EVALUATION OF PHYTOCHEMICAL ANALYSIS AND PHARMACOGNOSTICAL ACTIVITY OF THE LEAVES OF *PISONIA GRANDIS* R. Br.

Palanisamy.P¹, R.Margret Chandira¹, B.S.Venkateswarlu¹, B.Jaykar², A.Pasupathi¹, N. K. K. Sreedharan¹, J.Gomathi¹

¹Department of Pharmaceutics, Vinayaka Missions University Salem, Tamil Nadu, India.
²Department of Pharmaceutical Chemistry, Vinayaka Mission’s College of Pharmacy Salem, Tamil Nadu, India.

**ABSTRACT**

*Pisonia grandis* (Nyctaginaceae) is an evergreen tree, 9-12m height and widely distributed throughout India. The leaves of plant used in treatment of hepatoprotective, anti-inflammatory, fever and ulcer. The present study was carried out to investigate morphological, microscopical, physicochemical and phytochemical screening of leaves of *pisonia grandis*. Morphological studies of leaves showed the presence of various diagnostic characters. The preliminary phytochemical screening of alcoholic extract showed positive results for alkaloids, phenolic compounds, tannins, proteins, aminoacids and flavonoids. Morphological studies of leaves showed the presences of various diagnostic characters. The present studies pharmacognosy and phytochemical evaluation of *pisonia grandis*.

In the microscopical studies leaves showed the abaxial epidermis, abaxial side, adaxial epidermis, adaxial side, lamina phloemxylem,parenchymatous ground tissue, stomata raphide. The result of the study could be useful for identification and preparation of monograph of the plant.

**KEYWORDS:** *Pisonia grandis*, Phytochemical analysis, microscopy.
INTRODUCTION

*Pisonia grandis* R.Br syn. Shell bark, *Pisonia umbellifera*, etc., belonging to family Nyctaginaceae, 9-12m height and weidely distributed throughout India and tropical regions. The leaves are useful in treatment of hepatoprotective, anti-inflammatory, fever and ulcers. In other parts of the world the plant is considered wound healing effects, diuretics, purgactive and elephantoid. The leaves are used as green leaves for paralysis.\(^1\)\(^-\)\(^2\) The alcoholic extract showed hepatoprotective activity.\(^3\)\(^-\)\(^4\) In aqueous extract showed carbohydrates, tannins and flavonoids activitys. Plant have played a major role in the introduction of new therapeutic agents. In folk/tribal medical practice many diseases in south India. Most of these medicinal plants are not scientifically validated for their therapeutic efficacy and safety. A scientific study on this plant is likely to provide invaluable anti-inflammatory drug. In present work the plant, which is locally available in Salem district and have been used for long time in local folklore medicine, has been selected to study its hepatoprotective and anti-inflammatory activities.\(^5\)

MATERIALS and METHODS

**Plant materials**\(^6\)\(^-\)\(^8\)

The leaves of *Pisonia grandis* R.Br. were collected in the month of July 2014 from the shevaroyan hill, Salem District, Tamilnadu, India, identified and authentified by Mr.A.Balasubramanian, consultant, central siddha research, salem(D.T), Tamil nadu, India. The fresh leave were used to study macroscopy and microscopy where as shade dried powder was used for the determination of physicochemical parameters and phytochemical screening.

**Macroscopical studies**

The fresh leaves were subjected to macroscopic studies which comprised of organoleptic characteristic of the drug viz., colour, odour, taste, fracture, etc.,

**Preparation of Slide For Microscopical studies**\(^9\)\(^-\)\(^11\)

The leaves were cut and removed from the plant and fixed in FAA (formalin- 5 ml + acetic acid – 5 ml + 70% ethyl alcohol – 90 ml). After 24 hours of fixing the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the schedule given by Sass.\(^12\) Infiltration of the specimens was carried by gradual addition of paraffin wax until TBA solution attained super satutraion. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned to a thickness of 10-12 μm with the help of
rotary microtome (LEICA RM, 2135, Germany). Dewaxing of the sections was done by customary procedures given by Johnson. The sections were stained with Toludine blue as per the method published by O’ Brien. The necessary sections were also stained with safranin-fast green and Iodine in potassium iodide for starch and other constitutes. Microscopic descriptions of tissue are supplemented with micrographs wherever necessary, photographs of different magnifications were taken with Nikon Labphot 2 microscopic unit. For normal observations bright filed was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property under polarized light, they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars.

