

EVALUATION OF PHYSICOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF CHITRAKA (*Plumbago zeylanica* Linn.) - An Experimental Study

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ABSTRACT

The objective of the study is to evaluate the Physicochemical and Preliminary Phytochemical investigations and TLC test of Chitraka moola churna. The drug was purchased from Kajarekar pharmacy, Belgaum. It was authenticated by experts in this field and is used for the study. The physicochemical tests included determination of foreign matter, total ash, acid soluble, water soluble etc. The preliminary phytochemical tests include the tests for carbohydrate, protein, tannins, saponins etc. The results establish the standard of the drug sample. An attempt is made to standardize the drug Chitraka (*Plumbago zeylanica* linn.) which may be useful for future study.

KEYWORDS: Physicochemical, phytochemical, Chitraka, Moola churna.

1. INTRODUCTION

Chitraka (*Plumbago zeylanica* Linn.) is a well-known medicinal plant in Ayurveda and has been traditionally used for the management of various disorders.^{1,2} Its therapeutic value depends on the quality, purity, and chemical composition of the raw drug. Therefore, proper standardization is essential to ensure its safety, authenticity, and consistent therapeutic efficacy.^{3,4,5}

With the increasing use of herbal medicines, scientific evaluation has become necessary to establish quality standards. Physicochemical analysis, preliminary phytochemical screening, and thin-layer chromatography (TLC) are useful tools for assessing the identity and quality of medicinal plants.^{3,4} The present study was undertaken to evaluate these parameters in Chitraka root and generate baseline data that may support its quality control and future research.

2. STUDY DESIGN

The study was designed under the following heading,

- 1) Preparation of the drug.
- 2) Physicochemical analysis of Chitraka.
- 3) Preliminary phytochemical analysis of Chitraka.
- 4) TLC Test of Chitraka.

2.1) Preparation of the drug

a) Source of drug:

The genuine quality roots of Chitraka (*Plumbago zeylanica*) were purchased from Local Market Kolkata. A botanist and other experts verified the root and its identification were confirmed.

b) Purity, Identity and Strength:

For knowing the Purity, identity, strength and genuinity of the crude drug, it was subjected to physicochemical tests according to the API (Ayurvedic Pharmacopeia of India) standards and was confirmed.

c) Drying and Powdering :

The work was carried at Pharmacy and Dravyaguna Department of Belley Sankarpur Rajib Gandhi Memorial Ayurvedic College and Hospital, North 24 Parganas, West Bengal.

- i) The drug was subjected to powdering using pulverizer and 20 number sieves were used to get coarse powder of Chitraka. Then the powder was stored in an airtight container.
- ii) Fine powder of Chitraka was prepared using 120 number sieves and was stored in air tight container.

d) Preparation of Extracts:

Alcoholic extraction procedures of Chitraka were carried out in the Department of Belley Sankarpur Rajib Gandhi Memorial Ayurvedic College and Hospital, North 24 Parganas, West Bengal.

Alcohol extract:

Hot continuous extraction method was followed using soxhlet apparatus to get the alcohol extract of Chitraka. Then the powdered drug held in porous bag or a “thimble” made up of strong filter paper is placed in the sample chamber of the soxhlet. The suitable solvent is taken in a round bottom flask and the apparatus is assembled. When the mixture of the drug and the solvent is heated in a heating mantle, its vapours get condensed in the condenser and drip on the thimble containing the crude drug, extracting it by contact. When the level of the liquid in the sample chamber rises to the top of the siphon tube, the liquid siphons into the flask, this process is continuous and is done until drug is exhausted. Then the extract is collected and solvent is removed under water bath. The extract thus obtained was stored in the glass bottle, sealed and kept in refrigerator.

2.22) Physicochemical analysis of Chitraka⁶:

a) Determination of Foreign matter:

Materials: Sample & physical balancer.

Procedure:

- 100-500 g. of drug sample to be examined was spread in a thin layer.
- The foreign matter was detected by inspection with the unaided eye or the use of lens.
- Foreign matter was separated and weighed.
- Percentage of foreign matter present in air dried drug was calculated.

b) Determination of Total ash:

Materials: Silica crucible, physical balance, desiccators, electric bunsen burner & air dried drug.

