



PHYTO-CHEMICAL SCREENING AND STANDARDISATION OF LEAF PART OF THE TREE PLANT *LEUCAENA LEUCOCEPHALA* OF SIKKIM HIMALAYAN REGION

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ABSTRACT

The medicinal plants are sources of important therapeutic aids for alleviating human health and improving the quality of life. The present research study was undertaken with an objective to investigate the phyto-chemical and standardization of the leaf part of the plant- *Leucaena leucocephala lam.* of Sikkim Himalayan region. It is locally called *Subabul* in hindi, an ethno-medicinally important plant belonging to the family-Fabaceae. The phyto-chemical analysis was performed by using different types of solvents system ranging from non-polar to polar solvents in the well equipped laboratory and revealed the presence of maximum phyto-constituents in the leaf part of the plant such as Alkaloids, Sterols, Tannins, Proteins, Terpenoids, Carbohydrates, flavenoids, Sugar Moieties and Cardiac glycoside. On other hand, the loss on drying of the powder of *Leucaena leucocephala* (Lam.) was found to be 9.8% w/w. The ash value of powder leaf was determine as total ash, water soluble ash and acid insoluble ash was found to be 17.33%, 7.73% and 6.8% respectively. The Extractive Value was found to be 1.82%, 1.6%, 1.83% and 0.83% in Hexane, chloroform, Ethyl acetate and Methanol extract.

KEYWORDS: Standardization, Phytochemical analysis, *Leucaena leucocephala*, Sikkim Himalayan region.

1. INTRODUCTION

Plants have been associated with the human health from time immemorial and they are the important sources of medicines since the dawn of human civilization.^[1] Phyto-chemical screening refers to the extraction, screening and identification of the medicinally active substances found in plant. Some of the active compound found in plants is flavonoids, alkaloids, tannins, carotenoids, antioxidants and phenolic compound. Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert component by using selective solvents in standard extraction procedure. The so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.^[2] The evaluation of crude drug means confirmation of its identity and determination of its quality and purity.

Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious important that different species plant

would have different chemical constituents. However, these differences can extend to different varieties or even the same variety grown in different location or harvested at different time. Phytochemical screening of plant is essential to study the pharmacological study. It can be done by qualitative chemical analysis using specific reagents for specific constituents followed by confirmation with different chromatographic techniques like TLC, HPLC, HPTLC, Gas chromatography etc. Hence, the plant authentication and phytochemical evaluation of the crude drugs is an essential criteria, before proceeding for its pharmacological and toxicological studies. Standardisation is the process of evaluating the quality and purity of crude drugs by various parameters like morphological, physical, chemical and biological observations is called standardisation. A standardised extract means that the manufacturer has verified that the active ingredient believed to be present in the herb is present in the preparation and that the potency and the amount of the active ingredients are assured in the preparation.^[3]

