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COMPARATIVE STUDY ON PHARMACOGNOSTIC AND PHYTOCHEMICAL COMPOSITION OF SEED COAT AND COTYLEDON OF *CAJANUS CAJAN* L.

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Keywords:

Cajanus cajan, Cotyledon, Pharmacognostic, Phytochemical, Seed coat

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ABSTRACT: *Cjanus cajan* (L.) Millsp belonging to the family Fabaceae commonly known as pigeonpea or arhar. The present study conducted to assess the phytochemical and pharmacognostic in terms of organoleptic, fluorescence analysis and physicochemical parameters of seed coat and cotyledon of *C. cajan.* Differential extraction yielded of seed coat extracts of petether, chloroform, ethanol and aqueous shows 6.3%, 1.14%, 11.36% and 9.32% and cotyledon extracts of pet ether, chloroform, ethanol and aqueous shows 7.34 %, 4.25%, 10.45 % and 8.45 % respectively. Physicochemical parameters such as total ash, acid insoluble ash and water soluble ash of seed coat and cotyledon were found to be 14.5% and 11.5%, 8.35% and 7.36%, 2.11% and 3.39% respectively. Further the mineral elements like zinc, magnesium and copper content were found more in seed coat whereas, maximum content of iron were observed in cotyledon. The fluorescent analyses of powdered drug play an important role in the determination of quality and purity of the drug. Phytochemical analysis revealed the presence of glycosides, tannins, flavonoids, phenols, lignins, alkaloids, steroids and glycosides in seed coat and cotyledon. The maximum content of phenols, flavonoids, tannins, lignins, alkaloids, steroids were found in seed coat compared to cotyledon, whereas glycosides content was more in cotyledon. The pharmacognostic study revealed the purity of the sample and helps to differentiate the plant sample from the adulterants.

INTRODUCTION: The dietary plants are also considered to be medicinally which are going to reduce the risk of several chronic diseases. In recent years a lot of research has been done in the development of chemopreventive agents derived from foods that constitute integral parts of the human diets [1].

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Legumes, which play a crucial role in many diets worldwide are thought to be related with beneficial health implications in chronic diseases such as certain cancer types (colon, breast, prostate) [2] and diabetes [3]. Except from their known high nutritive value, significant quantities of phytochemical compounds are identified in legumes and considered to be responsible for their beneficial effects [4]. *Cajanus cajan* (L.) Mill sp (In english: red gram, hindi: tur or arhar, kannad: togari, sanskrit: adhaki) is a perennial member of the family Fabaceae. Other common names are pigeon pea, congo pea, gungo pea and no-eye pea [5].

Among the "Pigeon pea" is the globally popular name that was coined by Plukenet [6] in Barbados, where the crop was in barren lands for feeding its seeds to pigeons. It is originated in Asia and is being cultivated over 3000 years. *C. cajan* is grown throughout the tropical and subtropical countries especially in South Asia, Eastern and southern Africa, Latin America and Australia. About 90% of the world production of *C. cajan* is contributed by India where it is cultivated in semi- arid regions as kharif. It cultivated mainly Karnataka, Maharastra, Tamilnadu, Rjasthan states. In Karnataka *C. cajan* is grown in an area of 5.83 lakh hectares with a production of 2.57 lakh tonnes. It is largely grown in the northern parts of the state especially in Kalaburgi district, which is called "Pulse bowl of Karnataka". *C. cajan* is very prominent crop in this district and covers approximately an area of 2.5 lakh hectares occupying 65% of the total area under *C. cajan* cultivation in Karnataka state [7]. Besides its high nutritional value, *C. cajan* is also used as traditional folk medicine in India, China, Philippines and some other nations. The seeds are astringent, acrid, sweet, cooling, anthelmintic, resolvent, expectorant and constipation [8]. Further, the extract from seeds showed hypolipidemic, antioxidant and antimicrobial activities [9].

The pharmacognostic studies of leaf, stem, root and seeds of *C. cajan* have been reported by other workers [10, 11, 12, 13]. The *C. cajan* seed coat and cotyledon proper and detailed pharmacognostical studies have not been reported so far. So an attempt was made to standardize the drug on the basis of parmacognostic and phytochemical parameters. The objective of the present study to evaluate various pharmacognostical standards like organoleptic, ash values, mineral contents fluorescence analysis and phytochemical analysis of *C. cajan* seed coat and cotyledon.

