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PHARMACOGNOSTIC, PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF *CAESALPINIA BONDUCELLA [L.] Roxb. SEED*

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ABSTRACT

Objective: *The study was intended to investigate requisite detail about pharmacognostic characteristic and phytochemical profile of Caesalpinia bonducella (L.) Roxb seeds, a critical plant in the Indian system of medicine.* ***Methods:*** *Pharmacognostic investigation of the anatomical section of the dried seeds as well as powder microscopy was carried out to determine its morphological, anatomical and phytochemical diagnostic features. The qualitative microscopy, quantitative microscopy and other WHO suggested parameters of standardization of dried seeds and powder were carried out as per standard procedures.* ***Results:*** *Pharmacognostic assessment of Caesalpinia bonducella seeds shows two parts, namely epicarp or epidermis which is the septa of the layer. The epicarp is sclerotic and columnar layer of palisade zone is wide parenchymatous thin-walled tissue forming the sacrotesta. Branched vascular strands are observed which are spread across inner seed coat. Preliminary phytochemical examination demonstrated the presence of steroids, saponins, flavonoids, alkaloids, and tannins. The previously stated phytochemicals were further confirmed by Thin-layer chromatography (TLC).* ***Conclusions:*** *It can be presumed that the pharmacognostic profile of the Caesalpinia bonducella (L.) Roxb seeds might be supportive in setting some diagnostic indices for the identification and the preparation of the monograph of the plant.*

KEY WORDS

C. bonducella Microscopical characters, phytochemical Screening.

INTRODUCTION

Genus of *Caesalpinia* is widespread in 500 species that has medicinal benefits based on their pharmacological activity. One of the medicinal plants from this genus is *Caesalpinia bonducella Linn. (C. bonducella)* an Indian herb belonging to Family Caesalpiniaceae. It is found throughout India and other tropical countries of the World. Name 'Bonducella' of the species is derived from the Arabic word "Bonduce" which means a "little ball" that indicates the globular shape of the seed. *C. bonducella* also distributed in other tropical and subtropical parts of Asia, such as India, Srilanka, Vietnam, China, Myanmar and Bangladesh. In Indonesia, *C. bonducella* known as Bagore, Kalici, Tinglur and Areuy Matahiang [1-2]. It is a common shrub that is scandent prickly in the southern parts of India and Sri Lanka which is often grown as a hedge plant [3]. Review of the literature reveals the presence of some imperative phytoconstituents such as bonducellin, phytosterinin, β-sitosterol, furanoditerpenes, flavonoids, aspartic acid, arginine, citrulline, β-carotene [4]. The seed kernel of plant *C. bonducella* essentially contains bonducin, sulfur containing compounds and seed moreover comprises of unsaturated fats [5-6]. *C. bonducella seeds also contain* alkaloid as caesalpinine and a bitter principle such as bonducin [7]. Triterpenoid, fatty acid triglycerides, and sterols isolated from seeds may possibly render herb its therapeutic properties [8-9]. The pharmacological activities exhibited by C.

bonducella seed are attributed to the presence of numerous valuable bioactive phytoconstituents. *C. bonducella* seed are traditionally used in the treatment of intermittent fever, asthma, colic, antiperiodic, in dyspepsia, dentrifice and filariasis. Seed kernel isused in the treatment of orchitis, ovaritis, scrofula, useful for dispersing swellings, restraining hemorrhage in hydrocele leprosy and keeping off infectious diseases [10-12]. In Ayurveda, *C. bonducella* (Gajjaga) has been acknowledged to treat various diseases and disorders, including diabetes [13]. In view of the various medicinal constituents and uses credited to *C. bonducella* L. endeavor is made to examine anatomical and other physicochemical parameters required for quality control of the crude drug material. Thus, this pharmacognostic research was undertaken with an aim to assess various parameters like macroscopic, microscopic, physicochemical and phytochemical properties of *C. bonducella* L.

MATERIALS AND METHODS:

Procurement of plant materials:

Mature seeds of *C. bonducella* were collected from nearby cantonment territory of Sangamner, Ahmednagar district, cleaned and dried at room temperature in shade, away from direct sunlight. Identification of the plant was done by Dr. T. Chakraborty, Joint Director, Botanical Survey of India, Koregaon Road, Pune, authenticated plant by comparing morphological features and a sample voucher specimen of plant is deposited for future reference (Voucher number: BSI/CAEB7PRAK)

Drying and Size reduction of plant

The seeds of *C. bonducella* were cleaned to evacuate the adhered foreign material and were washed under tap water, air dried, homogenized to powder and stored in hermetically sealed bottles.

