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Original Research Article

Pharmacognostical and Phytochemical Studies on *Spondia pinnata* Leaf

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Abstract: *SpondiaS. pinnata* (L.F.) Kurz belongs to the family Anacardiaceae. Present study carried to study the pharmacognostical and phytochemical characterestics of *S. pinnata* leaf. The standardization of the plant material (leaves of *S. pinnata*) was done and leaf constants like stomata number, stomatal index, vein islet number, vein termination number, palisade ratio was determined. Physical parameters like foreign matter, loss on drying, ash values and different extractive values were determined. The fresh leaves of *S. pinnata* were extracted with aqueous alcohol. The percentage yields were found to be 20.20%w/w. The extraction is done to determine the biological activity and phytochemical screening of extract. The preliminary phytochemical investigation revealed the presence of carbohydrates, alkaloids, glycosides, tannins and phenolic compounds and vitamin C. Also it had been seen that majority of the phytoconstituents were extracted in both water and ethanol extract of fresh leaves of *S. pinnata*. These constituents may be responsible for the reported pharmacological activities of this specific part of plant.

Keywords: Microscopical and chemomicroscopical analysis, Palisade ratio, Vein islets number and vein termination number, Stomatal index and stomatal number.

Introduction

A medicinal herb contains a number of chemical compounds, some of which are responsible for medicinal activity and are called secondary metabolites. Since ancient time, plant based product has been used for health care, search is continued for new plant material and their interactions with biological systems. Whenever such plant material is found to be useful it is taken up for further investigation, as regards to the constituents present for its biological action. On confirmation of its biological activity, the suitable extracts or isolated phytoconstituents are prepared from the plant material and put into usage [1].

S. pinnata belongs to the family Anacardiaceae in English it is called hogplum [2]. It is distributed throughout India. Its wood is employed for packing cases, tea chests and match-splints. The fruits are eaten as a vegetable when green and as a fruit when ripe. Essential oil from the pulp yielded carboxylic acids and esters, alcohols, aromatic hydrocarbons. The major compounds were 9, 12, 15-octadecatrien-1-ol (36.78%), hexadecanoic acid (25.27%) and furfural (19.77%) [3]. Fruits are very nutritious and rich in vitamin C, minerals and iron content. It is astringent and anti-scorbutic. It is stated to be useful in bilious dyspepsia. *S. pinnata* leaves have been used in dried or fresh form to treat wounds caused by poisonous arrows. The bark of *S. pinnata* tree is aromatic in nature and have astringent taste. It is useful in dysentery, diarrhea and is also given to prevent vomiting. A decoction made from the bark can be used to treat gonorrhea. The bark is also used for tanning. The root is considered useful in regulating menstruation. The plant is reported to have anti-tubercular properties. The leaves are aromatic, acidic and astringent. They are used for flavoring. The flowers are sour and used in curry as a flavouring and also eaten raw. The juice is applied in earache [4]. The present study includes Pharmacognostical characterization of leaves of *S. pinnata* and identification of phytochemical constituents by chemical tests.

Materials and Methods

In the present study, the leaves of *S. pinnata*, the following macroscopic characters for the fresh leaves were noted: type, size and shape, apex, margin, venation, base, petiole, surface, phyllotaxy, colour and taste. The transverse sections of the fresh leaves through the lamina and the midrib were mounted and observed after staining with phloroglucinol and dilute hydrochloric acid. Examination of the powder for starch grains and calcium oxalate prism were carried out.

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Microscopical and chemomicroscopical analysis

Put the sample powder in a test tube and add sufficient potassium hydroxide solution. Boil the sample over a Bunsen flame for a few minutes. This will soften the sample and help in obtaining fine characters [5].

Quantitative investigation

The quantitative leaf microscopical examinations were performed on epidermal strips to determine: Palisade ratio, Vein islets number and vein termination number, Stomatal index and stomatal number.

Palisade ratio

Palisade Ratio is the average number of palisade cells under one epidermal cell. Place leaf fragments of about 5×5 mm in size in test tube containing about 5ml of potassium hydroxide solution and heat in a boiling water bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in potassium hydroxide solution and put a small drop of glycerol solution on one side of the cover glass to prevent the preparation from drying. Examine with 40X objective and 6X eye piece to which a microscopical drawing apparatus is attached. Trace 4 adjacent epidermal cells on a paper, focus gently downward to bring the palisade into view and trace sufficient palisade cell to cover the area of the outline of the 4 epidermal cells. Count the palisade cells under the 4 epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath the epidermal cell, dividing the count by 4; this is the Palisade Ratio. For each sample of leaf make not fewer than 10 determinations and calculate the average number [6].