MATERIAL AND METHODS:

Plant collection: The seeds of *Cajanus cajan* L. varieties Maruti (ICP-8863) were collected from the field of Gulbarga District in Karnataka. Seeds were moistened for 1h and then dried in oven at 55^0C overnight. The hull or seed coat was removed mechanically means by using hand grinder. The two fractions like seed coat and cotyledon were made into fine powder using grinder.

Organoleptic studies of crude extract: For the determination of organoleptic characters such as color, nature, taste and yield of the extracts, 100g of dried and powdered plant material was successively extracted in the Soxhlet extractor using petroleum ether, chloroform, ethanol (95%, v/v) and distilled water solvents in the increasing order of polarity for 18h. The resulting liquid extracts were evaporated to dryness under reduced pressure. The yield of the extracts were calculated using the following formula [14, 15].

$$\text{Extractive value (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Determination of total ash and acid insoluble ash content: Two grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incarnated by gradual increasing of the temperature (400-500^0C) in the muffle furnace for 6h. The crucible were cooled to room temperature in a desecrator and the weight of ash content was weighed in as electronic digital balance (Anamed make).

The total ash content of the plant material thus obtained was boiled for 15min, after adding 25ml of 25 %(v/v) HCl and was allowed to cool. It was filtered through a Whatman filter paper No. 44 (ash less). The insoluble ash thus retained on filter paper along with paper was ignited in a pre-weighed sintered crucible (100^0C). Then the crucible along with the residue was weighed and calculated the total ash and acid insoluble ash content using the following formula [14].

$$\text{Total ash content (\%)} = \frac{Z-X}{Y} \times 100$$

Where, Z= Weight of the crucible; X = Weight of the crucible with ash; Y = Weight of the plant material taken (g).

Estimation of minerals: Estimation of minerals element by the method of AAS (Atomic Absorption Spectrometer) [16]. Five gram of seed coat and cotyledon ash was dissolved in 25ml of dilute HCl (1:1v/v) in the china dish and covered with a watch glass and incubated 20-25 minute on hot water bath, gradually cooled and filtered through a whatman filter paper No.44 (ashless).

10ml of filtrate was taken in 100ml volumentric flask and made its final volume to 100ml by adding distilled water. This solution was diluted to 10 times using distilled water, this stock ash solutions were used for the detection and estimation of elements using Atomic Absorption Spectrometer. The mineral content of the sample was calculated using the following formula

$$\text{Mineral content (ppm)} = \frac{Z \times S \times A}{Y \times W \times 100}$$

Where, Z= Mineral content of the standard solution, S=Reading of the test sample of a mineral, Y= Reading of the standard solution of the mineral, A= Dilution factor, W= weight of plant material (g), 100= volume of the stock solution.

Fluorescent studies powder drugs: A pinch of dried and powdered plant material was taken in a clean test tube with about 10ml of solvent like acetone, benzene, petroleum ether, chloroform, ethanol, glacial acetic acid, HCl, HNO_3, methanol and distilled water. All the tubes were shaken well and incubated for about 30min. The colors of the drug solutions thus obtained were observed for their characteristic color reaction under the visible light (fluorescent tube) and ultra violet light (UV366nm) and were recorded by comparing with a standard color chart [17].

Phytochemical studies:

Qualitative Phytochemical test: Qualitative phytochemical analysis was carried out on the extracts (*viz*, petroleum ether, chloroform, alcohol, and aqueous) to determine the presence or absence of metabolites such as alkaloids, flavonoids, glycosids, saponins, tannins, phenols, lignins, steroids and saponins as described by Harborne [18].

Quantitative phytochemical analysis: The quantitative estimation of secondary metabolites such as phenols was estimated by the method of Malick and Singh [19], flavonoids content was estimated by the method of Jia *et al.* [20], the alkaloid was estimated by the method of Harborne [21], the total glycoside was estimated according to the method of Huguchi and Hanssen [22], the total steroids were estimated according to the method of Scanchez *et al*. [23]. Further, the total tannins were estimated by Folin Denis method [24] and the total lignins were estimated according to the method of Stafford [25].

