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Preliminary Phytochemical Investigation of Commonly Used Ethnomedicinal Plant

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Abstract:
The use of medicinal plants in traditional healthcare systems has gained renewed interest due to the presence of bioactive compounds known as phytochemicals. This study aims to conduct a preliminary phytochemical screening of three ethnomedicinally important plants: *Phyllanthus acidus, Madhuca longifolia,* and *Cinnamomum tamala*. These species have been extensively used in various traditional systems of medicine for the treatment of ailments such as fever, inflammation, gastrointestinal disorders, and diabetes. Methanolic extracts of the leaves and bark were subjected to qualitative tests to detect the presence of major phytochemical groups including alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and terpenoids. The results revealed the presence of multiple bioactive compounds across all three plants, indicating their potential pharmacological relevance. This preliminary study provides a scientific basis for further investigation and validates the traditional uses of these plants.

Keywords: Phytochemical screening, Ethnomedicinal plants, *Phyllanthus acidus, Madhuca longifolia*, *Cinnamomum tamala*, Secondary metabolites, Medicinal plants, Bioactive compounds

Introduction:

Medicinal plants have long served as a cornerstone of traditional medicine, especially in developing countries where access to modern healthcare is limited. Ethnomedicinal knowledge, passed down through generations, has led to the identification of numerous plant species used for treating a wide range of diseases. Modern phytochemical studies aim to validate such traditional claims by identifying and characterizing the bioactive compounds present in these plants.

Among the numerous ethnomedicinal plants, *Phyllanthus acidus* (star gooseberry), *Madhuca longifolia* (mahua), and *Cinnamomum tamala* (Indian bay leaf) are commonly used across various regions in India and Southeast Asia. These plants are traditionally utilized for their anti-inflammatory, antidiabetic, and antimicrobial properties. However, despite their extensive use in folk medicine, limited scientific data is available regarding their phytochemical composition.

This study aims to conduct a preliminary phytochemical screening of these three plant species to detect the presence of various secondary metabolites. Such an investigation not only supports traditional uses but also opens avenues for further pharmacological studies and potential drug development.

Figure 1: a) *Pyllanthus acidus* leaves, b) *Madhuca longifolia* leaves c) *Cinnamomum tamala* leaves

Materials and method:

Pyllanthus acidus, Madhuca longifolia and *Cinnamomum tamala* leaves were collected from Local area of Indore and authenticated by Dr SN Dwivedi, Dept. of Botany, Janata PG College, APS University, Rewa. Voucher specimens of the plant parts were submitted in Department of Pharmacognosy, SAGE University, Indore, with voucher specimen No. J/Bot./PAL-07, J/Bot./MLL-08 and J/Bot./CTL-09 for reference purpose.

Extraction of dried leaves

50gms of dried shaded leaves powder will be exhaustively extracted with chloroform, ethanol, water and hydroalcohol (1:1) using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Preliminary phytochemical screening

The powder extracts will be individually evaluated for the presence of different phytoconstituents as per the below mentioned methods:

The powder extracts will be individually evaluated for the presence of different phytoconstituents as per the below mentioned methods:

- **Test for terpenes:** To the 5ml of the extract, 2ml of chloroform and 3ml of conc. H2SO4 will be added. The formation of a reddish brown ring confirmed the presence of terpenes.
- **Test for flavonoids:** A few drops of conc. HCl will beadded in the small amount of the prepared extracts. The red colour will be immediately developed, which confirmed the presence of flavonoids.
- **Test for saponins (Frothing test):** 0.5ml of the extract will be taken into a test tube and dissolved in distilled water. Frothing will be persisted on warming, which preliminary shows as evidence of saponins.
- **Test for steroids (Liebermann–Burchard reaction):** 2ml of acetic anhydride and 2 ml conc. H2SO4 will be added into 5ml of the extract in a test tube. Change of colour from violet to blue confirms the presence of steroids.
- **Test for glycosides:** 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1 ml of conc. H2SO4 will be added into 5ml of the extract in a test tube. The appearance of a brown ring indicates the presence of glycosides.
- **Test for proteins (Biuret test):** 4% of NaOH and few drops of 1% CuSO4 solution were added into 3ml of the extract in a test tube. Formation of violet or pink color indicates the presence of proteins.
- **Test for reducing sugars (Fehling test):** 1ml of Fehling's A and Fehling's B solutions will be mixed in a test tube, boiled for one minute then added an equal volume of test solution (2ml extract). The

mixed solution will be then heated on boiling water bath for 5–10 min. First a yellow then a red brick precipitate will be observed.

