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R E S E A R C H A R T I C L E

Pharmacognostical and phytochemical studies of Black and White seeds of *Kevach* (Mucuna pruriens)

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A B S T R A C T

The genus *Mucuna*, belonging to the Fabaceae family, sub family Papilionaceae, includes approximately 150 species of annual and perennial legumes, commonly known as Velvet bean, Cowitch, Cowhage in English and Kawaanch, Kavach in Hindi. *Mucuna pruriens* is widespread in tropical and sub-tropical regions of the world. It is considered a viable source of dietary proteins due to its high protein concentration (23–35%) in addition its digestibility. The present study deals with hydroalcoholic extract of Mucuna pruriens black seed and white seed. The pharmacognostic evaluation of two seeds of Mucuna pruriens i.e. *black seed and white seed* including the morphological, microscopical characters and different physicochemical standard has been developed. Physicochemical constants of *Black and White seed of M.P*including determination of loss on drying, ash values and extractive values. The preliminary phytochemical screening of various leaf extracts was also carried out and phytoconstituent of both seeds was revealed.

Keywords: Mucuna, Phytocontituents, Physicochemical, Hydroalcoholic

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The seers of Ayurveda were able to understand and record the various aspects regarding the drugs that even today are difficult to understand with modern available parameters. The

medicinal plant products, which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action on the human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.[1] *Mucuna pruriens* Linn. belongs to the family fabaceae, commonly known as cowhage plant or kapikacho or kevach in Hindi, is the most popular drug in Ayurvedic system of medicine. *Mucuna pruriens* is a twinning herb found all over tropical parts of India. It is an Indian indigenous leguminous plant, is well known for producing itch.

This property is attributed to the trichomes (hair) present on the pods. It has been established that this unique property is accounted by the presence of 5-hydroxy tryptamine (5-HT) in the hair. It is also likely that histamine and kinin like substances may also be responsible. Some reports show that anti-histaminics afford protection against the itch[2].It is one of the most popular green crops currently known in the tropics; velvet beans have great potential as both food and feed as suggested by experiences worldwide[3]Itching bean *Mucuna pruriens*isan underutilized legume species grown predominantly in Asia, Africa and in parts of America.It is attributed to the presence of high levels of 3, 4- dihydroxy-L-phenylalanine, L-Dopa, the aromatic non-protein amino acid.[1]

The use of the plant in treating PD was practiced in Ancient system of medicine in China, India and Amazon. The plant is found in early Sanskrit incunabula for treating various ailments since 1500 BC. In Ayurveda Kampavata, a nervous malady similar to Parkinson's syndrome, have been treated by Mucuna. The Mucuna seed preparations are employed for the treatment of PD in India[4]The seed, root and stem of MP possess valuable medicinal properties. It has been reported to contain analgesic, anti-neoplastic anti-inflammatory, anti-epileptic, anti-microbial and learning and memory enhancing properties. Further, some studies, including those conducted in the present laboratory, have demonstrated MP's potent neuroprotective properties in PQ-induced Parkinsonian mice and it also shows anticonvulsant properties too[2,5] . Interestingly, MP seed extract contains L-DOPA, the dopamine precursor that is used as a therapeutic agent against PD. Although the antioxidative properties of MP are well reported, the exact mechanism of MP's antioxidative action remains unknown.[6]

M. pruriens is a popular Indian medicinal plant, which has long been used in traditional Ayurvedic Indian medicine, for diseases including parkinsonism. This plant is widely used in Ayurveda, which is an ancient traditional medical science that has been practiced in India since the Vedic times (1500–1000 BC). The beans have also been employed as a powerful aphrodisiac in Ayurveda and have been used to treat nervous disorders and arthritis. The bean, if applied as a paste on scorpion stings, is thought to absorb the poison. The non-protein amino acid-derived L-dopa (3,4-dihydroxy phenylalanine) found in this under-utilized legume seed resists attack from insects, and thus controls biological infestation during storage. According to D'Mello (1995), all anti-nutritional compounds confer insect and disease resistance to plants.[3]

2. Materials and Methods

Black and White seeds of Mucuna Pruriens were taken after proper examination. The plant part of both types of Kapikacchu seeds (black and white) were taken from field after proper identification. The plant material was identified and authenticated.

