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RESEARCH ARTICLE

Pharmacognostical & Phytochemical Evaluation of Karanja patra (*Pongamia pinnata* linn.)

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Abstract:

The study was undertaken on the basis of classical references in Ayurvedic literature. Kranaja Patra was studied for its prelimnary Pharmacognostical and Phyto chemical aspects. The study was conducted on ethanolic extract of Karanja Patra (*Pongamia pinnata Linn*)

Key Words: Karanja patra, Ethanlic extract.

INTRODUCTION

Ayurveda, the knowledge of life science bestowed health and longevity in the form of preventive and curative measures. The curative aspects are mainly covered by Dravya Chikitsa (treatment using drugs). As diseases are born with human, there is always a search for safest curative drugs. Acharya Charaka identified the necessity of complete knowledge on herbs and their utility in therapeutics. He gave the simile between the poisons with in discriminately used drug. On other hand drug used with perfect knowledge can act as nectar and very good drug can become a deadly poison, if the knowledge is properly and improperly used respectively.

In the present era, the attraction towards Ayurveda is increasing day by day due to fewer side effects. Ayurveda considered every dravya in the nature as

Aushadhi. The Aushadhi here includes raw or processed drugs obtained from natural resources as plants, animals and minerals. Ayurveda deals with thousands of natural sources as medicine

and has given freedom to utilize them for curing ailments.

बहुतातत्रयाग्येत्वंअनेकविधकल्पना।

संपच्चेतिचतुष्कोऽयंद्रव्याणांगुणउच्यते॥ च. सु. ९/७

Means drug should have the following qualities in it, Such as it should have free availability, effectiveness, capability of being subjected to various pharmaceutical processing and it should be excellent in condition.

Karanja Patra is one of the plant origin drug, which had been mentioned for its varied benefits in the literature of Ayurveda. Its leaf, flower are being used in many disease such as kasa, krimi, shotha, vrana, kustha etc.

On this scientific background the present drug Karanja Patra- *Pongamia pinnata* Linn .was subjected for different studies to know its pharmacognostical characters, chemical constitution in the selected part of plant.

RESEARCH ARTICLE

OBJECTIVE

Pharmacognostical and Preliminary phytochemical analysis of Karanja patra.

REVIEW OF LITERATURE:

Charakoktaganas[1]:

LekhaneeyaDashaimaneeya, BhedaneeyaDashaimaneeya, Kandughna Dashaimaneeya, Katuskandha.

Sushrutokta ganas[2]:

Aragvadhadigana, Salasaradigana, Varunadigana, Shyamadigana, Shiroveerechaneeyagana.

Asthanga Hrudayam[3]:

Vamaka, Aragvadhadigana, Varunadigana, Shyamadigana.

NIGHANTU KALA:

Nighantu	Vargas
Dhanawantari Nighantu[4]	Amradi Panchamovarga.
Raj Nighantu[5]	Prabhadradivarga.
Bhavaprakasha Nighatu[6]	Guduchyadi
NighantuAdarsha[7]	Chirabilvadi
ShaligramNighantu[8]	Guduchyadi

COLLECTION:

The leaf of Karanja is collected during the Varsha and Vasantaritu, after the blossoming of the flowers and before ripening of the fruits.

According to Acharya Sushruta, the leaf of Karanja is collected in the Varsha ritu[9]

SANSKRIT NAME: KARANJA

BOTANICAL NAME:

***Pongamia pinnata* Linn.**

Etymological Derivation of Botanical name:[10]

Pongamia- Tamil name pungam,

Pinnata - Indicates pinnately compound leaf.

FAMILY: Leguminosae.

SUB Family: Papilionaceae.

BOTANICAL CLASSIFICATION:

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Leguminoseae
Genus:	*Pongamia*
Species:	*Pinnata*

ETYMOLOGY OF SYNONYMS[11]:

1. करञ्ज (भा. प्र.नि.): कंजलंरञ्जयतिनीलाभत्वात ।

It imparts bluish colour to water.

2. उदकीर्य (अ. नि.):उदकेकीर्यन्तेपुष्पण्यस्य ।

Flowers scattered in water.

3. करज (भा. प्र. नि.):नखःतदाकृतिपुष्पत्वात् ॥

Flowers are shaped like nail.

4. गुच्छपुष्पक (कै. नि.):गुच्छेपुष्पाण्यस्य ॥

Flowers are present in bunches.