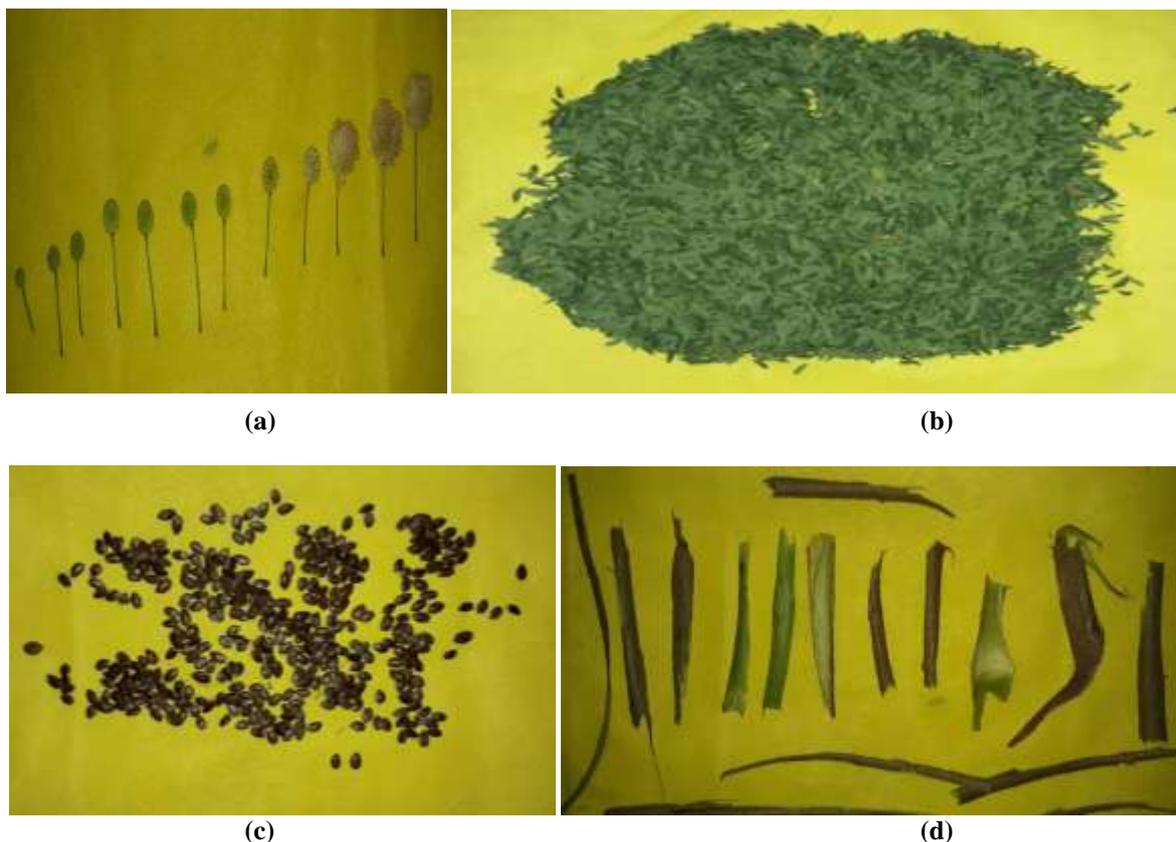


Fig. 1: (a)flowers; (b)leaves; (c)seeds; (d)bark.

2. MATERIALS AND METHODS

2.1 Collection of plants

The leaves of matured *Leucocaena leucocephala* were collected from Bardang, Singtam of Himalayan region, East Sikkim. Collected leaves were washed with running tap water in order to remove the dirt and soil and kept for sun drying for almost 2 hours and then further kept for shade drying until the constant weight is achieved and ground to a powder with the help of an electric blender.^[4]

2.2 Preparation of Plant Extract

- To 50gm of leaf powder, 250ml of each solvent, viz. hexane, chloroform, ethyl acetate and methanol was added serially for preparing extract in increasing solvent polarity.
- Extraction with each solvent was done for three days at room temperature.
- After that the supernatant of each solvent was recovered by filtering through Watmann filter paper.
- This process was done thrice and the respective solvent from the supernatant was evaporated in the rota vapour to obtain crude extract which are to be stored in 4°C until used for evaluation. [Coded as I-hexane extract, II- chloroform, III-Ethyl acetate, IV-Methanol respectively].



Fig. 2: Powdered leaves.

3. Preliminary Phytochemical Screening

The chemical tests for each individual constituent are discussed below one by one.^[5-7]

3.1 Test for alkaloids

i) Mayer's test: To 2ml of the plant extract, 2ml of hydrochloric acid was added. Few drops of the Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

ii) Dragendroff's test: To 2ml of the plant extract, 2ml of hydrochloric acid was added. Add few drops of Dragendroff's reagent. The sample will react with reagent and produce orange or orange red precipitate which indicates the presence of alkaloids.



Fig. 3: Test for alkaloids: (a) Dragendorff's Test; (b) Meyer's Test.

3.2 Test for sterol

i) Salkowski test

A few milligrams of plant extract was dissolved in chloroform and then 2ml of conc. Sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. Red layer development in the layer of chloroform layer indicates the presence of sterols.

ii) Lieberman Burchardt test

Chloroform solution of the extract with few drops of acetic anhydride and 1ml of conc. H₂SO₄ from the side gives reddish ring at the junction of two layers.



