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A COMPARATIVE PHARMACOGNOSY STUDY OF BLACK AND WHITE SEEDS OF KAPIKACCHU (*MUCUNA PRURIENS* (L.) DC.)

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ABSTRACT: Background: Kapikacchu (*Mucuna pruriens* (L.) DC.) belonging to the family Fabaceae is commonly known as Velvet bean, Cowitch, Cowhage in English and Kawaanch, Kavach in Hindi. It is mainly distributed in Asia, Africa, Pacific Islands and the United State. Kapikacchu is very famous plant for its aphrodisiac effect. Traditionally it is being used as a potent antiparkinsonian, anti-inflammatory, aphrodisiac and nervine tonic. In market two types of seeds (black and white) are available and are being used simultaneously in the name of Kapikacchu. No data is yet available in context of the comparative pharmacognosy of black and white seeds of Kapikacchu. The aim of present article is to put forward the comparative pharmacognostical analysis of both types of Kapikacchu seeds. **Methods:** Macroscopic evaluation, microscopic evaluation, physicochemical evaluation, extractive values, phytochemical analysis, T.L.C. study were carried out using both varieties of Kapikacchu seeds and comparative data was obtained. **Results:** Data pertaining to the above cited evaluations was recorded for both varieties of seeds of Kapikacchu. **Conclusion:** All the values hence obtained were subjected to comparison with their corresponding standard values as mentioned in API. It was observed that all the values were under their normal range.

INTRODUCTION: Kapikacchu (*Mucuna pruriens* (L.) DC.) is a very well-known drug used in *Ayurvedic* classics. It is used as Balya, Vrishya, Brihankaraka and for Vata shaman [1]. It is described as Vrishya Dravya in Samhita [2] as well as Nighantus [1]. Kapikachhu seeds contain L-Dopa which is indicated in Parkinson's disease and used as aphrodisiac. L-Dopa is precursor of the neurotransmitter Dopamine. In market two types of Kapikacchu seeds (black and white) are available and are being used simultaneously in the name of Kapikacchu.

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These are different germplasms of cultivated variety of kapikacchu and botanically both are *Mucuna pruriens* var. utilis.

Mucuna pruriens is a tropical twining herb commonly known as velvet bean belongs to the family Fabaceae. It is commonly known as Cowitch, Cowhage in English and Kawaanch, Kavach in Hindi. The plant is famous for the extreme itchiness it produces on contact, particularly with the young foliage and the seed pods due to the presence of 5- hydroxytryptamine (5-HT) [3].

It is grown predominantly in Asia, Africa, and many parts of America [4]. The beans of the *M. pruriens* are known to produce the unusual non protein amino acid L-dopa, a potent neurotransmitter.

From the ancient times Kapikacchu has been used in Ayurvedic medicine for the treatment of Parkinson's disease associated with progressive degeneration of dopaminergic neurons in specific areas in the brain which is a common age-related neurodegenerative disorder. But the researches discover that it is also used in many other diseases such as for treating arthritis, anxiety, cancer, cough, diarrhoea, dysentery, diabetes, dysmenorrhea, delirium, gonorrhoea, gout, impotence, muscular pain, parasitic infections, rheumatic disorders, as analgesic and antipyretic, to induce vomiting, to treat snakebite and scorpion stings, sexual debility, sterility, tuberculosis and its direct application on skin can help to stimulate surface blood flow in conditions that involve paralysis. In India, it is considered an aphrodisiac, diuretic, nerve tonic and uterine stimulant. In Central America, it is known as Nescafe as the seeds are roasted and ground to make a coffee substitute for decades [5].

MATERIALS AND METHODS: We took both types of Kapikacchu seeds (black and white) from field after proper identification. The plant material was identified and authenticated from Botany Department, University of Rajasthan, Jaipur.

(1) Macroscopic study:
The collected drugs i.e (black and white) seeds of Kapikacchu were dried and studied organoleptically, with naked eye and magnifying lens, with the help of Pharmacognostical procedure i.e. Appearance, size, shape, colour, and odour and findings were recorded.

(2) Microscopic study: All fresh samples were cut in very thin slices with the help of blade and were dipped in water for some time to make them soften. After that staining was done with safaranine. After staining, mounting was done on microslides. In this process, sections were transferred on slides & glycerine was added on sections. Then coverslip was put on sections, excess water was wiped out & then the slides were observed in microscope & photos were taken.

