methanol (MEMP) using a Soxhlet apparatus, followed by solvent fractionation. Phytochemical screening, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) were quantified. Chromatographic profiling was conducted using various Chromatographies. Results: Physicochemical standardization revealed a Total Ash content of 6.12% and a low moisture content of 4.55%, confirming high purity and stability. Methanol and aqueous solvents yielded the highest extractable matter. MEMP exhibited substantial phenolic (71.1 mg GAE/g) and flavonoid (46.0 mg QE/g) content, which was further enriched in the Ethyl Acetate Soluble Fraction (EASF). HPTLC fingerprinting of MEMP revealed a dominant marker at an Rf of 0.80, accounting for 34.8% of the total peak area. Gradient elution column chromatography successfully isolated this primary compound as a pure, white crystalline solid designated as MEMP-A (melting point 202-208°C). Conclusion: The crude leaves of M. philippensis meet standard pharmacopeial purity limits. The methanolic extract and its ethyl acetate fraction are densely concentrated with polyphenols and flavonoids. The successful chromatographic isolation of the major constituent (MEMP-A) provides a foundation for further spectroscopic characterization and targeted pharmacological formulation.

INTRODUCTION

The global reliance on botanical therapeutics has expanded significantly, driven by the structural diversity and multifaceted pharmacological

***Corresponding Author:** Arjun Kashnia
Address: *APEX University, Jaipur, Rajasthan*
Email ✉: arjunkashnia@gmail.com

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potential of plant-derived secondary metabolites. However, to ensure safety, efficacy, and reproducibility, the World Health Organization (WHO) mandates rigorous pharmacognostic standardization as the foundational step in botanical drug development.[1] Establishing precise physicochemical parameters such as ash values, moisture content, and solvent extractive profiles is critical for authenticating raw botanical materials and establishing baseline quality control limits prior to downstream processing.[2] The evaluation of physicochemical parameters serves as a standard reference to establish quality control limits, prevent adulteration, and ensure the stability of botanical materials. *Mallotus philippensis* contains a rich density of secondary metabolites, specifically polyphenols, flavonoids, and tannins, which are known to dictate its biological activity. [3-5]

M. philippensis (Lam.), a prominent member of the Euphorbiaceae family, has a well-documented history in traditional medicinal systems.[6] While the red glandular powder from its fruits (commonly known as Kamala) has been extensively studied for its anthelmintic and purgative properties, the pharmacological potential of its leaves remains a subject of growing scientific interest. Preliminary literature and traditional practices suggest that the leaves harbor a dense concentration of bioactive secondary metabolites, which are increasingly recognized for their ability to neutralize systemic oxidative stress and modulate metabolic pathways.[7-8] The therapeutic efficacy of M. philippensis leaves is fundamentally linked to its phytochemical architecture, particularly its rich reserves of polyphenols, flavonoids, and tannins. [9] This study details the comprehensive pharmacognostic standardization of *M. philippensis* leaves, followed by sequential solvent extraction, quantitative phytochemical profiling, and the targeted chromatographic separation of its primary bioactive constituents to establish a robust chemical fingerprint for future therapeutic application. [1]

2. MATERIALS AND METHODS

2.1 Physicochemical Standardization

Physicochemical properties, including foreign organic matter (FOM), loss on drying (moisture content), ash values, and solvent extractive values, were measured according to WHO guidelines for the quality control of herbal drugs. Extractive values were determined using a range of solvents (Petroleum Ether, Chloroform, Ethyl Acetate, Methanol, and Aqueous) to assess the distribution of polar and non-polar constituents.

2.2 Extraction and Fractionation

Soxhlet extraction apparatus:

To remove non-polar, lipophilic substances (fats, waxes, fixed oils, chlorophyll), 100 g of coarse leaf powder was first defatted with 250 mL of petroleum ether (50-60°C) using a continuous hot Soxhlet extraction method. The resulting marc was subsequently extracted with 250 mL of methanol for 72 hours. The methanolic extract (MEMP) was concentrated, and its percentage yield was calculated. MEMP was then fractionated based on a polarity gradient into Chloroform (CSF), Ethyl Acetate (EASF), and Aqueous (ASF) soluble fractions. [11]

2.3 Phytochemical Analysis

Qualitative chemical tests were performed on the powdered drug, crude methanolic extract, and solvent fractions to identify the presence of alkaloids, glycosides, flavonoids, saponins, proteins, and tannins/polyphenols. Quantitative estimation of Total Phenolic Content (TPC) was

determined & expressed as mg Gallic Acid Equivalents (GAE)/g of dried extract. Total Flavonoid Content (TFC) was determined via the Aluminum Chloride colorimetric method and expressed as mg Quercetin Equivalents (QE)/g of dry extract. [12-13]

