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ANALYTICAL STUDY OF KARANJA BEEJA POWDER

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ABSTRACT
In adequate knowledge of drug identification by plant collector, Vaidyas or Pharmacies are often leads to wrong identity or inferior quality of drugs. Adulteration and substitution are rampant in Ayurvedic drug trade. To detect all such foreign material in drugs whether they are entire or in powdered form wide knowledge of plant histology and microscopic is very essential for establishing the correct identities. Accurate determination of drug identity, its sources and evolving standards is an important and essential part of investigation. Microscopic identity of drug material is indispensable for the identification of entire, broken or powdered material for assessing the quality of drug further needs other evaluated factors like phytochemical investigation, evaluation of active constituent, profiling and estimation of physical pharmacopeal standard.

KEYWORDS: Karanja beeja powder, Analytical Study, *Pongamia pinnata Linn.* Linn.

INTRODUCTION[1]
Adulteration and substitution is one of the major problems encounter while assessing the quality of herbal drugs. Seeds of plants are also constitute an important source of raw drugs used in medicine. In adequate knowledge of drug identification by plant collector, Vaidyas or Pharmacies are often leads to wrong identity or inferior quality of drugs. Adulteration and substitution are rampant in Ayurvedic drug trade. To detect all such foreign material in drugs whether they are entire or in powdered form wide knowledge of plant histology and microscopic is very essential for establishing the correct identities. Accurate determination of drug identity, its sources and evolving standards is an important and essential part of investigation. Microscopic identity of drug material is indispensable for the identification of entire, broken or powdered material for assessing the quality of drug further needs other evaluated factors like phytochemical investigation, evaluation of active constituent, profiling and estimation of physical pharmacopeial standard. In Ayurveda primary identification of drug is done by organographic method by assessment of taste, odor etc.

AIMS AND OBJECTIVES
1. To Study the drug analytically.

MATERIAL AND METHODS
Pharmacognostical Study
50 gm powder of seeds was prepared and was stored in air tight container. The seed powder was prepared for noticing the diagnostic microscopic powder character and for subjecting the powder to physio chemical test.

Macro and Microscopic Studies[2]
Morphological characters of all the seeds were studied by observing under the dissecting microscope. The powder was also evaluated for their organoleptic characters.

For microscopic characters the T.S of the seeds were taken separately after soaking the seed overnight in water. Observing the section under the dissecting microscope by keeping the scale along with the seeds drew the diagrammatic sketches.

Free hand section of the soaked seeds were taken. They were cleared with chloralhydrate and stained with various reagents for their histochemical studies.

The powder was also treated similarly for observing the diagnostic microscopic characters. The drawings were made with the help of Camera Lucida as well as

photographs of the T.S were taken by the photomicrography unit.

Color Test
To differentiate the seed powders they were subjected to various color tests by treating them with various concentrations of acids and alkalies. The colors developed were noted down.

Histochemical Test
To locate the region for certain constituent of the drug few histochemical test were also performed for the presence of, rheuthenium red for mucilage, $FeCl_3$ for tannin, iodine for starch & aleurone grains, sudan III for fixed oil, pholoroglucinol and HCl for lignified elements.

Analytical Study[3]
For judging the quality of the seed drugs and to find out if there is any difference in the closely resembling species and species used by the same common name but differ in their appearance. So 4 seeds separately under 2 groups are subjected for the following parameters.
1) Physico chemical constants.
2) Qualitative chemical tests.
3) TLC.

Physico Chemical Constants
Using the procedure mentioned in "Quality control methods for medicinal plant material" WHO and "Ayurvedic Pharmacopoeia of India" carried out determinants of various physicochemical constant as mentioned below.

A) Determination of foreign matter
Medicinal plant materials should be entirely free from visible signs of contamination, by moulds or insects, and other animal excreta. No abnormal odor, discoloration, slim or signs of deterioration should be detected.