5. घृतपर्णक (नि. आ.): घृतवत्स्निग्धानिपर्णान्यस्य ॥

Leaves are glossy and unctuous.

6. घृतपूर्ण (भा.प्र.नि.):घृतवत्स्नेहेनपूर्णानिबीजन्यस्य ॥

Seed yields ghee like substance.

7. नक्तमाल (भा. प्र. नि.):नक्तंरात्रौ आ समन्तातअलतिभूषयतियथा ॥

Flower blossoms during night.

8. पूतिपत्रक (सो. नि.):पूतीनिदुर्गन्धीनिपत्राण्यस्य॥

Leaves are foetid in nature.

9. लाजपुष्पक (अ. नि.):लाजाकृतीनिपुष्पाण्यस्य ॥

Flowers resembles like Laja (Parched paddy).

10. श्लीपदारि (नि. आ.):श्लीपदहर्तृत्वात् ॥

It is an effective drug for Filaria.

11. स्निग्धपत्र (कै.नि.):स्निग्धानिपत्रण्यस्य ।|

Leaves are unctuous in nature.

MORPHOLOGY:

Habit: A medium sized semi-evergreen plant growing up to the height of 20-50 feet. Bark greyish green or brown, very often mottled with dark brown dots, specks, lines or streaks.

Phyllotaxy: Alternate.

Leaves: Imparipinnately compound, petiolated, Leaflet are 4-5 and about 1-5 inches long, shape- ovate- elliptic, margin- wavy, Apex- acuminate, glabrous on both the surface. Colour: Shining with dark green colour to bright green in colour.Gandha: ugragandha after crushing.

Inflorescence: Axillary raceme or panicle. **Flowers**: Complete, Regular, Bisexual, pinking white, fragment, mainly blossoms during night.

Calyx: Sepals are 5 and Gamosepalous.

Corolla:Petals are five and polypetalous purple to white.

Androecium: Stamens are 10 and dialephous.

Gynaecium: Capels are unicarpillary.

Ovary: Superior.

Fruits: Legume.

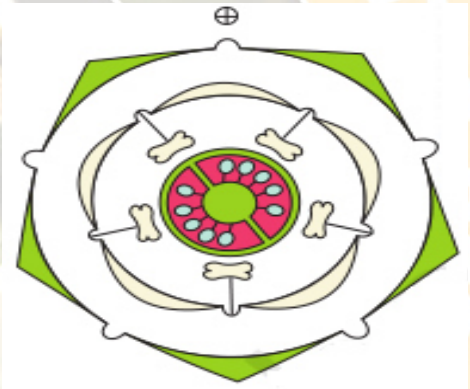
Seeds: Reniform, 1.2-1.8 cms wide.

Floralformula:

K(5)C(5)A(9+1) G_1

9. Floral diagram:

Fig. 1. Showing Floral formula of *Pongamiapinnata*Linn.



MATERIALS AND METHOD

The present study was carried out under following two headings;

A) Pharmacognostical study of Karanjapatra.

B) Phytochemical study of Karanjapatra.

A) Pharmacognostical study of Karanjapatra:

Aim: - The aim of present study was to see *Morphological*, *Microscopic*, *Physical evaluation* of Karanja patra (*Pongamia pinnata* Linn).

1) Morphological study:

Aim: - To study morphological features of Karanjapatra.

Materials: The materials required for study are –

Drug: Karanja(*Pongamiapinnata*Linn.).

Part: Patra (Leaves).

Collection of materials: The leaves of Karanja(*Pongamiapinnata*Linn.) were collected freshly from Herbal garden of N.K.J. Ayurvedic medical college and PG centre Bidar.

Equipment: Sense organs

Methods:

i) Organoleptic method

ii) Extra features.

(i)Organoleptic characteristics: In this method nature of the leaves, colours, taste, size, shape, odour, etc characteristics were studied with the help of sense organs.

(ii) Extra features: The arrangement of leaves and special characteristics of leaves were studied.

(2) Microscopical study[12]:

Materials: The materials collected for the studies were.

Drug: Fresh leaves of *Pongamiapinnata*Linn. (Karanjapatra).

Equipment: Compound Microscope, Eye Piece, Camera Lucida, Glass Slides, Cover Slips, Watch Glass, Camel Brush, Mountain Brush, Filter Paper, Blades, Spirit Lamp, Pipettes.