Vein islets number and vein termination number

Take pieces of leaf lamina with an area of not less than 4 square millimetres from the centre portion of lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing potassium hydroxide solution on a boiling water bath for 30-60 minutes or until clear and prepare a mount in glycerol solution. Place the stage micrometer on the microscope stage and examine with your objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimetres. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islet and vein termination within the square with those overlapping on two adjacent slides and excluding those intersected by the other two sides. The result obtained is the number of vein-islet and vein termination in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islet and vein termination per square millimetre [6].

Stomatal index and stomatal number

a) **Stomatal index**

The Stomatal Index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Place leaf fragments of about 5×5 mm in size a test tube containing about 5ml of potassium hydroxide solution and heat in a boiling water bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in potassium hydroxide solution and put a small drop of glycerol – ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (X) for each epidermal cell and a circle (0) for each stoma. Calculate the result as follows:

$$\textbf{Stomatal Index} = S \times 100 / (E+S)$$

Where, S → the number of stomata in a given area of leaf and E → the number of epidermal cells (including trichomes) in the same area of leaf. For each sample of leaf make not fewer than ten determinations and calculate the average index. [6].

b) *Stomatal number*

Place leaf fragments of about 5×5 mm in size a test tube containing about 5ml of potassium hydroxide solution and heat in a boiling water bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in potassium hydroxide solution and put a small drop of glycerol – ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (X) for each stomata and calculate the average number of stomata per square mm for each surface of the leaf [6]. Other parameters determined for the powdered leaves were: Moisture content, Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive values, Water soluble extractive values.

Moisture content

Place about 10g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01g) it in a tarred evaporating dish. For example, for underground or unpowdered drug, prepare about 10g of the sample by cutting shredding so that the parts are about 3mm in thickness. After placing above said amount of the drug in a tarred evaporating dish dry at 105^{O}C for 5 hours and weigh [6].

Total ash

Incinerate about 2 to 3g accurately weighed, of the ground drug in a tarred platinum or silica dish at a temperature not exceeding 450^{0}C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhausted the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450^{0}C. Calculate the percentage of ash with reference to the air dried drug [6].

Acid insoluble ash

Boil the total ash obtained for 5 minutes with 25ml of dilute hydrochloric acid, collect the insoluble matter in an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid insoluble ash with reference to air dried drug [6].

Water soluble ash

Boil the total ash obtained for 5 minutes with 25ml water, collect the insoluble matter in an ash less filter paper, wash with hot water and ignite for 15 minutes at a temperature not exceeding 450^{0}C. Subtract the weight of the insoluble matter from the weight of ash; the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug [6].

Alcohol soluble extractive values

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing standing for 18 hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105^{0} C to constant weight and weigh. Calculate the percentage of alcohol soluble extract with reference to the air dried drug [6].

Water soluble extractive values

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of chloroform water of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing standing for 18 hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{0}C to constant weight and weigh. Calculate the percentage of alcohol soluble extract with reference to the air dried drug [6].

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for determining the presence of carbohydrate (Molisch test), alkaloids (Dragendorff's test), Glycosides (Keller- Kiliani test), tannins and phenolic compounds (Lead acetate test).

Molish's test (Test for carbohydrate):

To 2-3 ml of aqueous- alcoholic extract added few drops of α-naphthol solution in alcohol. Shake and added concentrated sulphuric acid from sides of the test tube.

Biuret test (Test for proteins):

To 3ml of aqueous-alcoholic extract, added 4% sodium hydroxide solution and few drops of 1% copper sulphate solution.

Salkowski reaction (Test for steroid):

To 2ml of the aqueous- alcoholic extract, added 2ml of chloroform and 2ml of concentrated sulphuric acid. Shake well.

Solubility test (Test for volatile oil):

Oils are soluble in ether benzene and chloroform, but insoluble in 90% ethanol and in water.

Dragendorff's test (Test for alkaloids):

To 2-3ml of the filtrate added few drops of Dragendroff's reagent.

Keller kiliani test (Test for glycosides):

To 2ml of the extract, added glacial acetic acid, 1 drop 5% ferric chloride and concentrated sulphuric acid.

Ninhydrin test (Test for amino acids):

Heat 3ml of the extract and 3 drops of 5% Ninhydrin solution in boiling water bath for 10 minutes.

Lead acetate test (Test for tannins and phenolic compounds):

To 2-3ml of the aqueous- alcoholic extract added lead acetate solution.

Test for Vitamin C

Diluted 1ml of the extract with 5ml of water and added 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2ml of dilute sodium hydroxide solution. Added 0.6 ml of hydrochloric acid dropwise and stirred. Added 2ml of the extract to a few ml of 2, 6- dichlorophenolindophenol solution, the solution is decolourised.

