



Preliminary Pharmacognostic and Phytochemical Analysis on *Phyllanthus simplex* Retz.

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ABSTRACT

The present study was undertaken to evaluate the pharmacognostic and phytochemical profile of *Phyllanthus simplex* Retz. to access the quality standards for pharmaceutical industries. Various parameters like macroscopic and microscopic characters of stem leaf and root, fluorescence characteristics of the powder after treatment with different chemical reagents were studied. Preliminary phytochemical analysis has also been performed for the presence of sugars, proteins, Amino Acids, Steroids, Tannins and phenolic compound and Organic acid. Powder microscopy revealed the presence of spiral, pitted, and annular xylem vessels, phloem fibers, tannin cells and calcium oxalate crystals. These studies will help in future for identifying this plant for further research.

Keywords: calcium oxalate crystals, Microscopic characters, *Phyllanthus simplex* Retz., Phytochemical screening, Powder analysis.

INTRODUCTION

Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are regarded as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds¹.

Phyllanthus simplex Retz. belongs to the family Euphorbiaceae. This family comprising about about 300 genera and 7500 species². It is a glabrous protuberant herb, which has a taproot and its branches are compressed. It's flowers are solitary and leaves are distichous. The siddha and ayurvedic medicines text showed that the Hindus used equal parts of the fresh leaves, flowers, fruit and cumin seeds with sugar, made into an electuary for the treatment of gonorrhoea by taking a tea spoonful for twice a day.

The fresh leaves, bruised and mixed with buttermilk, make a wash to cure itches in children. The root is used as an external application for abscesses³⁻⁵. Leaves also contains vanillin, β -daucosterol, β -sitosterol and rutin⁶. The plant also shows antioxidant and anti-inflammatory activities⁷.

There is no detailed investigation on pharmacognostic and phytochemical aspects of *Phyllanthus simplex* Retz. The objective of the present study is to evaluate various pharmacognostic parameters for analysis of these findings would be helpful for authentication, purification, quality control of *Phyllanthus simplex* Retz. for better use in pharmaceutical herbal formulations. Similar work has been reported in different plant species such as *Andrographis paniculata*⁸, *Portulaca quadrifida*.⁹

MATERIALS AND METHODS

Collection and Authentication

Phyllanthus simplex Retz was collected from the Karnatak University campus, Dharwad during the months of July to September 2013. The plants were identified by one of the authors and the voucher specimen is kept in the department herbarium for future references.

Pharmacognostic Studies

The collected plant material was shade dried for 8-10 weeks in the laboratory and dried material was coarsely powdered mechanically with the help of grinder, passed through 20 mesh sieve and stored in air tight container for further use. Fresh plant material is used to study anatomical features.

Microscopical characters, physiochemical analysis were carried out using the powder and for macroscopical studies, transverse section of the stem, root and leaf were prepared and stained according to standard methods^{10,11}.

Organoleptic and Microscopic Analysis

Various sensory parameters such as color, odor, taste, size, shape, texture were studied by the organoleptic evaluation. Microscopic characters were studied by taking hand sections from the fresh plant material and stained according to standard procedures^{10,11}.

Stomatal type, Stomatal number and Stomatal index

Stomatal type was determined based on the classification of stomata on the ground of nature and number of subsidiary cells¹².

Stomatal number is the average number of stomata per square millimeter of leaf and stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being



counted as one cell and it can be calculated by using following equation:

$$SI = \frac{S}{E+S} \times 100$$

Palisade Ratio

The palisade ratio is the average number of palisade cells beneath one epidermal cell of the leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells.

Physicochemical and Fluorescence Analysis

Physicochemical parameters such as percentage of total ash value, acid insoluble ash, water soluble ash, were investigated^{10,11}. Stem powder was subjected to Fluorescence behavior and tested after 24 hours, treating with freshly prepared chemical reagents which were exposed to visible light and UV light (short and long wavelength)^{12,13}.

Extraction and Phytochemical Analysis

Powdered material was subjected to soxhlet extraction, where 25gm of powdered plant material was taken and extracted for 18 hours successively with different solvent like petroleum ether, chloroform, acetone, ethanol and water. Preliminary phytochemical analysis was carried out by standard procedure^{10,11}.

RESULTS AND DISCUSSION

Macroscopic Characters

It is annual glabrous, branches compressed, leaves distichous, stipules peltate subsagittate are arranged in rows so that each branch resembles with a compound leaf, flowers on short or long pedicels and are solitary, axillary, unisexual filaments free, anthers didymous, ovary smooth or pubescent granular, styles short free reflexed arms recurved, its fruit is capsule, seeds minutely tubercled.

Powder Characteristics

Organoleptic evaluation revealed the following characteristic the powder was Ceylon green in color with characteristic odor and light pungent in taste. Powder microscopic characters showed the presence of spiral, pitted, and annular xylem vessels (Fig. 2C, 2G, 2H), phloem fibers (Fig. 2F), tannin cells (Fig. 2D) and calcium oxalate crystals (Fig. 2E).