Foreign matter is material consisting of any of the following,
Parts of the medicinal plant material or materials other than those named with limits specified for the plant material concerned.

Any organism, part or product other then that the name in the specification and description of the plant material concerned.

Mineral admixtures not adhering to the medicinal plant material plant materials, such as soil, sand, and dust.

As the seed (fruits) were collected from the plants they were devoid of admixture of foreign or extraneous material. Hence this test was not performed.

B) Loss on drying
The moisture content of a drug should be determined for the percentage of its active chemical constituents because its percentage depends upon air dried basis. So the moisture content of the drug should be minimized in order to prevent decomposition of the crude drugs either due to chemical change or microbial contamination.

Procedure
2 gram of drug sample was taken in a pre weighed dried Petri dish. It was dried in an oven at 105°C until reaching a constant weight. The Petri dish was taken out, self cooled and weighed immediately. The weight loss i.e. loss on drying was calculated and expressed as % w/w.

C) Determination of Ash
This test was conducted to evaluate the percentage of inorganic salts, naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration.

Procedure
2 gram accurately weighed sample was taken in a pre weighed dried crucible. It was incinerated in a muffle furnace up to 450°C. The crucible was taken out, self cooled and weighed immediately. From the weight of the ash, the ash value was derived with reference to the air dried drug. It was calculated and expressed as %w/w.

D) Determination of water soluble extractive value
This test was carried out to determine the water soluble extractive and approximate measures of their chemical constituents of the test drug.

Procedure
5 gm. of the sample was weighed accurately. To it 50 ml of distilled water was added and kept covered overnight. It was stirred intermittently in the initial period. Next day, it was filtered. 20 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as% w/w.

E) Determination of n-hexane extractive value
About 5 gm of drug and 100 ml of n-hexane on a water bath for 2hrs. filter and remove the solvent. Dissolve 100 mg of the residue in 5 ml of n-hexane.

F) Determination of Alcohol soluble extractive value
About 5gm accurately weighed. Sample was macerated with 100ml of alcohol (methanol) in a closed conical flask for 24hr, shaking frequently during 6hr and allowed to stand for 18 hours. It was filtered rapidly to prevent loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottom evaporating dish and dried at 105ºC to constant weight. From the weight of the residue the percentage of alcohol soluble extractive value was calculated with reference to air dried sample.

J) Determination of Chloroform soluble extractive value

About 5gm accurately weighed samples was macerated with 100ml of chloroform in a closed conical flask for 24hrs, shaking frequently during 6 hrs and allowed to stand for 18 hrs. It was filtered rapidly to prevent loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottom evaporating dish and dried at 105ºC to constant weight. From the weight of the residue the percentage of chloroform soluble extractive value was calculated with reference to air dried sample.

K) Determination of Petroleum ether extractive value

This value gives the amount of fixed oil present in the seed. As seeds are important source of fixed oil used in food industry. The percentage of fixed oil in a particular amount of sample is necessary to be studied. The continuous cold extraction method is used to extract the fixed oil from the seed powder.

5gm of air dried sample was weighed and then transferred in a paper pocket which was placed in extraction thimble. Then it was extracts with petroleum ether (60ºC to 80ºC) in a soxhlet extraction till complete extraction. The extract was transferred in a previously weighed tarred flat-bottom evaporating dish and evaporated to dryness on water bath. Then the residue was dried in oven at 105ºC till consecutive two constant weights. The value was calculated with reference to air dried sample.

I) pH

The pH value of an aqueous liquid may be defined as negative log of hydrogen ion concentration. The pH of the filtrate of a particular concentration of aqueous solution of the sample is often used as one of the parameters.

A 10% w/v aqueous solution of the sample was prepared. It was then filtered and the pH of the filtrate was noted in Elico‟s digital pH meter using combined glass electrode.

Preliminary Phytochemical Investigations

The phytochemical studies of the samples were carried out to have an idea about the chemical profile of the sample. The details of the parameters and methods employed are presented below.