Procurement of Chemicals:

All the chemicals utilized were of analytical grade and were obtained from different vendors. Petroleum ether AR (PCL, India), Potassium hydroxide AR (Merck, India), Clonidine (Unichem, India), Haloperidol (Sunpharma, India), Chlorpheniramine maleate (Unimark Remedies Ltd., India), Sodium cromoglycate (Lupin, India) were purchased from commercial sources.

Pharmacognostic Studies

Organoleptic evaluation

Diverse sensory parameters of the plant material (Colour, Odor, Size, Shape, and Taste) were examined by organoleptic evaluation.

Macroscopic evaluation

Diverse macroscopic characters of *C. bonducella* seeds such as the type of seed, presence or absence of kernel and characters of seeds were documented.

Qualitative microscopy.

In this study, transverse sections of seeds were examined under the microscope (10X and 40X). Staining reagents Phloroglucinol- Hydrochloric) were employed according to standard techniques. The different distinguishing characters were observed with or without staining and images were recorded [14-17].

Physiochemical analysis

The physicochemical constant for example percentage of total Ash value, acid-insoluble ash value, Water-soluble ash value, water-soluble extractive value, alcohol soluble extractive value and pet-ether soluble extractive value were determined. Moisture content determination was performed according to the WHO guidelines [18-20].

Preliminary Phytochemical Screening

The seeds of *C. bonducella* were procured, dried in the shade and subsequently powdered in a homogenizer. The powdered seeds were used for extraction. Powder drug was passed through 120 mesh to remove the fine powder. Coarse powder material (500g) was employed for successive extraction with petroleum ether and 70% ethanol in water in Soxhlet apparatus. Crude extract obtained was vacuum dried to get solvent-free dry extract. All fractions, including the aqueous fraction, were concentrated under reduced pressure using a rotary evaporator and dried in vacuum and subjected to phytochemical screening. Pet-ether and ethanolic extracts of seeds were subjected to preliminary phytochemical screening for the detection of various class of phytochemicals [21-24].

Chromatographic evaluation [25-26]

On the basis of preliminary phytochemical tests, ethanolic extract and fractions were subjected to Thin layer chromatography (TLC). Precoated silica gel plates were used for development of chromatograph. Different solvent systems used were Benzene: Glacial Acetic Acid: Methanol (20:20:60) for detection of carbohydrates, Toluene: Ethyl acetate: Diethylamine

(70:20:10) for alkaloids, Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26) for flavonoids and Chloroform: Methanol: Water (65:35:10) for glycosides. After development, initially spots were visualized in the Ultraviolet (UV) chamber (254, 365 nm) the present study freshly prepared vanillin-sulphuric acid and anisaldehyde solution was used as visualizing agent to detect the bands on the TLC plates. The distance travelled by phytochemicals was noted by calculating its retention factor (Rf) value. After visualized using visualizing agents, the spots were observed in different colours. The R_f values were measured and the chromatogram was photographed and described in the tabulated form.

RESULTS AND DISSCUSION:

Pharmacognostic characteristics

Macroscopy

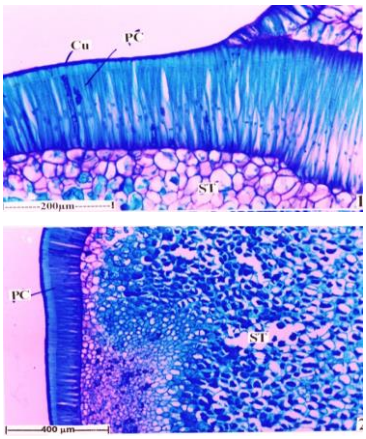
The shape of *C. bonducella* seeds is globous to round smooth and glossy (Table 1.). Slightly compressed on one side because of close squeezing of adjacent seed. It shows Hilum and micropyle closed to each other. Hilum encompassed by a dark area typically with a whitish remnant to funicle. Micropyle is close to the outskirts of a dark region. It contains a seeds coat that is dark greenish to grayish and somewhat dark bluish in nature. [Figure 1].

Figure 1: Morphology of *C. bonducella seed.*

Microscopy

C. bonducella seed has a thick cylindrical straight funicle attached on the hilum part of the seed and near the Micropyle, the seed part consists of two parts, namely epicarp or epidermis which is the septa of the layer. The epicarp is sclerotic which is made up of a thin compact layer of columnar cells. Sclereids are observed like palisade in sectional view. The palisade layer is 300 µm thick in the hilar region and 180 µm thick in the lower region. The outer surface of the palisade layer has the Cuticle. The columnar layer of palisade zone is wide parenchymatous thin-walled tissue forming the sacrotesta [Figure 2a].