**Physico-Chemical Constants Determinations**

The collected leaves were washed with water, dried, powdered and subjected to the following physico-chemical constant determination.

**Determination of Ash Values**

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituent by making use of water-soluble ash and acid insoluble ash.

**Total Ash**

**Procedure**

Incinerate about 2 to 3 gms accurately weighed of air dried powder of *Pisonia grandis* R.Br. in a tared platinum or silica crucible at a temperature not exceeding 450°C until free from carbon, cooled and weighed. Calculated the percentage of total ash with reference to the dried powdered drug.

**Acid Insoluble Ash**

The ash obtained in the above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The residue was collected on a ashless filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.
Water Soluble Ash
Boiled the ash for 5 minutes with 25 ml of water. The insoluble matter collected in a Gooch crucible or on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Sulphated Ash
Heat a silica or platinum crucible to redness for 10 mintues allow to cooled in a desicator and weighed. Put 2 gms of substance, accurately weighed, into the crucible, ignitly at first, until the substance is thoroughly charred. Cooled, moisten the residue with 1 ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignited at 800°C ± 25°C until all black particles have disappeared. Allowed the crucible to cooled, add a few drops of sulphuric acid and heated. The percentage of sulphated ash with reference to the air dried drug was calculated.

The results are expressed in table no.3

PRELIMINARY PHYTOCHEMICAL STUDIES
PRELIMINARY PHYTOCHEMICAL TESTS[7-9]
All the extracts of *Pisonia grandis* were subjected to qualitative tests for the identification of various active constituents.

Test For Carbohydrates And Glycosides
A small quantity of various extracts were dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's test
The filtrate was treated with 2 - 3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Fehling's test
The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates.
Another portion of extracts were hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the presence of glycosides.

**Legal's test**
To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

**Borntrager’s test**
Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

**Detection of Fixed Oils and Fats**

**Filter paper test**
Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicated the presence of fixed oils.

**Saponification test**
Few drops of 0.5M alcoholic potassium hydroxide was added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

**Detection of Proteins Ao and Free Aminoacids**
Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

**Million’s test**
The above-prepared extracts were treated with Million’s reagent. Red colour formed shows the presence of proteins and free amino acids.

**Biuret test**
To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.
Ninhydrine test
The extracts were treated with Ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids.

Detection of Saponins
The extracts were diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Detection of Tannins and Phenolic Compounds:
Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.
1) 5% Ferric chloride solution - violet colour
2) 1% solution of gelatin containing
   10% sodium chloride - white precipitate
3) 10% lead acetate solution - white precipitate
Above findings shows the presence of phenolic compounds and tannins.

Detection of Phytosterols
Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski test
To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

Libermann Burchard test
The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection of Alkaloids
Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.
1) Mayer's reagent - cream precipitate
2) Dragendroffs reagent - orange brown precipitate
3) Hager's reagent - yellow precipitate
4) Wagner’s reagent - reddish brown precipitate

Detection of Gums and Mucilages
A small quantity of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage.

Detection of Flavonoids
1) Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.
2) To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.
3) Shinoda’s test
Small quantities of the extracts were dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

The phytochemical constituents present in various extracts were presented in table no.5

RESULTS AND DISCUSSION
The plant *Pisonia grandis* R.Br. is an indigenous tree which was chosen for this study. The plant belongs to the family Nyctaginaceae. The scanty availability of information on this plant facilitates the study on it. The attempt is made to study the pharmacognostical and phytochemical activities of leaves of *Pisonia grandis*. The study was divided into three major parts. Viz.

- Pharmacognostical studies
- Phytochemical screening

Macroscopical studies
The macroscopic charater was useful inquick identification of plantn material and also serves as an important standardization parameter. The all parts glabrous or the young shoots minutely puberulous. Leaves ovate or ovate oblong, 15-25 cm long, usually unequal and obtuse at the base.
Microscopical Studies

The leaf has dorsiventrail symmetry with prominent lateral veins and midrib (Fig 1.1, 1.2 and 2.1). The lamina is uniformly thin and even on both abaxial and adaxial surfaces/The lateral vein is planocortex with flat adaxial side and cortex abaxial side (Fig 1.2). The adaxial epidermis of the lateral vein is thin with small, Square shaped thick walled cells. The abaxial epidermal cells are slightly papillate. The ground tissue of the lateral vein is parenchymatous, circular and compact. The vascular bundle is single, collateral and ovate in outline.