Results and Discussions

Standardization parameter of fresh leaves of *S. pinnata*

Macroscopical Analysis

In the present study, the leaves of *S. pinnata*, the following macroscopic characters for the fresh leaves were noted: type, size and shape, apex, margin, venation, base, petiole, surface, phyllotaxy, colour and taste.

Fig.1: Photograph of Spondia*S. pinnata*

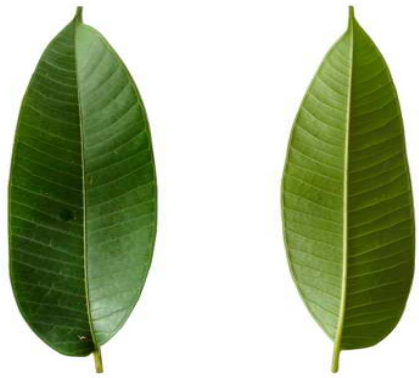

Fig.2: Leaf of Spondia*S. pinnata*

Table 1: Result of macroscopy of *S. pinnata* leaf

Morphological parameter	Observation
Condition	Fresh
Type	Simple
Size: Length	15-17 cm
Width	6.5-7.5 cm
Shape	Ovate
Apex	Acuminate
Margin	Serrate
Venation	Pinnately reticulate
Base	Asymmetric
Petiole	Short petiole
Surface	Thick and glabrous
Phyllotaxy	Simple and opposite
Colour: Outer	Dark green in colour
Inner	Light green in colour
Odour	Characteristic
Taste	Sour

The simple fresh leaf of *S. pinnata* has an average length of 15-17 cm and 6.5-7.5 cm in width. Ovate shaped reticulately pinnate leaves have serrate margin with acuminate apex. Thick and glabrous surfaced leaves have short petiole and are dark green in outer surface and light green in inner surface. *S. pinnata* leaves have characteristic odour with sour taste. The results are narrated in Table 1.

Microscopical Analysis

The transverse sections of the fresh leaves through the lamina and the midrib were mounted and observed after staining with phloroglucinol and dilute hydrochloric acid.

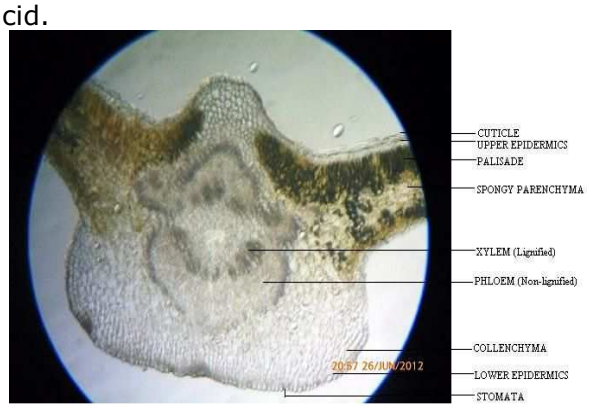

Fig.3: Transverse Section of Spondia*S. pinnata* Leaf

Chemomicroscopic examination

Microscopic examination of the powder done and observed starch grains ,calcium oxalate prism and xylem vessel .The photographs of same are given as fig.4,5 and 6.

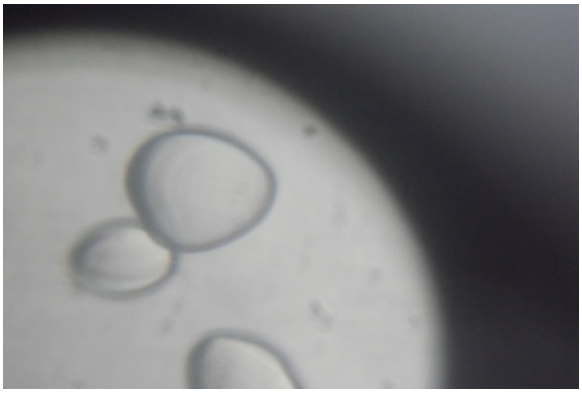

Fig. 4: Starch Grains

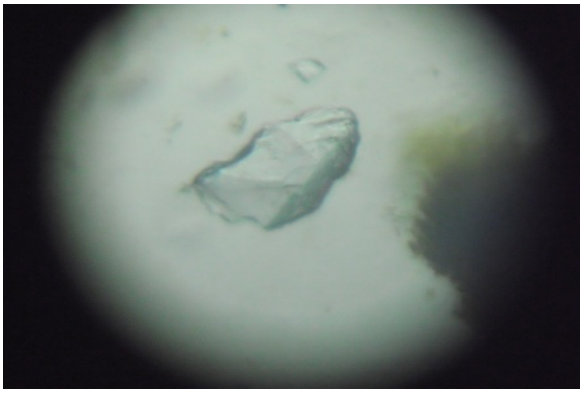

Fig. 5: Calcium Oxalate Prism

Fig.6: Xylem Vessel

Quantitative investigation

Quantitative leaf microscopy was performed on epidermal strips to determine palisade ratio, vein islet number and veinlet termination number and the type of stomata. Other parameters determined for the powdered leaves were moisture content, total ash, acid insoluble ash, water soluble ash, alcohol and water soluble extractive values. (4)

