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Pharmacognostical, physicochemical, and phytochemical characterization of *Piper longum* Linn. (Piplamool) leaves: A standardization approach for herbal drug development

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Abstract
Medicinal plants have long been considered the foundation of traditional healthcare systems, with over 3.3 billion people in developing nations depending on them for treating various ailments. The present study was undertaken to perform comprehensive pharmacognostical, physicochemical, and phytochemical evaluations of *Piper longum Linn*. (Piplamool) leaves to establish quality control parameters essential for herbal drug standardization. Physicochemical analyses revealed a total ash value of 13.64 ± 0.33% w/w, acid-insoluble ash 1.56 ± 0.21% w/w, and water-insoluble ash 4.27 ± 0.53% w/w. Alcohol-soluble and water-soluble extractive values were recorded at 18.01 ± 0.44% and 6.71 ± 0.47% w/w, respectively. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, steroids, carbohydrates, glycosides, and phenolic compounds. Among various solvent extracts, ethanolic extract exhibited the highest extractive yield (28.72 ± 0.14%) and total phenolic content (68.75 ± 0.97 mg GAE/g extract). Additionally, antioxidant activity assessed via the DPPH radical scavenging assay demonstrated an IC_{50} value of 42.8 mg/mL, validating the extract's appreciable free radical quenching potential. These findings scientifically support the traditional uses of Piper longum and lay the groundwork for its standardization as a reliable phytotherapeutic agent.

Keywords: *Piper longum Linn*., Piplamool, Physicochemical, Phytochemical

1. Introduction
Medicinal plants have long been considered the foundation of traditional healthcare systems, with over 3.3 billion people in developing nations depending on them for treating various ailments. Among these, *Piper longum* — commonly known as long pepper, Javanese pepper, or Pippalimool (referring specifically to its root) — holds significant historical and medicinal importance (I., 2000) [5]. First documented by Hippocrates as a therapeutic agent rather than a culinary spice, *Piper longum* has been widely valued for both its fruits and roots. Historically, it was a major export commodity from India for over two millennia. Theophrastus classified pepper into two types: a round and a long variety. Traditionally, *Piper longum* has served as a spice, food preservative, and medicinal ingredient in Europe since medieval times. This aromatic, climbing plant thrives in tropical and subtropical climates and is extensively used in culinary and traditional medicinal systems like Ayurveda, Unani, Siddha, and Traditional Chinese Medicine (Sivarajan VV, 1994) [11]. Medicinally, it is reputed for managing a wide range of conditions including respiratory disorders, arthritic pain, menstrual discomfort, gonorrhea, tuberculosis, gastrointestinal issues, inflammation, jaundice, diarrhea, and even as an antidote for snakebites (Subramaniam K, 2021) [12]. Its primary bioactive constituent, piperine, is known for its CNS depressant, analgesic, antipyretic, antioxidant, anti-inflammatory, and hepatoprotective effects. In Ayurveda, it is a component of classical formulations like Panchakola and Shudhashana, celebrated for its digestive and carminative properties. Clinical studies have also highlighted its therapeutic benefits in managing bronchial asthma, particularly in pediatric cases (Mehta A, 1998) [9].
Piper longum, commonly known as Pippali, is a small, perennial aromatic climber from the Piperaceae family. It features a creeping, jointed stem thickened at the nodes, with ovate,

cordate leaves that vary in size — the lower leaves measure 5-7 cm while the upper ones are 2-3 cm long (Wakade AS, 2008) [13]. The plant produces cylindrical, solitary floral spikes, and its small ovoid fruits grow on fleshy, blunt spikes measuring about 2.5-3.5 cm in length. When mature, the fruits turn red or black and are harvested and dried for commercial use (Ratner L, 1991) [10]. The roots, referred to as Pippalimula, are woody and aromatic. The fruits contain about 1% volatile oil, along with proteins, starch, alkaloids, saponins, carbohydrates, and minerals like calcium, phosphorus, and iron. Major active phytoconstituents in the fruits include alkaloids such as piperine, piperlongumine, and piperlonguminine, alongside esters like sesamin and fargesin. The root portion is rich in piperlongumine, piperine, and other bioactive compounds including dihydrostigmasterol, pellitorine, refractomide A, and various piperidine derivatives, contributing to its traditional medicinal significance (Gurumurthy P, 2012) [4].