Qualitative tests

The methods employed to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. Using the methanol, water and chloroform soluble extracts of samples carried out qualitative tests. These tests indicate the types of phyto-constituents present in the sample.

A) Test for Carbohydrates

Molish's test (General test)

To 2-3ml aq extract, few drops of alpha-naphthol solution in alcohol was added and shaken and conc H_2SO_4 from the sides of test tube was added. Violet ring was formed at the junction of the two liquids confirmed the presence of carbohydrates.

(i) Test for Mucillage

Seed powder was kept in water for few hr that got swelled in water showing the presence of mucilage.

B) Tests for Proteins

(i) Millions reagent test

To 3ml of test solution 5ml of Million‟s reagent was added. White ppt indicates the presence of proteins.

(ii) Precipitation test

The test solution gives white colloidal ppt when following reagents were added.

a) Absolute alcohol.
b) 5% $HgCl_2$ solution
c) 5% $CuSO_4$ solution
d) 5% lead acetate
e) 5% ammonium sulphate.

Test for Saponins

(i) To an aqueous solution of sample add solution of lead acetate, formation of white precipitate indicates the presence of saponin.
(ii) Add some water to sample and shake vigorously formation of a stable froth with honeycomb structure indicate the presence of saponins.

Test for starch

Aqueous extracted was treated with iodine solution will give blue color indication the presence of starch.

Test for alkaloid

(a) Dragendoff's reagent test

A small amount of methanol extract was taken in a watch glass; solvent was evaporated and to the residue added few drops of dilute HCl followed by few drops of Dragendoff's reagtent (Potassium Bismath Iodine Solution. Orange colored precipitate will indicate the presence of alkaloids.

(b) Mayers reagent test

A portion of the methanolic extract was taken in a watch glass and was evaporated, to the residue add few drops of dilute HCl and few drops of Mayer's reagent (Potassium mercuric iodine solution) were added. The creamish white precipitate will indicate the presence of alkaloid.

Test for fixed oil

Press small quantity of petroleum ether extracts separately between two filter papers. Oil stains on the paper indicates the presence of fixed oil.

TLC

TLC can readily ascertain the presence of the essential chemical constituents of a medicinal plant and detection of their presence in a preparation or extracts. Even a semi quantitative assessment of a chemical constituent in a preparation is possible by TLC. It is readily a component tool to standardize a drug and more relevant than mere estimation of ash content etc, which may confirm to the prescribed values even if an essential medicinal plant is absent in a preparation. No doubt, more sophisticated techniques like HPLC, GLC, HPTLC etc, have been developed but TLC is relatively simple, easy to handle, quick, convenient and inexpensive method for quick, assessment of the quality of most of the herbal preparations.

OBSERVATIONS AND RESULTS

Table No 1: Macroscopic characters of the seeds of *Pongamia pinnata Linn.*

Characters	***Pongamia pinnata Linn.***
Shape	Eliptic, reniform,oblong, sub Reniforms
Size (cm)	1.8 x 2.4, 1 x 1.8, 0.3 x 1.2
Surface	Smooth and shiny hilum located near the depression acute end of seeds
Texture	Leathery
Color	Light brownish
Hardness	Coraiceous leathery
Micropyle	Above the hilum on the ventral side
Hilum	Distinct

Table No 2: Organoleptic characters of the seed powder.

Characters	*Pongamia pinnata Linn.*
Color	Yellowish brown
Texture	Fine
Odor	Nausating, not
	Characteristics
Taste	Bitter with slightly
	tingling sensation

Table No 3: Microscopic characters of T.S. of seeds of *Pongamia pinnata Linn. Linn.*

Cuticle	**Thin**
Palisade cells	Covered with a thick cuticle composed of vertical & columnar cells
Parenchyma cells	Thick walled cells 6-9 long, 5-7 broad & badad cells with starch grain
Crystals	Present in cotyledon(abundant in the inner side)
Epidermis cells	Cyllindrical, flat 9.2 - 12.8 - 18.4 long x 4.6 - 6 -9.2
Starch grain	Simple
Oii cavity	Present

Macroscopic structure of *Pongamia pinnata Linn. Linn.* :

Two type of seeds (1) seeds of single seeded pods (2) Seeds of double seeded pods (pods containing two seeds), ovate to oblong.

