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Preliminary phytochemical screening of leaf extracts of *Moringa oleifera* using various solvents

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Abstract
Moringa oleifera, belonging to the family Moringaceae, is a medicinally significant tree that originated in the Indian subcontinent and has now spread widely across tropical and subtropical regions of the world. Valued for its nutritional richness and therapeutic potential, it has long been integrated into traditional healthcare practices. In this study, preliminary phytochemical screening was performed on leaf extracts prepared with solvents of different polarity, namely petroleum ether, chloroform, methyl acetate, ethanol, methanol, and water. The qualitative assays confirmed the occurrence of diverse classes of phytoconstituents such as carbohydrates, alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, saponins, steroids, and glycosides. These findings reinforce the medicinal importance of M. oleifera and provide a basis for further investigations focused on isolating and characterizing its bioactive metabolites for pharmacological applications.

Keywords: *Moringa oleifera*, leaf extract, phytochemical analysis, solvent-based extraction, bioactive metabolites

Introduction
The drumstick tree, *Moringa oleifera* (family Moringaceae), is globally recognized for its rich nutritional profile and diverse therapeutic applications, often earning it the title of "Miracle Tree." Traditional medicinal systems make use of nearly every part of the plant leaves, seeds, roots, bark, flowers, pods, and fruits demonstrating its wide-ranging value.
Botanically, *M. oleifera* is a fast-growing tree, typically 10-12 m tall, which may remain evergreen or become deciduous depending on environmental conditions. It is characterized by a corky stem, brittle slender branches, and finely divided tripinnate leaves. The foliage is particularly significant for its high concentrations of vitamins and minerals, surpassing many conventional food sources, and has therefore been promoted as a tool against nutritional deficiencies.
In addition to macronutrients and micronutrients, the plant synthesizes a diverse spectrum of secondary metabolites. Phytochemical investigations have reported the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phenolic compounds, and glycosides. Of particular pharmacological interest are glucosinolates and their hydrolysis products such as isothiocyanates, which have been linked with chemopreventive effects.
Ethnopharmacological records, supported by experimental evidence, attribute numerous biological activities to *M. oleifera* preparations. These include cardioprotective and circulatory benefits, antipyretic and anti-inflammatory actions, neuroprotective effects in seizure conditions, gastroprotective roles in ulcerative states, and potential in regulating hypertension and lipid disorders. Leaf carotenoids are also traditionally used to alleviate ocular conditions, including conjunctivitis.
Given this therapeutic diversity and phytochemical richness, systematic characterization of its bioactive constituents remains essential. The present study focused on the preliminary qualitative screening of secondary metabolites in leaf extracts obtained using solvents of varying polarity (petroleum ether, chloroform, methyl acetate, ethanol, methanol, and water). This solvent-based fractionation enables mapping of metabolite distribution and sets the groundwork for future isolation, quantification, and pharmacological exploration of *M. oleifera* constituents.

Plant Profile and Classification
- Botanical name: *Moringa oleifera* Lam.
- Kingdom: Plantae

- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Capparales
- **Family:** Moringaceae
- **Genus:** *Moringa*
- **Species:** *oleifera*
- **Common names:** Drumstick tree, Miracle tree, Sahjan, Munga

Fig 1: *Moringa oleifera* Plant

Moringa oleifera is notable for its rapid growth and ability to withstand dry environmental conditions, making it well suited for tropical and subtropical regions. The tree exhibits a straight but relatively slender stem and light, spreading branches. Its foliage is divided into multiple fine leaflets arranged on a common stalk, giving the leaves a delicate, feather-like appearance. The plant produces elongated, cylindrical pods that resemble drumsticks, a feature responsible for its most common vernacular name. With its wide ecological tolerance and multipurpose applications in food, nutrition, and traditional medicine, *M. oleifera* has become an important subject of research in the fields of pharmacognosy and ethnomedicine.

Materials and Methods

Collection and identification of *Moringa oleifera*

To collect an identify *Moringa oleifera* plant from local garden Chhindwara Madhya Pradesh the species was identified by the local people during the time of collection and letter on the authentication by Mr Mahesh Ghaywat botanist danielson degree College Chhindwara Madhya Pradesh India.