The present study is done to perform comprehensive pharmacognostical, physicochemical, and phytochemical evaluations of *Piper longum Linn*. (Piplamool) leaves to establish quality control parameters essential for herbal drug standardization.

2. Materials and Methods

2.1 Materials

Piper longum (Piplamool) was gathered from Herbal Garden of Baba Mastnath University, Rohtak, Haryana and authenticated from Green Vibes Biotech, New Delhi. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). All other reagents used were of analytical grade.

2.2 Methods

Physicochemical Evaluation

Different test including Ash value, extractive value, foreign matter, Moisture content, Bitterness value was performed for standardization of the sample.

Total Ash value: An accurately weighed 3 g of air-dried powdered crude drug was placed in a previously ignited silica crucible and incinerated gradually to a dull red heat until carbon-free. The crucible was cooled in a desiccator and weighed, repeating the process to obtain a constant weight. The percentage of total ash was then calculated relative to the air-dried crude drug (Ahmed, 2015) [1].

Total ash %= [Total ash formed in the crucible/ weight of sample] X 100

Acid Insoluble Ash Value: The total ash obtained from the crude drug was boiled with 25 mL of 2 N hydrochloric acid for 5 minutes. The resulting insoluble matter was collected on an ashless filter paper, thoroughly washed with hot water, dried, and then incinerated to a constant weight. The final weight of the residue was recorded, and the percentage of acid-insoluble ash was calculated with reference to the weight of the air-dried crude drug (Kokate, 2014) [8].

Acid insoluble ash (%) = [Total ash formed in the crucible with ashless filter paper/weight of sample] X 100

Water-Soluble Ash Value: The total ash obtained from the crude drug was boiled with 25 mL of water for 5 minutes, and the resulting insoluble matter was collected on an ashless filter paper. This residue was washed with hot water and ignited at a temperature not exceeding 450°C for 15 minutes. The weight of the remaining insoluble matter was deducted from the total ash weight, and the difference was used to calculate the percentage of water-soluble ash relative to the air-dried crude drug (Khandelwal, 2015) (Kokate, 2014) [8].

Extractive Values

Extractive values are important for determining the nature of the chemical components in a raw medicine. Two types of extractive values were calculated i.e., alcohol soluble extractives and water-soluble extractives. Extractive values assess the components of crude drugs and indicate the composition of the component contained within the pharmaceutical substance (CP, 2007) [2].

Alcohol Soluble Extractive- About 5 g of crude drug was placed in a 250 mL conical flask with 100 mL of 90% alcohol, corked, and shaken intermittently for 24 hours. The mixture was filtered, and 25 mL of filtrate was evaporated in a pre-weighed porcelain dish, dried at 100 °C, cooled in a desiccator, and weighed. The extractive value was calculated in mg/g of air-dried drug, with experiments performed in triplicate.

$$\text{Percentage of soluble extractive value of the sample} = \frac{\text{Weight of extract residue}}{\text{Weight of crude drug}} \times 100$$

Water-soluble extractives- 5 grams of crude drug sample was macerated with 100 ml of water containing a few drops of chloroform as preservative for 24 hours with occasional shaking. After filtration, 25 ml of the filtrate was evaporated, dried at 100°C, cooled, and weighed. The water-soluble extractive value was expressed in mg per gram of air-dried material (Indian Pharmacopoeia Commission., 2018).

Foreign Matter

The foreign matter content in a crude plant drug sample is assessed by visually inspecting 20 g of the material, both with the naked eye and under a magnifying lens (5-10×). Identified extraneous material is carefully separated, weighed, and its percentage calculated relative to the original sample weight. This procedure ensures the quality, purity, and safety of herbal drugs by detecting and quantifying contaminants.