Method:

- The clean and dry crucible was weighed. Accurately weighed 2 gms of drug was taken and placed in this crucible and this was kept in electric Bunsen burner.
- The temperature was gradually increased up to 500° C until white colored ash was obtained, including the absence of carbon, cooled in desiccators and weighed.
- If carbon free ash is not obtained in this manner, then the crucible was allowed to cool and the residue was moistened with about 2ml of water or a saturated solution of Ammonium nitrate R. Dried on water bath, then on a hot plate and ignited to constant weight. Residue was allowed to cool in suitable desiccators for 30 minutes and then weighed without delay. The content of total ash in mg per gm of air-dried material was calculated.

c) Determination of Acid insoluble ash:

Materials: Physical balance, crucible, electric bunsen burner, desiccators, air dried drug and ash less filter paper.

Method:

- To the crucible containing the total ash, 25 ml of diluted Hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible and filtered. The insoluble matter on an ash less filter paper was collected and washed with hot water until the filtrate was neutral.
- The filter paper containing the insoluble matter was transferred to the original crucible and dried on a hot plate and subjected to constant heat.
- The residue was allowed to cool in suitable desiccators for 30 minutes and then weighed again.
- The content of acid insoluble ash in mg per gram of air-dried material was calculated.

d) Determination of Alcohol soluble extractive:

Materials: Physical balance, powdered air dried drug, glass stopper conical flask, ethyl alcohol, vacuum filter, hot plate, water bath, beaker and desiccators.

Method:

- Accurately weighed 4gm of coarsely powdered air dried drug was placed in a glass stopper conical flask. Macerated with 100ml of ethanol for 6 hours shaking frequently. Then allowed to stand for 18 hours.
- Filtered rapidly taking care not to lose any solvent.
- 25ml of the filtrate was taken in a tarred flat-bottomed dish and evaporated to dryness on a water bath.
- Dried at 105°C for 6 hours and cooled in a desiccators for 30 minutes and weighed without delay. The content of alcohol extractable matter in mg/g of air dried drug was calculated.

e) Determination of Water- soluble extractive:

Materials: Powdered air-dried drug, physical balance, glass stopper, conical flask, water, reflux condenser, filter paper, water bath, beaker and desiccators.

Method:

- Accurately weighed 5gms of coarsely powdered air- dried material was placed in a glass stopper conical flask.
- This was macerated with 100 ml of water and weighed to obtain the total weight including the flask.
- The flask was shaken well and allowed to stand for 24 hours.

- The flask was attached to a reflux condenser and boiled gently for one hour, cooled and weighed and readjusted to the original weight by adding water. This was filtered rapidly through a dry filter.
- 25 ml of the filtrate was transferred to a tarred flat bottomed dish and evaporated to dry on a water bath.
- This was dried at 105°C and cooled in desiccators for 30 minutes and weighed without delay. The content of water extractable matter in mg / gm of air-dried material were calculated.

Preliminary Phytochemical investigations of Chitraka:

Qualitative chemical tests were conducted for aqueous, alcoholic and chloroform extracts of Chitraka (*Plumbago zeylanica*) to identify the various Phyto constituents. The various tests and reagent used are given below and observations are recorded.

Place of work: Preliminary phytochemical investigations of Chitraka were carried out at Biogenics, Research and Training Center in Biotechnology, Hubli.

Material:

Drug: Aqueous, Alcoholic & Chloroform extractive sample of Chitraka (*Plumbago zeylanica*)

Equipments: Test tube, holder, stand, spirit lamp, pipette, glass rods, beaker 50 ml to 250 ml, conical flask, water bath.

3. Methods:

3.1) Test for carbohydrates:

- **Benedict's test:** Test solution when treated with Benedict's reagent and boiled on water bath if it shows reddish brown precipitate.
- **Molisch test:** To the 2ml of sample, 2 drops of Molisch reagent is added and mixed carefully, run down the concentrated H₂SO₄ along the walls of tubes carefully. The purple violet color at the junction of two layers is observed.
- **Barfoed's test:** Test solution treated with Barfoed's reagent on boiling water bath shows brick red precipitate.

3.2) Test for Alkaloids:

- **Mayer's test:** Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream precipitate.
- **Wagner's test:** Test solution treated with Wagner's reagent (Iodine in potassium iodide) gives brown precipitate.
- **Dragendroff's test:** The filtrate when added with few drops of Dragendroff's reagent gives orange red color.

3.3) Test for Proteins:

- **Million's test:** Sample solution is added with million's reagent and heated on a water bath; protein is stained yellow on warming.
- **Ninhydrin test:** To 1ml of test solution add 5 drops of Ninhydrin solution and boil for 2 minutes. Violet or purple colored solution indicates the presence of amino acids and proteins.

3.4) Test for tannin:

- **Ferric chloride test:** Test solution treated with few drops of ferric chloride solution gives dark color.