1. Macroscopic study:

The collected drugs i.e (black and white) seeds of Kapikacchu were dried and studied for its organoleptic property, with naked eye and magnifying lens, with the help of pharmacognostical procedure i.e. Appearance, size, shape, colour, and odour and findings were recorded.

3. Microscopic study:

The micro chemical test of powdered seeds.Microscopic slides were preparedeither by soaking a pinch offine powder indistilled water for 1 hr. and staining with saffranin for 2-4 minutes or treating withsolution of chloral hydrate for 1 hour.[7]It reveals the absence or presence of lignified cells, cuticle, crystals of calcium oxalate, mucilaginous cells, Hemicellulose, stone cells, endodermal starch grains.[8]

Physico-chemical Analysis:

The parameter studied were loss on drying total ash, water soluble ash, acid insolubleash, extractive value.

3. Loss on Drying:

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. 2 gm. of air-dried drug reduced to powder was placed in a crucible of silica. Originally the crucible was cleaned and dried and weight of empty dried crucible was taken. The powder was spread in a thin uniform layer. The crucible was then placed in the oven at 105^{o} C. The powder was dried for 4 hours and cooled in a desiccator to room temperature and weight of the cooled crucible plus powder was noted.

4. Total Ash:

Place about 2-4g of the ground air-dried material,accurately weighed, in a previously ignited and tarredcrucible (usually of platinum or silica). Spread thematerial in an even layer and ignite it by graduallyincreasing the heat to 450°C until it is white,indicating the absence of carbon. Cool in a desiccatorand weigh. Ash value can be calculated by usingformula:-

$$\text{Ash value} = \frac{\text{Initial weight - Final weigt}}{\text{Initial weight}} \times 100$$

5. Water soluble Ash:

The total ash obtained above was boiled with 25 mlof distilled water for 5 minutes. The insoluble matterwas collected on an ash less filter –paper, washedwith hot water and ignited to constant weight at lowtemperature. The weight of the insoluble matter wassubtracted from the weight of total ash, represents thewater soluble ash. The percentage of water solubleash was calculated with

reference to the air drieddrug. The result was calculated with reference to theair dried drug.

6. Acid Insoluble Ash:

The total ash obtained was boiled with 25 ml ofdilute hydrochloric acid for 5 minutes. The insolublematter was collected on tarred grouch crucible,washed with hot acidulated water, ignited, cooled andweighed. The percentage acid insoluble ash wascalculated with reference to the air dried drug. Thesame procedure was repeated with other ashobtained.[9]

7. Determination of Extractive Value

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

Method:-

7.1. Hot extraction:

7.1.1 Water Soluble Extractive Value: Place about 15gm of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gm. of air dried material.

7.1.2 Alcohol Soluble Extractive Value: Place about 15gm of coarsely powdered air-dried material,accurately weighed, in a glass-stoppered conical flask. Add 300ml of water and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Dry the extracted powder in oven till the weight is constant. Calculate the content of extractable matter in mg per gm. of air-dried material by using this formula:-[10]

$$\text{Extractive value} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

8. Preparation of Extract

Seeds were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds were shade dried for 7–12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered material was extracted with hydroalcoholic solvent (1:1 ethanol and water) by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract. The percentage yield of the extract was found. The extract was stored in airtight container. Desired concentration of stock solution was prepared using the solvent for the following studies and then Preliminary phytochemical investigations were done.[1,11]

9. Tests for phytochemical Screening: [12,10]

A. Tests for Carbohydrates:

Molish's test (General test):

To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing Sugars:

a) Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.

b) Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharides:

Barfoed's test:

Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Red precipitates were observed.

Tests for Non-Reducing Sugars:

a) Test solution does not give response to Fehling's and Benedict's test.

b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins:

a) **Biuret test (General test):** To 3 ml T.S added 4% NaOH and few drops of 1% $CuSO_4$ solution observed for violet or pink colour.

b) Million's test (for proteins): Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.

c) Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.

d) **Test for protein containing sulphur:** Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to PbS formation was observed.

e) **Precipitation test:** The test solution gave white colloidal precipitate with following reagents:

i) Absolute alcohol
ii) 5% $HgCl_2$ solution
iii) 5% CuSO4 solution
iv) 5% lead acetate
v) 5% ammonium sulphate

B. Tests for Steroid:

a) Salkowski Reaction: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shaked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

C. Tests for Amino Acids:

a) Ninhydrin test (General test):- 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.

b) Test for Tyrosine: Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.

c) Test for tryptophan: To 3 ml T.S. added few drops glycoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

D. Tests for Glycosides:

Tests for Cardiac Glycosides:

a) Baljet's test:- A test solution observed for yellow to orange colour with sodium picrate.

b) **Legal's test (For cardenoloids):** To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

c) **Test for deoxysugars (Kellar Killani test):** To 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.

