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Research Article

PHARMACOGNOSTICAL & PHYTOCHEMICAL STUDY OF “KRISHNA TILA”

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ABSTRACT

Considering present day's life style oxidant stress is more on each individual. So anti-oxidants are in demand. Ayurveda has provided rational basis of such thoughts & medicines like *Krishna tila* mentioned in samhitas for maintaining good health & to treat diseased condition. An attempt was made to study in detail regarding the pharmacognostical & phytochemical estimation of *Krishna tila*.

Keyword: *Krishna tila*, pharmacognostical study, phytochemical study, microscopical study, observations, results.

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.

So the pharmacognostical & phytochemical study of *Krishna tila* was undertaken to understand its genuinity & originality so that it can be recognized easily from the adulterants & substitutes which can bring good results in the form of treatment

AIMS AND OBJECTIVES:

- 1) Morphological & microscopical study of *Krishna tila* beeja (*sesamum indicum*, Linn)
- 2) Preliminary phytochemical analysis of (*Sesamum indicum*, Linn) beeja

MATERIALS AND METHODS

PHARMACOGNOSTICAL STUDY:

Aim: The aim of study was to analyze morphological microscopically evaluation of *Krishna tila* beeja (*sesamum indicum*, Linn)

MORPHOLOGICAL STUDY:

Materials: The material collected for the study were

Drug	: <i>Krishna tila</i>
Parts used	: Beeja
Collection of material	: The beeja of <i>Krishna tila</i> were collected from Kanvi (Karnataka)
Equipments	: Sense organs

METHODS :

1) **Organoleptic Method:** In this method the colour taste, size, shape, odour, characteristics of *Krishna tila* beeja were studied with the help of sense organs.

2) **Extra features:** The special characteristics of beeja were studied.

MICROSCOPICAL STUDY¹:

Materials: The materials collected for the study were

Drug: The seeds of *Sesamum indicum*, Linn

Chemical: Sudan red 3, picric acid, chloral hydrate, conc. HCl, Glycerin, Iodine.

METHODS:

1) Transverse Section Method:

- (a) Put the sample in a test tube add sufficient water so that sample remains submerged.
- (b) Keep it for half an hour. This will soften the hard sample for getting fine section.
- (c) For section the sample is kept in potato slit (sample which are difficult to hold) and with help of new blade thin transverse section were taken.
- (d) Thick oblique sections were rejected.
- (e) With help of mountain hair brush, selected sections were transferred to watch glass containing water.

2) Staining method:

- a) Selected the thin Transverse section of the sample was taken and transferred on a slide with the help of mountain hairbrush.
- b) Drop of water was added.
- c) Sudan red 3 reagent was added & washed with ethanol to remove excess stain & observe
- d) picric acid is added & observe

- e) Finally a drop of glycerine was added and the section was covered avoiding air bubble carefully with cover slip.
 f) With the help of a blotting paper excess water present outsidess were wiped off.
 g) The section was focused under microscope and the arrangements of cells were studied.

POWDER MICROSCOPY:

METHOD:

A specimen was prepared by placing a little amount of powder on a watch glass with the camel hair brush and stained as same as mentioned in above said transverse section method. The section was focused under microscope and the arrangements of cells were studied.

B) MATERIALS FOR PHYTOCHEMICAL STUDY:

Aim: To know the physicochemical chemical constituents in a trial drug subjecting to different test like fixed oil determination.

SOLUBILITY OF KRISHNA TILA BEEJA²:

METHODOLOGY:

The pinch of fine powder of *Krishna tila* was added 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 few residues was considered that the drug was more soluble in that solvent.

EXTRACTION³:

Material – Drug’s coarse powder of *Krishna tila beeja*

Equipments: Soxhlet apparatus of 1000ml round bottom flask

CHEMICALS: Petroleum Ether

Methods: The coarse powder of *Krishna tila beeja* was subjected to exhaustive extraction by soxhlet apparatus with ether. After extraction the solvent was distilled off to obtain semisolid extract. Then kept petridish for further evaporation and weight of each batch extract were recorded.

