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A Comprehensive Phytochemical and Pharmacognostic Study of *Anogeissus latifolia* leaves

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ABSTRACT

Anogeissus latifolia is one of the most important medicinal plants useful in traditional system of medicine. It is used in the treatment of wound, diarrhoea, dysentery and diabetes in Hamirpur and Una, Himachal Pradesh. Due to its growing importance in ethno medicinal field, proper identification and evaluation are essential. This research article presents detailed Pharmacognostical, physicochemical and phytochemical evaluation on leaves of *Anogeissus latifolia* which will be used in its identification and resulting standardization. The fresh and powdered leaves were examined for their macroscopic and microscopic properties. Physicochemical properties and phytochemical screening were carried out as per WHO guidelines. *Anogeissus latifolia* is a simple, opposite to alternate type of leaf, stipulate, Petiolate with reticulate venation, entire margin and Cuniate base. The total ash, water soluble ash, acid insoluble ash values along with the moisture content, water soluble extractive and alcohol soluble extractive values also obtained by following WHO guidelines. Phytochemical screening showed presence of alkaloids, tannins, terpenoids and flavonoids as the major phytochemical constituents. The Pharmacognostical measures of the leaves of *Anogeissus latifolia* were determined and serve as quality control standards for identification and purity control of the drug.

Keywords: *Anogeissus latifolia*, morphology, microscopy, phytochemical analysis

INTRODUCTION

The plant *Anogeissus latifolia*, commonly known as Dhava, belongs to the family Combretaceae [1]. It is commonly found in central India and much of the Indian Peninsula, typically growing at elevations around 1200 meters [2]. It thrives in various forest types including moist Shiwalik forests, dry Shiwalik forests, southern dry mixed deciduous forests, central peninsular forests and northern dry deciduous forests [3]. This species is among the most widespread in India. Its leaves are rich in tannins, which are widely utilized. The tree is valued for its bark and leaves due to their high tannin content- leaves can contain 9% to 95% Gallotannin, young leaves and shoots have 5% to 30% tannins [4]. It has been revered in traditional medicine systems across various cultures for its wide array of therapeutic properties. Medicinally, *A. latifolia* is known to help with urinary tract infections. Liver problems, fever, epilepsy, heart conditions, stomach disorders and various other ailments. Its effectiveness is largely due to its high levels of tannins, flavonoids, terpenes and saponins, which offer various health benefits [5]. Pharmaceutical companies rely on such plants for the extraction of secondary metabolites used in health products and medicines [6]. This work is aimed at evaluating in detail the Pharmacognostical standards of *Anogeissus latifolia* hence developing standardization parameters of this plant.

The Plant Taxonomy of *Anogeissus latifolia* represented by Figure 1.

MATERIALS AND METHODS

Collection, identification, authentication of plant materials

The leaves of *Anogeissus latifolia* commonly known as Gum Ghatti were collected from Morni hills, Panchkula, Haryana (India) at an altitude of 1136.8 m from sea level during the month of July. The plant was identified and authenticated by Dr. Sunita Garg, Former Chief Scientist, Head at Raw Materials Herbarium and Museum, CSIR-NIScPR, New Delhi. A sample of the plant material was deposited in the herbarium under voucher no. NIScPR/RHMD/Consult/2023/4570-71 for reference. The leaves were cleaned, dried under shade and used for further studies.

Chemicals, Reagents and Solvents

All chemicals, reagents and solvents used in this study were of analytical grade.

Macroscopic examination of leaves

The fresh leaves of *Anogeissus latifolia* were examined. The Macroscopic and Organoleptic properties were evaluated based on standard procedure. Organoleptic properties include color, taste and odor of the plant drug while the macroscopic properties include shape, size, apex, margin, lamina, venation, type of leaf; midrib and petiole were noted [7].