Chemicals: Phloroglucinol, Chloral hydrate, Conc. HCl. Glycerine, Iodine.

Methods:

1) Section Method

2) Staining process method:

Thin transverse section of the sample was taken and transferred it on a slide with the help of mountain hairbrush. Add a drop of water. Added few drops of chloral hydrate solution and allowed to heat for two to three minutes. Added equal proportions of phloroglucinol and conc. HCl, warm gently on a flame and cool it. Finally added a drop of glycerine and covered the section avoiding air bubble carefully with cover slip. Focused the section under microscope and the arrangements of cells were studied.

3) Physical (Microscopical) evaluation:

Aim: To know the physical constituents of the trial drug and to see their different values subjecting to the different tests as described in the Ayurvedic pharmacopeia of India.

Material:

Drug: *Pongamia pinnata* Linn- Karanja patra

Parts: Leaves of Karanja.

Equipments: Compound Microscope, Eyepiece, Camera Lucida, Glass Slides, Cover Slips, Watch Glass, Camel Hairbrush, Mountain Brush, Filter Paper, Blades, Spirit Lamp, Micrometer.

Chemicals: Phloroglucinol, Chloral hydrate, Conc. HCl, Glycerine.

1) **Methods:**Stomatal number, Stomatal index, Vein islet number, Vein termination number andPalisade ratio.

2) Staining Process Method

1) Section Method:

A fresh healthy, non-infected leaf of Karanja was selected. It was cut at its mid rib and taken the sample into small square section of potato, then hold the sample vertically in between the thumb and fore finger, with the help of new blade, 10 to15 sufficient thin transverse sections were taken; thick and oblique sections were rejected. Then with the help of mountain hairbrush, the thin selected sections were transferred to the watch glass containing water. Put the Samples in a test tube, added sufficient quantity of chloral hydrate.

Methodology:

3-4 pieces of the fresh leaf were cut from the middle portion of the lamina avoiding midrib and margin. These sections were taken in a test tube and boiled with chloral hydrate solution in a water bath, until they were clean enough for observation. Different cleaning methods are applied for individual leaves that mainly have very thick lamina. The leaf Sections were taken in watch glass and one of them was mounted on a glass slide in chloral hydrate solutions with lower surface of the leaf facing up wards so that the veins, which are more prominent in the lower surface, are seen clearly under the microscope. For this study, 6x eyepiece and low power objectives were used. The stage micrometer was focused (1mm) and the camera lucida was fixed in such a way that the aperture of it is in the same line with that of the eyepiece. Black drawing sheet was placed on the same side of the microscope where camera lucida was fixed. Using a white pencil draw a square of 10×10 cm length.

Then the stage micrometer was removed & the slide was mounted with the leaf specimen & focus in the same way. The square drawn in the paper was adjusted in such a way that it lies exactly in the middle of the field of vision and the image of the leaf piece mounted appears to be superimposed on the square of the drawing sheet. Starting from one side all the vein islets inside the square as well as on the boundary was traced. The vein let termination within the square only was taken into account.

Stomatal number:

Methodology: For determining the Stomatal index fragments

Cleared the pierce of the leaf by boiling in chloral hydrate solution.

Peeled out the upper & lower epidermis by using forceps.

Kept it on slide and mount in glycerine water.

Drawn a square of 1 mm by means of stage micrometer.

Fixed the camera Lucida.

Placed the slide with cleared leaf on the stage, trace the epidermal cell & stomata.

Counted the numbers of stomata present in the area of 1sq. mm.

Included the cell of at least half of its area lies within the square.

‥Record the result for each of the ten fields and calculated the average number of stomata per sq.mm.

Stomatal index:

For determining the Stomatal index, the fragment of leaf of 5x5 mm2 in size was taken in a test tube containing 5 ml of chloral hydrate solution. It was heated in a water bath until the fragment become transparent.

Then peeled out the upper and lower epidermis by means of forceps.

The cleared one is mounted on the glass slide by adding few drop of chloral hydrate solution.

Then draw a square of 10x10 cm on a black drawing sheet.

Fixed the camera lucida, placed the mounted slide on stage of microscope.

Counted the number of stomata & epidermal cells in each field.

Calculated the Stomatal index by using the formula.

I = S E + S X100

I - Stomatal index

S – Stomatal Number

E – No. of Epidermal cells

Palisade ratio:

Methodology:

Taken a healthy leaf, the lamina of the leaf avoiding the midrib was cut into 4-5 pieces.