Fig. 4: Test for Sterol.

3.3 Test for tannins

i) Ferric chloride test

The test sample of each reagents were taken separately in water, warmed and filtered. To a small volume this filtrate, a few drop of 5% W/W solution of ferric chloride prepared in 90% alcohol were added. Appearance of dark green colour or deep blue colour indicated the presence of tannins.

ii) Gelatin test

Extracts are mixed with few drops of 1% solution of gelatin containing 10% sodium chloride which gives white precipitate.



Fig. 5: Ferric Chloride test for Tannins.

3.4 Test for proteins

A few milligrams of plant extracts was dissolved in 2ml of water and conc. HNO₃ was added in it. Yellow colour indicates the presence of protein.



Fig. 6: Test for Protein.

3.5 Test for sugars

About 0.5 gm of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volume of Fehling's solution A and B. Formation of red precipitate of cuprous oxide was in indication of the presence of reducing sugars.

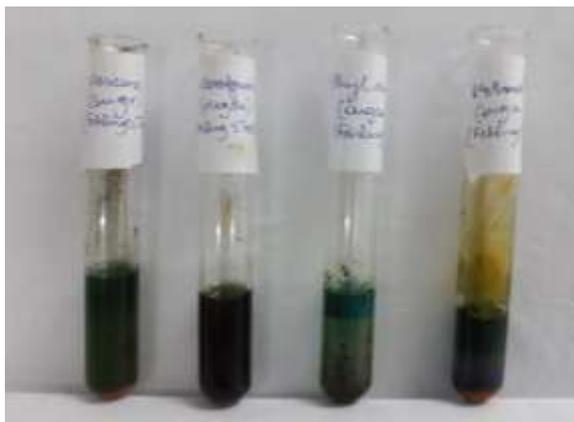


Fig. 7: Test for Sugar.

3.6 Test for flavonoids

About 0.5 gm of each extract was boiled with 5ml of distilled water and then filtered. To 2ml of this filtrate, add few drops of 10% ferric chloride solution was added. A green-blue colour or violet colour indicated the presence of a phenolic hydroxyl group.



Fig. 8: Test for Flavonoids.

3.7 Test for saponins

One gram of each extract was boiled with 5ml distilled water and filtered. To the filtrate, 3 ml of distilled water was further added and shaken vigorously for another five minutes. Frothing with persisted warming was taken as an evidence for the presence of saponins.



Fig. 9: Test for Saponin.

3.8 Test for anthraquinones

An aliquot of 0.5 gm of extract was boiled with 10ml of sulphuric acid (H_2SO_4) filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted out in another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for the colour change.

3.9 Test for terpenoids (Salkowski test)

To 0.5g of each extract, 2ml of chloroform was added, followed by further addition of 3ml of conc. Sulphuric acid to form a layer. A reddish brown coloration in the interface indicates the presence of terpenoids.

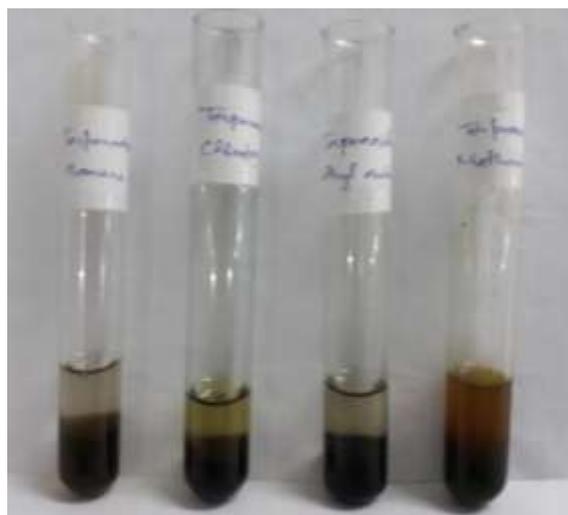


Fig. 10: Test for Terpenoids.