Microscopic Characters

T.S of Stem

Detailed transverse section of stem (Fig.1A) was circular in outline. Outer epidermis is followed by cortex, endodermis, pericycle, vascular bundle and central parenchymatous large pith. The outer most single layer compactly arranged with interruption of stomata. Epidermis covered with thick cuticle. Cortex made up of 3-5 layers of simple parenchymatous cells without any intercellular spaces. Inner cells loaded with chlorophyll

and crystals of calcium oxalate, Cortex is followed by single layer of endodermis. Pericycle is made up of 3-5 layered, discontinuously arranged beneath the endodermis. Vascular bundles are conjoint collateral and open type. Metaxylem is facing towards cortical region and protoxylem towards central pith region with xylem parenchyma and fibres. Xylem is separated by uni-serrate medullary rays. Phloem is situated above the xylem with sieve elements and fibres. Larger region of the section is occupied by pith; parenchymatous cells are compactly arranged in pith region. Pith cells are with starch grains and calcium oxalate.

T.S of Root

Detailed transverse section of root (Fig.1B) showed the outer most 3-5 layers are composed of tangentially elongated cork cells, Cortex region is found to be reduced, loosely arranged and made up of 3-5 layers of simple parenchymatous cells which are isolated with simple starch grains and tannin content. Cortex ends with single layered of parenchymatous endodermis. Vascular bundles radially arranged. Xylem consists of xylem parenchyma and fibres. Phloem is situated above the xylem.

T.S of Leaf

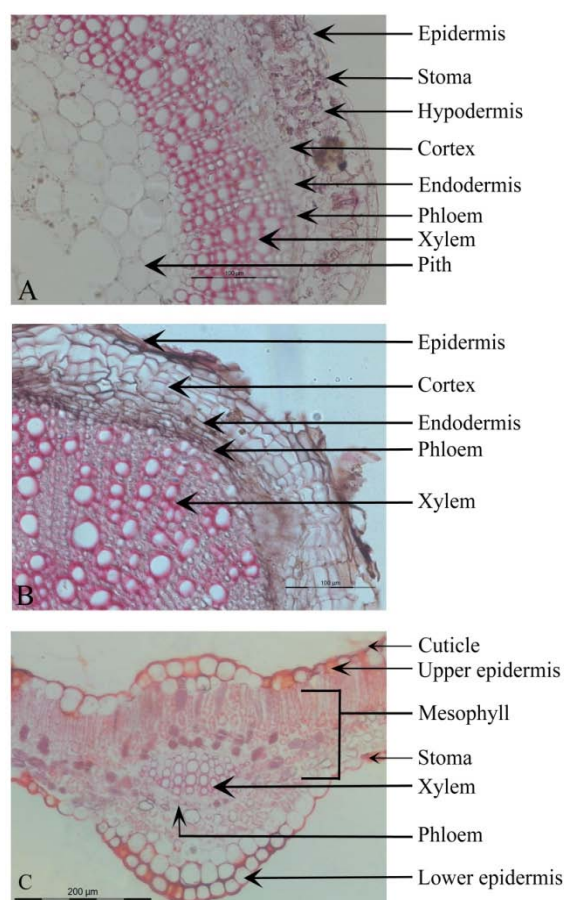


Figure 1: A. T.S of Stem. B. T.S of Root C. T.S of Leaf.

Detailed transverse section of leaf (Fig.1B) is dorsiventrally differentiated. Lamina is flat and reduced. Epidermis on both the surfaces uniseriate, composed of

compactly arranged rectangular cells with cuticle on the surface and stomata are present on both the surfaces (**Fig.2A and 2B**). Upper epidermal cells are larger than lower epidermis. Mesophyll tissue is made up of upper palisade parenchyma and lower spongy parenchyma, which are filled with chlorophyll pigment. Mid rib consists of epidermis, collenchyma, mesophyll and vascular tissue.

An arc of xylem lies in the middle of round tissue. Xylem vessels are arranged in radial rows and phloem lies on abaxial side.

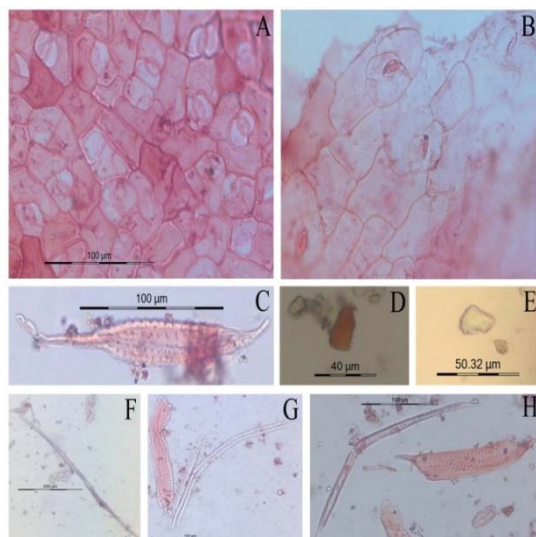


Figure 2: A. stomata on upper surface of leaf B. stomata on lower surface of leaf. C. Tracheid, D. Tanin cell. E. Calcium oxalate crystal F. G. Fibers, H. Trachied

Physiochemical Analysis

Moisture content, ash value, extractive values are mentioned below.