(3) Determination of Moisture Content: Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105ºC for 5 hours, and weight of sample was calculated for every 30 minutes, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing [6].

(4) Determination of pH: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidity or basic nature of a solution. pH value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India [7].

(5) Determination of Extractive values:
Determination of Alcohol Soluble Extractive: Alcohol-soluble extractive value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India [8].

Determination of Water Soluble Extractive: Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

(6) Determination of Total Ash: The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface. Total ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India [9].

(7) Determination of Acid Insoluble Ash: Acid insoluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India [10].

(8) Determination of Water Soluble Ash: Water soluble ash value was calculated as per the methods mentioned in the Ayurveda Pharmacopeia of India[11].

(9) Phytochemical screening [12]: Freshly prepared extracts were subjected to preliminary phytochemical screening. Presence of carbohydrates (Molisch's Test, Benedict's test, Barfoed's test, Fehling solution test), alkaloids (Mayer's test, Dragondroff's test, Wagner's Test, Hager's Test), amino acids (Ninhydrin test),

proteins (Biuret test, Xanthoprotic test, Millons test), saponins (Foam test), glycosides (Borntragor's test), Phenolic Compound, flavonoids (Shinoda test), steroids (Salkowski test) and tannins (Ferric chloride solution, Lead acetate test, Pot. Dichromate test) were tested.

(10) Determination of Heavy metals:- Presence of Arsenic compound, Nickel compound, Cobalt compound, Lead compound, mercury compound, Silver compound and Zinc compound were tested by different methods.

Arsenic compound: 10 mg of ash was taken in a test tube and dissolved while gently heating with 5 ml of slightly acidic water and hypophosphorus reagent was added, a brown precipitate was formed.

Nickel Compound: Dissolved 20 mg of the ash of the drug in about 0.5 ml of water, acidified with a few drops of dilute hydrochloric acid, and then Added drop by drop a dilute solution of sodium hydroxide. A blue precipitate is formed which turns green on warming.

Cobalt Compound: Dissolved 20 mg of the ash of the drug in about 0.5 ml of distilled water, and acidified with a few drops of dilute hydrochloric acid. Added a few drops of dilute solution of sodium hydroxide. A blue precipitate is formed which turns pink on warming.

Lead Compound: Dissolved 0.1 gm of the substance being examined in 1ml of dilute acetic acid or use 1ml of the prescribed solution. Added 2 ml of potassium chromate solution; a yellow precipitate insoluble in 2ml of 10M sodium hydroxide was produced.

Mercury Compound: Dissolved 20 to 25 mg of the ash of the drug in 1 ml of distilled water and added potassium iodide solution. A red precipitate is formed that dissolved in an excess of the reagent.

Silver Compound: Dissolved 20 to 25 mg of the drug in 2-3 ml of distilled water and added 0.2 ml of 7 M hydrochloric acid. A curd white precipitate is formed that is soluble in 3 ml of 6 M ammonia. Added a few drops of a 10% W/V aqueous solution of potassium iodide a yellow precipitate is developed.

Zinc Compound: Dissolved 20 to 25 mg of the ash in 2 to 3 ml of distilled water and added 0.2 ml of 10 M sodium hydroxide. A white precipitate is formed which is dissolved in 2 ml of 10 M sodium hydroxide solution. Added about 5 ml of 2 M ammonium chloride followed by 0.1 ml of sodium sulphide solution. A flocculent, white precipitate is produced.

(11) Chromatography [13]: Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Chromatography plates: T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F_{254}: Plates were dried in hot oven at 105^0 C for one and half hour.

Preparation of mobile solution: Toluene: Ethyl acetate: Formic acid 14: 5: 1

Sample application: Sample was applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached 1cm below the top of the T.L.C. plate.

Visualization: Under day light, under Iodine vapor and under Dragondroff's reagent.

R_f Value: Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots with the distance travelled by the front of the mobile phase.