Microscopic examination of leaves

Transverse section of leaf

Microscopic examination of leaf sample was performed by taking transverse section (T. S) through midrib adhering to standardized histological protocols. Observation for epidermal cells (upper and lower), trichomes, type and distribution of stomata, xylem and phloem was also done [8].

Quantitative microscopy

Stomata number and stomata index

A section from the middle of a leaf was treated by boiling in chloral hydrate solution for 5 minutes. The top and bottom epidermis layers were separated. The outer skin of the leaf was placed on a slide, cleaned with water, and observed under a microscope fitted with a measuring scale. The number of stomata in different fields of view was counted, and the average number was recorded [9,10].

The **stomatal index** was then calculated using this formula:

$$\textbf{Stomatal Index (\%)} = \frac{\textbf{Number of stomata (S)} \times 100}{\textbf{Number of epidermal cells I + Number of stomata (S)}}$$

Palisade Ratio

To determine the palisade ratio, a thin piece of leaf (about 2 mm thick) was softened by boiling it in chloral hydrate solution for 5 minutes. It was then mounted on a slide, and four clear areas of the epidermis were chosen. The number of palisade cells directly underneath these epidermal cells was counted by focusing on

the palisade layer. This process was repeated in different parts of the leaf to calculate an average, which gave the palisade ratio.

Vein- Islet number

A section of leaf was made transparent by boiling in chloral hydrate solution. Using a microscope with stage micrometer, a square area of one square millimeter was marked on the leaf. This prepared leaf was mounted on a slide with a drop of glycerin and covered with a slip. Under the microscope, the average number of small vein- enclosed areas (called vein- islets) within four different squares was counted, and the average was recorded.

(A)

(B)

(C)

Figure 1: (A) Arrangement of branches in *A. latifolia* **(B)** Leaves of *A. latifolia* and **(C)** *A. latifolia* showing flowers and fruits

Vein Termination Number

The number of vein endings was counted. This count was done within four different one- square- millimeter areas under the microscope, and the average was taken to determine the vein termination number per square millimeter.

Chemo-microscopic examination

The presence or absence of cell inclusions including starch grain, protein, lignin, fats/oil, calcium carbonate and calcium oxalate crystals was assessed through Chemo-microscopic analysis following standard protocols [11].

Determination of analytical standards

Analytical standards and physicochemical constants of the leaf includes total ash value, water insoluble ash value, acid insoluble ash value, sulphated ash value, extractive values and moisture content that was determined to evaluate the quality and purity of the drug. For determination of different ash values, the powdered sample was passed through sieve no.120 and only fine powder was used.

Total ash value

A tarred silica crucible was placed in muffle furnace for about 15 minutes at 450ºC until free from carbon, cooled in a desiccator for about one hour and the crucible was weighed (W_1). 3.0g of the powered material was placed into the silica crucible and heated gently until all the moisture exhausted and the plant material was completely charred (W_2) and the sample turns grey white. The crucible was removed with crucible tong, cooled in a desiccator, and weighed again (W_3).

The percentage ash content was determined as:

% Ash = Final weight of crucible (W_3) – Initial weight of crucible (W_1) x 100

Weight of sample and crucible (W_2) – Initial weight of crucible (W_1)

Water soluble ash value

To determine the water- soluble portion of the ash, the total ash obtained was mixed with 25 mL of distilled water and brought to a gentle boil. The mixture was filtered through ashless filter paper, and the residue was thoroughly rinsed with hot water. The filter paper containing the residue was then dried and ignited in a muffle furnace at a temperature not exceeding 450ºC until carbon- free. The weight of the residue was recorded as water- insoluble ash.

% Water Soluble Ash = % Total Ash - % Water Insoluble Ash

Acid insoluble ash

The ash obtained from total ash was transferred into a beaker containing 25 ml of dilute hydrochloric acid and was boiled for 5 minutes. The resulting mixture was filtered using an ash-less filter paper, and the residue was rinsed repeatedly through the filter paper with hot water until it was free from acid. The filter paper and residue were then dried and incinerated at 500ºC in a muffle furnace until carbon free. The remaining ash represents the acid- insoluble ash content.