3.10: Test for cardiac glycosides (Keller – Killiani test)

To 0.5g of the extract diluted to 5ml of water, 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayered with 1ml of conc. Sulphuric acid. The brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. The violet ring may appear below the brown ring, while in the acetic acid layer greenish ring may form just above the brown ring and gradually spread over throughout the layer.

Using this methods, the presence of several phytochemicals sterols, tannins, proteins, sugars, and other secondary metabolites was evaluated.

3.11 Test for carbohydrates

i) Benedict's test

Take 1ml of test sample in dry test tube; add 2ml of *Benedict's reagent* to all the tubes. Keep in water bath for 5 mins, development of brick red precipitate occurs.

ii) Barfoed's test

Take 1ml of test sample, add 2ml of *Barfoed's reagent* to all the tubes and keep in boiling water bath. Look for the development of brick red precipitate.



Fig. 11: Test for Carbohydrates; (a) Benedict's test; (b) Barfoed's test.

4. Standardization of Powdered Leaves

4.1 Determination of Moisture Content

The moisture content was determined by taking 5gm of powdered drug in previously weighed dried petri dish and dried at hot air oven at 105-110⁰c, till the two consecutive weights was acquired. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % W/W with reference to the air-dried sample.^[8]

$$\begin{aligned} \text{Loss on drying} &= 58.97\text{gm} - 58.48\text{gm} \\ &= 0.49\text{gm} \end{aligned}$$

5gm of powder contains = 0.49gm of moisture.

$$\begin{aligned} \text{Percentage \% of moisture content} &= [0.49/5] 100 \\ &= 9.8\% \text{ W/W.} \end{aligned}$$

4.2 Extractive Values

The extractive values are used for the evaluation of crude drugs when they cannot be estimated by any other method. The extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.

4.2.1 Extractive value of Hexane

Weight of the powder taken = 50gm.
Yield of the product = 0.91gm
Percentage % yield = $[0.91/50] 100$
= 1.82%

4.2.2 Extractive value of Chloroform

Weight of the powder taken = 50gm.
Yield of the product = 0.8gm
Percentage% yield = $[0.8/50] 100$
= 1.6%

4.2.3 Extractive value of Ethyl-acetate

Weight of the powder taken = 48gm.
Yield of the product = 0.592gm
Percentage% yield = $[0.592/48] 100$
= 1.23%

Extractive value of Methanol

Weight of the powder taken = 48gm.
Yield of the product = 0.4gm
Percentage % yield = $[0.4/48] 100$
= 0.83%

4.3 Determination of ash value of a crude drug

Ash values are helpful to determine the quality as well as the purity of a crude drug, especially when the drug is present in powdered form.^[9]

They are determined by the following methods:

- Determination of the total ash.
- Determination of acid insoluble ash.
- Determination of water soluble ash.

4.3.1 Determination of total ASH

The powdered drug was weighed accurately about 5gm in silica crucible. The powdered drug was incinerated by increasing the heat (not exceeding 450⁰c) gradually until the sample was free from carbon and cooled. The weight of the ash was taken and the percentage of the total ash in contrast to the air dried sample was calculated.

Weight of empty crucible = 34.22gm.

$$\begin{aligned} \text{Weight of sample with crucible} &= [34.22+3] \\ &= 37.22\text{gm.} \end{aligned}$$

Weight of crude drug after incineration = 36.70gm

$$\begin{aligned} \text{Weight of total ash} &= 37.22-36.70 \\ &= 0.52\text{gm} \end{aligned}$$

3gm of crude drug gives = 0.52gm of total ash.

$$\begin{aligned} \text{Percentage \% of the crude drug} &= [0.52/3]100 \\ &= 17.33\% \text{ W/W.} \end{aligned}$$

4.3.2 Determination of acid insoluble ASH

The total ash obtained as above the procedure was boiled for 5 minutes and was mixed with 25ml of dilute HCL acid. The product was filtered and the insoluble matter was collected on a ash less filter paper, the filter paper was washed with hot water, ignited in tarred crucible at temperature not exceeding 450⁰c and cooled and was kept in a desiccators. The obtained residue was weighed and the acid insoluble ash of the crude drug was calculated with reference to the air dried drug.