Tests for Saponin Glycosides:

a) Foam test: The drug extract or dry powder was shaked vigorously with water. Persistent foam was observed.

Tests for Coumarin Glycosides:

Test solution when made alkaline, observed for blue or green fluorescence.

E. Tests for Flavonoids:

a) To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.

b) Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.

c) Ferric chloride test: - Test solution, added few drops of ferric chloride solution observed for intense green colour.

F. Tests for Alkaloids:

a) Dragendroff's test: To 2-3 ml filtrate added few drops Dragendroff's reagent observed for orange brown precipitate.

b) Mayer's test:- 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate.

c) Hager's test:- 2-3 ml filtrate with Hagers reagent observed for yellow precipitate.

d) Wagner's test:- 2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate.

G. Tests for Tannins and Phenolic Compounds:

To 2-3 ml test solution, added few drops of whether showed following was observed:

a) 5% $FeCl_3$ solution: Deep blue-black coloured.

b) Lead acetate solution: White precipitate.

c) Acetic acid solution: Red colour solution.

d) Potassium dichromate: Red precipitate.

e) Dilute iodine solution: Transient red colour.

f) Dilute HNO_3 : Reddish to yellow colour.

3. Results and Discussion

Morphological Parameters: Morphological characteristics of *Black and white seed of Mucuna pruriens* have been described in Table 1. The powders of both types of M.pruriens seed was observed under microscope. Aleurone grains were observed in both the powdered seed in yellow colour. The needle shaped crystal was found in the powder microscopy resembling the presence of calcium oxalate crystal. Vascular bundle was observed in both the seeds.

Physical Parameters

Results of foreign matter analysis, loss on drying, ash values, extractive values in different solvents, pH determination, swelling index and foaming index are given in Table 2.

Loss on drying is used to determine the moisture content of the drug. The moisture content of black seed of M.pruriens was found to be less than the white seed of M.pruriens i.e. 5% and 6% respectively. Total Ash is the residue remaining after incineration. It is a quantity analysis technique to determine siliceous material and inorganic substance in a sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of inorganic substance in Ash. Black seeds had Total Ash 2%, Acid Insoluble Ash 0.3% and Water Soluble Ash 1%. White seeds had Total Ash 3.33%, Acid Insoluble Ash 0.03% and Water Soluble Ash 2.67%.

Extractive value show soluble content present in sample. Aqueous extractive value was found to be 3% in black seed and in white seeds was 5.28%. The hydroalcoholic extractive value was found to be 24.47% in black seed and in white seeds was 17.84%.

Carbohydrate was present in both the extracts of drug i.e. black and white seed, showing the presence of reducing sugar. The test for sulfur containing protein had positive results for both samples.Amino acid and steroids was found to be absent in both the sample. Cardiac glycoside, tannin and phenolic compound was present in black seed but was found to be absent in white seed extract. Flavonoids were present in both the drug extracts.