2.4 Chromatographic Separation

- **Thin Layer Chromatography (TLC):** Pre-coated Silica-gel 60 plates were used as the stationary phase. A mobile phase of Methanol:Chloroform (5:5) was employed, and spots were detected using Vanillin-Sulfuric acid reagent at 366 nm and 254 nm. [14]

- **High-Performance Thin-Layer Chromatography (HPTLC):** Densitometric scanning was performed on the methanolic extract using the established mobile phase to quantify the relative peak areas of the separated phytoconstituents. [15]

- **Column Chromatography:** A glass column (25-30 cm height, 2.5-3 cm diameter) packed with Silica gel-G was used. Gradient elution was performed starting from 100% Methanol, transitioning through Methanol:Chloroform mixtures, to 100% Chloroform. Fractions of 30 mL were collected, monitored by TLC, and identical fractions were pooled and crystallized. [16]

3. RESULTS AND DISCUSSION

3.1 Physicochemical Evaluation

The determination of extractive values provides insight into the total extractable phytoconstituents based on polarity. The highest yields were observed in highly polar solvents, indicating that the majority of the active constituents in the leaf matrix are hydrophilic or of intermediate polarity.

Table 1: Extractive Values of *M. philippensis* Leaf via Successive Extraction

Solvent	Color of Extract		Extractive value (% w/w)
	Normal Light	UV Light 254nm	
Pet ether	Greenish Brown	Brownish	3.28
Chloroform	Reddish brown	Brownish Black	5.35
Ethyl Acetate	Greenish Brown	Brownish	8.90
Methanol	Reddish brown	Dark Brownish	9.25
Aqueous	Reddish brown	Dark Brownish	11.16

The proximate analysis (Table 2) confirms the purity and proper handling of the crude drug. A Total Ash Content of 6.12% is well below the critical 10% threshold, indicating freedom from significant inorganic contamination (e.g., sand or brick dust). This is reinforced by a negligible Acid-insoluble ash value (1.15%), which specifically quantifies siliceous matter. The optimal moisture content (4.55%) is highly significant, as maintaining moisture below 10% prevents microbial proliferation and halts enzymatic degradation during storage.

Table 2: Proximate Analysis of Crude Drug

Physicochemical Parameter	Value in % w/w	Significance
Total Ash Content	6.12 ± 0.12 %	High values (>10%) suggest sand or brick dust.
Water-soluble ash value	1.28 ± 0.32 %	Measures the salt content of the drug
Acid-insoluble ash value	1.15 ± 0.08 %	Measures siliceous matter

Sulphated-ash value	1.05 ± 0.05 %	Measures siliceous matter
Moisture content	4.55 %	Low moisture prevents microbial growth
Volatile oil	0.84 %	Total volatile matter at 105°C
Foreign matter	1.26 %	More FOM cause contamination

3.2 Extraction Yields and Solvent Fractionation

Following defattation (which yielded a 3.35% sticky greenish-black mass), the methanolic extraction of the marc produced a robust yield of 9.2% (w/w). Because methanol is a highly efficient broad-spectrum solvent, it penetrated the cellular membrane to extract a wide variety of polar compounds. Subsequent fractionation of the methanolic extract demonstrated that the bulk of these compounds partitioned into the aqueous phase (35.30%), followed by the chloroform phase (27.6%) and the ethyl acetate phase (20.75%).

3.3 Qualitative and Quantitative Phytochemical Analysis

The crude methanolic extract (MEMP) tested positive for a broad spectrum of active secondary metabolites, specifically heavy concentrations of glycosides, flavonoids, and tannins/polyphenols.

The presence of phenolic compounds, flavonoids, and tannins is highly significant. These classes of phytochemicals are well-documented for their electron-donating capabilities, making them potent free radical scavengers. The high concentration of these polyphenols in the methanolic leaf extract strongly supports the plant's traditional therapeutic use in managing oxidative stress and diabetes conditions.

- **Fraction specificities:** The Chloroform fraction (CSF) showed highly selective enrichment of tannins and polyphenols. The Ethyl Acetate fraction (EASF) retained significant flavonoids and alkaloids. The Aqueous fraction (ASF) primarily captured hydrophilic primary metabolites like carbohydrates and proteins.

Table 3: Report of Chemical Test on Powder Materials & Various Extracts

Plant constituents	Powdered drug	Chloroform fraction	Ethyl Ace fraction	Methanol Crude Ext	Aqu. Fraction
Alkaloids	+	–	+	+	–
Glycoside	++	++	+	+++	+
Carbohydrates	+	–	–	+	+
Fixed Oil	–	–	–	–	–
Flavonoids	++	++	+	++	+
Saponins	+	–	+	+	–
Protein	+	–	–	+	+
Tannins/ PolyPhenol	++	+++	++	+++	+
Gums	+	+	–	+	–

Table 4: Total Phenolic (TPC) and Total Flavonoid Content (TFC)

Extracts	Total phenolic content (mg of GAE/g of extract)	Total Flavonoid content (mg of QUE/g of extract)
MEMP	71.1 ± 2.08	46.0 ± 1.2
EASF	85.25 ± 1.72	64.12 ± 2.15
CSF	54.52 ± 0.65	30.4 ± 1.25

ASF	26.35 ± 0.24	17.28 ± 0.62

The quantitative analysis supports the qualitative screening. The EASF is the most densely concentrated with polyphenols (85.25 mg GAE/g) and flavonoids (64.12 mg QE/g), making it the prime candidate for antioxidant-driven pharmacological applications.