Table 1: Physiochemical analysis

Parameters	values (w/w)%	
Moisture content	08.25 ± 0.60	
Ash values	Total ash	05.69 ± 0.18
	Sulphated ash	12.92 ± 0.13
Extractive values	Ether soluble	09.64 ± 0.19
	Alcohol soluble	11.26 ± 0.30
	Water soluble	20.15 ± 0.49

Average of three readings, SD, on dry weight of samples

Table 2: Leaf constants

Parameters	Values	
Stomatal type		
paracytic		
Stomatal number	Upper epidermis	24.8 ± 0.60
	Lower epidermis	41.4 ± 0.30
Stomatal index	Upper epidermis	21.2 ± 0.25
	Lower epidermis	12.2 ± 0.14
Palisade ratio	07.23 ± 0.4	

Average of five readings per microscopic view

Fluorescence analysis

Powdered material was tested with different chemical reagents and its fluorescence behavior with different wavelength of light mentioned below.

Table 3: Fluorescence Analysis

Material with Chemical	Day light	UV light (short)	UV light (long)
P + Phloroglucinol:HCl	Dark green	Dark green	Black
P + Methanol	Green	Green	Saffron
P + Ethanol	Light green	Green	Saffron
P + Pet. Ether	Brownish green	Green	Brick red
P + Acetone	Dark green	Green	Brick red
P + Chloroform	Green	Dark green	Brick red
P + 50% H ₂ SO ₄	Green	Dark green	Black
P + 50% HNO ₃	Chrome yellow	Parrot green	Reddish brown
P + 50% HCl	Brown	Dark green	Black
P + 10% NaOH	Brown	Dark green	Dark brown
P + Ammonia	Brown	Dark green	Black
P + Glacial Acetic acid	Greenish Brown	Dark green	Carnoy's red
P + Picric Acid	Greenish yellow	Chrome yellow	Brown
P + 5% FeCl ₃	Dark green	Green	Black

Preliminary Phytochemical analysis

Phytochemical analysis (Table 4) revealed the presence of sugars, proteins, Amino Acids, Steroids, Tannins and phenolic compound and Organic acid.

CONCLUSION

Pharmacognosy has been generally pursued for utilitarian ends and may thus be called an applied science. It has played an important role in the development of the pure sciences. In descriptive botany, plant classification (taxonomy) and plant chemistry (phytochemistry).

The study has revealed the presence of various phytochemicals in the extracts known to produce therapeutic and physiological activities.

This may serve as a justification for the traditional usage of the plants in the treatment of different ailments. The phytochemicals screened for the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids.

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Table 4: Preliminary Phytochemical Analysis

Chemical constituents	Chemical test	Petroleum ether	Chloroform	Acetone	Alcohol	water
Sugars	Molish test	-	-	-	+	+
Reducing sugars	Fehling test	-	+	+	+	+
	Benedict test	-	-	+	+	+
Test for monosaccharides		-	-	+	+	+
Galactose	Phloroglycinol	-	-	+	+	+
Non reducing sugar	Iodine test	-	-	-	-	-
Gums	Fehling test	-	+	+	+	+
	Benedict test	-	-	+	+	+
Mucilage		-	-	-	-	-
Proteins	Biuret test	-	-	+	+	+
	Millions test	-	-	+	+	+
	Xanthoprotein test	-	-	-	+	+
Amino Acids	Precipitation test	+	-	-	-	-
	Test solution+ absolute alcohol					
	Test solution + HgCl ₂	-	-	+	+	+
	Test solution + CuSO ₄	-	-	+	+	+
	Test solution + NH ₄ OH	+	-	-	+	+
Amino Acids	Ninhydrin test	+	+	-	-	-
	Tyrosin test	-	+	-	+	+
Steroids	Salkowski reaction	+	+	-	+	-
	Deoxysugar	+	+	-	+	+
Antraquinoneglycosides	Borntrangers test	-	+	+	+	+
	Modified Borntrangers test	+	+	+	+	+
Saponin glycosides	Foam test	-	-	-	-	-
Coumarin glycosides		-	-	+	+	+
Flavonoides	Shinoda test	-	-	-	+	+
	Sulphuric acid	-	-	-	-	+
Alkaloides	Mayer's test	-	-	-	-	-
	Wagner's test	+	+	+	+	+
Purine	Murexide test	-	-	-	+	+
Tannins and phenolic compound	Extract + 5% FeCl ₃	-	-	+	+	+
	Extract + Gelatin	-	+	-	-	-
	Extract + acetic acid	-	-	-	-	-
	Extract + Potassium dichromate	-	-	-	-	+
	Extract + Dil Iodine					
	Extract + Dil HNO ₃	+	+	+	+	+
	Extract + 1 drop NH ₄ OH + potassium ferricyanide	-	-	+	-	-
Organic acid	White ppt immediately	-	-	+	+	+
	Ppt on shaking	-	-	+	+	+
	Ppt on boiling and cooling	-	-	+	+	+
	Ppt on adding absolute alcohol	-	-	+	+	+

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