Elliptical or reniform centrally swollen 1.8 to 2.4 cm long, 1 to 1.8 cm broad and 0.3 to 1.2 cm in maximum thickness, central portion, transversally wrinkled wrinkles arising from a small depression from one end where lies the hilum, a small projection above the represents the position of micropyle; a ridge running along the peripheral surface indicates the margins of two cotyledons, Testa copper brown, smooth, wined, brittle and separable very easily, exposing, pale yellowish white cotyledon taste bitter, mucilaginous and oily; odor not characteristics.

Powder characters of seeds of *Pongamia pinnata Linn. Linn.*

Shows fragments of epidermis of testa in sectional and surface view, in surface view the cells are polygonal to homogonal in shape with thick dark brown colored walls and in sectional view the cells are dome shaped; narrow, cylindrical, brown pigment cells; cells of cotyledons filled with oil globular and starch grains and fragments of outer cotyledons tissue embedded with oil cavity.

Microscopic characters of seeds of *Pongamia pinnata Linn. Linn.*

LS of seed is oval in outline and shows outer copper brown colored testa encircling the inner whitish yellow cotyledons and a small, thick radicle near the micropylar edge of seed. TS of the seed is compared of an outermost layer, an epidermis of testa, made up of three sides thickened dome shaped cells, covered with thin cuticle, followed by 3 to 4 rows of subrectangular thin walled parenchymatous cells, underneath this lies a row of

narrow, thick walled, tangentially running cells pilled with dark brown pigment followed by 2 to 3 rows of collapsed celled parenchymatous tissue. The epidermis of cotyledon is made up of small sized, squarish cells, underneath of which lies a layer of parenchyma filled with aleurons grains, the remaining tissue of the cotyledons, occupying the major portion of the section are compared of oval, radially arranged big sized parenchymatous cells filled with oil globules and simple starch grains, circular to oval tangentially running isolated oil cavities often exhibiting cellular structure in undeveloped state filled with greenish oil globules are embedded in the peripheral zone of cotyledons.

The cotyledons are protected with in testa when fresh, they are swollen, freshly and in close contact with the testa, throughout the entire perimeter. In outline they comfirm to that of the testa and are 1.8 to 2.4 cm long and 1.8 – 1 cm broad. On drying, the cotytedons shrinks and become detached from the testa at several places. Under a thin cuticular layer, the epidermis of the cotyledon is present, the cells are cylindrical, of the flat surface and from 9.2 – 13.9 – 1.8 µ long x 4.6 x 6 x 9.2 µ. The cells of the mesophyll are filled with round starch grains and rounded to oval colorless shining oil globules. The cells of the mesophyll are filled with round starch grains and rounded to oval colouless shining oil globules. The cells of mesophyll are rectangular to polygonal and measure 9.2 – 20.2 - 46µ x 6.9 – 14.26 – 23 µ (Israili and Issar, 1971).

Table No 4: Physiochemical Parameters.

Sr. No.	*Parameters*	*Pongamia Pinnta*
1	Foreign matter w/w	-
2	Loss on drying % w/w	11.4% w/w
3	Ash Value % w/w	3.55% w/w
4	Water soluble extractive value % w/v	25.5% w/v
5	Methanol soluble extractive value % w/v	59.7% w/v
6	Petroleumether soluble extractive value % w/v	30.5% w/v
7	Chloroform soluble extractive value % w/v	17.3% w/v
8	pH of 10% w/v aq. solution of seeds	6.04

Table No 5: Qualitative Test.