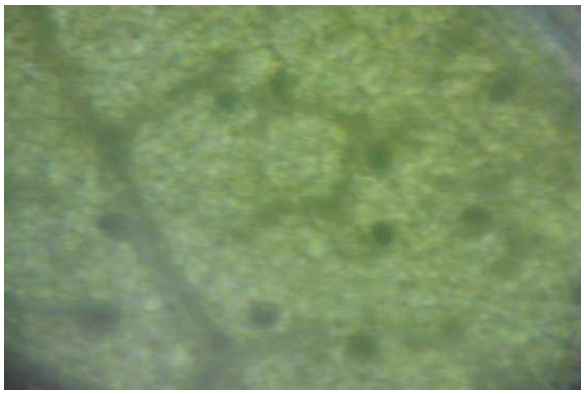

Fig. 7: Vein islet & Termination number

Fig. 8: Paracytic type stomata

Table 2: Result of standardization parameter of leaf drug-leaf constants

Sl no.	Leaf constants	Estimated value
1	Vein islet number	6-15
2	Vein termination number	18-29
3	Stomata number	6
4	Stomatal index	11.5
5	Type of Stomata	Paracytic
6	Palisade ratio	1:6

S. pinnata leaf have palisade ratio 1:6, and the stomata are paracytic in nature and are six in average number. Vein islet number is in the range of 6-15 and found to have around 18-29 vein terminations.

Standardization parameter of powdered drug

In the present study, powdered leaves of *S. pinnata* were investigated for the different standardization parameter. The results were recorded in table 3:

Table 3: Result of standardization parameter of powdered drug

S. no.	Physical constant	Estimate percentage
1	Foreign matter	Nil
2	Loss on drying	0.39%w/w
3	Total ash value	9%w/w
4	Acid insoluble ash value	1%w/w
5	Water soluble ash value	8%w/w
6	Alcohol soluble extractive value	49.6%w/w
7	Water soluble extractive value	10.4%w/w

Extraction

The percentage estimated yield of aqueous alcoholic extraction of fresh leaves *S. pinnata* were 20% and recorded in table 4:

Table 4: Result of percentage yield of extract

Sl no.	Nature of extract	Estimated percentage yield
1	Aqueous alcoholic extract	20.20 %w/w

Preliminary phytochemical investigation

Preliminary photochemical investigations were conducted for the ethanol and water extracts of leaves of *S. pinnata* to identify the various phytoconstituents. The presence or absences of various phytoconstituents were recorded in table 5:

Table 5: Result of phytochemical investigation

Sl no.	Constituent	Aqueous alcoholic extract
1	Carbohydrate (Molisch test)	++
2	Proteins (Biuret test)	--
3	Steroid (Salkowski reaction)	--
4	Volatile oil (90% alcohol)	--
5	Alkaloids (Dragendorff's test)	+
6	Glycosides (Keller Kiliani test)	++
7	Amino acid (Ninhydrin test)	--
8	Tannins and phenolic compounds (Lead acetate test)	++
9	Test for Vitamin C	++

(+)-presence of constituent, (-)-absence of constituent

The results indicate that the aqueous and alcoholic extract mainly contains carbohydrate, alkaloids, glycosides, vitamin C, tannins and phenolic compounds.

In the present study macroscopic characterestics of the entire leaf of *S. pinnata* revealed. Transverse sections of the leaf through the midrib have shown all characteristic cells like phloem, xylem, lower and upper epidermal cells. Stomata were also present. Presence of cuticle also revealed. Chemomicroscopic examination of dried powder of *S. pinnata* clearly indicated the presence of starch grains, calcium oxalate crystals and individual xylem vessels. From the quantitative study of the *S. pinnata* leaf various parameters like stomatal number, stomatal index, veinlet number, veinlet termination number, palisade ratio found out. Different standard parameters like ash values and extractive values specific to *S. pinnata* determined. Phytochemical analysis of dried powder shown the presence of plant metabolites like carbohydrate, alkaloids, glycosides, vitamin C, tannins and phenolic compounds are enriched in *S. pinnata* leaves.

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