The total ash taken = 0.38gm.

Amount of acid insoluble = 0.026gm.

$$\begin{aligned} \text{Percentage \% of acid insoluble ash} &= [0.026/0.38]100 \\ &= 6.8\% \text{ W/W.} \end{aligned}$$

*Acid insoluble ash value of crude drug is always less than the total Ash value of the same crude drug.

4.3.3 Determination of water soluble ASH

It is the difference in weight between the total ash and the residue obtained after boiling the total ash in the water.

The total ash content was boiled with water for 5 minutes. The water insoluble matter was collected in ash less filter paper and was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^oc. The weight of the insoluble matter from the weight of the total ash was subtracted and the difference

of the weight represented the water soluble ash. The percentage of the water soluble ash with reference to the air dried drug was calculated.

The total ash taken = 0.38gm

Weight of water insoluble ash = 0.086gm

Amount of water soluble ash = 0.38 - 0.086
= 0.294gm.

Percentage % of water soluble ash = $[0.294/0.38]100$
= 7.73% W/W.

5. Report

Table: 13.1: Preliminary photochemical analysis of powdered leaf of *leucaena leucocephala* (Lam.) is summarised below.

Phytochemical tested	Test Used	Hexane	Chloroform	Ethyl-Acetate	Methanol
Alkaloids	(i) Mayer's test	++	+	-	+
	(ii) Dragendroff's test	++	+	-	+
Sterols	(i) Salkowaski test	++	-	++	-
	(ii) Libermans Burchats test	++	-	++	-
Tannins	(i) Ferric chloride test	+	+++	++	+++
	(ii) Gelatin test	+	+++	++	+++
Proteins	(i) Xanthoproteic test	+	+	+	+++
Terpenoids	(i) Salkowaski test	+	+++	++	+++
Carbohydrates	(i) Benedicts test	++	-	+	++
	(ii) Barfoeds test	++	++	-	++
flavenoids	(i) Ferric Chloride test	-	++	++	+
sugar	(i) Fehling's Solution test	++	+	++	+++
Cardiac Glycoside	(i) Keller killani test	+	++	-	+++
saponin	(i) foam test	-	-	-	-

+ = Slightly Present, ++ = Moderately Present, +++ = Significantly present, - = Absence

Table 13.2: Standardization Report of proximately analysis.

Sl. No.	Experimental Studies	Observations for Powdered leaf part of crude drug- <i>Leucaena Leucocephala</i>
1.	Hexane Extractive Value	1.82% w/w
2.	Chloroform Extractive Value	1.6% w/w
3.	Ethyl Acetate Extractive Value	1.83% w.w
4.	Methanol Extractive Value	0.83% w/w
5.	Total Ash Value	17.33% w/w
6.	Acid-Insoluble Ash	6.8% w/w
7.	Water Soluble Ash	7.73% w/v
8.	Moisture Content	9.8% W/W

The above data was calculated at least three times to determine each standard parameter and express in mean value \pm 0.7 SEM.

6. CONCLUSION

The pharmacopoeial standards in Ayurvedic Pharmacopoeia of India are not adequate enough to ensure the quality of plant materials since the materials received in the manufacturing premises are not in a condition that effective microscopic examination can be done. Therefore chemical, methods, instrumental methods and then layer chromatographic analysis would determine the proper quality of plant material. Non standardized procedures of extraction may lead to the degradation of the phyto-chemical present in the plants

and may lead to the variations thus leading to the lack of reproducibility. Efforts should be made to produce batches with quality as consistent as possible (within the narrowest possible range) and to develop and follow the best extraction processes. Phytochemical Screening of Hexane, chloroform, ethyl acetate and methanolic extract of leaf of *Leucaena leucocephala* (Lam.) showed the presence of Alkaloid, Glycoside, Carbohydrate, tennins, Amino Acids, Sterols and Terpenoids but saponin is totally absent in leaf part of the plant.

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