The midrib is very thick and has wide and thick abaxial part and slightly raised hump. The midrib has papillate epidermal cells (Fig 2.1, 2.2). The ground tissue consists of outer four or five layers of collenchyma and remaining portion with thin walled wide and compact parenchyma.The vascular system of the midrib is complex (Fig 2.1, 2.2). It has a deep and wide are of about 10 discrete vascular bundles and an adaxial accessory bundle.
The are of main bundles are collateral and wedge shaped of the are of bundles, the abaxial median bundle is the largest, those along the two lateral area become gradually small towards the adaxial part.

The accessory adaxial bundle is large rectan and horizontally oriented. All vascular bundles have several parallel file of angular, thick walled wide xylem elements. Phloem is in the form of wide plate with radial file of sieve elements and parenchyma cells (Fig 2.2).

**Lamina (Fig 3.1)**

The lamina has thick adaxial and abaxial epidermal layers. The cells are rectangular and have thick cuticle.

The mesophyll consists of a single row of palisade cells which short, wide and loosely arranged. The spongy parenchyma consists of four or five layers of lobed cells and wide air chambers.

Calcium oxalate druses are wide spread in the palisade cells (Fig 3.2). The druses are small, solitoy and diffuse in distribution stomata and epidermal cells (Fig 4.1, 4.2).

The stomata occur only the abaxial epidermis. They are anomocytic type. The guard cells are ellipical or circular. The abaxial epidermal cells are smaller with slightly wavy anticlinal walls (Fig 4.1).

The adaxial epidermis has wider cells which polygonal in outline. Their anticlinal walls are fairly thick and strainght (Fig 4.2).

**Venation (Fig 5.1)**

The lateral veins are thick and straight. They have parenchymatous bundle sheath cells. The vein islets are district and wide. They vary in shape and orientation. The vein terminations are district; they are long slender and straight; they may be simple or branched.

**Raphides**

Calcium oxlate needles forming thick raphide bundles are abundant in the mesophyll. They are random in distribution as well as orientation (Fig 5.1, 5.2). The bundles are spindle shaped with conical ends.
Palanisamy et al.

World Journal of Pharmaceutical Research

Powder microscopic results of the leaf (Fig 6.1, 6.2, 6.3): The leaf powder shows the following structures.

i) Raphides
Calcium oxalate raphides are abundant in the powder. They appear as dark spindle shaped bodies scattered freely or embedded in the mesophyll (Fig 6.1, 6.2).

ii) Abaxial epidermis
Pieces of abaxial epidermis are seen in the powder. They possess dense stomata and wavy epidermal cells (Fig 6.1).

iii) Adaxial epidermal cells are also seen in the powder. They are apostomatic. The cells are wide and have thin, wavy anticlinal walls (Fig 6.3).

PHYSICO-CHEMICAL PARAMETERS
The result of various physical constants Viz. ash values and extractive values of the leaves of *Pisonia grandis* are shown in table.

Ash values
The physicochemical analysis of the leaves powder was carried out. In this study ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash) were determined. The total ash value, acid insoluble ash value, water soluble ash value and Sulphated ash value was found to be 3.0232% w/w, 2.6932% w/w, 1.3992 % w/w and 3.4236 % w/w respectively.

Extractive values
Extractive values (water soluble and alcohol soluble extractive values) were determined. The alcohol soluble extractive value (16.72% w/w) is more then that of water soluble extractive value (12.92% w/w). The water soluble and alcohol soluble values indicate the presence of amount of constituents which are water and alcohol soluble.

Table. No: 1 Ash values and Extractive values of *Pisonia grandis* R.Br.

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Total ash (% w/w)</th>
<th>Acid insoluble ash (% w/w)</th>
<th>Water soluble ash (% w/w)</th>
<th>Sulphated ash (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pisonia grandis</em> R.Br.</td>
<td>3.0232</td>
<td>2.6932</td>
<td>1.3992</td>
<td>3.4236</td>
</tr>
</tbody>
</table>
Extraction of plant material

The dried and coarse powdered leaves of *Pisonia grandis* was extracted with solvents of increasing polarity successively by soxhlet apparatus whereas aqueous extract was obtained by cold maceration.