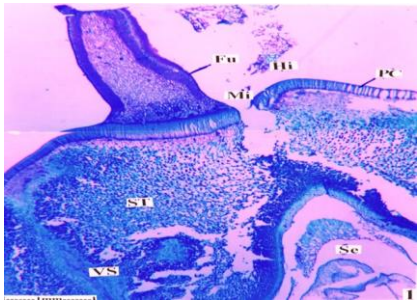
Figure 2a: Transverse Section of C*. bonducella* seed coat.



Cu: Cuticle, PC: Palisade Cells, ST: Sarcotesta

The cells of the sarcotesta have a dense accumulation of tannin. The freely branched vascular strands are spread within the inner seed coat [Figure 2b].

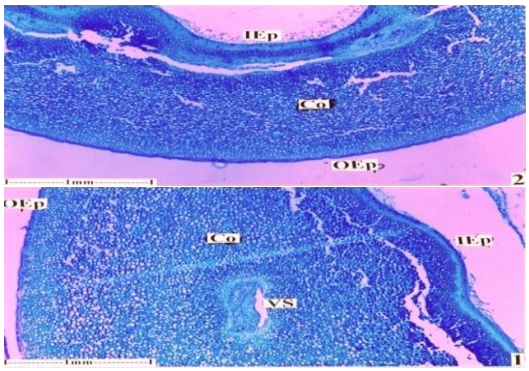
Figure 2b: Longitudinal Section of C. *bonducella* seed coat.



PC: Palisade Cells, ST: Sarcotesta, Fu: Funicle, Hi: Hilum, Mi: Micropyle, Se: Sclereids; VS: Vascular Strands

There are also lobed and stellate sclereids in the inner sacrotesta region. Cotyledons: There are two large cotyledons occupying the entire interior of the seed. The cotyledons have thin, darkly stained inner and outer epidermal layers and homogeneous, small, compact ground parenchymatous tissue. Less distinct vascular strands are spread across the cotyledonous tissue [Figure 3].

gure 3: Longitudinal Section of Lateral part of C.*bonducella* seed coat.



LS: Lobed sclereids, Co: Cotyledons, VS: Vascular Strands, IEp:Inner Epidermal Palisade, OEp:OuterEpidermal Palisade

Powder microscopy

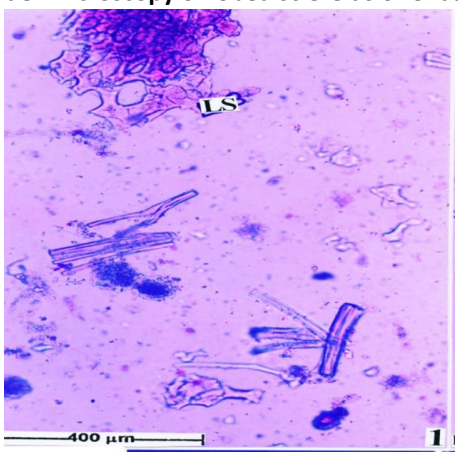
Powdered microscopy of *C. bonducella* seed shows the presence of sclereids of the seed coat. Columnar sclereids are vertically elongated, thin, palisade-like cells [Figure 4a].

Figure 4a: Powder microscopy of columnar sclereids of C. *bonducella* seed

LS: Lobed sclereids, CS: Columnar sclereids

They have thick walls and a wide lumen. The walls are lignified and appear bright under polarized light. These sclereids are also called macro sclereids which are 230 µm long and 30 µm thick. Lobed sclereids are irregular shaped, lobed thick walled sclereids with a wide lumen [Figure 4b].

Figure 4b: Powder microscopy of lobed sclereids of C. *bonducella* seed



LS: Lobed sclereids

The thin-walled parenchyma cells are also observed scattered in the powder. Powder microscopy of seed shows lobed sclereids and columnar sclereids which are observed under polarized light [Figure 4c].

Figure 4c: Powder microscopy of thin walled small parenchyma cells of *C. bonducella* seed

Physico-chemical analysis

In the physicochemical analysis, endeavor is made to evaluate various parameters of *C. bonducella* seeds such as loss on drying, ash value and extractive value which are mentioned in Table 2. Foreign matter of powdered seed indicates that there is no contamination by any unnecessary matters. Loss on drying (LOD) was found to be in an acceptable range that indicates the amount of moisture and volatile matter present in *C. bonducella* seeds. Ash value of seeds predicts that *C. bonducella* seeds comprise of a massive amount of calcium oxalate crystals. Extractive values display that seed contains non-polar as well as polar constituents. Generally, extractive values are indications of constituents present in the drug and are useful in the determination of adulterated drugs and exhausted drug [Table 2].

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out to detect the presence of phytoconstituents in various extracts. Phytochemical screening confirmed the presence of carbohydrates, alkaloids, flavonoids, triterpenoids, proteins, saponins, steroids, tannins and glycosides (Table 1). Phytochemical screening indicates that *C. bonducella* seeds contain different polar and non-polar phytochemicals present in the drug.