RESULT AND DISCUSSION:

TABLE 1: MACROSCOPICAL EXAMINATION OF SEEDS

S. No.	Observed for	Black seeds	White seeds
1	Shape & structure	Oval	Oval
2	Colour	Black	White
3	Odour	Odourless	Odourless
4	Touch	Smooth	Smooth
5	Taste	Sweetish-bitter	Sweetish-bitter

TABLE 2: MACROSCOPICAL EXAMINATION OF SEEDS POWDER

S. No.	Sample	Colour	Taste	Odour	Texture
1.	Black seeds Powder	Pale cream	Sweetish-bitter	Non irritant	Fine powder
2.	White seeds Powder	Off white	Sweetish-bitter	Non irritant	Fine powder

Microscopic evaluation:

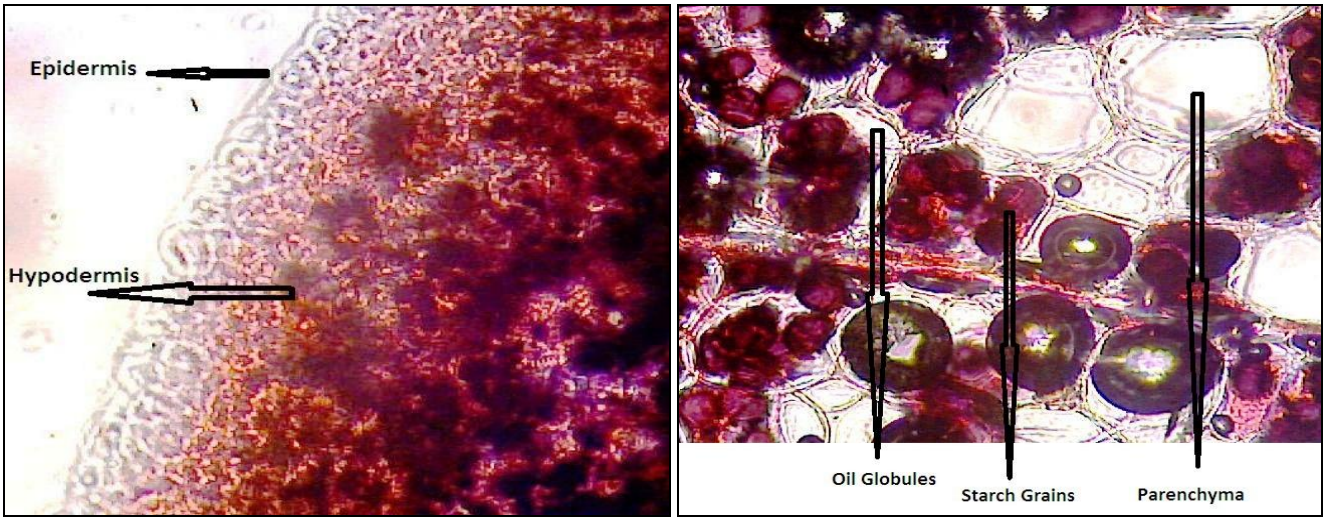


FIG. 1: MICROSCOPY OF BLACK SEEDS

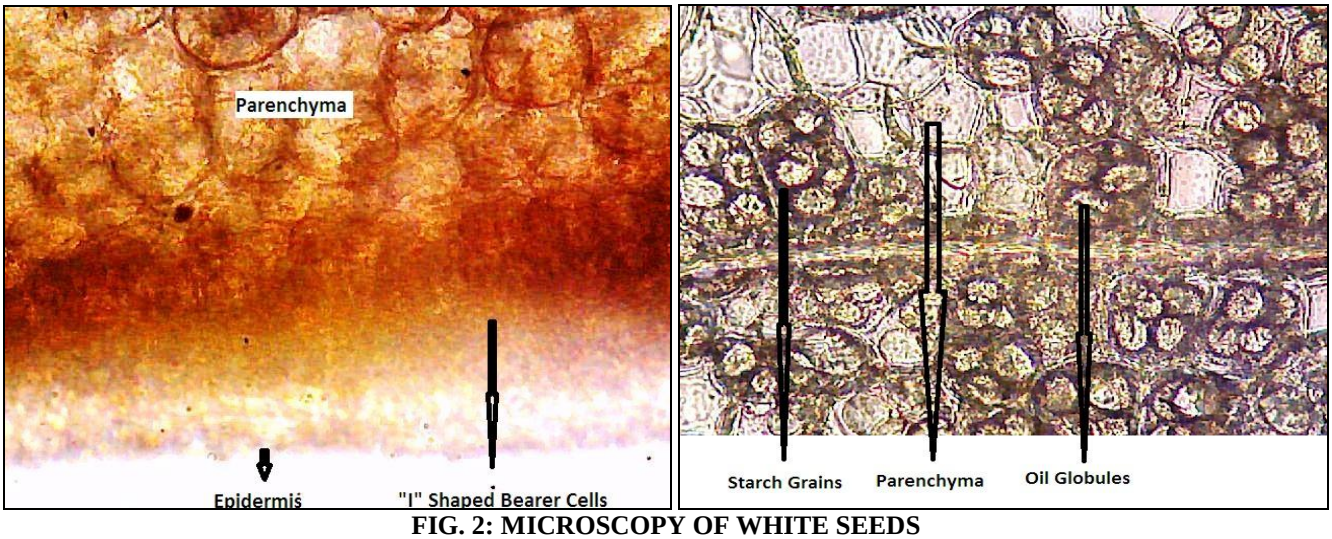


FIG. 2: MICROSCOPY OF WHITE SEEDS

Mature seed shows a thin seed-coat and two hard cotyledons; outer testa consisting of single layered palisade-like cells; inner testa composed of 2 or 3 layers, outer layer of tangentially elongated, ovoid, thin-walled cells, inner 1 or 2 layers of dumb-bell or beaker shaped, thick-walled cells; tegmen composed of a wide zone of oval to elliptical, somewhat compressed, thin-walled, parenchymatous cells; some cells containing starch grains; cotyledons composed of polygonal, angular, thin-walled, compactly arranged, parenchymatous cells, containing aleurone and starch grains; starch grains small, simple, rounded to oval measuring 6-41 µin dia., but not over 45 µin dia.; a few vascular bundles with vessels showing reticulate thickening or pitted were present [14].

TABLE 3: PHYSICO-CHEMICAL ANALYSIS

S. No.	Tests	Black seeds	White seeds
1	Moisture content	2.83 %	1.69%
2	pH	5.05	5.09
3	Alcohol Extractive Value	5.95%	7.58%
4	Aqueous Extractive Value	24.36%	23.72%
5	Total Ash	2.41%	3.00%
6	Acid Insoluble Ash	1.93%	0.46%
7	Water Soluble Ash	0.90%	0.81%