Statistical analysis: The data of all measurements are means from three replications. Data and statistical significance of difference were evaluated with analysis of variance (ANOVA) using SPSS 10.0 package

RESULTS AND DISCUSSION:

Organoleptic studies: The organoleptic characters such as extractive values, colour, nature, taste of the crude drugs of different parts of *C. cajan* and their results are presented in **Table 1**.The extraction yield of seed coat of different solvents varied from 5.14% to 11.36% and could be ranked from high to low *i.e.*, ethanol > aqueous > pet ether > chloroform. Similarly, the yield of cotyledon extracts were 4.25% to 10.45% and could be ranked from high to low *i.e.*, ethanol > aqueous > pet ether > chloroform respectively. The maximum extraction yield was observed in seed coat compared to cotyledon. The results revealed that ethanol shows higher extractive values may be its unique feature of dissolving all polar and nearly all new polar constituents. Similarly, ethanol extract shows higher extractive value in croton seed [26]. The crude extracts of seed coat and cotyledon of *C. cajan* have exhibited a wide range of colour.

The pet-ether extracts shows dark brown colour in seed coat and yellowish in cotyledon, whereas, chloroform extracts shows dark brown colour in seed coat and yellowish in cotyledon respectively. The ethanolic extracts were dark brown colour in seed coat and golden yellow colour in cotyledon. Whereas, the aqueous extract of seed coat and cotyledons were brown in colour. Further, the extracts of *C. cajan* seed coat and cotyledon have either pungent bitter or bitter in taste. Of these, the pet-ether and chloroform extracts were pungent bitter while the remaining was bitter in taste. Similarly, the nature of these extracts varies from sticky (pet-ether), waxy (chloroform), resin (ethanol) and powdery (aqueous) respectively.

Total Ash, Acid insoluble ash and Water soluble ash: In the present investigation the total ash and acid insoluble ash value of seed coat and cotyledon of *C. cajan* were recorded in **Table 2.** The highest content of total ash 14.5%, acid insoluble ash 8.35% and water soluble ash 2.11% were recorded

in seed coat followed by cotyledon, which had 11.5% of total ash 7.36% of acid insoluble ash 3.39% of water soluble ash respectively. Similarly, the 3.70% of total ash have been reported in stalk of *C. Cajan* [27]. The variation in the percent of ash is probably due to the nature of metabolites they possess. The ash value is useful in determining authenticity and purity of the drug and also these values are important quantitative standards.

TABLE 1: ORGANOLEPTIC CHARECTERS OF SEED COAT AND COTYLEDON EXTRACTS OF *C. CAJAN*

Characters	Successive extracts	Plant parts	
		Seed coat	Cotyledon
Extractive value (%w/v)	Pet-ether	6.3%	7.34%
	Chloroform	1.14%	4.25%
	Ethanol	11.36%	10.45%
	Aqueous	9.32%	8.45%
Colour	Pet-ether	Dark Brown	Yellowish
	Chloroform	Dark Brown	Yellowish
	Ethanol	Brown	Brown
	Aqueous	Brown	Brown
Taste	Pet-ether	Pungent Bitter	Pungent Bitter
	Chloroform	Pungent Bitter	Pungent Bitter
	Ethanol	Bitter	Bitter
	Aqueous	Bitter	Bitter
Nature	Pet-ether	Sticky	Sticky
	Chloroform	Waxy	Waxy
	Ethanol	Resin	Resin
	Aqueous	Amorphous Powder	Amorphous Powder

TABLE 2: TOTAL ASH, ACID INSOLUBLE ASH AND WATER SOLUBLE ASH VALUE OF *C. CAJAN*

Physical constant	Plant parts	
	Seed coat	Cotyledon
Total ash	14.5%	11.5%
Acid insoluble ash	8.35%	7.36%
Water soluble ash	2.11%	3.39%

