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Botanical and pharmacognostic evaluation of *Caesalpinia bonduc* seed, a prevalent Indian traditional drug having vivid therapeutic prospects

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Abstract

Caesalpinia bonduc (L) Roxb. is a vital remedy for treating several ailments in Indian traditional systems of medicine. This perennial medicinal plant grows as a hedge plant up to 15 m tall and is found worldwide, particularly in Sri Lanka, India, Burma, and the Andaman and Nicobar Islands. In the current study, we attempted to assess the botanical and pharmacognostic properties of *C. bonduc* with special reference to the seeds. The pharmacognostic study of the anatomical section of the seeds as well as microscopic studies of the powder was conducted to determine their morphological and anatomical features. The qualitative and quantitative microscopy as well as physicochemical properties and TLC profile of the seed sample were carried out as per standard procedures recommended by WHO. Macroscopic and microscopic examinations revealed the characteristic features of *C. bonduc*. The transverse section also revealed the characteristic features of the *C. bonduc* seed, including the cotyledon, epidermis, osteosclereids, parenchyma cells, sclereids, starch grains, sub-epidermis; and testa. A quantitative pharmacognostic analysis of the powder of the seed revealed a moisture content of 6.61%. Physicochemical investigation of the seed recorded total ash, water-soluble ash and acid insoluble ash as 3.26, 1.69 and 0.39 %w/w, respectively. The highest extractive value was recorded for ethanol, followed by hexane. The residence of toxic heavy metals was conducted using an Inductively coupled plasma optical emission spectrometry and was found to be within the permitted limit, proving the safety profile of the seed material tested. The TLC profile of the raw seed extract recorded various spots in visible, short UV and long UV lights, which gives a clear idea of the abundance of various phytochemicals. Diverse pharmacognostic features recorded in this study will surely help to identify and standardize *C. bonduc* in quality control and drug formulation in the future.

Keywords: *Caesalpinia bonduc*, Pharmacognosy, Physicochemical, Extractive value, Microscopical characters

Introduction

Medicinal herbs have long been used by humans as a natural remedy for a variety of maladies and disorders. Numerous pieces of evidence, especially through scientific studies illustrate the immense potential of medicinal plants utilized in various traditional systems. Due to this, the globe has experienced a significant growth in plant research in recent years [1]. Researchers are paying more attention to these than ever before because they have a great potential to benefit society, or indeed all humanity, particularly in the formulation of medicines and other beneficial pharmacological studies [2]. Due to their cultural and historical factors, the worldwide acceptance and reliance on medicinal plants have become a hot topic of global concern in both developed and developing countries. Medicinal plants have emerged as a major problem in the world due to a lack of data on safety and efficacy, as well as poor regulation and control systems. The general public and healthcare practitioners alike require current, authoritative facts on the efficacy and safety of medicinal plants used in treatment. The World Health Organization (WHO) is now urging nations to incorporate safe and beneficial traditional remedies and practices into their health care systems, including public and private. Thus, monographs are primarily intended to promote harmonization in the use of herbal medicines in terms of safety, efficacy, and quality control [3].

The dearth of safety documentation and strict quality assurance, on the other hand, has hampered the acceptability of alternative medicines in developed countries. Due to this, there is a need for documentation of traditional medicine research [4]. With this backdrop, it becomes critical to make an attempt to standardize the plant parts that will be used as drugs. Stepwise pharmacognostic investigations of the raw materials can help with the standardization process.

These investigations aid in the correct identification and authentication of plant parts. The precise identification and quality control of the plant materials is pivotal to guaranteeing the reproducible quality of herbal-based drugs, which will finally ensure their efficacy and safety.

Traditional/herbal remedies are promoted and encouraged by the WHO in national health care programs because they are accessible, economical and safe so that ordinary people can rely on them [5]. Undisputed documentation, quality assurance, and the inception of authentic pharmacognostic standards are vital parameters for assessing medicinal plants. The primary steps in the pharmacognostic evaluation of medicinal plants are macroscopic and microscopic characteristics, physicochemical studies, and fluorescence analysis [6]. The WHO also states that the macroscopical and microscopical accounts of a medicinal plant are the primary steps in determining its identity and degree of purity [5].