QUANTIFICATION OF EXTRACTIVE YIELDS

Alcohol soluble extractive value

A 5.0 g of air- dried material was weighed accurately and coarsely powdered, placed with 100 ml of 95% ethanol for 24 hours in a closed conical flask. The flask and its contents were shaken mechanically for about 6 hours and was allowed to macerate for another 18 hours and then filtered. The filtrate was collected and evaporated to dryness in a tared flat-bottomed shallow dish at 105ºC and weighed. Then the percentage of ethanol- soluble extractive with reference to the air-dried drug was calculated.

Water soluble extractive value

A 5.0 g of air- dried material was weighed accurately and coarsely powdered, placed with 100 ml of chloroform- water for 24 hours in a closed conical flask. The flask and its contents were shaken mechanically for about 6 hours and was allowed to macerate for another 18 hours and then filtered. The filtrate was collected and evaporated to dryness in a tared flat-bottomed shallow dish at 105ºC and weighed. Then the percentage of ethanol- soluble extractive with reference to the air-dried drug was calculated.

Determination of moisture content

A preheated, tarred porcelain crucible with a lid was weighed and recorded as (W_1). A measured amount of the dried sample was added to the crucible and the total weight was recorded as (W_2). The sample was then heated in an oven at the 65ºC for 12 hours, with periodic weight checks at 6-, 3-, 2- and 1-hour intervals until a constant weight was achieved. After heating, the crucible was cooled in a desiccator and weighed again to obtain the constant weight (W_3).

PHYTOCHEMICAL PROFILING

Qualitative phytochemical Profiling of the crude extract

Qualitative phytochemical tests were performed to detect the presence of various secondary metabolites as important plant compounds in the crude plant extract by following standard procedures [7,12].

RESULTS

Macroscopic analysis of *Anogeissus latifolia* Leaf

The macroscopic characteristics of *Anogeissus latifolia* leaves, including their general appearance, texture and taste were observed. These include features like the plant's organoleptic traits (such as color and smell) as well as other morphological aspects, both qualitative and quantitative. These findings are summarized in Table 1.

Quantitative leaf microscopy

Microscopic measurements such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were taken from the leaf and listed in Table 2. These measurements are useful for identifying the plant.

Microscopic analysis of leaf

The microscopic characteristics of *Anogeissus latifolia* are shown in Figure 2-3.

Table1: Macroscopic examination of the leaf of *Anogeissus latifolia*

S. No	Macroscopical features	Description
	Organoleptic characters	
1.	Color	Adaxial surface is dark green as compared to Abaxial surface
2.	Texture	Smooth and glossy
3.	Taste	Bitter
4.	Odour	non-distinct
	Macromorphological Characters	
5.	Margin	Entire and glabrous
6.	Apex	Ovate to elliptic
7.	Shape of Lamina	Elliptic or elliptic oblong
8.	Venation	Reticulate
9.	Base	Cuniate/ obtuse, round or acute
10.	Type of leaf	Simple, opposite to alternate
11.	Leaf arrangement	Opposite to alternate
12.	Stipule/Non-stipule	Stipulate
13.	Petiole/ Non-Petiole	Petiolate leaf
14.	Mid- rib	Raised at abaxial surface compared to adaxial surface
	Quantitative Macroscopy	
15.	Leaf length	6-7 cm
16.	Leaf width	2.5- 5 cm

Table 2: Quantitative analysis of *Anogeissus latifolia* leaves

Analytical Profile of *A. latifolia* leaf

The leaf of *A. latifolia* was assessed for various analytical parameters, including total ash, water- soluble ash, acid- insoluble ash, alcohol soluble extractive, water- soluble extractive and moisture content. All results were recorded in percentage and are detailed in Table 5.