Extraction of leaves of *Moringa oleifera*

The fresh plant material was washed with running tap water to remove other undesirable material and dried under shade. The air - dried leaves (75 gram) of *Moringa oleifera* were crushed. The crushed leaves extracted with petroleum ether 25 gram of the Powder leaves separately extracted in 500 ml conical flask with 98% ethanol (ethanol extraction) and methanol (methanolic extraction) methyl acetate (acetate extraction) chloroform and water (aqueous extraction). The conical flask were plugged with rubber cork than chicken for 20 minutes and allowed to stand at room temperature for 48 hours and 4 days. The resultant extract was drained. Extract were collected in air tight container extraction yield of all extracts were calculated using the following equation below the result.

Fig 2: *Moringa oleifera* Leaves

Qualitative Phytochemical Analysis of Plant Extracts

The different solvent extracts of *Moringa oleifera* leaves were subjected to standard qualitative phytochemical screening in order to determine the presence or absence of major classes of bioactive compounds, including carbohydrates, alkaloids, terpenoids, flavonoids, steroids, tannins, phenolic compounds, saponins, proteins, amino acids, fats, oils, and glycosides.

Detection for Carbohydrates

- **Alcoholic Alpha Napthol:** two milliliters of the extract were mixed with two drops of Molisch's reagent (prepared by dissolving α-naphthol in ethanol). Concentrated H_2SO_4 was then carefully added along the side of the test tube to form a separate layer. The appearance of a violet ring at the junction of the two liquids confirmed the presence of carbohydrates.
- **Fehling's Test:** Equal volumes (1 mL each) of Fehling's solution A and B were combined with 1 mL of the extract. The mixture was heated in a boiling water bath for about 10 minutes. Formation of a brick-red precipitate indicated reducing sugars.
- **Benedict's Test:** Equal volumes of Benedict's reagent and extract were mixed and heated in a water bath for 5-10 minutes. A color change from green to yellow to red suggested the presence of reducing sugars, depending on the concentration.
- **Copper Acetate Reagent Test:** Two milliliters of Barfoed's reagent were added to 2 mL of extract and the mixture was boiled for 4-5 minutes. Development of a red precipitate indicated monosaccharides.
- **Seliwanoff's Test:** Three milliliters of Seliwanoff's reagent were mixed with 1 mL of the extract and heated in a water bath for about 1 minute. A rose-red coloration confirmed the presence of ketose sugars.

Detection for Alkaloids

The extracts were dissolved in distilled water, acidified with dilute hydrochloric acid, shaken thoroughly, and filtered. The clear filtrate was then tested using the following reagents**:**

- **Potassium Mercuric Iodide Solution Test:** Addition of Mayer's reagent to 2 mL of the filtrate produced a white or creamy precipitate, indicating the presence of alkaloids.
- **Potassium Bismuth Iodide Solution Test:** When 2 mL of the filtrate was treated with Dragendorff's reagent

along the side of the test tube, an orange to reddish-brown precipitate was observed, confirming alkaloids.
- **Picric Acid Solution Test:** To 1 mL of the filtrate, a few drops of Hager's reagent were added. Formation of a yellow precipitate signified alkaloids.
- **Iodo Potassium Iodide Test:** Treatment of 1 mL of the filtrate with 2 mL of Wagner's reagent (iodine solution in potassium iodide) resulted in a reddish-brown precipitate, further confirming the presence of alkaloids.

Detection for Terpenoids and Steroids
- **Salkowski's Test:** The extract was dissolved in chloroform and filtered. To the filtrate, concentrated H_2SO_4 was carefully added. A red interface confirmed sterols, whereas a reddish-brown coloration suggested triterpenes.
- **Liebermann-Burchard's Test:** The extract was dissolved in chloroform, followed by sequential addition of acetic anhydride (3 mL) and glacial acetic acid (3 mL). After gentle warming and cooling, concentrated H_2SO_4 was added dropwise. Development of a brown ring at the junction and a green coloration of the upper layer indicated steroids, while a deep red coloration confirmed triterpenoids.

