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Comparative Pharmacognostic, Physicochemical and Phytochemical Studies of Different Samples of *Plumbago zeylanica* L. Roots

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ABSTRACT

The objective of the present study is to evaluate the quality of the marketed and self collected samples of *Plumbago zeylanica* L. roots on the standardization parameters. This study is planned mainly to confirm changes with quality of drug. Now a day's more demand of herbal drugs for disease treatment, lack of knowledge of proper methodology and availability are promoting the practices of adulteration and substitution. Thus, the standardization of the plant crude drugs is necessary to maintain their therapeutic efficacy. Comparative studies were carried out to evaluate the standards of *P. zeylanica* L. with emphasis on organoleptic evaluation, physicochemical and phytochemical analysis. Samples were procured from local market and self collected to determine the qualitative and quantitative variations. The result indicates that self collected sample showed significant results with comparison to marketed sample.

Keywords: *Plumbago zeylanica* L., Physicochemical studies, Phytochemical screening, Standardization.

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INTRODUCTION

Plants serve as important source for new drug discovery for many diseases. Many researchers are working worldwide on the plant to search active lead molecules. Plant provides lead structure for synthetic modification and bioactivity optimization. The use of medicinal plants increased enormously worldwide as they are consider safe with comparative to synthetic drugs. Traditional medicine systems like ayurveda, siddha, unani, chinese etc. are using whole plants, crude extracts and purified constituents of plants for cure and prevention of diseases. [1-2]

Plumbago zeylanica (Family Plumbaginaceae) commonly known as *chitrak* is found throughout the India. It grows wild and as a garden plant in East, North and Southern India and Sri Lanka (Ceylon). *P. zeylanica* has been formulated into different dosage forms that are implicated in indigestion, skin diseases, gynecological disorders etc. *Citrakadi vati, Citrakaharitaki* and *Citrakadi churna* are the marketed formulations containing *P.*

zeylanica. [3] It is used in indigestion and is considered to be an antidote to the undesirable metabolites produced during digestion. It is also used in diarrhea, dysentery, gastroenteritis, chronic skin diseases and gynecological disorders. [4]

Pharmacological studies performed by a number of researchers have indicated that *P. zeylanica* extract and its constituents have therapeutic properties including antiplasmodial [5], antimicrobial, antihyperglycemic, insecticidal, antimalarial [6], antiallergic [7], central nervous system stimulating properties [8], anti-atherogenic, cardiotonic, neuroprotective, hepatoprotective, anti-inflammatory, anti-diabetic [9], anticancer and anti-hyperlipidemic activities [10-11], anti-helicobacter pylori activity [12], antileishmanial activity. [13] Plumbagin, the major constituent of this plant, possesses anti-fertility [14-15], anti-viral [16], anti-bacterial [17-18], hypolipidaemic and anti-inflammatory activities. [19]

For the standardization and quality assurance purpose, authenticity, purity and assay of crude drug must be verified. The objective of the present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies to provide more information, scientific validation and determine the quality of the drug based on marketed and self collected sample of *P. zeylanica* roots for its highly praised medicinal use.

MATERIALS AND METHODS

Plant material

The fresh roots of chitrak plant were collected in early morning during month of April from medicinal plant garden, Vedica college of B. Pharmacy, Bhopal, Maghya Pradesh, India. Second sample were purchased from local market of Bhopal. Fresh roots were dried in shade till constant weight was obtained. Self collected sample of *P zeylanica* roots were coded as sample P1 and market collected sample was coded as P2. Fresh root used for microscopic characterization and root powder had been used to determine the physicochemical parameters such as ash values, extractive values, powder microscopic characters as well as qualitative analysis.

Chemicals

The standard compound plumbagin was purchased from Sigma Aldrich and its purity was confirmed by chromatographic methods. All chemicals and solvents were of analytical grade (AR Grade) and were purchased from Sigma Aldrich, Ranbaxy fine chemicals Ltd., LOBA chemicals Ltd., CDH, S. D. fine chemicals Ltd.

Instruments

Plant material was extracted by soxhlet extractor (Perfit India Ltd.). Extracts were concentrated by vacuum rotary evaporator (Buchi R-114, Switzerland).