Moisture content is water holding capacity of a sample, high moisture content in a sample indicates that it may decrease the stability of the sample. Moisture content in black seeds was 2.83% and in white seeds was 1.69%.

pH is a method of quantity analysis of acidic and basic nature of drug. pH of black seeds was 5.05 and of white seeds was 5.09. Both are acidic in nature.

Extractive value show soluble content present in sample. Water soluble content present in black seeds was 24.36% and in white seeds was 23.72%. Alcohol soluble content present in black seeds was 5.95% and in white seeds was 7.58%.

Total Ash 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 2.41%, Acid Insoluble Ash 1.93% and Water Soluble Ash 0.90%. White seeds had Total Ash 3.00%, Acid Insoluble Ash 0.46% and Water Soluble Ash 0.81%.

TABLE 4: QUALITATIVE PHYTOCHEMICAL TESTS OF EXTRACTS OF KAPIKACCHU SEEDS

Sr. no.	Name of test	Black seeds of Kapikacchu			White seeds of Kapikacchu		
		Aqu. ext.	Alc. ext.	Pet. ether	Aqu. ext.	Alc. ext.	Pet. ether
1. Carbohydrate test							
A.	Molisch test	-ve	-ve	-ve	-ve	-ve	-ve
B.	Benedict test	-ve	+ve	-ve	-ve	+ve	-ve
C.	Barfoed's test	+ve	+ve	-ve	+ve	+ve	-ve
D.	Fehling test	+ve	-ve	-ve	-ve	-ve	-ve
2. Alkaloids							
A.	Dragondroff test	-ve	+ve	-ve	-ve	+ve	-ve
B.	Wagner's test	-ve	-ve	+ve	+ve	-ve	+ve
C.	Hager's test	+ve	-ve	-ve	+ve	-ve	-ve
3. Amino acids							
A.	Ninhydrine test	-ve	+ve	-ve	+ve	-ve	-ve
4. Proteins							
A.	Biuret test	-ve	+ve	-ve	+ve	+ve	-ve
B.	Xanthoprotic test	+ve	+ve	-ve	-ve	+ve	-ve
C.	Millon's test	-ve	-ve	-ve	-ve	-ve	-ve
5. Saponins							
A.	Foam test	+ve	-ve	-ve	+ve	-ve	-ve
6. Glycosides							
A.	Borntragor's test	-ve	-ve	-ve	-ve	-ve	+ve
7. Phenolic compound							
A.	Phenolic test	+ve	+ve	-ve	+ve	+ve	-ve
8. Steroids							
A.	Salkowski test	+ve	+ve	-ve	-ve	-ve	-ve