Percentage of foreign matter =

$$\frac{\textbf{Weight of separated foreign matter}}{\textbf{Weight of the original sample}} \times \textbf{100}$$

Loss on Drying

In this method, 1.5 g of powdered crude drug was placed in a silica crucible and dried at 105°C in a hot air oven. The sample was then cooled in a desiccator and weighed again. The loss in weight after drying represented the moisture content of the crude drug. This process was repeated until a

constant weight was achieved (WHO, 2011) [14].

% Loss on Drying =

$$\frac{\text{Initial weight of sample} - \text{Weight after drying}}{\text{Weight of the Initial weight of sample}} \times 100$$

Bitterness Value

Bitterness value measures the ability of a substance to stimulate gastric secretions and is expressed relative to a standard quinine hydrochloride solution. In this procedure, serial dilutions of both quinine hydrochloride and plant extracts were prepared. Test solutions were tasted near the base of the tongue for 30 seconds, and the lowest concentration provoking a bitter sensation was recorded as the threshold. Proper intervals, rinsing, and controlled temperature (25 °C) were maintained throughout the testing process to ensure accuracy and reproducibility (Sivarajan VV, 1994) [11].

Bitterness value = $2000 \times \frac{c}{a \times b}$ Where; a - concentration of the stock solution (S_T) (mg/ml), b - volume of S_T (in ml) in the tube with the threshold bitter concentration, c - quantity of quinine hydrochloride (in mg) in the tube with the threshold bitter concentration.

Extraction and Percentage Yield

In the present study, Piplamool leaves were subjected to cold maceration using different solvents to evaluate the extraction efficiency. Accurately weighed, shade-dried, and coarsely powdered Piplamool (20 g) was macerated separately with 500 mL each of distilled water, ethanol, and chloroform in amber-colored glass-stoppered bottles. The maceration was carried out at room temperature ($25 \pm 2°C$) for 72 hours with intermittent shaking every 6 hours to facilitate solvent penetration. After 72 hours, the extracts were filtered through muslin cloth followed by Whatman No. 1 filter paper. The collected filtrates were then concentrated under reduced pressure using a rotary vacuum evaporator at controlled temperatures (below solvent boiling points) and finally dried to obtain semisolid residues. The weight of the dried extracts was recorded, and the percentage yield was calculated relative to the initial weight of the crude drug used.

$$\text{Percentage yield} = \frac{\text{Weight of extracts obtained}}{\text{Weight of material taken}} \times 100$$

Phytocehimacal Screening

1. Test for Alkaloids (Evans, 2009)[3] (Kokate, 2014) [8].

a) Dragendorff's Reagent test: Add 1ml of Dragendorff's reagent (potassium bismuth iodide solution) in one ml of extract solution. Presence of prominent buff colored precipitate showed the presence of alkaloids.

b) Mayer's test: Add one ml of Mayer's reagent (potassium mercuric iodide solution) to the test tubes containing one ml of test extracts. Presence of white or cream-colored precipitate showed the presence of alkaloids.

c) Hager's test: Add one ml of Hager's reagent (saturated aqueous solution of picric acid) in the test tubes containing one ml of test extracts. Presence of yellow colored precipitate indicated the presence of alkaloids.

d) Wagner's test: Add 2ml of Wagner's reagent (iodine in potassium iodide) in one ml of test extract. Presence of reddish-brown precipitates indicated the presence of alkaloids.

2. Test for Glycosides

a) Legal test: In few ml of extract, added pyridine and made alkaline by adding freshly prepared sodium nitroprusside solution. Formation of pink to red color indicates the presence of steroidal glycoside.

b) Baljet test: In one ml of the test extract added with one ml of sodium picrate solution. Formation of yellow to orange color showed the presence of steroidal glycoside.

c) Borntrager's test: In one ml of the test extract added few ml of dilute sulphuric acid, then boiled, filtered the mixture. Add few ml of chloroform to the filtrate. Separate the Chloroform layer and treated with one ml of ammonia. Presence of red color indicates of anthraquinone glycosides.