3.5) Test for Glycosides

- **Conc. H₂SO₄ test:** 1ml of conc. H₂SO₄ solution was added to the 1ml of solution and was allowed to stand for 2 minutes. Formation of reddish color indicates presence of glycosides.

3.6) Test for Flavonoids:

- **Alkaline reagent test:** Test solution when treated with sodium hydroxyl solution shows increase in the intensity of yellow color which becomes colorless on addition of few drops of dilute acid.
- **Lead acetate solution:** Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

3.7) Test for saponins:

- **Foam test:** Sample solution mixed with saponins and shaken, if there is formation of stable foam for one minute it indicates the presence of saponins.

3.8) Test for sterols:

- **Salkowski test:** A few drops of concentrated sulphuric acid was added to the 5 ml of sample solution (extract) shaken and allowed to stand and observed the lower layer. If it turns red indicates the presence of sterols.
- **Liebermann Burchard test:** The test solution is treated with a few drops of acetic anhydride and mixed well. When conc. Sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and upper layer turns green.

Identification by T.L.C

Drug: Extraction of sample (Aqueous, Alcoholic & petroleum ether) which is treated with 1:10 ml solute, solvent like ethyl alcohol with dilution method.

Equipment: Silica gel, TLC kit, hot air oven, standard glass, wattaman glass plate, beakers, sprayer.

Chemicals: Dragendroff's reagent, Silica gel, ethyl alcohol.

Method: T.L.C. of the ethyl alcohol extract, aqueous extract, petroleum ether extract of the sample was carried out as follows.

The silica gel powder mixed with water and made thin slurry, and then with the help of glass slide, the silica gel was spread on glass plates uniformly. After some times the air dried plate were kept in a hot oven at 110-120^o C. The samples were loaded on one end of the plate with the help of capillary tubes, leaving 5 cm from the edge. The spots were carefully done without allowing them to spread. The spots were air dried and the spotted plate was gently immersed in presaturated closed, TLC chamber for development. The development was stopped when solvent front reached to 3/4th of the plate. The plate was then removed from TLC chamber and the solvent front was immediately marked with a pencil line. Then the plate was air dried and observed under UV transilluminator to note the fluorescing spots. Then Dragendroff's solution is sprayed on the plates.

R_f value of the spots were found out by using the formula,

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Distance traveled by the solvent

4. OBSERVATIONS AND RESULTS

The observations and results are recorded under following headings,

- ❖ Observations made during the preparation of the drug.
- ❖ Results of physicochemical analysis.
- ❖ Results of phytochemical analysis.
- ❖ Observations and results of Thin layer chromatography (TLC).

4.1 Observations regarding preparation of drug:

Preparation of Chitraka churna:

- ❖ The dried, cleaned roots of Chitraka weighing 4 kg was procured, then 4 kg of drug was subjected to coarse powdering under 20 number mesh and 4 kg of dry drug yielded 3.75kgs of coarse powder.
- ❖ The other 500 gm of drug was subjected to fine powdering under 120 number mesh and yield was 300gms of fine powder.
- ❖ 1 k of coarse powder was used to prepare the alcohol extract and remaining kept unused in airtight container.

Observations of prepared Chitraka churna:

The fine powder of Chitraka prepared using 120 number mesh was brown in color with pungent taste and irritant odor.

Observations during preparation of alcohol extract:

- ❖ By appropriate technique the coarse powder of Chitraka was filled in the round fold of filter paper i.e. in thimble in soxhlet apparatus. So that it cannot obstruct any path ways of soxhlet apparatus and uniform temperature is maintained.
- ❖ During each batch, the cycles were continued up to extractive factors of the powder were get completely extracted in to the solvent, then and then only every batch was stopped. It was observed that it took around 30 cycles for complete extraction.
- ❖ After extraction solvents were distilled off.
- ❖ Liquid extraction taken off was concentrated on hot water bath until it became semisolid.
- ❖ 1 kg coarse powder of Chitraka yielded 140 gms of (14%) alcohol extract, which was thick in consistency, dark brownish colored with characteristic smell and pungent taste. When alcohol extract was mixed with water the color of the mixture was brownish in appearance.

4.2 Results of physicochemical analysis:

The prepared coarse powder of Chitraka (*Plumbago zeylanica*) was undertaken for physicochemical analysis and the following results were noted.