Then fragments of the leaf were boiled in test tube containing chloral hydrate solution.

The upper & lower epidermis is peeled out by using forceps.

Cleared one fragment of leaf was mounted on glass slide as upper epidermal layer kept upper most.

Arranged the camera lucida and drawing board.

Traced off the outline of four cells of epidermis.

Then by using high power objective, focused down to palisade layer and traced off sufficient cells to cover epidermal cells.

Counted the palisade cells under the four epidermal cells.

Included the palisade cells in the count when more than half is within the area of epidermal cell and exclude it when less than half was within the area of epidermal cells.

Palisade ratio was calculated by dividing the total number of palisade cell by 4.

Vein islet Number:

Methodology:

3-4 piece of fresh leaf were cut from the middle portion avoiding the mid-rib.

These pieces of leaf are taken in a test tube containing chloral hydrate and heated in a water bath for 30 min.

Peeled out the upper and lower epidermis by using forceps.

Palisade ratio:

Methodology:

Taken a healthy leaf, the lamina of the leaf avoiding the midrib was cut into 4-5 pieces.

Then fragments of the leaf were boiled in test tube containing chloral hydrate solution.

The upper & lower epidermis is peeled out by using forceps.

Cleared one fragment of leaf was mounted on glass slide as upper epidermal layer kept upper most.

Arranged the camera lucida and drawing board.

Traced off the outline of four cells of epidermis.

Then by using high power objective, focused down to palisade layer and traced off sufficient cells to cover epidermal cells.

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Vein islet Number:

Methodology:

3-4 piece of fresh leaf were cut from the middle portion avoiding the mid-rib.

These pieces of leaf are taken in a test tube containing chloral hydrate and heated in a water bath for 30 min.

Peeled out the upper and lower epidermis by using forceps.

Then mounted a piece of leaf fragment on glass slide adding chloral hydrate solution. Placed a cover slip with lower surface facing upward.

For this study, 6x eyepiece and low power objective were used.

Camera Lucida was fixed and 10 x 10 cm square was drawn.

Counted the number of vein islet starting from one side of the square as well as on the boundary.

Vein let termination:

Methodology:

The number of vein-let termination present within the square was counted.

4) Determination of pH:

Materials:

Drug: Karanjapatra extract.

Equipment: Digital calibrates pH.

Method:

50ml of Normal saline water was taken in beaker; digital pH meter was immersed up to the maximum immersion level. Allowed the reading to stabilize and using a screwdriver turned the pH calibration trimmer to read 7.0. Then 5gms of *Pongamia pinnata* Linn. extract was added with 50ml of Normal saline water in a beaker. It was gently stirred well with glass rod. At uniform suspension, digital pH meter was immersed, observed for maximum immersion and reading was recorded[13].

B. PHYTOCHOCHEMICAL STUDY:

Aim:To know the chemical constituent in a trial drug, subjecting to different tests like extraction, preliminary phytochemical analysis.

Materials for Phytochemical Test:

Solubility of *Pongamiapinnata*Linn

Materials: Funnels, beaker, filter paper, test tube, fine powder of *Pongamia pinnata* Linn.

Solvents: 1) Ethyl alcohol 2) Ethyl acetate 3) Petroleum Ether 4) Chloroform

5) Methane 6) Normal saline water 7) Solvent ether 8) Acetone 9) Benzene 10) Toluene

11) Xylene 12) Carbon tetrachloride.

Methodology:

Take a 1gm of fine powder of *Pongamiapinnata* Linn. Andadded to the different solvent taken in a test tube and mixed well and allowed to stand for certain period. Then the mixture was filtered through filter paper kept in different funnels. The filter paper which contains fewer residues, it was considered the drug was more soluble in that solvent.

I. Extraction[14]:

Materials:

Drug: Coarse powder of leaves of *Pongamia pinnata* Linn.

Equipments required:

Soxhlet apparatus of 1000ml, round bottom flask, water condenser with distillation apparatus. Beaker's of 500ml, measuring cylinder, weighing machine, filter paper, magnetic stirrer, porcelain glass chips (boiling chips).

Chemical: 90% Ethyl alcohol.