Mineral Elements of *C. cajan*: Results of element analysis of seed coat and cotyledon by AAS (Atomic absorption spectrophotometer) are recorded in **Table 3**. The highest content of zinc (0.0244±0.0012ppm), magnesium (0.0230 ±0.0131 ppm) and copper (0.1811±0.0511ppm) were recorded in seed coat compared to cotyledon whereas; the maximum amount of iron (0.0247±0.0121ppm) was recorded in cotyledon compared to seed coat. The very negligible amount of lead was detected in cotyledon (0.0001±0.0001 ppm) followed by seed coat (0.0003±0.0002ppm). Similarly, *C. cajan.* leaves contain zinc, iron magnesium, calcium and copper [28, 29].

TABLE 3: MINERAL ELEMENTS OF SEED COAT AND COTYLEDON OF *C. CAJAN*

Elements	Plant parts	
	Seed coat	Cotyledon
Zinc (ppm)	0.0244±0.0012	0.0181±0.0025
Iron (ppm)	0.0134 ±0.0034	0.0247±0.0121
Magnesium (ppm)	0.0230 ±0.0131	0.1011±0.0123
Copper (ppm)	0.1811±0.0511	0.0230±0.0012
Lead (ppm)	0.0003±0.0002	0.0001±0.0001

Note: ND-Not detected
Each value is expressed as mean ± S.D (n=3) and statistically significant at P<0.05

Fluorescent studies of *C. cajan*: The fluorescent study of dried powder of seed coat and cotyledon of *C. cajan* were observed in both under the visible and ultra-violets (UV) light by treating with various solvents showed characteristic colours were recorded in the **Table 4.** Among various solvents tested acetone, hydrochloric acid, petroleum ether did not show any fluorescence in both seed coat and cotyledon powder. Whereas, distilled water, methanol, acetone, chloroform, glacial acetic acid,

ethanol, sulphuric acid, nitric acid showed characteristic colouration in both seed coat and cotyledon powder. These results are supportive with fluorescent studies performed with *Lens culinaris* seed coat and cotyledon powders [30]. The *C.cajan* leaf and seed powder showed characteristic colouration in distilled water, benzene, chloroform, HCl, H_2SO_4, HNO_3, CH_3COOH, 5% $FeCl_3$, 5% I_2, 1N NaOH, picric acid and 1N NaOH+methanol solvents [13].

TABLE 4: FLUORECENT STUDIES OF SEED COAT AND COTYLEDON POWDERS OF *C. CAJAN*

Sl. No.	Treatment	Seed coat		Cotyledon	
		Visible Light	UV light	Visible Light	UV Light
1	Distilled water	Light Yellow	Brown	Yellowish	Light Yellow
2	Ethanol	Light Yellow	`Colourless	Colourless	Light Yellow
3	Methanol	Light yellow	Colourless	Colourless	Light Yellow
4	Acetone	Colourless`	Colourless	Colourless	Colourless
5	Chloroform	Light yellow	Brown	Colourless	Light Yellow
6	Glacial acetic acid	Light Yellow	Light Brown	Colourless	Light Yellow
7	Sulphuric acid	Light Yellow	Brown	Brownish	Light Brown
8	Nitric acid	Yellowish	Light Brown	Yellowish	Light Yellow
9	Hydrochloric acid	Black	Black	Black	Black
10	Petroleum ether	Colourless	Colourless	Colourless	Colourless

Preliminary screening of phytochemicals: The phytochemicals detected in *C. cajan* L. seed coat and cotyledon extracts are listed in **Table 5.** Test for flavonoid, tannins, phenols, glycosides, alkaloids, steroids and lignins were positive in all extracts except pet ether and chloroform extract alkaloids and steroids are not detected in both seed coat and cotyledon. The saponine is absent in all extracts of seed coat and cotyledon. Similarly, *C. cajan* seed extracts showed the presence of alkaloids, glycosides, resins, phenols, steroids, lignins, fats and oils in acetone, aqueous, benzene, chloroform, diethyl ether, methanol extracts in varying quantities [13]. The alcohol and water extracts of *Cicer arietinum* seeds shows the presence of phytosterols, flavonoids, tannins, phenolic compounds [31].