Detection for Flavonoids
- **Lead Acetate Test:** Addition of 10% lead acetate solution to the extract produced a yellow precipitate, confirming flavonoids.
- **Shinoda Test:** To 5 mL of extract, magnesium turnings and concentrated HCl were added. A pink coloration indicated flavonoids.
- **Alkaline Reagent Test:** The extract was treated with sodium hydroxide solution, producing deep yellow to red coloration, which became colorless upon addition of dilute HCl.
- **Sulfuric Acid Test:** Addition of concentrated H_2SO_4 to the extract resulted in orange coloration, confirming flavonoids.

Detection for Tannins and Phenolic Compounds
- **Ferric Chloride Test:** A portion of the extract was dissolved in distilled water and treated with 2 mL of 5% ferric chloride solution. Development of a blue-green or violet coloration confirmed the presence of tannins or phenolics.
- **Lead Acetate Test:** When a few drops of lead acetate solution were added to the aqueous extract, formation of a white precipitate indicated phenolic compounds.
- **Gelatin Test:** Mixing the extract solution with 1% gelatin solution containing 10% sodium chloride produced a precipitate, demonstrating the presence of tannins.
- **Alkaline Reagent Test:** Treatment of 2 mL of extract with 1N sodium hydroxide led to yellow or red coloration, further supporting the presence of tannins.
- **Dilute Iodine Test:** Addition of a few drops of dilute iodine solution resulted in a transient red coloration, confirming phenolics.

Detection for Saponins
- **Froth Test:** Extract diluted with distilled water was shaken in a graduated cylinder for 15 minutes. Persistent froth indicated saponins.
- **Lead Acetate Test:** Addition of 1% lead acetate to 1 mL extract gave a white precipitate, confirming saponins.
- **Honeycomb Froth Test:** One milliliter of extract mixed with 5% sodium bicarbonate solution was shaken vigorously. A honeycomb-like froth indicated saponins.
- **Emulsion Test:** To the froth solution, 2-3 drops of oil (e.g., olive or coconut) were added and shaken. Formation of a stable emulsion confirmed saponins.

Detection for Fats and Oils
- **Acrolein Test:** Extract heated with potassium bisulfate released a pungent, irritating odor, confirming fats.
- **Solubility Test:** Extract was tested for solubility in chloroform and 90% ethanol. Solubility indicated the presence of oils.

Detection for Proteins and Amino Acids
- **Biuret Test:** Extract was treated with 1 mL 10% NaOH and a drop of 0.7% $CuSO_4$ solution. Violet or pink coloration indicated proteins.
- **Ninhydrin Test:** Three drops of 5% ninhydrin were added to 3 mL extract and heated in a water bath for 10 minutes. A blue color confirmed amino acids.
- **Xanthoproteic Test:** Extract treated with concentrated nitric acid and heated produced a yellow color, confirming aromatic amino acids.
- **Sodium Nitroprusside Test:** Two milliliters of extract mixed with sodium nitroprusside and alkalized with NaOH produced a red or purple color, confirming proteins.
- **Millon's Test:** Addition of Millon's reagent produced a white precipitate that turned brick red on boiling, confirming proteins.

Detection for Glycosides
- **Borntrager's Test:** Extract hydrolyzed with dilute H_2SO_4, filtered, and shaken with benzene/chloroform. The organic layer was treated with ammonia, producing pink/red coloration indicative of anthraquinone glycosides.
- **Legal's Test:** Extract dissolved in pyridine was treated with sodium nitroprusside, followed by 10% NaOH. A pink to red coloration indicated cardiac glycosides.
- **Keller-Killiani Test:** Extract was treated with glacial acetic acid, ferric chloride, and concentrated H_2SO_4 along the tube wall. A blue color in the acetic acid layer indicated cardiac glycosides.
- **Baljet's Test:** Extract solution treated with picric acid and alkalized with NaOH gave a yellow to orange coloration, confirming glycosides.

Results and Discussion

The powdered leaves of *Moringa oleifera* (75 g) were sequentially extracted with solvents of progressively increasing polarity, namely petroleum ether, chloroform, methyl acetate, ethanol, methanol, and water. The total extractive yield obtained was 30.65 g. Further fractionation of the methanolic extract produced distinct fractions with the following yields:
- **Methyl acetate:** 6.32 g
- **Methanol:** 5.23 g
- **Water:** 5.78 g
- **Chloroform:** 5.23 g

- **Ethanol:** 5.19 g
- **Petroleum ether:** 5.75 g

The qualitative phytochemical screening of these solvent extracts demonstrated that the leaves of *Moringa oleifera* contain a wide array of bioactive secondary metabolites. Compounds such as alkaloids, terpenoids, flavonoids, tannins, carbohydrates, phenolic compounds, saponins, and glycosides were detected across the extracts.