Methods:

The air dried leaves of *Pongamia pinnata* Linn. was subjected to exhaustive extraction by Soxhlet apparatus around 18 hrs with 90 % ethyl alcohol. Extraction was done in two batches of this one batch of coarse powder with ethyl alcohol. The extraction process was carried out for about 18 hrs to each batch. After the extraction, the solvent was Normal saline off to obtain semisolid extract and it was concentrated on magnetic stirrer. The weights of each batch extract were recorded.

Preliminary phytochemical test[15]:

Materials:

Drug: Extractive sample of *Pongamia pinnata* Linn.

Equipments:

Test tube, test tube holder, test tube stand, spirit lamp, pipette, glass rods, beakers 50ml -250ml,conical flash, water bath, burner, stand.

Chemicals :

10 % conc. H2SO4, Chloroform solution, Acetic anhydride, Sulphar powder, Soda lime, Million's reagent, Mercuric sulphate, 10 % Sulphuric acid, 1 % Sodium nitrate, 5 % Sodium hydroxide, 1 % Copper sulphate, 10 % Tannic acid, Acetic anhydride, Acetyl chloride. Zinc chloride, Mayer's reagent, Wagner's reagent, Hager's reagent, Dragendorff's reagent (potassium bismuth iodide) ,Ammonium Renikate, Molish's reagent, Barford's reagent, Benedict reagent, Saponin, Ferric chloride, fragments pieces of Magnesium ribbon and conc. Hydrochloric acid, Zincdust, Sodium hydroxide, 10 % Lead acetate, Bromine water, Ferric chloride, Lead acetate.

Methods:

i) Test for sterols:

a) Salkowski's test: To 2ml extract added 2ml Chloroform and 2 ml Cone H2So4, shake well.

b) Liebermann – Burchardreaction: To 2ml extract few drops of Chloroform + 2ml Acetic Anhydride + 2 drops Conc. H2So4 from side of test tube.

c) Sulphar test: Added a pinch of Sulphar powder to the solution of extract.

ii) Test for proteins

Preparation of test solution:0.5 gm of sample extract was added to 100ml of water and heated. This solution was used for following tests.

a) Biuret test (General test): To 3 ml of test solution added 4 % sodium hydrate and few drops of copper sulphate solution.

b) Million's test: To 3 ml of test solution added 5 ml of Million's reagent.

c) Xanthoproteintest: To 3 ml of test solution added 1 ml conc. H2So4 and boiled the precipitate and added few drops of ammonium hydroxide.

iii) Test for Triterpenoids:

a) Tschugajewtest: To 2ml extract in a test tube, added 2 ml acetyl chloride and pinch of zinc chloride, boiled in water bath.

iv) Test for Alkaloids:

Preparation of test solution: Evaporated the alcoholic extract, to residue added dilute HCl, shaked well and filtered by using the filtrate the following test are performed.

a) Mayer's test: To 2ml of filtrate in a test tube added few drops of Mayer's reagent

b) Wagner's reagent test: To 2 ml of filtrate in a test tube added few drops ofWagner's reagent.

c) Hager's test: To 2ml of filtrate in a test tube added few drops of Hager's reagent

d) Dragendorff'stest: To 2ml filtrate in a test tube added few drops of Dragendorff's reagent.

v) Test for carbohydrate:

a) Molish's test (General): To 2ml extract in a test tube added few drops of Molish's reagent, shaked well and added few drops of H2So4from the side of test tube.

b) Barfoed's test (Monosaccharides): Added equal volume of test solution and Barfoed's reagent in a test tube and heated for 2 min in water bath.

c) Benedict's test: (Reducing Sugar): Mixed equal volume of test solution and Benedict reagent in a test tube and heated for 5 min in water bath.

vi)Test for Saponin's:

a) Foam test: The drug extract was mixed with water and shaked vigorously.

b) Haemolytic test: To one drop of blood taken on the glass slide and added drug extract.

vii) Test for Tannins: a) Ferric chloride test: To 2ml extract added few drops of 5 % Fecl3 solution in a test tube

b) Lead acetate test: To 2ml extract in a test tube added few drops of lead acetate.

c) Bromine water test: To 2ml extract in a test tube added few drops of Bromine water.

viii) Test for Flavonoid's:

a) Shinoda test: To 2ml extract in a test tube added 5ml of 95 % ethanol and few drops of conc. HCl and 0.5gm magnesium turnings.

b) Lead acetate test: To 2ml of extract in a test tube added few drops of lead acetate solution.

c) Alkaline reagent test: To 2ml of extract in a test tube added increasing amount of sodium hydroxide, yellow ppt disappears after addition of acid.

d**) Zinc –HCl-reduction:**To 2ml extract in a test tube added a pinch of zinc dust & few drops of conc. HCl and allowed to stand

OBSERVATIONS

A) Observation of Pharmacognostical study:

The pharmacognostical study includes:

1) Morphological observation

2) Microscopical observation

3) Microscopical evaluation(Leaf constituents)

4) Determination of pH

1) Morphological observation:

In this study, the morphological characteristics were observed by organoleptic method.