The *Caesalpinia* genus comprises more than 500 diverse species, many of which have several beneficial effects on humans based on their interesting pharmacological properties [7]. One of the significant medicinal plants from this particular genus was *C. bonduc* (L.) [syn. *C. bonducella* (L.) Flemming, *C. crista* (Linn)] from the family of Fabaceae [8, 9]. This medicinal plant was widely seen Asian continent, including India, Sri Lanka, Indonesia, etc. [10]. Other tropical and subtropical Asian countries where *C. bonduc* can be found include Vietnam, China, Myanmar, and Bangladesh [11, 12]. *C. bonduc* was mentioned as an important plant in Ayurvedic classics. Different parts of the plant, such as seeds, roots, bark, and leaves, are used by health workers to treat various ailments colic fever, intermittent fever, malaria, menstrual complaints, pneumonia, skin diseases, swelling, fever, pulmonary tuberculosis, and edema [13]. *C. bonduc* was also found in hotter parts of India and Sri Lanka and also found at elevations of more than 1,000 meters and other tropical countries worldwide. It has been reported to have several interesting pharmacological activities such as adaptogenic [14], immunomodulatory [15], antiulcer [16], anti-diabetic [17], and anticonvulsant properties.

The current investigation focuses on the botanical and pharmacognostic characteristics of *C. bonduc* with special reference to the seeds with the help of macroscopic and microscopic features, physicochemical parameters including ash values, extractive values, presence of heavy metals and TLC finger printing. These parameters, in turn, can enable the quality assurance of *C. bonduc* and be helpful for the assemblage of a pertinent monograph for its accurate identification before going for the formulations.

Materials and methods

Plant collection and authentication

For our studies, healthy and disease-free *C. bonduc* plants along with seeds were collected from the Ernakulam area of Kerala, India. The *C. bonduc* samples were correctly identified and deposited at JNTBGRI, Thiruvananthapuram, Kerala, India. The plant was identified by Dr. Dan Mathew, a Senior Scientist at JNTBGRI. For future reference, a sample voucher specimen of *C. bonduc* was deposited at JNTBGRI. Throughout the research, the collection of the plants was done with extreme care.

Processing of the seeds

C. bonduc seeds were cleaned to remove any adhered foreign material, then washed several times with tap water, air-dried,

further homogenised to powder, and then stored in sealed bottles for future studies.

Taxonomical classification of *C. bonduc*

Taxonomical classification of *C. bonduc* was conducted based on the standard system recommended by Bentham and Hooker [18].

Botanical identification of *C. bonduc* through macroscopic evaluations

The macroscopic investigation involved the morphological description of plant sections using only the naked eye by placing the plant material on a white paper surface.

Detailed pharmacognostic evaluation of the seed

Microscopic evaluation

A microscopy examination of the seeds was conducted based on qualitative and quantitative analysis. All examinations were conducted on a compound microscope (Labomed Lx 3000 LED binocular microscope, India).

Qualitative microscopy

For qualitative microscopic examination, transverse sections of seeds were made using a microtome. The staining technique of the seed sections was performed as per the standard procedure and several unique identifying characters were recorded with the help of stained slides.

Powdered microscopy

The dried *C. bonduc* seeds were powdered finely and examined under a Labomed Lx 3000 LED binocular microscope (India). A small quantity of finely powdered seed was taken in a test tube and then boiled in chloral hydrate solution for a few minutes. After that, the solution containing powder was smeared on a slide-mounted with phloroglucinol, followed by the addition of a few drops of concentrated HCl [19]. Different cell components in the seed powder were carefully recorded and after that photography was taken using a digital camera.

Organoleptic evaluation

The seed of *C. bonduc* was subjected to organoleptic evaluation based on taste, color, odor, shape, texture, etc. [19].