The percentage yield of dried and coarse powdered leaves of *Pisonia grandis* was found to be 9.4%, 7.9%, 1.72% and 2.64% respectively with alcohol, aqueous, chloroform and petroleum ether.

The percentage yield of the aqueous extract of leaves of *Pisonia grandis* was found to be lower than alcoholic extract.

Table. No: 2 Data showing the percentage yield value of various extractions of *Pisonia grandis* R.Br. leaves

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Part used</th>
<th>Method Of Extraction</th>
<th>Yield in Percentage w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Petroleum ether</td>
<td>Chloroform</td>
</tr>
<tr>
<td><em>Pisonia Grandis R.Br.</em></td>
<td>Leaves</td>
<td>Continuous hot Percolation</td>
<td>2.64</td>
</tr>
</tbody>
</table>

Phytochemical screening

The various extract of the plant of *Pisonia grandis* were subjected to phytochemical screening which reveals the presence of various pharmacological active compounds showing in table below. Petroleum ether extract- Carbohydrates, fixed oils, Flavonoids Chloroform extract - Alkaloids and Flavonoids.

Alcoholic extract- Alkaloids, Phenolic compounds, Tanins, Protein and Amino acids, Saponins, Sterols and Flavonoids

Aqueous extract – Carbohydrates and Tanins

Table. No: 3 Phytochemical studies of various extracts of *Pisonia grandis* R.Br. leaves

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant Constituents</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Alcoholic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Gums and Mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Fixed oils and fats</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Legend for figures

**Fig. 1. Anatomy of the leaf**

![Image of Anatomy of the leaf](image1)

*Fig. 1.1: T.S of leaf through lateral vein with lamina*

![Image of Lateral vein with lamina enlarged](image2)

*Fig: 1.2: Lateral vein with lamina enlarged*

- [ABE- Abaxial Epidermis, ABS- Abaxial Side, ADE- Adaxial epidermis, ADS- Adaxial Side, La-Lamina, LV- Lateral Vein, Ph-Phloem].
Fig: 2: Anatomy of the midrib

![Anatomy of the midrib](image1)

**Fig: 2.1: T.S of Midrib:**

![T.S of Midrib](image2)

**Fig: 2.2: T.S of Midrib - Vascular bundle enlarged:**

- [ABS- Abaxial bundle, ABS- Abaxial Side, ADB- Adaxial Bundle, ADS- Adaxial Side, Col- Collenchyma, X- Xylem, Epidermis, GT- Ground Tissue, Phloem, PG- Parenchymatous Ground tissue].

Fig: 3: Anatomy of the Lamina:

![Anatomy of the Lamina](image3)

**Fig: 3.1: T.S of Lamina**
Palanisamy et al. World Journal of Pharmaceutical Research

[ABE – Abaxial Epidermis; ADE – Adaxial Epidermis; Dr – Druses; Cu – Cuticle; LV – Lateral Vein; PM – Palisade Mesophyll; SM – Spongy Mesophyll; Ra – Raphide; St-Stomata]

Fig: 4: Epidermal Morphology:

Fig: 4.1: Abaxial Epidermis with Stomata:

Fig: 4.2: Adaxial Epidermis:

[EC – Epidermal Cells; St – Stomata]

Fig: 5: Venation Pattern and Raphide distribution:

Fig: 5.1: Vein islets and vein-Termination:
Fig: 5.2: Raphides in the mesophyll tissue [under polarized light microscope]:

Fig: 5.3: Two Raphides enlarged:
[Ra – Raphide; VI – Vein Islets; VT – Vein Termination].

Fig: 6: Powder microscopy of the leaf:

Fig: 6.1: Fragment of abaxial epidermis with Raphide:
CONCLUSION

In present investigation various standization parameter such as microscopy, microscopy, physio-chemical parameter and phytochemical screening was carried which could helpful in authentification of *Pisonia grandis*. The result of present study will also as reference material in preparation of monograph.

ACKNOWLEDGEMENTS

Authors are thankful to Prof.(Dr.) B.Jayakar, Principal, Vinayaka mission’s college of pharmacy, Salem, Tamilnadu, India providing all the facilities for this research work.

REFERENCE