Test	*Pongamia Pinnata*
Alkaloid	-
Protein	+
Carbohydrates	-
Starch	+
Mucilage	+
Amino acids	-
Saponin	+

(1) TLC for *Pongamia pinnata Linn.*
Extract: Chloroform extract
Solvent: Toluene: Ethyl acetate(10: 2)

Table No 6

No. of Spot	*Color of Spot*
1	Blue
2	Pale blue
3	Blue
4	Pale blue
5	Blue
6	Blue
7	Blue

Table No 7: Visualization using - Vanillin Sulphuric acid.

No. of Spot	*Color of Spot*	*Rf*
1	Grey	0.36
2	Yellow	0.43
3	Grey	0.48
4	Yellow	0.52
5	Grey	0.57

DISSCUSSION ON DRUG
Microscopic characters of karanja seed
LS of seed is oval in outline and shows outer copper brown colored testa encircling the inner whitish yellow cotyledons and a small, thick radicle near the micropylar edge of seed. TS of the seed is composed of an outermost layer, an epidermis of testa, made up of three sides thickened dome shaped cells, covered with thin cuticle, followed by 3 to 4 rows of subrectangular thin walled parenchymatous cells, underneath this lies a row of narrow, thick walled, tangentially running cells pilled with dark brown pigment followed by 2 to 3 rows of collapsed celled parenchymatous tissue. The epidermis of cotyledon is made up of small sizedsquarish cells, underneath of which lies a layer of parenchyma filled with aleurons grains, the remaining tissue of the cotyledons, occupying the major portion of the section are compared of oval, radially arranged big sized parenchymatous cells filled with oil globules and simple starch grains, circular to oval tangentially running isolated oil cavities often exhibiting cellular structure in

undeveloped state filled with greenish oil globules are embedded in the peripheral zone of cotyledon.

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Powder Microscopy of Karanja
Shows fragments of epidermis of testa in sectional and surface view, in surface view the cells are polygonal to hexagonal in shape with thick dark brown colored walls and in sectional view the cells are dome shaped, narrow, cylindrical, brown pigment cells; cells of cotyledons filled with oil globules and starch grains and fragments of outer cotyledons tissue embedded with oil cavity.

Analysis of physical constants revealed absence of foreign matter in seeds.

- Loss on drying is 11.4% w/w
- Ash value is 3.55% w/w
- Water soluble extractive value is 25.5% w/v
- Methanol soluble extractive value is 59.7% w/v
- Petroleum ether soluble extractive value is 30.5% w/v
- Chloroform soluble extractive value is 17.3%w/v

The ph value is 6.04

Qualitative tests performed for the seed powder reveals the absence of protein, starch, alkaloid, Carbohydrates, amino acids & Saponin in *Pongamia pinnat. Only* Mucilage is present in *Pongamia pinnata Linn.*

The TLC for chloroform extract of *Pongamia pinnata Linn.* gave seven spots with Rf value 0.18, 0.24, 0.31, 0.38, 0.48, 0.54, and 0.62 respectively. After spraying with vanillin sulphuric acid gave five spots with Rf values 0.36, 0.43, 0.48, 0.52 and 0.57 respectively.

CONCLUSION

About 95% of medicinal plants used by the industries are collected from wild and over 70% of the plant collectors involve destructive harvesting because of the use of parts like root, bark, wood, seed and whole plant in case of herb.

For centuries many of the seed drugs are utilized by man and animal as an article of food for dietary supplement.

*Pongamia pinnata Linn. Linn.*belongs to same family Fabaceae familly under sub family papilionaceae. Macro-microscopic, physiochemical and preliminary phytochemical studies has been throughout investigated in the present work.

Though some works have been carried out on this medicinal raw drugs, the present work will add further to the existing knowledge on it, especially towards the microscopic profile which is highly essential for identification, authentication and standardization of seed drugs.

REFERENCES

1. Available from www.who.com
2. Ramnik Sood : Medical Laboratory Technology, 5th edition.
3. Indian Journal of Pharmacy, June 1980; 12(2): 123.127.