Evaluation of physicochemical properties

Various physicochemical properties such as loss on drying (LOD), percentage of ash values and extractive values of the powdered seed samples were conducted as per standard protocols recommended by the WHO [20]. The extractive values were measured with different solvents, i.e. hexane, toluene, petroleum ether, ethyl acetate, chloroform, acetone, ethanol, methanol and water. The heavy metal contents of the seeds were estimated using an Inductively coupled plasma optical emission spectrometry.

High Performance Thin Layer Chromatography (HPTLC) of seed extract

The *C. bonduc* seed powder was dissolved in HPLC grade methanol and centrifuged at 3000 rpm for 10 minutes. After that, the supernatant was collected and used for HPTLC analysis using CAMAG (Applicator- Linnomat V and Camag TLC scanner, Switzerland). Before HPTLC analysis, various mobile phases were tried to determine the maximum separation. Here, Toluene: Chloroform: Methanol (8:3:1) recorded most appropriate mobile phase. The supernatant was

spotted onto pre-coated TLC silica gel plates (silica gel 60 F254, Merck, Germany). After the spotting of the samples, the plates were placed into the developing chamber and then allowed to run until they reached a height of approximately 10 cm from the point of application. The developed chromatograms were observed under UV, daylight and after spraying with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 10 min to perceive the presence of

different phytoconstituents. Photographs were taken using a CAMAG TLC visualizer and the Rf values were calculated.

Results

Taxonomy of *C. bonduc*

The detailed taxonomical classification of *C. bonduc* is provided in Table 1.

Table 1: Taxonomic classification of *C. bonduc*

Kingdom	<i>Plantae - Plants</i>
Subkingdom	<i>Tracheobionta</i>
Super division	<i>Spermatophyta</i>
Division	<i>Magnoliophyta</i>
Class	<i>Magnoliopsida</i>
Subclass	<i>Rosidae</i>
Order	<i>Fabales</i>
Family	<i>Fabaceae / Leguminosae</i>
Genus	<i>Caesalpinia</i>
Species	<i>Caesalpinia bonduc</i> (L.) Roxb.

Botanical characters of *C. bonduc*

C. bonduc is a vine-like shrub that can grow about 6 m long and scrambles over other vegetation (Fig 1). Stems are covered with curved spines. It has about 2 cm length grey seeds called nicker nuts that are buoyant and long enough to be dispersed by ocean currents. It is an extensive climber. Branches are armed with hooked and straight hard yellow prickles. Leaves are 36-60 cm long; petioles are prickly; stipules are a pair of reduced pinnae at the base of each leaf, each furnished with a long mucronate point; pinnae are about 6-8 pairs and about 5-7.5 cm long with a couple of hook stipular spines at the base (Fig 1). Leaflets are 6-9 pairs, membranous, elliptic-oblong, obtuse, strongly mucronate,

glabrous on top and puberulous on the bottom. Petiolules are extremely short, and stipels are sharp hooked spines. Flowers are dense (usually spicate) long-peduncled terminal and supra-axillary racemes, 15–25 cm long, dense at the top and lax downwards (Fig 1). The pedicles are brown-downy and very short in bud, elongating to 5 mm in flower and 8 mm in fruit; the bracts are squarrose, linear, acute, reaching 1 cm in length, and fulvous-hairy. The calyx is fulvous-hairy and about 6-8 mm long. The lobes are obtuse and obviate-oblong. The petals are yellow and oblanceolate. Declinate filaments are flattened at the base and covered in long white silky hairs. Pods are oblong, with densely armed faces covered in wiry prickles (Fig 1).



Fig 1: Botanical features of *C. bonduc* (1) Entire plant; (2) Stem; (3) Leaves (4) Flowers, (5) Fresh fruits and (6) Dried seed

The seeds are exalbuminous. The seed coat is tough, glossy, and ranges from greenish to ash grey in color. It was traversed by cracks with faint circular and vertical markings, forming uniform rectangular to squarish reticulations all over the surface. At the narrow edge of the seed, a raised hilum with the stalk remains in the centre of the dark spot. A faintly colored, circular to oval-shaped elevated micropyle lies adjacent to the hilum. In the case of dry seeds, the kernel frequently separates from the testa. The testa comprises three layers that are approximately 1–1.25 mm thick. The outer layer was tinny and fragile, while the middle layer was extensive, fibrous, and colored dark brown, whereas the interior layer appeared white and papery.