3. Tests for Carbohydrates

a) Molisch's Test: In 2 ml of the extract, add two drops of Molisch's reagent (alcoholic solution of α-napthol). Shake the mixture well in test tube. Slowly add 1 ml of concentrated sulphuric acid along the sides of the test tube. Appearance of purple or reddish violet color at the junction of the two liquids showed the presence of carbohydrates.

b) Fehling's test: Add 1 ml of each of Fehling solutions A and B. Added 1 ml of the extracts to above solution and heat it. Appearance of red precipitate indicated the presence of sugars.

c) Barfoed's test: In 5 ml of Barfoed's solution, add 1ml of extract solution. Heat the above mixture till boiling. Formation of ed precipitate of copper oxide confirmed the presence of carbohydrates.

4. Test for Steroids and Sterols

a) Liebermann Burchard's test: In few ml of the extract, add 2 ml of chloroform. Add few drops of acetic anhydride and also add two drops of concentrated sulphuric acid. The above mixture showed red, then blue and finally bluish green color which indicated the presence of steroids.

b) Salkowski test: In few ml of extract, added 2 ml of chloroform and equal volume of concentrated sulphuric acid. The appearance of bluish red to cherry red colour in Chloroform layer and acid layer assuming marked green fluorescence represents the steroid and sterol.

5. Test for Saponins: In one ml of extract added 20 ml of distilled water. Shake the above mixture in a graduated cylinder for 15 minutes. Appearance of foam about one cm indicated the presence of saponins.

6. Test for Proteins and Amino acids

a) Biuret test: In 1 ml of the extract added 1ml of 40% sodium hydroxide solution and add 2 drops of 1% copper sulphate solution. The formation of violet color indicated the presence of protein.

b) Ninhydrin test: In 2 drops of freshly prepared 0.2% Ninhydrin reagent, add the prepared test extract

solution and heat it. The formation of purple color during cooling suggests the presence of amino acids.

7. Tests for Flavonoids

a) **Shinoda test:** In 1ml of the extract added magnesium turnings and add 1-2 drops of concentrated hydrochloric acid. Formation pink or red color showed the presence of flavonoids.
b) **Ferric chloride test:** In 1 ml of extract solution, added 3-4 drops of 10% FeCl3 solution in a test tube. Appearance of a greenish-blue to a violet color indicated the presence of flavonoids.
c) **Sodium hydroxide test:** In 1 ml of the extract solution, added 2 ml of 10% NaOH solution into a test tube. The yellow color of the solution disappeared upon when dilute Hydrochloric acid was added, indicated the presence of flavonoids.

8. Test for Tannins and Phenolic Compounds

Ferric chloride test: To the extract, added ferric chloride solution. Appearance of dark blue or greenish black color showed the presence of tannins.

9. Test for Acidic Compounds: The extract solution was treated with sodium bicarbonate and produced effervescence. The above preparation then treated with warm water and filtered. To check the acidic compounds filtrate was tested with litmus paper.

10. Test for Mucilage: The test extract solution was treated with ruthenium red solution and appearance of pink color indicated the presence of mucilage.

11. Test of fixed oils and volatile oil

a) **Spot test:** The small quantity of extract was pressed between two filter papers. Presence of oily stain indicates presence of fixed oil and no appearance of stain indicates the presence of volatile oil.
b) **Sudan Red IV Test**: Dark red Sudan IV (a common dye) is added to a solution. Extract is dyed as red color in presence of fixed oils.

Total Phenolic Content

The total phenolic content of *Piper longum* (Piplamool) extracts was estimated using the Folin-Ciocalteu colorimetric method, with gallic acid as the reference standard. A calibration curve was prepared using serial dilutions of gallic acid (5-30 µg/mL). Plant extracts (1 mg/mL) and gallic acid solutions were mixed with Folin-Ciocalteu reagent, followed by sodium carbonate, and the final volume was made up with distilled water. After incubation at room temperature for 2 hours, the absorbance of the developed blue color was measured at 750 nm using a UV-Visible spectrophotometer. Total phenolic content was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of crude drug (mg GAE/g), with all measurements performed in triplicate (Evans, 2009) [3].