TABLE 5: PHYTOCHEMICAL SECREENING OF EXTRACTS OF SEED COAT AND COTYLEDON OF *C. CAJAN*

Secondary metabolites	Name of the test	Seed coat				Cotyledon			
		PE	$CHCl_3$	Et-OH	Aq	PE	$CHCl_3$	Et-OH	Aq
	Ellagic acid test	+	+	+	+	+	+	+	+
Phenols	Phenol test	+	+	+	+	+	+	+	+
Tannins	Gelatin test	+	+	+	+	+	+	+	+
Flavonoids	Pews test	+	+	+	+	+	+	+	+
	Shinoda test	+	+	+	+	+	+	+	+
	NaOH test	+	+	+	+	+	+	+	+
Lignins	Labat test	+	+	+	+	+	+	+	+
	Lignin test	+	+	+	+	+	+	+	+
Steroids	Libermann-burchard test	-	-	+	+	-	-	+	+
	Salkowski test	-	-	+	+	-	-	+	+
Alkaloids	Iodine test	-	-	+	+	-	-	+	+
	Dragendroff's test	-	-	+	+	-	-	+	+
	Wagner's test	-	-	+	+	-	-	+	+
Glycosides	Kellar-kilani test	+	+	+	+	+	+	+	+
	Conc. H_2SO_4 test	+	+	+	+	+	+	+	+
Saponins	Foam test	-	-	-	-	-	-	-	-

'+': Present, '-': Absent; PE: Pet ether, $CHCl_3$: Chloroform, Et-OH: Ethanol, Aq: Aqueous extract

Phytochemical constituents: The results shows that seed coat contained higher amount of phenols (3.15±0.85 mg/100 mg), flavonoids (290.13±0.62 mg/100), tannins (190.0±0.98 mg/100 mg), lignins (200.0±0.82 mg/100 mg), alkaloids (0.82±0.12 mg/100 mg) and steroids (0.63±0.05 mg/100 mg) whereas, the maximum amount of glycosides (115.34 ±0.81 mg/100 mg) was observed in cotyledon

(**Table 6**). Phenolic compounds which inhibit the activities of α- amylase, α- glucosidase and protease, provide an attractive target for the development of potential therapeutic agents to treat diabetes, pancreatitis, coagulation and neoplastic diseases [32]. The *C. cajan* leaves crude extracts are high contents of phenol and flavonoids and are traditionally used in treatment of jaundice and diabetes [33]. The highest value of tannins was observed in seed coat which serves as astringent properties for healing of wound, inflaming mucous membrane and source of antioxidant [34]. The results shows that highest value of lignin 200.0±0.82 mg/100 mg was observed in seed coat compared to cotyledon. Similarly, brown cow pea seed coat shows 236.0 gm Kg^{-1} lignins [35] and lignins also act as antioxidants [36]. The seed coat contains high amounts of these bioactive compounds; it is reliable to possess large number of medicinal values.

TABLE 6: CONSTITUENTS OF SECODARY METABOLITES IN SEED COAT AND COTYLEDON OF *C. CAJAN*

Metabolites	Plant parts	
	Seed coat	Cotyledon
Phenol (mg/100mg)	308.15±0.85	3.7±0.18
Flavonoids (mg/100mg)	190.13±0.62	0.18±0.10
Tannins (mg/100mg)	290.0±0.98	1.92±0.15
Lignins (mg/100mg)	400.0±0.82	0.15±0.06
Steroids (mg/100mg)	0.21±0.08	0.63±0.05
Glycosides (mg/100mg)	82.0±0.12	115.34±0.81
Alkaloids (mg/100mg)	0.09±0.01	0.82±0.12

Each value is expressed as mean ± S.D (n=3) and statistically significant at P<0.05

CONCLUSION: The present study on Pharmacognostical and Phytochemical evaluation of *C. cajan* seed coat and cotyledon will provide useful information for its identification and authentication of the drug in future. Phytochemical studies revealed that presence of phenols, flavonoids, alkaloids, steroids, tannins, lignins and glycosides. The outcome of this phytochemical screening thus suggest that seed coat are rich source of secondary metabolites are highly recommended in everyday diet of man and health benefits.

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