Colour	Brownish green
Taste	Astringent, bitter
Size	Length-7.5cms, Width-2.5cms
Shape	Ovate-Lanceolate,
Odour	Bitter
Nature of leaf	Glabrous
Touch	Smooth ,Unctuous

a) Shape of leaf:

Shape	Ovate – Lanceolate
Length and width	Length-7.5cms, Width-2.5cms.
Midrib	Prominent on ventral surface, divided in to vein, Veinlets.
Phyllotaxy	Alternate
Apex	Acuminate.
Base	Petiolate.
Venation	Midrib, vein, vein lets.
Dorsi-Ventral leaf	Dorsally glabrous, veins are prominent on ventral surface. Deep greenish in colour.
Margin	Wavy.

2) Microscopical observations:

a) Epidermis:

It is the outer most layer of leaf. It is single layered on both side, covered by thick striated cuticle hairs. It is polygonal in shape. The epidermal cells of the leaf are having two surfaces. A different type of stomata based on the arrangement of epidermal cells has been seen. Trachoma's are variable outgrowth of epidermal cells. It is observed that the epidermis of Karanja having unicellular trachoma cells.

b) Upper cuticle:

It is thick, mostly unicellular with pointed apex, glandular. It has cylindrical compact cells, which continue as collenchymas around the midrib.

c) Lower cuticles: It has rounded clustered cells.

d) Parenchyma:

It occurs as general tissue in this plant. 4-6 layers of parenchyma present towards lower epidermis. It is acidimetric, thin walled and the simplest type of cells and they have intracellular spaces.

e) Sclerenchyma:

It is hard supporting tissue with heavy secondary thickenings. They are roundly isodiametric, it found in bundles covering the vascular bundles on either side.

f) Collenchyma:

It is composed of cellulose. It is 2-5 layered, pericycle represented by slightly lignified small fiber group. These are found towards lower surface that is around the circumference of the midrib.

g) Xylem: They are arranged in vertical series and separated by phloem. The cell of xylem shows pink colouration after staining. The structural elements observed in xylem are as follows:

i) **Tracheids**: It has lignified thickened and pitted cell wall.

ii) **Vessels:** It consists of a vertical series of trachied like segments. Whereas the type of vessels shows complete dissolution of the end wall to give slit like opening.

h) Phloem:

It shows reddish brown colouration after staining. They are arranged in a vertical series. The cell phloem is oval and small. The xylem and sclerenchymal sheath surrounds them.

i) Starch:

The small granules of starch are seen in chloroplast by the condensation of sugar. It looks like bluish black colour after staining with N/50 Iodine solution.

3) Microscopical evaluation (Leaf constituents):

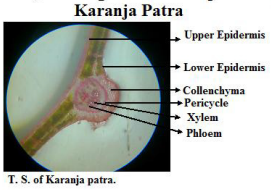
i) Observation of Stomatal number and Stomatal index: Each Stomata consists of two guard cells and the spore was counted as a single unit. Stomatal index was the percentage proportion of stomata on one side and epidermal cells plus stomata on other side.

ii) Vein islet: The number of vein islet per square mm was termed as vein islet number. This number per unit area of leaf was constant.

iii) Vein let termination: Starting from one side all the vein islets inside the square as well as on the boundary is to be traced. The vein-let termination within the square only was taken into account. To get exact values it is necessary to take reading from four such squares and trace the vein islet within it. The value obtained from vein islet and vein let termination was calculated as an average.

i) Observation of Palisade ratio: The average number of palisade cells present beneath each upper epidermal cells.

Photo Plate 2, Showing the Microscopical study of Karanja Patra



T. S. of Karanja patra.

Stomatal number | Vein Islet& Vein terminstion | Stomatal Index

4) DETERMINATION OF pH:

Observation of pH value:

50ml of distilled water was taken in a beaker.

Digital pH meter is immersed up to maximum immersion level.