Macroscopic and Organoleptic characters

The macroscopic study of crude drug was helpful in quick identification and also plays an important role in standardization of drug. Both samples were subjected to studies organoleptic characters viz., color, odour, appearance, taste, texture etc. [20]

Microscopic studies

All samples were cleaned and boiled separately. Their transverse sections were cut, stained, mounted and observed under microscope. [21]

Physicochemical analysis

Dried, powdered drug (2 g) was weighed in a tarred silica crucible and incinerated at temperature not exceeding 450°C until free from carbon. After cooling, the crucible was weighed to get the total ash value and then the ash was subjected for determining the acid insoluble and water soluble ash. Percentage of total ash, acid insoluble and water soluble ash were calculated with reference to the air-dried drug. [22-23]

Total Ash value

Dried, powdered drug (2 g) was weighed in a tarred silica crucible and incinerated at temperature not exceeding 450°C until free from carbon, then cooled and weighed. Percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value

Dried, powdered drug (2 g) was weighed in a tarred silica crucible and incinerated at temperature not exceeding 450°C until free from carbon, then cold and weighed. The ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected on ash less filter paper, washed with hot water, ignited, cooled in desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug. [22-23]

Determination of extractive values

Considering the diversity and chemical nature of the constituents present in crude drug, four solvents of different polarity viz. hexane, chloroform, methanol and water were used for determination of extractive values. Dried, coarsely powdered drug (5 g) was subjected continuous soxhlet extraction with 100 ml of hexane, chloroform, methanol as solvents and maceration process was used with water extraction. The extracts were concentrated in rotary evaporator and dried in vacuum desiccator. The extractive values were calculated as percentage w/w of solvent soluble extractive with reference to the air dried drug.

Determination of moisture content

The moisture content of crude drug was determined by loss of weight on drying (LOD) method. Dried, coarsely powdered drug (5 g) was taken in a tarred petri dish and kept in oven at 105°C till constant weight was obtained. Amount of moisture content present in sample was calculated as a reference to the air dried drug.

Preliminary phytochemical screening

Plants synthesize secondary metabolites which are responsible for various therapeutic activities. Therefore systematic preliminary phytochemical screening of plant material is necessary for identifying class of secondary metabolite and to establish a chemical

profile of a crude drug for its appropriate evaluation. For preliminary phytochemical screening extracts were subjected using the standard procedure for identifying various phytoconstituents. [24-25]

Fig. 1: Morphology of *Plumbago zeylanica* L. root.

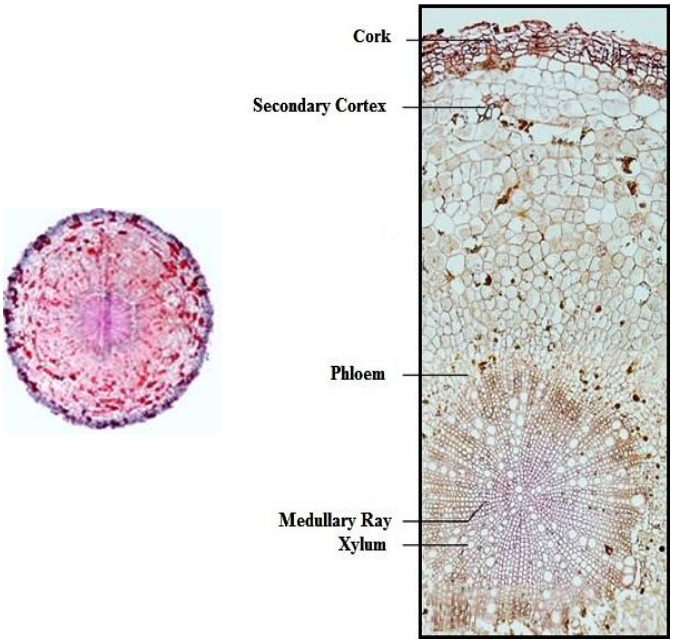

Fig. 2: Transverse section of root of *Plumbago zeylanica* L.

Table 1: Organoleptic identification of *Plumbago zeylanica* L. roots

S. No.	Parameters	Observations	
		Self collected sample (P1)	Market sample (P2)
1	Shape	1 cm diameter cylindrical	1.2 cm diameter cylindrical
2	size	10-15 cm long	15-20 cm long
3	touch	Rough	Rough
4	odour	Characteristics	Characteristics
5	taste	Acrid	Acrid
6	colour	Dark brown	Light brown
7	Foreign organic matter	No adultrants have been found	Slightly adultrants have been found

RESULTS

Macroscopic and Organoleptic studies

Roots are brown in color. The root surface is rough and firm due to scaling off of longitudinal striation. Inner side of root is creamy white, soft and collapsed. The results of organoleptic studies are presented in Table 1 and Figure 1.

Microscopic studies

Transverse section of root samples P1 and P2 showed outer most layer cork with 5-6 rows of light brown cells, rectangular in shape; starch grains compactly packed in the cortex region, phloem well developed with phloem fibers. Groups of phloem fibers were present near the phloem. Cambium single layered, xylem was well developed with xylem vessels. Medullary ray was single to multilayered and loaded with simple to compound starch grains (Figure 2).