Table 1: Morphological and organoleptic Characteristics of *C. bonducella* seed.

Parameter	Characteristics of seed
Color	Green
Odor	Characteristic
Taste	Bitter
Size	2-4 cm long,1-2 cm diameter
Shape	Globular

Table 2: Physicochemical parameters *of C. bonducella seed.*

Parameters	Value
Loss on drying	5.5 ± 0.25
Total ash	4.9±0.58
Water soluble ash	1.8 ± 0.54
Acid insoluble ash	2.2 ± 0.62
Petroleum ether soluble extractive value	2.49 ± 0.50
Acetone soluble extractive value	6.17±0.93
Alcohol soluble extractive value	8.45±0.23
Aqueous soluble extractive value	9.8±0.54

Table 3: Qualitative phytochemical analysis of ethanolic extract of *C. bonducella seed.*

Phytochemicals Test Crude powder	Pet-ether Extract	Ethanolic Extract
Alkaloids		
Dragendroff's test	-	+
Mayer's test	-	+
Wagner's test	-	+
Hagers's Test	-	-
Proteins and Amino acids	-	+
Flavonoids Alkaline reagent	-	+
Flavonoids Shinoda Test	-	+
Tannins FeCl3 test	-	+
Phlobatanins Hcl test	-	+
Triterpenes H2SO4 test	+	-
Steroids Liebermann and Burchard test	+	-
Saponins Frothing test	+	-
Cardiac glycosides Keller-kilianni test	-	_

+: indicates presence of constituents
-: indicates absence of constituents

Thin layer chromatography (TLC): TLC profile of ethanolic extract of *C. bonducella seeds* revealed the presence of five compounds (Table 4) having R_f values of 0.81, 0.42, 0.50, 0.85 and 0.54 after derivatization with visualizing agents. It was also observed in the UV chamber at wavelength 254 nm and 365 nm which is shown in Figure 4.

Figure 4. TLC of *C. bonducella* seed extracts.

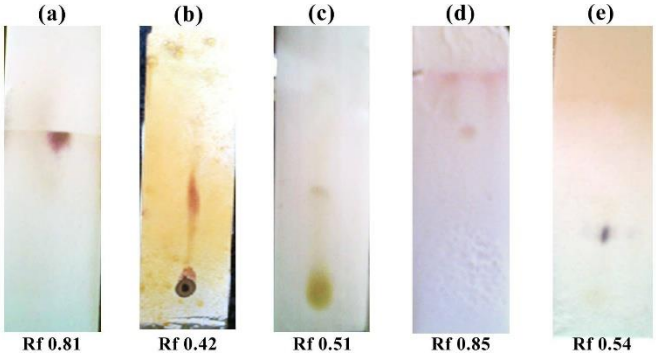


Table 4: Qualitative phytochemical analysis of ethanolic extract of *C. bonducella seed.*

Mobile Phase	Spraying reagent	Color of spot	R_f values for extract
Benzene: Glacial acetic acid: Methanol (20:20:60)	Anisaldyde-Sulphric acid	Violet	0.81
Toulene: Ethylacetate: Diethylmine	Dragendorffs Reagent	Orange Brown	0.42
Ethyl acetate: Formic acid: Glacial acetic acid: water (100:11:11:26)	Anisaldehyde-sulphric acid	Green	0.50
Chloroform: Methanol: Water (65:35:10)	Sodium Nitroprusside (5%)	Pink	0.85
Toluene: Ethyl acetate (9:1)	Vanillin – H_2SO_4	violet	0.54

R_f: Refractive index

CONCLUSION

With a specific end goal to standardize an herbal medication with various parameters like macroscopic, Microscopic, TLC, Phytochemical examination was Microscopic, TLC, Phytochemical examination was can be concluded that it can serve as a valuable resource of pharmacognostic and phytochemical information. The present work was embraced with a viewpoint to set down benchmarks which could be valuable in recognizing the authenticity of this medicinal herb. Microscopical studies have demonstrated the presence of epicarp with a sclerotic and columnar layer of palisade cells with wide parenchymatous thin- walled tissue by observed that spread across inner seed coat forming the sacrotesta. Branched vascular strands were observed that spreaded across the inner seed coat. Phytochemical screening showed the presence of steroids, saponins, flavonoids, alkaloids, and tannins as phytoconstituents. The TLC analysis of seeds can provide standard fingerprints and it can be used as a reference for the standardization and quality control of the drug. Physicochemical parameters established importance in detecting adulteration and mishandling of the crude drug. This pharmacognostical studies will provide helpful inputs for standardizing crude drugs and can also be useful in detecting and differentiate closely related species. In future, isolation and identification of individual phytochemicals, *in-vivo* studies are essential for better understanding of their mechanism of action.

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