THE DETERMINATION OF FIXED OIL IN DRUG⁴:

Materials – Drug coarse powder of *Krishna tila beeja*

Chemicals: Petroleum Ether

Method:

- (a) 10gm of drug in the form of the powdered drug in the condition in which it was received with 300ml of ether and a few small pieces of porous earthen ware in the distillation flask.
 (b) The flask was connected to the still head stopper k and water was run into the orifice k until it overflows at B. The flask was heated with frequent agitation until ebullition began and distillation continued at a rate which leaves the lower part of the condenser and it was cooled at the point G. The flask was rotated occasionally to wash down any material adhering to the upper part of the walls. The heating was discontinued at the end of the specified time and after at least five minutes the volume of oil was recorded in the graduated portion of the tube.
 The distillation was continued for further period of one hour and again the volume of oil was read off as before. If necessary again the distillation was continued until successive readings of the volume of oil do not differ the measured yield of fixed oil was taken to be the content of fixed oil in the drug.

DETERMINATION OF P^H5:

Materials:

Drug : *Krishna tila* beeja extract & choorna

Equipments : Digital calibrates P^H

Method :

- 50ml of distilled water was taken in beaker.
- Digital P^H meter was immersed up to the maximum immersion level.
- Allowed the reading to stabilize and using a screwdriver turned the P^H calibration trimmer to read 7.0
- Then 5gm of *Krishna tila* beeja extract was added with 50ml of distilled water in a beaker.
- Stirred well with glass rod gently.
- At uniform suspension, Digital P^H meter was immersed.
- The maximum immersion level was observed.
- Reading was recorded.
- The same procedure was repeated even for *Krishna tila* beeja choorna.

DETERMINATION OF MOISTURE CONTENT:

Materials: Drug: 5gm of *Krishna tila beeja* powder

Equipments: Weighing machine, Tared evaporating dish

Method:

- 5gm of *Krishna tila* beeja churna after accurately weighing was placed in a tared evaporating dish.
- It was dried at 105⁰ for 5 hours and it was weighed.
- The drying and weighing was continued at one hour interval until differences between two successive weighing corresponds to not more than 0.25%.
- Constant weight was reached when two consecutive weighing after drying for 30 minutes in a desicator.

Determination of ash value:

- 2gms of fine powder taken in silica crucible.
- Kept in muffle furnace and observed for its fumes.
- It has kept there unless the fume formation get stops.
- Observed for its ash coloration after it get cool itself.
- The weight of the ash taken and ash value get calculated.

PRELIMINARY PHYTOCHEMICAL TEST⁶:

Materials:

Drug: Sample of *Krishna tila* oil.

METHODS:

1. Test for Carbohydrates:

a) Molisch’s test : 2ml extract with few drops of Molisch reagent were taken and shook, then 2ml of concentrated 10% H₂SO₄ added slowly to the sides of the test tube.

2. Test for Proteins:

a) Millon’s test: 3ml test solution and 5ml millon’s reagent was added.

3. Tests for Fixed Oils:

a) Solubility test: The sample was dissolved in various solvents.

b) Dip test: Allow 0.05ml of substance being examined to fall onto a filter paper.

c) Odour and taste – Mix 0.15ml with 5ml of Ethanol (90%) and stir in 10gm of sucrose in powder and odour and taste are observed.

4. Test for Sterols:

a) Salkowski’s test: 2ml extract, 2ml chloroform and 2ml conc. H₂SO₄ were added, shook and allowed to stand

b) Liebermann – Buchard reaction: mix 2ml extract with chloroform . Add 1-2 ml acetate anhydride & 2 drops conc H₂SO₄ from the side of test tube

c) Libermann’s reaction: mix 3ml extract with 3ml acetic anhydride . Heat & cool. Add few drops of Conc H₂SO₄

5) Test for Saponins:

a) Foam test: The drug extract shake vigorously with water.