Table 2: Physicochemical parameters of *Plumbago zeylanica* L. root powder.

S. No.	Physicochemical parameter values (% w/w)	P1	P2
1	Total ash	2.01 ± 0.09%	2.51 ± 0.12%
2	Water soluble ash	0.49 ± 0.17%	0.42 ± 0.15%
3	Moisture content	1.91 ± 0.09%	2.3± 0.12%
4	Foreign organic matter determination	1 % w/w	2.0 % w/w
5	Swelling index determination	0	0
6	Foam index determination	0	0

Table 3: Solvent extractive values (%w/w) of *Plumbago zeylanica* L. root powder

S. No.	Name of extract	Color	Extractive value (P1)	Extractive value (P2)
1	hexane Extract	Yellowish	1.51 % w/w	1.32 % w/w
2	Chloroform Extract	Yellowish red	4.6 % w/w	3.98 % w/w
3	Methanol Extract	brownish red	14.4 % w/w	13.06 % w/w
4	Water Extract	Dark brown	16.1 % w/w	15.2 % w/w

Physicochemical analysis

Physicochemical parameters like ash values, extractive values, and moisture content were investigated and the results are presented (Tables 2-3). Ash values of crude drug provide an idea about the inorganic composition or earthy matter and other impurities present in drug. The extractive values are mainly useful for the determination of adulterated or exhausted drug. All the tests were performed in triplicate and result is presented in mean ± SEM.

Preliminary phytochemical screening

Extracts obtained by continuous soxhlet were subjected to qualitative phytochemical tests to identify the presence of secondary metabolite (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical screening exhibited the presence of steroids and triterpenes in hexane extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, tannin and glycoside in methanol extract & carbohydrate & tannin in aqueous extract (Table 4 and Table 5).

DISCUSSION

Plumbago zeylanica root (Chitrak) is most common plant used in Ayurvedic system of medicine. Evaluation of organoleptic, physicochemical parameters and phytochemical analysis can be useful to conclude difference in storage conditions, collection process and age of plant.

Table 4: Phytochemical analysis of *Plumbago zeylanica* L. root extracts of sample P1

S. No.	Chemical class	Chemical test	Hexane extract	Chloroform extract	Methanol extract	Water extract
1	Alkaloids	Dragendorff's test	-	-	-	-
2	Steroids	Salkowaski test	-	-	-	-
3	Carbohydrate	Molish test	-	-	+	+
4	Triterpenes	Vanillin-sulphuric acid test	+	+	-	-
5	Tannin	Ferric chloride test	-		+	+
6	Glycoside	Keller-killani test	-	+	+	+
7	Proteins	Biuret test	-	-	-	-
8	Flavonoids	Shinoda Test	-	-	-	-
9	Saponins	Lead acetate test	-	-	-	-

Where + is Present and – is Absent

Table 5: Phytochemical analysis of *Plumbago zeylanica* L. root extracts of P2

S. No.	Chemical class	Chemical test	Hexane extract	Chloroform extract	Methanol extract	Water extract
1	Alkaloids	Dragendorff's test	-	-	-	-
2	Steroids	Salkowaski test	-	-	-	-
3	Carbohydrate	Molish test	-	-	+	+
4	Triterpenes	Vanillin-sulphuric acid test	+	+	-	-
5	Tannin	Ferric chloride test	-		+	+
6	Glycoside	Keller-killani test	-	+	+	+
7	Proteins	Biuret test	-	-	-	-
8	Flavonoids	Shinoda Test	-	-	-	-
9	Saponins	Lead acetate test	-	-	-	-

Where + is Present and – is Absent

The samples were found almost uniform in organoleptic properties. The variation observed may be due to the difference in storage conditions, collection process and age of plant. The physicochemical parameters like extractive value, ash value indicates the quality and purity of drugs. Extractive values are representative of the polar or nonpolar extractable compounds present in plant material. The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiological ash and nonphysiological ash. The variation in these parameters from standard value indicates the low quality of the samples. Preliminary phytochemical screening showed the presence of steroids and triterpenes in hexane extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, tannin and glycoside in methanol extract and carbohydrate glycosides and tannin in aqueous extract of both sample.

By the above study, it can concluded that there is variation in uniformity in marketed and self collected sample, which can result into variation in quality, safety and efficacy of same formulation manufactured in different area. Therefore importance to be laid on collection process, sources, storage conditions and standardization of raw materials in order to maintain the quality aspects of the product throughout worldwide.

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