- **Test for carbohydrates (Molisch test):** 2–3ml of the aqueous extract, 2 drops of Molisch's reagent (10% alcoholic solution of -naphthol) will be added in a test tube. After mixing, a small amount of conc. H2SO4 is slowly added down the sides of the sloping test-tube, without mixing, to form a layer. Violet ring is formed at the interface between the acid and test layers.
- **Test for tannin and phenol (Ferric Chloride Test):** 3ml of extract, 3ml of 5% w/w of the FeCl3 solution will be added in a test tube. The blue-black colour indicates the presence of tannins and phenols.
- **Test for alkaloids**: In 10g of dried extracts 20ml of dilute HCl solution will be added with vigorous shaking and then filter. In the filtrate, the following tests will beperformed.

Mayer's Test: 3ml of the filtrates, 1ml of Mayer's reagent (potassium mercuric iodide) will be added in a test tube. The appearance of white precipitate confirmed the presence of alkaloids.

Wagner's Test: 3ml of the filtrate, 1ml of Wagner's reagent (iodine in potassium iodide) will be added in a test tube. The emergence of reddish-brown precipitate at the surface indicates the presence of alkaloids.

Dragendroff's Test: 3ml of the filtrate, 1ml of Dragendroff's reagent (potassium bismuth iodide) will be added in a test tube. The appearance of red brick precipitate indicates the presence of alkaloids. [1-5]

Results and discussion:

The preliminary phytochemical screening of leaf extracts of Phyllanthus acidus, Cinnamomum tamala, and Madhuca longifolia revealed the presence of a wide range of secondary metabolites across all solvent systems used—chloroform, ethyl acetate, ethanol, water, and hydroalcoholic (1:1) extracts. These phytoconstituents are known to contribute significantly to the therapeutic potential of medicinal plants.

1. Carbohydrates: All three plants tested positive for carbohydrates across all solvent systems in both Molisch's and Fehling's tests, indicating a strong presence of reducing and non-reducing sugars.

This uniformity suggests carbohydrates are abundantly distributed and easily extractable across polar and non-polar solvents.

2. Glycosides: Baljet's test, a general test for glycosides, was universally positive for all extracts and plants.

Borntrager's and Legal's tests showed variations. For example, Borntrager's test (indicative of anthraquinone glycosides) was negative in some non-polar solvents like chloroform in C. tamala, suggesting solvent polarity significantly affects glycoside extraction.

P. Acidus and M. longifolia showed more consistent glycoside presence compared to C. tamala.

3. Fixed Oils and Fats: All three plants tested positive for fixed oils in most solvents (especially in non-polar ones like chloroform and ethyl acetate).

Notably, C. tamala had a negative result in the saponification test with ethanol, water, and hydroalcoholic extract, possibly due to low oil content or interference in the reaction medium.

4. Proteins and Amino Acids: Biuret test results varied, with P. acidus showing negative results in chloroform and hydroalcoholic extracts, unlike C. tamala and M. longifolia, which had more widespread protein presence.

This suggests that proteins in P. acidus may be less extractable or present in lower concentrations in certain solvent systems.

5. Saponins: All three species tested positive in the foam test in most solvents, indicating their potential as surfactants or emulsifying agents due to saponin content.

Hydroalcoholic extracts sometimes showed reduced detectability, possibly due to saponin solubility preferences.

6. Phenolic Compounds and Tannins: Consistently present across all solvent systems in all three plants, indicating their abundance and solubility in both polar and non-polar solvents.

Their universal presence supports the antioxidant potential of the plants.

7. Steroids: Detected in most ethanol and aqueous extracts, but not consistently in chloroform or hydroalcoholic ones.

C. tamala was least positive in this category, showing limited detection in non-polar and mid-polar solvents.

8. Alkaloids: Detected consistently across the plants using Dragendorff's, Mayer's, and Wagner's tests

However, some solvents failed to show positive results in specific plants (e.g., Wagner's in water for P. acidus), possibly due to alkaloid precipitation or masking by other compounds.

9. Terpenes: Present in most extracts of all plants except water extracts, indicating they are predominantly soluble in non-polar to moderately polar solvents.

10. Flavonoids: All three plants showed strong flavonoid presence across all solvent systems and tests (Lead acetate, conc. H_2SO_4, $FeCl_3$).

This indicates robust antioxidant and potential anti-inflammatory properties.