6) Test for flavonoids :

To dry powder or extract add 5ml 95% ethanol , few drops of conc HCl & 0.5 gm magnesium turnings.

7) Test for fats & oils:

a) Saponification test: Evaporate extract to get 10 ml oil. To oil add 25ml of 10% NAOH. Boiling in boiling water bath for

30 min , cool . Add excess Na₂SO₄ solⁿ soap forms & rise to top filter. To filtrate add H₂ so₄ . Evaporate collect residue.

8) Test for Tannins :

To 2ml of aqueous or alcohol extract add few drops of 5% Fecl₃ solution.

OBSERVATIONS:

A) PHARMACOGNOSTICAL STUDY

1) Morphological study

2) Microscopical study

1. MORPHOLOGICAL OBSERVATION: In this study the morphological characteristics were observed by organoleptic method.

Table 1: Morphological study of Krishna tila:

COLOUR	Black
TASTE	Madhura ,Tikta, Kashaya
SIZE	App. 2.5-3mm long,1.5mm broad
SHAPE	Flattened ovate
ODOUR	Pleasant
NATURE OF SEED	One side slightly concave with faint marginal lines & an equally faint central line
TOUCH	Smooth

2. MICROSCOPICAL OBSERVATION OF KRISHNA TILA:

a) Transverse section of seed shows:

Testa – Of seed shows single layered palaside like thin walled, yellowish coloured cells & rest of the testa composed of collapsed cells

Endosperm – 3 layered, rarely 2 layered , consisting of cellulose polygonal cells of Parenchyma containing fixed oils & small aleurone grains

Cotyledons - Two externally covered with thin cuticle, single layered epidermal cells

followed by single row of palaside like cells

Rest of the tissue consist of polygonal, parenchyma cells containing fixed oil & aleurone grains.

- **Reagent – Sudan red 3 – one drop added to the sections of different thickness & then later washed with ethanol to remove excess stain**

- **Observation – Red colored globules seen of oil in the endosperm & cotyledon**

- **Reagent - Picric acid – one drop of picric acid is added to the sections of different thickness & observed under microscope**

- **Observation – Yellow color indicates aleurone grains present in the cells of endosperm & cotyledon.**

b) POWDER MICROSCOPY:

Blackish colored shows palaside like cells in surface view , parenchyma

cells , aleurone grains & oil globules

B) PHYTOCHEMICAL OBSERVATION

a. OBSERVATION OF SOLUBILITY TEST:

Petroleum ether – 0.13 gms

Acetone - 0.28 gms

➤ The residue was minimum in petroleum ether

b. OBSERVATION OF EXTRACTION:

During determination of extraction following things were observed such as

- By appropriate technique 100gms coarse powder of Krishna tila (Sesamum indicum Linn.) inside the round fold of filter paper i.e. thimble in soxhlet apparatus. Uniform temperature was maintained, that means the heat was gradually increased from 10-25⁰C.

- Observed the changes in colour of solvent from slight white to milky white. After extraction solvent was distilled off.

- Observation was done, so that solvent was completely distilled off from total extraction. Extraction was taken off in a petridish and kept at room temperature for concentration of extraction.

c. OBSERVATION OF COLLECTION OF FIXED OIL:

- By proper technique the extracted solution of Krishna tila was taken in round flask and connected to special still head. The uniform temperature was maintained.

- The heating was done with frequent agitation until ebullition began.

- Observed the changes in colour, from milky white to yellow colour.

- After distillation the oil which was recorded in the graduated portion of the tube was collected in air tight bottle.

d. OBSERVATION OF PH VALUE:

- 50 ml of distilled water was taken in a beaker.

- Digital pH meter was immersed up to the maximum immersion level.

- Allowed the reading to stabilize and used a small screw driver, then the pH 7 calibration treamer to read 7.0

- Then 0.5ml of Sesamum indicum Lin. extract was added with 50ml of distilled water in a beaker.