As depicted in Figure 1, the kernel surfaces are furrowed and ridged, firm, pale yellowish-white, circular to oval, flattened, and approximately 1.23–1.75 cm in diameter. The ascar of the micropyle is located at one end of the kernel, from which a prominent ridge separates the embryo's two cotyledons. The plumule's radical axis is thick, cylindrical, and straight.

Pharmacognostical Evaluation of the seed

Macroscopic

Seeds are globous or rounded, smooth, shiny, and 1-2 cm diameter. They are somewhat flattened on one side due to the close pressing of adjacent seeds. Hilum and micropyle are seen close together. About 4 mm in diameter, a dark circle

surrounds the hilum, mostly with a pale remnant to the funiculus and the micropyle at the periphery of the dark area. The seed coats are seen greenish to bluish-gray in color and are linear and shiny. The kernel or embryo is filled in the seed

and detaches easily from the seed coat when it becomes dry. The kernel is pale white in color and makes up a significant portion of the seed. The weight of a single seed was approximately 2 gm [Fig. 2].



Fig 2: Morphology of *C. bonduc* seeds

Microscopic:

The transverse section of the seed reveals an outermost compact and single-row arrangement of very narrow, translucent, radially elongated cells, which form the palisade or, in other words, the malpighian layer. In normal view, these cells will appear as hexagonal shapes and have thick walls containing a rich quantity of pectin. Subsequently, 2 or 3 layers of sub-epidermis consisting of thick-walled bearer cells which were stemmed by multi-layered osteosclereids. The size of osteosclereids cells slowly increases and elongates laterally and the intercellular spaces toward the inner side also increase. A brown substance was detected in the outer few osteosclereids layers and laterally elongated vascular elements

in the tissues were also recorded in the lower part of this zone. There are two types of sclereids present in this region. Some are small, oval to circular-shaped and osteosclereids (bone or barrel-shaped elongated sclereids dilated at their ends) are filled with brown-colored contents. The cells within the vascular tissues gradually compact and round toward the inner margin. The cotyledons usually display a single outer layer of the epidermis containing small, isodiametric cells. Interestingly, the inner parenchymatous ground tissue cells are rich with fixed oil and are seen with uniformly distributed empty cavities. Cotyledons are also rich in starch grains [Fig. 3].