	9. Tannins						
A.	$FeCl_3$ test	+ve	+ve	-ve	+ve	+ve	-ve
B.	Lead acetate test	+ve	+ve	+ve	+ve	+ve	+ve
C.	Potassium dichromate test	+ve	-ve	-ve	+ve	-ve	-ve
	10. Flavonoids						
A.	Shinoda test	-ve	-ve	-ve	+ve	-ve	-ve

TABLE 5: CHROMATOGRAPHY OF ALCOHOL EXTRACT

Sample	Visualisation in day light (Rf value)	Visualisation Under Iodine vapour (Rf value)	Visualisation in Dragondroff's reagent (Rf value)
Black seeds	0.49	0.06, 0.12, 0.14, 0.25, 0.29, 0.73, 0.79	0.72, 0.77
White seeds	0.49	0.06, 0.12, 0.14, 0.25, 0.73, 0.79	-
TLC			

TABLE 6: CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACT

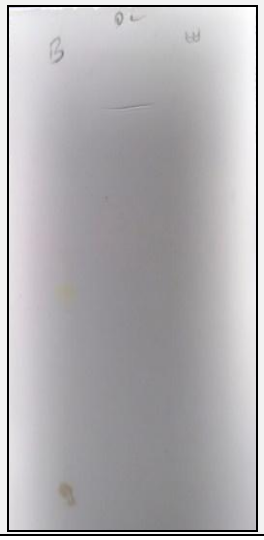
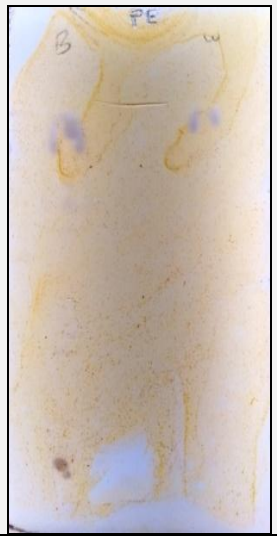
Sample	Visualisation in day light (Rf value)	Visualisation Under Iodine vapour (Rf value)	Visualisation in Dragondroff's reagent (Rf value)
Black seeds	-	0.10, 0.12, 0.20, 0.32, 0.60, 0.66, 0.72	0.62, 0.67
White seeds	-	0.20, 0.60, 0.66, 0.72	-
TLC			

TABLE 7: HEAVY METALS PROFILE OF KAPIKACHHU SEED POWDER

S. No.	Heavy metals	Black seeds	White seeds
1.	Arsenic compound	-ve	-ve
2.	Nickel Compound	-ve	-ve
3.	Cobalt Compound	-ve	-ve
4.	Lead Compound	-ve	-ve
5.	Mercury Compound	-ve	-ve
6.	Silver Compound	-ve	-ve
7.	Zinc Compound	-ve	-ve

CONCLUSION: T.S. of black seeds and white seeds show presence of parenchyma, starch grains and oil globules. Moisture content of both seeds (black and white) was closely same. No significant difference was observed in total Ash values of powders. Extractive values also showed little variation. The alcohol soluble extractive value of white seeds was more than black seeds powder. Water soluble extractive values were approx. same for both samples. All the values were found similar to the standard values mentioned in API.

Qualitative analysis of extracts is performed to evaluate general phytochemical profile. Carbohydrates, proteins, amino acids, alkaloids, tannins, saponins, phenolic compounds were present in each sample. Steroids were absent in each sample. Glycosides were present in Petroleum ether extract of white seeds. Flavonoids were present in aqueous extract of white seeds.

Heavy metal like Lead, Mercury, Silver, Arsenic, Nickel, Cobalt and Zinc were absent in both the samples.

CONFLICTS OF INTEREST STATEMENT: There are no conflicts of interest.

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