- At uniform suspension digital pH meter was immersed.
- The maximum immersion level was observed.
- Reading was recorded.

e. OBSERVATION OF MOISTURE CONTENT:

At the time of observation moisture content was evaporated through the hot air oven. It was kept up to 1 hour and taken out. It was cooled in desiccator up to half an hour. Weight was recorded and repeated half hourly until the two concurrent weight obtained were same.

f. OBSERVATIONS OF ASH VALUE:

- 2gms of fine powder taken in silica crucible.
- Kept in muffle furnace and observed for its fumes.
- It has kept there unless the fume formations get stops.
- Observed for its ash coloration after it gets cool itself.
- The weight of the ash taken and ash value get calculated.

C) OBSERVATIONS ON PRELIMINARY PHYTOCHEMICAL TESTS:

Table 2: Observations

TEST	OBSERVATION
	OIL
Test for carbohydrates a) Molish's test	Violet ring is formed at the junction of two liquids
2)Tests for proteins a) Million's test	White ppt, warm ppt turns brick red or the ppt dissolves giving red coloured sol ⁿ
3)Tests for fixed Oils a)Solubility test b)Dip test c) Odor & taste	Soluble in petroleum ether Filter paper permanently stained with oil Pleasant
4)Test for Steroids a)Salkowski reaction b) Liebermann- Buchard reaction c) liebermann's reaction	Chloroform layer appears red & acid layer shows greenish yellow fluorescence First red then blue & finally green color appears Blue color appears
5)Tests for saponins i) Foam test	Persistent foam is observed
6)Test for Flavonoids a) Shinoda Test	Pink color observed
7)Test for fats & oils a) saponification	It contains glycerol
8) Test for Tannins	Deep blue black color observed

RESULTS OF PHARMACOGNOSTICAL STUDY:

1. MORPHOLOGICAL STUDY:

Table 3: Observations

COLOUR	Black
TASTE	Madhura ,Tikta , Kashaya
SIZE	App. 2.5-3mm long,1.5mm broad
SHAPE	Flattened ovate
ODOUR	Pleasant
NATURE OF SEED	One side slightly concave with faint marginal lines & an equally faint central line
TOUCH	Smooth

2. MICROSCOPICAL STUDY

a) The results of T.S. of Krishna tila seed shows:

Testa - Of seed shows single layered palaside like cells & rest of testa composed of collapsed cells
Endosperm- 3 layered, consisting of polygonal cells of parenchyma containing fixed oils & small aleurone grains
Cotyledons- 2, externally covered with thin cuticle single layered epidermal cells, rest of tissue consists of polygonal parenchyma cells containing fixed oil & aleurone grains

POWDER MICROSCOPY:

Blackish colored showed palaside like cells, parenchyma cells, oil globules & aleurone grains

B. RESULTS OF PHYTOCHEMICAL STUDY:

Table No.4: Result of Solubility Test :

No.	Solvent	Soluble	Sparingly Soluble	Insoluble
1	Distilled water	+	-	-
2	Petroleum ether	+	-	-
3	Acetone	+	-	-
4	Benzene	-	+	-
5	Chloroform	-	+	-
6	Ethyl alcohol	-	-	+
7	CCl ₄	-	+	-
8	Methanol	-	-	+
9	Butyl alcohol	-	+	-

Table No. 5: Result of Extraction

Seed of Sesamum indicum, Linn. Of each	Solvent	Extract
Seed Coarse powder 100 gms.	Total Petroleum ether 800ml.	650ml

Table No. 6: Result oil Distillation

Seed of Sesamum indicum, Linn. Of each batch	Solvent	Oil
Extract 650ml	Total petroleum ether 600 ml.	30 ml.