Allowed the reading to stabilize and using a small screwdriver turned the pH 7 calibration trimmer to read 7.0.

Then 5mg of *Pongamia pinnata* Linn was added with 50ml of distilled water in a beaker.

Stirred gently it with glass rod.

At uniform suspension, digital pH meter was immersed.

The maximum immersion level was observed**.** Reading was recorded.

B) PHYTOCHEMICAL OBSERVATIONS:

1) Observation of solubility test: The residue was very minimum in 90% Ethyl alcohol; hence the solubility of the test drug in that solvent is maximum.

2) Observations of extraction: During extraction following things were observed such as,

By appropriate technique the coarse powder of Karanja patra was taken in the round fold of filter paper i.e. thimble in

Soxhlet apparatus, so that it cannot abstract any pathways of Soxhlet apparatus, uniform temperature was maintained, that means the heat was gradually increased from 20 0c -80 0c.

Observed the changes in colour of solvent, from dark green to light green.

After extraction, solvent were distilled off. Observation was done, so that whether solvent are completely distilled off from total extraction.

Extraction was taken off in a clean china dish and kept over magnetic stirrer for concentration of extraction.

3) Observations of preliminary phytochemical test:

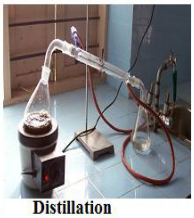
i)Test for sterols:	
i)Salkowski's test	Turns into red colour
ii)Liberman-Burchardt's	No change in colour
iii)Sulphar test	Sulphar powder sinks and settled at the bottom of test tube
ii) Test for proteins:	
i)Biuret test	Violet and pink colour observed.
ii) Million's Test:	First white precipitate by warming turns into brokered and dissolves giving red colour solution.
iii)Xanthoprotein Test:	White precipitate observed, it turns into yellow by boiling.
iii) Test for Triterpenoids	
i) Liebermann's Test:	No change in the colour.
ii) Tschugajew Test:	Zinc chloride powder sinks and settled at the bottom of test tube.
iv) Test for Alkaloids:	
i) Mayer's Test:	White coloured Ppt observed.
ii) Wagner's Test:	Reddish brown ppt observed.
iii) Hager's Test:	Yellow ppt seen.
iv)Dragendorff's Test:	Orange brown ppt observed.
v)Test for carbohydrates:	
i) Molish's Test:	Violet ring formed at the junction of two liquids.
ii) Barfoed's Test:	No change seen.
iii) Benedict's Test:	First appears green colour and after heating, it turns to yellow.
vi) Test for Saponins:	
i) Foam Test:	Persistence foam observed.
ii)Hemolytic Test	Hemolytic zone appears.
vii) Test for tannins:	
i) Ferric chloride test:	Deep blue-black colour was seen.
ii) Lead acetate test:	White ppt was seen after adding lead acetate.
iii) Bromine water test:	No change in bromine water colour.

viii) Test for Flavonoids:	
i) Shinoda Test:	Pink colour observed.
ii) Lead acetate:	Yellow coloured ppt observed.
iii) Alkaline reagent test:	Yellow coloured ppt observed.
iv) Ferric chloride test:	Deep blue-black Colouration was observed.
v) Bromine water test:	Discoloration of Bromine water was not seen.
vi) Zinc HCl reduction test:	Zinc dust sink and settled at the bottom of test tube.

Photo Plate 3 Showing Extraction and Phytochemical analysis

Dried Leaf of Karanja

Extraction of Karanja



Distillation

Phyto chemical analysis of Karanja patra Extract

RESULTS

A) RESULTS OF PHARMOCOGNOSTICAL STUDY:

1) Morphological study:

Colour	Brownish green
Taste	Astringent ,bitter
Size	Length- 7.5cms Width 2.5cms
Shape	Ovate-Lanceolate, Rhomboid-Oblong
Odour	Bitter
Nature of leaf	Glabrous
Touch	Smooth, unctuous.

2) Microscopical study: By the section method and by staining process method under the microscope following results were seen. Trachoma, parenchyma cells, xylem ,phloem, vascular bundles, stomatal cells, palisade cells, starch grains ,lower epidermis, upper epidermis. The basic types include different cellular parameters of plant cells as follows.