Chloroform, methyl acetate, ethanol, and methanol extract consistently tested positive for alkaloids, terpenoids, phenolic compounds, saponins, glycosides, flavonoids, tannins, and carbohydrates, suggesting that medium to high polarity solvents are particularly effective in extracting a broad spectrum of phytoconstituents.

In contrast, petroleum ether (non-polar) and aqueous (highly polar) extracts predominantly showed the presence of carbohydrates, alkaloids, flavonoids, saponins, phenolic compounds, and tannins. This indicates that both extremes of solvent polarity non-polar and highly polar are still capable of isolating certain classes of bioactive molecules, although with a narrower phytochemical profile compared to moderately polar solvents.

The detected secondary metabolites are well known for their diverse pharmacological and therapeutic potentials, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. Their presence in *Moringa oleifera* extracts provides scientific support for the plant's longstanding use in traditional medicine.

Overall, these findings reaffirm the medicinal value of *Moringa oleifera* and emphasize the importance of further work on the isolation, purification, and biological evaluation of its active phytochemical constituents. Such investigations could lead to the development of novel therapeutic agents from this plant resource.

Plant collection

Table 1: Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Moringa oleifera*	Leaf	75gm

Solubility determination

Table 2: Solubility determination of *Moringa oleifera*

S. No.	Solvent	Methyl acetate	Methanol	Ethanol	Petroleum ether	Chloroform	Water
1.	Water	Soluble	Soluble	Soluble	Insoluble	Slightly soluble	Soluble
2.	DMSO	Soluble	Soluble	Soluble	Slightly soluble	Soluble	Soluble
3.	Di ethyl Ether	Soluble	Soluble	Soluble	Soluble	Soluble	Slightly soluble
4.	Benzene	Soluble	Slightly soluble	Soluble	Soluble	Soluble	Insoluble
5.	Acetone	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble

Qualitative phytochemical analysis of *Moringa oleifera* extracts

Table 3: Phytochemical analysis of *Moringa oleifera*

S. No.	Experiment	Result					
		Methyl acetate	Methanol	Petroleum Ether	Chloroform	Ethanol	Water
Test for Carbohydrates							
1.	Molish's Test	+	+	+	+	+	+
2.	Fehing's Test	+	+	+	+	+	+
3.	Benedict's Test	+	+	-	+	+	-
4.	Bereford's Test	-	-	-	-	+	-
5.	Seliwanoff's Test	+	+	-	+	+	-
Test for Alkaloids							
1.	Mayer's Test	+	+	-	+	+	-
2.	Hager's Test	-	+	+	+	+	+
3.	Wagner's Test	+	+	+	+	+	+
4.	Dragendroff's Test	+	+	-	+	+	-
Test for Triterpenoids and Steroids							
1.	Salkowski's Test	-	+	+	-	+	-
2.	Libermann-Buchard's Test	-	-	-	+	+	+
Test of Flavanoids							
1.	Lead Acetate Test	+	+	+	+	+	+
2.	Shinoda Test	+	+	-	+	+	-
3.	Alkaline Reagent Test	+	+	+	+	+	-
4.	H2SO4 Test	-	+	-	-	+	-
Test for Tannins and Phenolics Compounds							
1.	Ferric chloride Test	+	+	-	+	+	-
2.	Lead Acetate Test	+	+	-	+	+	+
3.	Gelatin Test	+	-	-	-	+	-
4.	Alkaline Reagent Test	+	-	-	+	+	-
5.	Dilute Iodine Test	+	+	-	+	+	+
Test for Saponins							