Table No.7: Results of P^H Value

Distilled water normal P ^H	7.0
Acidic media P ^H	0-7
Alkaline Media P ^H	7-14
Sesamum indicum linn extract	7.7

e. RESULT OF MOISTURE CONTENT:

Weight of crucible = 19.30 gm (A)

Weight of sample = 5 gm (B)

$$\text{Moisture content} = \frac{A + B - \text{Concurrent values} \times 100}{B}$$

$$\text{Moisture content} = \frac{19.30 + 5 - 24.15 \times 100}{5}$$

$$\text{Moisture content} = \frac{24.30 - 24.15 \times 100}{5}$$

$$\text{Moisture content} = \frac{0.15 \times 100}{5}$$

$$\text{Moisture Content} = 3 \%$$

f. RESULT OF ASH VALUE:

Weight of empty crucible = 19.30gms (A)

Weight of sample powder taken (2gms) = 2.00gms (B)

Weight of empty crucible + drug taken =

$$A+B = (C) = 21.30\text{gm}$$

Weight of crucible + ash (D) = 21.1688gm

Weight of ash = C - D = 21.30 - 21.1688 = 0.1312gm

Therefore 100gms of ash value is = 0.1312 × 50 = 6.560%

Table 8: Result of Photochemical Study:

TEST	OBSERVATION
	OIL
1) Test for carbohydrates	
a) Molish's test	+
2)Tests for proteins	
a) Million's test	+
3)Tests for fixed Oils	
a)Solubility test	+
b)Dip test	+
c) Odor & taste	+
5)Tests for saponins	
i) Foam test	+
6)Test for Flavonoids	
a) Shinoda Test	+
7)Test for fats & oils	
a) saponification	+
8) Test for Tannins	+

DISCUSSION ON PHARMACOGNOSTICAL STUDY:

The pharmacognostical study suggests the procedure of organoleptic study, which composes of the morphological, microscopical and physical evaluation of the present undertaken official part of Krishna tila (*Sesamum indicum*, Linn.)

Organoleptic method expresses the appearances, texture, odour, taste. This plays an important role in identification and establishes a protocol of drug. The seed is black in colour, flattened, ovate in shape, smooth or reticulate, slightly concave with faint marginal lines.

The microscopical study reveals, testa of seeds shows single layered palaside like thin walled, yellowish coloured cells & the rest of the testa composed of collapsed cells, endosperm 3 layered, rarely 2 layered, consisting of cellulosic polygonal cells of parenchyma containing fixed oils & small aleurone grains, cotyledons two externally covered with the cuticle, single layered epidermal cell, followed by single row of palaside like cells rest of the tissue consists of polygonal, parenchyma cells containing fixed & aleurone grains.

DISCUSSION ON PHYTOCHEMICAL STUDY:

In the present study all the actives component of *Sesamum indicum*, Linn was tested quantitatively by employing specific chemical tests.

Before carrying out the phytochemical tests the drug was subjected for its solubility in different solvents like ethyl alcohol, chloroform, distilled water etc. Maximum solubility was observed in petroleum ether.

Before carrying out the Phytochemical tests the different forms of trial drug was prepared adopting proper method. For preparation of extract the drug was subjected for its solubility in different solvents like petroleum ether, acetone, distilled water etc. Maximum solubility was observed in 70-80% petroleum ether. Then the drug was subjected for exhaustive extraction with petroleum ether in soxhlet apparatus and extract was subjected for distillation. The distilled sample was

kept at room temperature to evaporate the petroleum ether residue present in the extract.

Then the sample oil was subjected for its phytochemical investigation by using specific methods. In present study it is observed that the trial drug shows presence of carbohydrates, proteins, steroid, fats & fixed oil.

CONCLUSION

The following conclusions from the study are:

- 1) Morphological characters of *Krishna tila beeja* are found similar to the classical references.
- 2) Microscopical study of *Krishna tila beeja* showed externally single layered epidermal cells followed by single row of palaside like cells & polygonal cells containing fixed Phytochemical test of *Krishna tila beeja* shown presence of carbohydrates, proteins, fixed oil, steroids, saponins, flavonides & tannins as phyto constituents.
- 3) Presence of flavonides & tannins in *Krishna tila beeja* dominates antioxidant property.

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