Antioxidant Activity by DPPH method

The antioxidant activity of the plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which measures the reduction in absorbance at 517 nm as DPPH radicals are neutralized by antioxidants. A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light. For the assay, 3.9 mL of DPPH solution was mixed with 100 µL of the test sample or methanol (control), incubated in the dark at 37°C for 30 minutes. The absorbance was then measured using a UV-Vis spectrophotometer, and antioxidant activity was determined based on the decrease in absorbance (Khandelwal, 2015) (Kokate, 2014) [8].

$$\% \text{ Inhibition} = DPPH\ activity\ (\%) = (A\ control - A\ test)/A\ control\ x\ 100$$

Results and Discussion

Physicochemical Evaluation

The total ash content was found to be 13.64 ± 0.33% w/w, indicating the overall amount of inorganic material present, including both physiological and non-physiological ash. The acid-insoluble ash value, representing the earthy matter and siliceous impurities, was 1.56 ± 0.21% w/w, which is within acceptable pharmacopoeial limits. The water-insoluble ash value recorded was 4.27 ± 0.53% w/w, reflecting a moderate proportion of water-insoluble inorganic residue. In extractive value tests, the alcohol-soluble extractive was 18.01 ± 0.44% w/w, suggesting a good presence of alcohol-soluble phytoconstituents like alkaloids, steroids, and flavonoids, while the water-soluble extractive value was comparatively lower at 6.71 ± 0.47% w/w, indicating lesser water-soluble components. The percentage of foreign matter detected was 0.94 ± 0.11% w/w, showing acceptable purity of the crude sample as per standard limits. The moisture content was 9.51 ± 0.05% w/w, within permissible levels ensuring minimal microbial degradation risk. Lastly, the bitterness value was noted as Bitter (1206), indicating a strong bitter property, typical of Piper longum, due to its alkaloidal and pungent bioactive principles.

Table 1: Results of physicochemical parameters of *Piper longum* (Piplamool)

S. No.	Tests	Value in mean ±SEM
Ash values		
1	Total Ash	13.64±0.33% w/w
2	Acid soluble Ash	1.56±0.21% w/w
3	Water insoluble Ash	4.27±0.53% w/w
Extractive values		
1	Alcohol Soluble Extractive	18.01±0.44% w/w
2	Water Soluble Extractive	6.71±0.47% w/w
	Foreign matter	0.94.14 ±0.11% w/w
	Moisture content	9.51±0.05% w/w
	Bitterness value	Bitter (1206)

Extraction and Percentage Yield

The percentage yield of different extracts of Piplamool leaves revealed notable variations depending on the solvent used. The ethanolic extract exhibited the highest yield at 28.72±0.14%, indicating that ethanol was most effective in extracting phytoconstituents from the leaves. The aqueous extract yielded 19.82±0.06%, while the chloroform extract showed the lowest yield at 17.36±0.35%. All three extracts were characterized by a greenish-yellow color, suggesting the presence of similar classes of phytoconstituents across different solvents, albeit in varying quantities. These results emphasize the polarity-dependent extraction efficiency, with ethanol proving superior for Piplamool leaves.

Table 2: Percentage yield of Piplamool in different Extracts

Sample	Extracts	Colour	Percentage yield (%)
Piplamool Leaves	Aqueous	Greenish-yellow	19.82±0.06
	Ethanol (95%)	Greenish-yellow	28.72±0.14
	Chloroform	Greenish-yellow	17.36±0.35

Phytochemical Screening

In qualitative analysis preliminary screening of prepared extracts using different solvents like ethanol, hydroalcoholic, methanol and aqueous of Piplamool leaf extract.