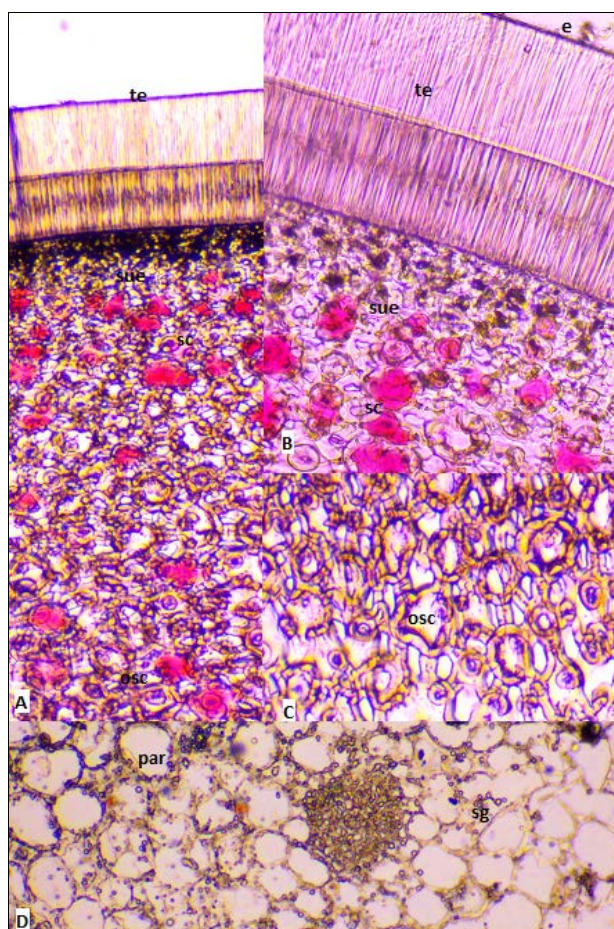


Fig 3: Microscopy of *Caesalpinia bonduc* seed: A: Transverse section of seed; B & C: A portion enlarged; D: Transverse section of cotyledon; e.: Epidermis; osc.: Osteosclereids; par.: Parenchyma cells; sc.: Sclereids; sg.: Starch grains; sue.: Sub-epidermis; te.: Testa

Powder

The powder color was usually light whitish-yellow, through mustard to brown. Moreover, the powder was coarse and free-flowing, tasting bitter and possessing a tamarind-like odor. They possessed palisade cells with a columnar shape and recorded bone-shaped, thick-walled empty parenchymatous cells with resinous contents. The thick-walled compressed parenchymatous cells comprise thick-walled small, roundish to polygonal bearer cells and in surface view, they are seen as dark brown cell contents in the center of the cell. Furthermore, thick-walled parenchymatous cells were slightly

larger than the bearer cells with brown cell contents, usually thin-walled, roundish to polygonal-shaped cells with round starch grains. In iodine staining, these usually appear as bluish-black color and record identical round-shaped colorless oil globules and yellowish-brown resinous masses of irregular shape and size while observed under the microscope. The powder analysis shows fragments of palisade like elongated cells of testa, fragments of subepidermal cells with or without the palisade cells, crystals, oil globules, osteosclereids, simple starch grains filed in the parenchymatous cells of the cotyledons [Fig. 4].