Parameters	Values (Mean + SD, n= 5)
Stomatal number	7.94 ± 0.18 mm^2
Stomatal index	11.62 ± 0.15 %
Vein islet number	5.40 ± 1.2 mm^2
Vein termination number	3.62 ± 0.06 mm^2
Palisade ratio	5.41 ± 0.5

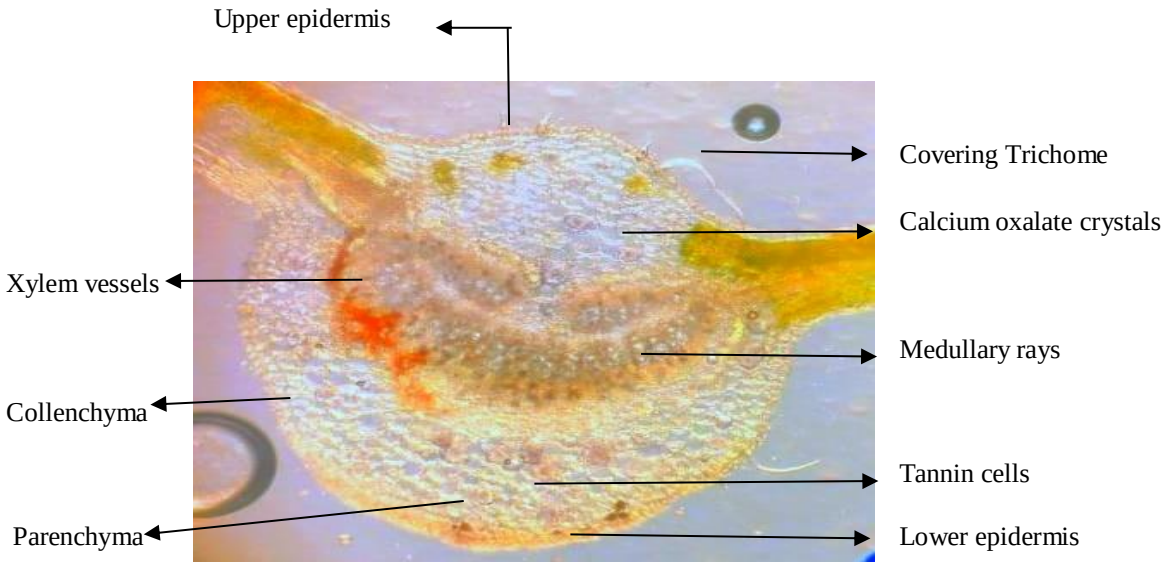

Figure 2: Photomicrograph showing the Transverse section of the *A. latifolia* leaf lamina across midrib

(a) (b)

Figure 3: Photomicrographs of *Anogeissus latifolia* leaf (a) Surface view under 10x magnification; (b) Lower epidermis under 10x magnification

Table 5: Analytical evaluation of *A. latifolia* leaf

S. No	Parameters	Values (% w/w)

1.	**Moisture content**	7.23
2.	**Ash values**	
	Total ash	8.17
	Acid insoluble ash	0.80
	Water soluble ash	2.47
3.	**Extractive values**	
	Water soluble extractive value	13.46
	Alcohol soluble extractive value	15.27

Table 4: Results of Phytochemical screening

S.No.	Phytochemical constituents	Pet. Ether	Benzene	Chloroform	Ethanol	Water
1.	Carbohydrates	-	-	-	+	+
2.	Reducing sugars	-	-	-	-	++
3.	Alkaloids	-	-	++	+++	-
4.	Glycosides	-	-	-	-	+
5.	Steroids	++	-	-	-	-
6.	Saponins	-	-	-	+	+
7.	Terpenoids	++	++	+++	+++	-
8.	Tannins	-	-	-	+++	++
9.	Flavonoids	-	-	+	+++	+