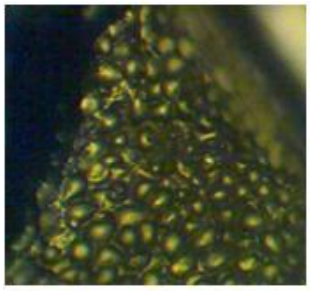

Fig.1: Aleurone grains of white seed

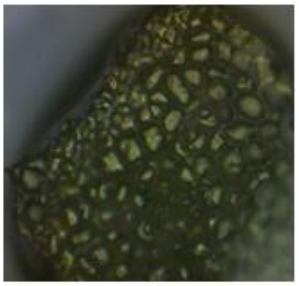

Fig.2 Aleurone grains of black seed

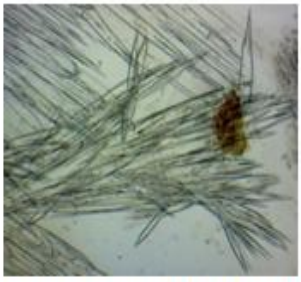

Fig.3: Calcium oxalate crystal of white seed

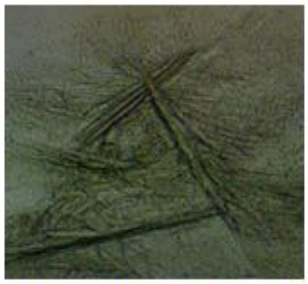

Fig.4: Calcium oxalate crystal of black seed

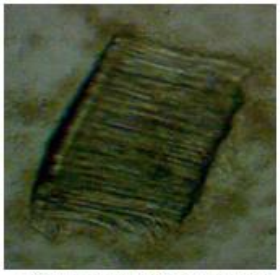

Fig.5: Vascular bundle of white seed

Fig.6: Vascular bundle of black seed

5. Conclusion

In appearance the shape of both the seeds are same but there is a difference in their size, white seed is bigger in size than the black seed. Other organoleptic characteristics are similar. The loss on drying of both the seeds was closely same. The ash value shows no significant difference. Extractive value showed slight differences. The hydro-alcoholic extractive value of white seed was less than the black seed. Aqueous extractive value of black seed was less than the white seed. All the values were found significant to the standard values of API. Hydro-alcoholic extract of powdered seed were subjected tophytochemical screening for the detection of various seed constituents, characterized for their possible bioactive compounds. Carbohydrate, protein, Flavonoid compounds were present in both the sample. Steroids and alkaloids showed negative results for both the samples. Saponin, Cardiac glycoside, Tannin and phenolic compounds were present only in the black seed extract.

Table 1: Results of Accuracy

Table 1: Observation of Organoleptic Characters of Black and white seed of Mucuna pruriens

S.no.	Parameter	Black seed	White seed
1	Colour	Black	White
2	Odour	Odourless	Odourless
3	Taste	Sweetish bitter	Sweetish bitter
4	Size	2.5 cm	3 cm
5	Shape	Oval	Oval

Table 2: Physical parameter

Parameters	Results (%)	
	Black Seed	White Seed
Loss on drying	5%	6%
Total Ash	2%	3.33%
Acid insoluble ash	0.3%	0.03%
Water soluble ash	1%	2.67%
Aqueous extractive value	3%	5.28%
Hydroalcoholic extractive value	24.47%	17.84%

Table 3: Qualitative Phytochemical Tests of Extracts of Black And White Seed of Mucuna Pruriens

TESTS	BLACK SEED	WHITE SEED
A. Test for Carbohydrate		
1. Molish's test	Positive	Positive
2. Fehling's test	Positive	Positive
3. Benedict test	Positive	Positive
4. Barfoed's test	Negative	Negative
5. Tannic acid	Negative	Negative
B. Test for Protein		
1. Biuret test	Negative	Negative
2. Million's test	Negative	Negative
3. Xanthoprotein	Negative	Negative
4. Protein containing sulphur	Positive	Positive
5. Precipitate test		
• 5% $HgCl_2$	Negative	Negative
• 5% $CuSO_4$	Negative	Negative
• 5% Lead acetate	Positive	Positive
C. Test for Steroids		
1. Salkowski reaction	Negative	Negative
D. Tests for amino acid		
1. Ninhydrin test	Negative	Negative
2. Test for Tyrosine	Negative	Negative
E. Test for Cardiac Glycoside		

1. Baljet test	Positive	Negative
2. Legal test	Negative	Negative
3. Kellar killani	Negative	Negative
F. Test for saponin		
1. Foam test	Positive	Negative
G. Test for Coumarin glycoside		
1. Alkaline solution	Negative	Negative
H. Test for Anthraquinone glycoside		
1. Borntrager test	Negative	Negative
2. Modified borntrager test	Negative	Negative
I. Flavanoids test		
1. Lead acetate	Positive	Positive
2. Sodium hydroxide	Positive	Positive
3. Ferric chloride	Positive	Negative
J. Tests for Alkaloids		
1. Dragendroff's	Negative	Negative
2. Mayer"s	Negative	Negative
3. Hager's	Negative	Negative
4. Wagner's	Negative	Negative
K. Test for Tannins and Phenolic compounds		
1. 5% $FeCl_3$ solution	Positive	Negative
2. Lead acetate	Negative	Negative
3. Acetic acid	Negative	Negative
4. Potassium dichromate	Negative	Negative
5. Dilute Iodine	Negative	Negative
6. Dilute Nitric acid	Negative	Negative

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