Table 1 Showing the Physico chemical values of Chitraka

Sl. no.	Test	Values % w/w	Standard values (API)
1	Foreign matter	1.26%	Not more than 3%
2	Total ash	2.65%	Not more than 3%
3	Acid –insoluble ash	0.55%	Not more than 1%
4	Water soluble extractive	16%	Not less than 12%
5	Alcohol soluble extractive	15%	Not less than 12%

Result of analysis: The sample is confirmed to API standards with respect to above tests and hence the sample can be declared as of 'Standard quality'.

Table 2 Showing phytochemical analysis of Chitraka

Sl. No.	Tests	Aqueous ext.	Alcoholic ext.	Chloroform ext.
1.	Test for carbohydrates a) Molisch's test b) Benedicts test c) Bradford's test	+ + +	+ + +	+ + -
2.	Test for proteins a) Ninhydrin test b) Millon's test	- +	- +	- +
3.	Test for alkaloids a) Mayer's test b) Dragendroff's test c) Wagner's test	+ - -	+ + +	- + -
4.	Test for glycosides a) Conc. Sulphuric acid test	+	+	+
5.	Test for tannin a) Ferric-chloride test	+	+	-
6.	Test for flavonoids a) Alkaline magnet test b) Lead acetate solution test	+ +	- +	- +
7.	Test for saponin a) Foam test	+	-	-
8.	Test for steroids a) Salkowaski test b) Libermann Burchardt test	- -	+ +	+ +

4.3 Observations of Thin layer chromatography (TLC):

- One gram of the Chitraka sample was refluxed with water, alcohol and petroleum ether (20ml) for an hour and filtered. The extracts were used for the analyses.
- The Silica gel slurry was spread on glass plates uniformly with the help of glass slide.
- The samples were spotted with the help of capillary tubes carefully, without allowing spreading. A space of 2cm was maintained between each spot.
- The chromatogram developed by the ascending technique. Development was allowed to proceed until the solvent front has traveled the 3/4th distance.
- First, plate was observed under UV transilluminator to note the fluorescent spots. Then Dragendroff's solution is sprayed on the plates.
- The distance traveled by the solvent front was noted to be 12.2cm. R_f value of the spots were then found out by using the formula

$$R_f = \frac{\text{Distance traveled by the Solute}}{\text{Distance traveled by the solvent}}$$

Results of TLC:

Stationary phase: **Silica Gel G**

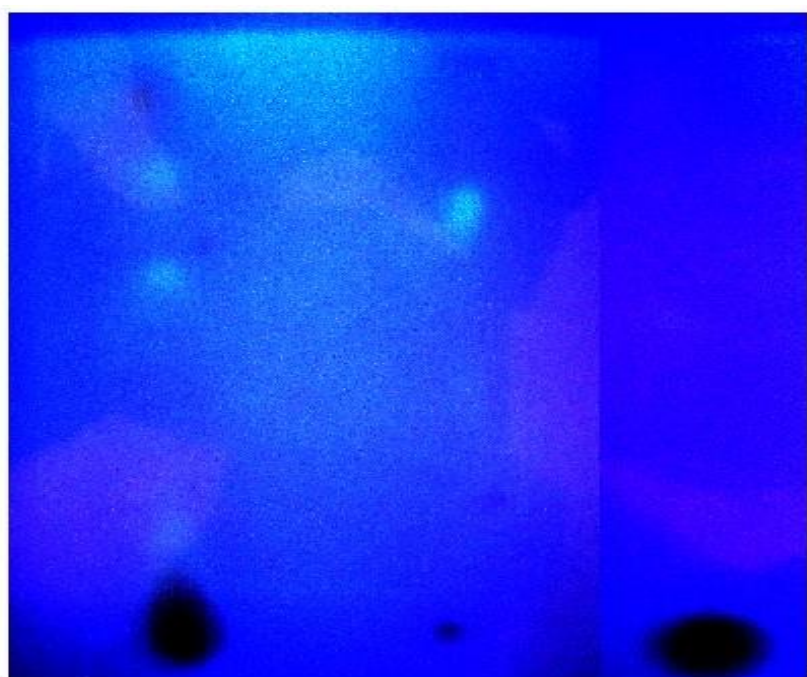
Mobile Phase: **Butanol: Acetic acid: water (4:1:5).**