Microscopical cells	Values
Trachoma	09-13/mm2
Parenchyma cells	22-25/ mm2
Xylem	15-20/ mm2
Phloem	15-20/ mm2
Vascular bundles	11-15 in bundles
Stomatal cells,	11-16/ mm2
Palisade cells	10-14/ mm2
Starch grains	08-14/ mm2
Lower epidermal cells	18-22/ mm2
Upper epidermal cells	21-25/ mm2
Fibers	25-30/ mm2
Collenchymal cells	10-15 mm2

3)Microscopical Evaluation: In Microscopical evaluation following results were seen such as**.**

Parameters	**Values**

1) Stomatal number	11-16/ mm2
2) Stomatal index	4.4m
3) Vein islet number	18/ mm2
4) Vein termination number	11/ mm2
5) Palisade ratio	20-24/ mm2

4) Results of pH value:

Distill water Normal PH	7.0
Acidic media PH	0 -7
Alkaline media PH	7-14
Pongamia pinnata linn extract PH	**5.6**

B) RESULTS OF PHYTOCHEMICAL STUDY:

i) Solubility tests:

Solvent	Soluble	Sparingly soluble	Insoluble
Distilled water	-	+	-
Solvent ether	-	-	+
Petroleum ether	-	+	-
Acetone	-	-	+
Benzene	-	+	-
Toluene	-	-	+
Chloroform	-	+	-
Ethyl alcohol	**+**	**+**	**-**
Xylene	-	-	-

ii) Extraction

Leaves of *Pongamia pinnata* Linn	**Solvent**	**Extract**
Coarse leaves powder 60gms	650ml ethyl alcohol	40gms

iii) Preliminary phytochemical

Tests:	**Results:**
i)Test for sterols:	
Salkowski's test	**+ ve**
Liberman-Burchardt's test.	**- ve**
Sulphar test	**+ ve**
ii) Test for proteins:	
Biuret test	**+ ve**
Million's Test:	**+ ve**
Xanthoprotein Test:	**+ ve**
iii) Test for Triterpenoids:	
Liebermann's Test:	**-ve**
Tschugajew Test:	**+ ve**
iv)Test for Alkaloids:	
Mayer's Test:	**+ ve**
Wagner's Test:	**+ ve**
Hager's Test:	**+ ve**
Dragendorff's Test:	**+ ve**
v)Test for carbohydrates:	
Molish's Test:	**+ ve**
Barfoed's Test:	**- ve**
Benedict's Test:	**+ ve**
vi)Test for Saponin's:	
Foam Test:	**+ ve**
Hemolytic Test	**+ ve**
vii) Test for Tannin's:	

Ferric chloride test:	**+ ve**
Lead acetate test:	**+ ve**
Bromine water test:	**- ve**
vii) Test for Flavonoid's:	
Shinoda Test:	**+ ve**
Lead acetate:	**+ ve**
Alkaline reagent test:	**+ ve**
Ferric chloride test:	**+ ve**
Bromine water test:	**- ve**
Zinc HCl reduction test:	**+ ve**

DISCUSSION

Pharmacognostic study:

Detailed TS of leaf of *pongamia pinnata* Linn. passing through midrib shows very broad elevation at the lower side, the midrib being covered by thick-walled epidermis bearing multi-cellular covering trichome with underlying layers of collenchyma. Trichomes are plenty on the lower side and the epidermal cells are papillate. Few trichomes shows collapsed cells Upper portion of midrib shows few layers of collenchymatous hypodermis and a concave elevation. The midrib shows a vascular bundle having concave upper surface and is formed by rows of vessels encircled by patches of phloem

Phytochemical study:

Karanja patra is treated with different solvents and found well dissolved in 90 % ethanol hence Karanja patra extraction is done in the same, and the extraction is used for further phytochemical analysis in the laboratory of Dravya guna department. Extracts were subjected to various preliminary Phytochemical tests for detection of the Phyto constituents. The positive tests of Karanja extract are- Sterols, proteins, Triterpenoids, Alkaloids, Carbohydrates, tannins, Saponins, and Flavonoids.

CONCLUSION

T.S. of leaf of *pongamia pinnata* Linn. passing through midrib shows very broad elevation at the lower side, the midrib being covered by thick-walled epidermis bearing multi-cellular covering trichome with underlying layers of collenchyma. The midrib shows a vascular bundle having concave upper surface and is formed by rows of vessels encircled by patches of phloem. Phytochemical analysis of ethanolic extract of Karanja patra shows presence of sterols, proteins, triterpenoids, alkaloids, corbohydrates, saponins, tannins and flavonoids.

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