Phytochemical evaluation of Phyllanthus acidus leaves extract

S. No.	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
1	Carbohydrate	*Molisch's test*	+	+	+	+	+
		Fehling's test	+	+	+	+	+
2	Glycosides	*Legal's test*	-	+	+	+	-
		Borntrager's test	+	-	+	+	+
		Baljet test	+	+	+	+	+
3	Fixed oil and Fats	*Spot test*	+	+	+	+	-
		Saponification test	+	+	+	+	+
4	Proteins & Amino acids	*Biuret test*	-	+	+	+	-
5	Saponins	*Foam test*	+	+	+	+	+
6	Phenolic Comp.& Tannins	*$FeCl_3$ test*	+	+	+	+	+
7	Steroids	*Libermann-bucchard test*	+	-	+	+	-
8	Alkaloids	*Dragendorff's test*	-	+	+	+	+
		Mayer's test	+	+	+	+	+

		Wagner's test	+	+	+	-	+
9	Terpines		+	-	+	+	+
10	Flavonoids	*Lead acetate test*	+	+	+	+	+
		Con. H_2SO_4 test	+	+	+	+	+
		FeCl3 test	+	+	+	+	+

Phytochemical evaluation of Cinnamomum tamala leaves extract

S. No.	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
1	Carbohydrate	*Molisch's test*	+	+	+	+	+
		Fehling's test	+	+	+	+	+
2	Glycosides	*Legal's test*	+	+	+	+	+
		Borntrager's test	-	-	+	+	+
		Baljet test	+	+	+	+	+
3	Fixed oil and Fats	*Spot test*	+	+	+	+	+
		Saponification test	+	+	-	-	-
4	Proteins and Amino Acids	*Biuret test*	+	+	+	+	+
5	Saponins	*Foam test*	+	+	+	+	+
6	Phenolic Comp. and Tannins	*$FeCl_3$ test*	+	+	+	+	+
7	Steroids	*Libermann-bucchard test*	-	+	-	+	-
8	Alkaloids	*Dragendorff's test*	+	+	+	+	+
		Mayer's test	-	+	+	+	+
		Wagner's test	+	+	+	+	+
9	Terpines		+	+	+	+	-
10	Flavonoids	*Lead acetate test*	+	-	+	+	+
		Con. H_2SO_4 test	+	+	+	+	+
		FeCl3 test	+	+	+	+	+

Phytochemical evaluation of Madhuca longifolia leaves extract

S. No.	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Water	Hydroalcoholic (1:1)
1	Carbohydrate	*Molisch's test*	+	+	+	+	+
		Fehling's test	+	-	+	+	+
2	Glycosides	*Legal's test*	+	+	-	+	+
		Borntrager's test	+	+	+	+	-
		Baljet test	+	+	+	+	+
3	Fixed oil and Fats	*Spot test*	+	+	-	+	+
		Saponification test	+	+	+	+	-
4	Proteins and Amino Acids	*Biuret test*	+	-	+	+	+
5	Saponins	*Foam test*	+	+	+	+	-
6	Phenolic Comp. and Tannins	*$FeCl_3$ test*	+	+	+	+	+
7	Steroids	*Libermann-bucchard test*	-	+	+	+	+
8	Alkaloids	*Dragendorff's test*	+	+	+	+	+
		Mayer's test	+	+	-	+	+
		Wagner's test	+	+	+	+	+
9	Terpines		+	-	+	-	+
10	Flavonoids	*Lead acetate test*	-	+	+	+	+
		Con. H_2SO_4 test	+	+	+	+	+
		FeCl3 test	+	+	+	+	+

Conclusion:

The comprehensive presence of carbohydrates, glycosides, alkaloids, flavonoids, and other phytochemicals across the extracts of Phyllanthus acidus, Cinnamomum tamala, and Madhuca longifolia validates their ethnomedicinal uses. This preliminary screening provides a foundation for further pharmacological and quantitative studies aimed at isolating bioactive compounds for therapeutic applications. All three plants demonstrated rich phytochemical diversity, supporting their wide use in traditional medicine. Polar solvents (ethanol, water, hydroalcoholic) tended to extract a broader range of phytoconstituents, especially phenolics, flavonoids, and glycosides. Non-polar solvents like chloroform were more selective, showing limited extraction in some cases (e.g., saponification in Cinnamomum tamala and flavonoids in Madhuca longifolia). Hydroalcoholic extracts offered a good balance between polar and non-polar compound extraction, making them especially valuable for medicinal formulation purposes.

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