Detection: **312 nm and Drangendroff's reagent.**

Table 3 Showing the results of TLC

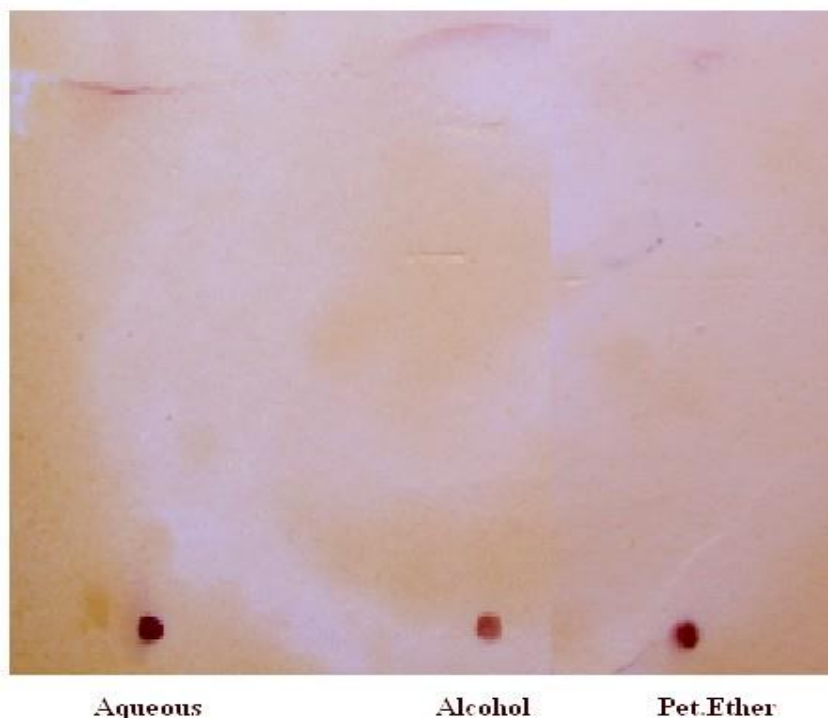
Sl. No	<i>Plumbago zeylanica</i> Linn Sample+solvent	TLC plate system	Detector	Rf Values	Spot colour	Result
1	Water extract	Silicagel G	Ultra violet (312 nm)	0.188, 0.655, 0.696	Fluorescent green	Present
2	Alcohol Extract	Silicagel G		0.662	Fluorescent green	Present
3	Petroleum ether extract	Silicagel G		No bands	--	Absent
4	Water extract	Silicagel G	Drangendroff's reagent	0.655	Orange	Present
5	Alcohol Extract	Silicagel G		0.696	Orange	Present
6	Petroleum ether extract	Silicagel G		0.672	Orange	Present

@ 312 nm



Aqueous Alcohol Petroleum ether

@ Drangendroff's reagent



DISCUSSION

The findings of the present study demonstrate that the Chitraka (*Plumbago zeylanica* Linn.) root sample complied with the physicochemical standards prescribed by the Ayurvedic Pharmacopoeia of India, indicating its good quality and authenticity. The preliminary phytochemical analysis revealed the presence of several bioactive constituents, including carbohydrates, alkaloids, glycosides, tannins, flavonoids, saponins, and steroids in different extracts, suggesting the therapeutic potential of the drug. Thin-layer chromatography (TLC) further confirmed the characteristic chemical profile of the extracts, supporting their identity and purity. Overall, these findings provide scientific evidence for the standardization of Chitraka and may serve as a useful reference for future quality control, research, and pharmaceutical applications.

CONCLUSION

Plumbago Zeylanica, commonly known as Chitraka is found in the tropical and subtropical areas of India. This long living herb has variety of effects on our body. Its tiksna, laghu and rukhsa guna works miraculously on digestive systems, skin disorders and so on. And further research on the drug for its efficacious results are on progress. A humble effort is made to study the physicochemical and preliminary phytochemical study of the drug to add onto the vast research areas.

REFERENCES

1. Sharma PV. Dravyaguna Vijnana. Vol. II. Varanasi: Chaukhambha Bharati Academy; Reprint 2018. p. 331–334. (For the therapeutic uses of Chitraka.)
2. The Ayurvedic Pharmacopoeia of India. Part I, Vol. II. New Delhi: Ministry of AYUSH, Government of India; 2008. p. 35–37. (For botanical identity and standards of Chitraka.)
3. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2021. (For physicochemical and phytochemical evaluation methods.)
4. Anonymous. Quality Control Methods for Herbal Materials. Geneva: World Health Organization; 2011. (For herbal drug standardization.)

5. Evans WC. Trease and Evans Pharmacognosy. 16th ed. Edinburgh: Elsevier; 2009. (*For pharmacognostic evaluation and phytochemical screening.*)
6. The Ayurvedic pharmacopoeia of India, part 1, vol 2nd. 1st Ed. New Delhi: Ministry of health and family welfare, Govt of India; 1999.P.190-193

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