1.	Froth Test	+	+	+	+	+	+	
2.	Lead Acetate Test	+	+	+	+	+	+	
3.	Honeycomb Froth Test	+	+	-	+	+	-	
4.	Emulsion Test	+	+	+	+	+	+	
Test for Fats and Oil's								
1.	Acrolein Test	+	-	+	+	+	-	
2.	Solubility Test	+	+	+	+	+	+	
Test for Protein and Amino Acids								
1.	Biuret's Test	+	-	+	-	+	-	
2.	Ninhydrin's Test	+	+	-	-	+	-	
3.	Xanthoproteic Test	+	-	-	+	+	-	
4.	Millon's Test	-	-	-	-	-	-	
5.	Sodium Nitroprusside Test	+	+	+	+	+	-	
Test for Glycosides								
1.	Borntrager's Test	-	+	-	+	+	-	
2.	Legal's Test	-	-	-	-	+	+	
3.	Keller-Killiani Test	-	+	-	-	+	+	
4.	Balget's test	-	-	-	-	+	-	

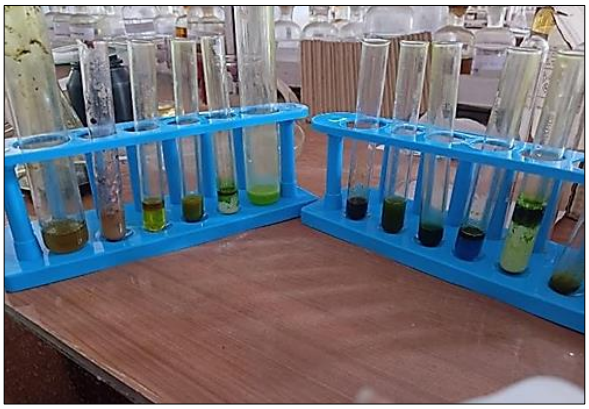

Fig 1: Methyl Extracts Result

Fig 2: Petroleum Ether Extracts Result

Fig 3: Chloroform Extracts Result

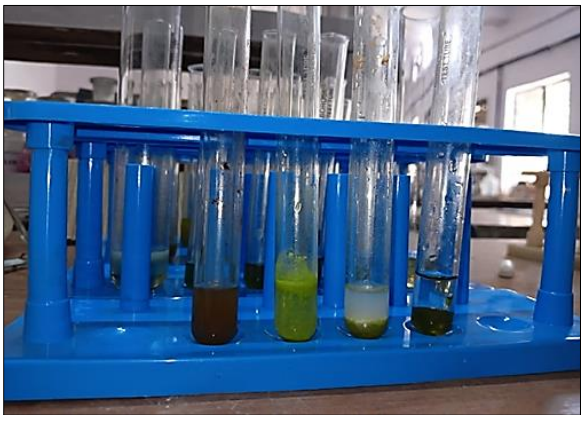

Fig 4: Water Extracts Result

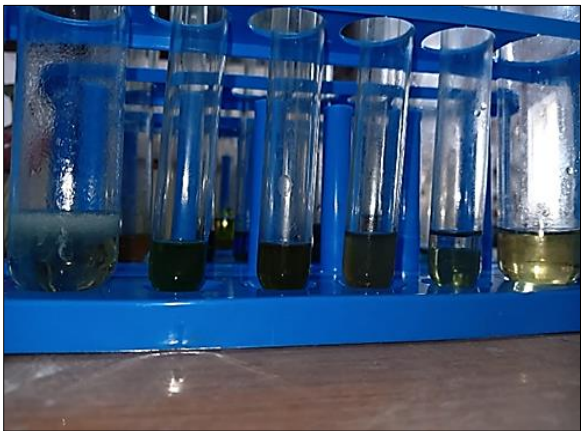

Fig 5: Ethanol Extracts Result

Fig 6: Methanol Extracts Result

Conclusion

The present investigation on the leaves of *Moringa oleifera* demonstrated the presence of a wide range of phytoconstituents, including alkaloids, carbohydrates, terpenoids, flavonoids, tannins, phenolic compounds, saponins, and glycosides, across extracts prepared with methanol, ethanol, methyl acetate, chloroform, petroleum ether, and water. These findings highlight *M. oleifera* as a valuable reservoir of bioactive secondary metabolites with potential therapeutic importance. Although the qualitative screening confirms the occurrence of these phytochemicals, further research is needed to isolate, characterize, and quantify the individual compounds. Establishing standardized extraction protocols and quality benchmarks will also be essential to ensure reproducibility and to support future pharmacological studies. Such efforts could pave the way for the development of novel plant-based medicines and functional formulations derived from *M. oleifera*.

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