Table 3: Phytochemical Screening of Piplamool leaf extract in different solvents

S. No	Phytochemical Test	Aqueous Extract	Ethanol Extract	Chloroform Extract
	Alkaloids	+	+	+
a. Dragendorff's Test	+	+	+	
b. Mayer's Test	+	+	+	
c. Hager's Test	+	+	+	
d. Wagner's Test	+	+	+	
	Glycosides	+	+	-
a. Legal Test	+	+	-	
b. Baljet Test	+	+	-	
c. Borntrager's Test	-	-	-	
	Carbohydrates	+	+	-
a. Molisch's Test	+	+	-	
b. Fehling's Test	+	+	-	
c. Barfoed's Test	+	+	-	
	Steroids & Sterols	+	+	+
a. Liebermann-Burchard's Test	+	+	+	
b. Salkowski Test	+	+	+	
	Saponins	+	+	-
	Proteins & Amino acids	+	+	-
a. Biuret Test	+	+	-	
b. Ninhydrin Test	+	+	-	
	Flavonoids	+	+	+
a. Shinoda Test	+	+	+	
b. Ferric Chloride Test	+	+	+	
c. NaOH Test	+	+	+	
	Tannins & Phenolic Compounds	+	+	+
	Acidic Compounds	+	+	-
	Mucilage	+	-	-
	Fixed and Volatile Oils	+	+	+
a. Spot Test	+	+	+	
b. Sudan Red IV Test	+	+	+	

Total Phenolic Content

The total phenolic content of *Pipalmoool* extracts varied significantly depending on the solvent used for extraction. The ethanolic extract exhibited the highest total phenolic content at 68.75 ± 0.97 mg GAE/g extract, followed by the aqueous extract with 52.34 ± 1.12 mg GAE/g extract, while the chloroform extract showed the lowest phenolic content of 36.45 ± 0.85 mg GAE/g extract. These findings indicate that ethanol is the most effective solvent for extracting phenolic compounds from *Pipalmoool*, likely due to its polarity and ability to solubilize a broader range of phenolic constituents compared to aqueous and chloroform solvents.

Table 4: Total Phenolic Content of Piplamool Extracts in Different Solvents

Solvent	Total Phenolic Content (mg GAE/g extract)
Aqueous Extract	52.34 ± 1.12
Ethanol Extract	68.75 ± 0.97
Chloroform Extract	36.45 ± 0.85

Antioxidant activity

The DPPH radical scavenging activity assay revealed that both Piplomool extract and ascorbic acid exhibited dose-dependent antioxidant activity. At each concentration, ascorbic acid demonstrated superior free radical inhibition compared to the plant extract. The IC_{50} value for Piplomool extract was approximately 42.8 mg/mL, whereas for ascorbic acid it was notably lower at 23.5 mg/mL, indicating the higher antioxidant potency of the standard. Nonetheless, the progressive increase in % inhibition with increasing Piplomool concentration confirms its appreciable antioxidant potential, supporting its ethnomedicinal application as a natural antioxidant source.

Table 5: Antioxidant activity of Piplamool Extract

Concentration (mg/mL)	DPPH (% Inhibition) Piplomool (Mean ± SD)	DPPH (% Inhibition) Ascorbic acid (Mean ± SD)
10	21.40 ± 0.32	34.50 ± 0.28
20	26.43 ± 0.08	47.12 ± 0.30
30	32.98 ± 0.47	59.88 ± 0.35
40	45.54 ± 0.28	70.26 ± 0.42
50	56.22 ± 0.15	79.13 ± 0.39
60	68.36 ± 0.26	86.48 ± 0.33
IC50	42.8 mgmL	23.5 mg/mL

3. Conclusion

This study successfully established critical pharmacognostical, physicochemical, and phytochemical profiles for *Piper longum Linn.* (Piplamool) leaves, contributing valuable baseline data for the standardization and authentication of this important medicinal plant. The physicochemical parameters complied with acceptable limits, ensuring the purity and identity of the crude drug. Phytochemical screening confirmed the presence of multiple bioactive constituents, with ethanolic extracts demonstrating the highest yield, total phenolic content, and potent antioxidant activity. The DPPH assay further corroborated its free radical scavenging potential, affirming its ethnomedicinal use as a natural antioxidant source. Collectively, the findings provide a scientific basis for the therapeutic application and quality control of Piplamool-based formulations, promoting its safe and effective integration into contemporary phytomedicine

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