Table 3: Chemo-microscopic characteristics of leaf of *Anogeissus latifolia*

S. No.	Constituents	Testing reagents	Observations	Inference
1.	Cellulose	N/50 iodine followed by almost dry 80% H_2SO_4 treatment	Blue-black coloration	++
2.	Tannins (Ferric chloride test)	70% methanol extract treated with dil. $FeCl_3$	Solution changed from dark green to olive green	+++
3.	Calcium carbonate (Effervescence test)	Acetic acid with 50% H_2SO_4	Effervescence produced with needle- like crystals	++
4.	Fats and oils	Sudan IV stain added	Brick-red droplets observed	+
5.	Starch (Iodine test)	N/50 iodine solution added	Blue- black coloration developed	+++
6.	Calcium oxalate	Clear in chloral hydrate sol. In glycerin and then dil.H_2SO_4 added	Shiny and irregular- shaped crystals observed	+++
7.	Proteins (Ninhydrin test)	Ninhydrin reagent added and gently warmed	Yellow ppt produced	++
8.	Lignin (Wiesner's test)	Phloroglucinol with conc. HCl added	Pink color produced	++

Inference: - Absent, + traces, ++ present, +++ significant

Discussion

The macroscopic and microscopic evaluation of the leaf of *Anogeissus latifolia* revealed that it is Petiolate, stipulate, simple and opposite to alternate leaf with reticulate venation, entire and glabrous margin and Cuniate base.

The Organoleptic evaluation of fresh leaves of *Anogeissus latifolia* revealed the dark green color of adaxial surface as compared to abaxial surface with smooth and glossy texture with bitter taste and non- distinct odour.

The sequential use of macroscopy followed by microscopy enhances the efficiency and effectiveness of sample analysis. Hence, the plant drug was identified, dried, in shade and then powdered.

The Transverse section of the leaf of *Anogeissus latifolia* showed dorsi- ventral structure of leaf. The lamina is supported by the midrib and veins with serrated or smooth margins. The leaf composed of elongated palisade mesophyll cells, located closer to adaxial surface while loosely arranged spongy mesophyll cells present beneath the palisade layer. Vascular bundles branch off from the midrib and extend into the leaf veins and form a network throughout the leaf lamina. The xylem and phloem are located within the interior of leaf veins.

Microscopic evaluation is essential in ensuring the identity and quality of crude plant materials. It forms an integral part of modern herbal Pharmacognostic research, especially for identifying and authenticating medicinal plants. The anatomical analysis of the leaf revealed significant structural features.

Table 3 and 4 presents analytical and phytochemical results, respectively. The ash content reflects the plant's total mineral residue, and includes both physiological and non- physiological ash. **Acid- insoluble ash,** which reflects silica and sand content, was 0.80, while the **Water- soluble ash** was also 2.47 indicating that only a small portion of the ash could dissolve in water.

The **Extractive values** serve to determine the suitability of different solvents for isolating active compounds. The leaves showed higher solubility in ethanol as 15.27% compared to water as 13.26% implying the presence of more alcohol- soluble phytoconstituents like phenols, flavonoids, steroids, tannins and terpenoids.

Lastly, the **moisture content** was 7.23 % shows the acceptable range for crude drug materials, indicating the stability and lower possibility for enzymatic degradation and microbial contamination of *A. latifolia* leaves.

The Chemo- microscopic examination detected the presence of calcium oxalate crystals, tannins, starch grains, cellulose, fats and oils and lignified cell walls (Table 5) and further supports the plant's medicinal potential.

CONCLUSION

The Pharmacognostical, Phytochemical and Physicochemical analysis of *Anogeissus latifolia* have been confirm the presence of important secondary metabolites like tannins and flavonoids, which are often responsible for the plant's therapeutic effects. Such detailed analysis supports the potential of *Anogeissus latifolia* in herbal medicine and highlighting its value in developing standardized plant- based drugs and official monographs.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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