3.4 Chromatographic Profiling (TLC and HPTLC)

TLC plate visualization under UV light:

Preliminary TLC of MEMP utilizing a Methanol:Chloroform (5:5) mobile phase provided sharp resolution with minimal tailing. Distinct spots were observed at Rf values of 0.22, 0.36, 0.44, 0.65, and 0.72, confirming the diversity of the extracted constituents.

This distinct TLC pattern establishes a preliminary chromatographic fingerprint for the MEMP extract, confirming the presence of multiple bioactive markers and providing the foundational solvent system required to perform quantitative and high-resolution HPTLC analysis. HPTLC densitometric scanning expanded upon this fingerprint, revealing a clear hierarchy in compound concentration:

Table 5: HPTLC fingerprint analysis of MEMP

Sample	Mobile Phase	Peak Number	Retention Factor (Rf)	% Area
MEMP	Methanol: Chloroform (5:5)	1	0.8	34.8
MEMP	Methanol: Chloroform (5:5)	2	0.32	14.65
MEMP	Methanol: Chloroform (5:5)	3	0.44	12.45
MEMP	Methanol: Chloroform (5:5)	4	0.62	23.68

- **Peak 1 (Dominant Marker):** Located at an Rf of 0.80, this constituent accounted for a substantial 34.8% of the total peak area, suggesting it is a primary secondary metabolite.
- **Peak 4 (Secondary Marker):** Located at an Rf of 0.62, representing 23.68% of the total area.

3.5 Column Chromatography and Isolation of MEMP-A

To isolate the dominant marker identified via HPTLC, gradient column chromatography was performed, yielding 48 total fractions. Fractions 25-32, eluted with a Methanol:Chloroform (6:4) solvent system, displayed a single distinct spot on TLC monitoring. These fractions were pooled and recrystallized using absolute methanol, yielding a highly pure compound designated as MEMP-A.

Sr. No	Pooled Fraction	Solvent system Composition	TLC Pattern	Color in UV	Code
1	1-4	Methanol 100%	3 spots	Green color- Not proceed for purification	---
2	5-12	Methanol: Chloroform (9:1)	2 spots	Yellow green consistency	-
3	13-24	Methanol: Chloroform (7:3)	3 spot	White Compound	--
4	25-32	Methanol: Chloroform (6:4)	1 spot	Solid Whitish Crystals	MEMP-A
Proceed for purification, Re-crystallization by absolute methanol					

5	32-40	Methanol: Chloroform (1:1)	2 spots	Reddish brown	-
6	41-48	Chloroform 100%	2 spots	Brown	-

Spectroscopic Characterization Profile of MEMP-A:

- **Description:** Solid Whitish Crystals
- **Solubility:** Soluble in Methanol, Chloroform
- **Rf Value:** 0.80 (Methanol:Chloroform 6:4)
- **Melting Point:** 202-208°C

4. DISCUSSION:

Proximate analysis confirmed that the raw botanical material met rigorous purity benchmarks. A total ash content of 6.12% falls well below the standard 10% threshold, indicating the absence of significant inorganic or earthy contamination (such as sand or soil). Qualitative and quantitative phytochemical profiling revealed that the pharmacological potential of M. philippensis is inextricably linked to its polyphenolic and flavonoid content. These compounds are well-documented for their electron-donating capabilities, making them potent free radical scavengers capable of mitigating systemic oxidative stress. The successful isolation of MEMP-A represents a significant step in deciphering the therapeutic matrix of M. philippensis leaves. Its physical characteristics, precise Rf value (0.80), and specific solubility profile provide the foundational data necessary for its imminent structural elucidation via advanced spectroscopic techniques (such as NMR, FTIR, and Mass Spectrometry) and opens the pathway for targeted in-vivo biological testing.

5. CONCLUSION:

The pharmacognostic standardization of *Mallotus philippensis* leaves confirms that the raw botanical material meets rigorous purity, cleanliness, and stability benchmarks. The extraction and fractionation data reveal a phytochemical matrix densely populated with polyphenols and flavonoids, particularly concentrated within the ethyl acetate fraction. Most significantly, optimized chromatographic techniques (HPTLC and Column Chromatography) facilitated the successful isolation of the plant's primary secondary metabolite—a pure, crystalline compound (MEMP-A). This baseline chemical fingerprint validates the traditional utility of the plant and paves the way for the specific structural elucidation and targeted in-vivo testing of its isolated bioactive constituents.

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