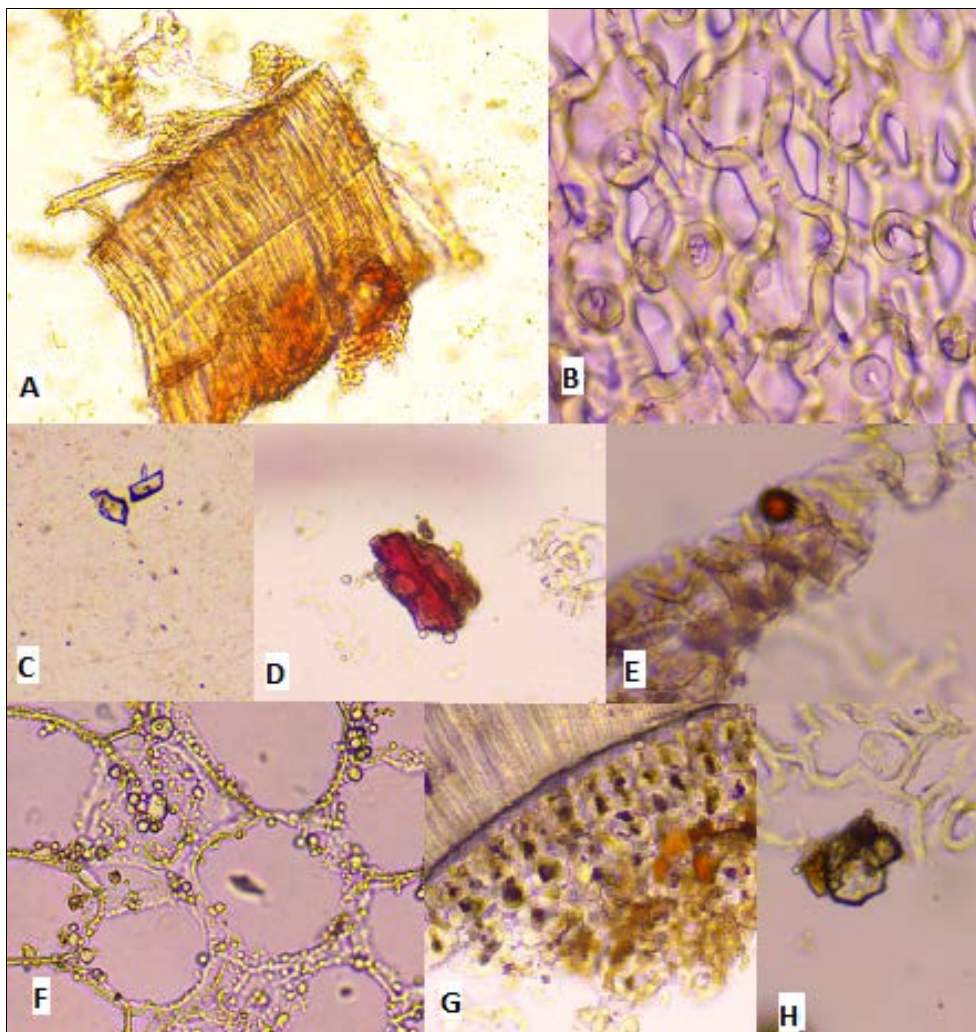


Fig 4: Powder microscopy of *C. bonduc*; A: Fragments of palisade like elongated cells of testa; B: Osteosclereids; C: Crystals; D: Sclereids; E: Oil globules; F: Parenchymatous cells of the cotyledons filled with starch grains; G: Fragments of sub epidermal cells with palisade cells; H: Brown content.

Organoleptic assessment of *C. bonduc* seed

The organoleptic description of the *C. bonduc* seed are presented in Tables 2.

Table 2: Organoleptic properties of *C. bonduc* seed

Parameters	Organoleptic properties
Color	Light brown
Odour	Characteristic
Taste	Astringent/bitter
Texture	Outer hard and inner soft

Physicochemical investigation

The result of the physicochemical investigation of the *C. bonduc*

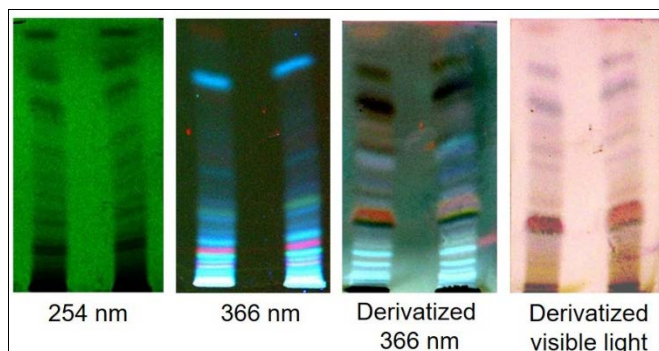
seed is shown in Table 3. The physicochemical study of powdered *C. bonduc* seed recorded parameters such as foreign matter, moisture content, values of total ash, acid insoluble ash and water-soluble ash. In addition to this, it also displayed the values of alcohol soluble extractive and water-soluble extractive (Table 2). Furthermore, the physicochemical analysis also includes the extractive values in different solvents. Among the different solvents used, ethanol extract recorded the maximum yield (11.28%), followed by hexane, methanol and water.

Table 3: Physicochemical properties of *C. bonduc*

Parameter	Results
Foreign matter (% w/w)	Nil
LOD (% w/w)	6.61
Ash values	
Total ash (% w/w)	3.26
Acid insoluble ash (% w/w)	0.39
Water soluble	1.69
Alcohol soluble extract (%)	13.05
Water soluble extract (%)	16.70
Extractive values (%)	
Hexane	4.9
Toluene	0.936
Pet ether (40:60 + 60:80; 1:1)	1.228
Ethyl acetate	1.04
Chloroform	0.496
Acetone	0.480
Ethanol	11.28
Methanol	3.224
Water	3.68
Heavy metals (mg/kg)	
Arsenic	0.14
Cadmium	BDL
Lead	0.08
Mercury	0.11

TLC profile

After developing the TLC profile, the spots were detected in both short (254 nm) and long (366 nm) UV. At 254 nm, three major spots along with several minor spots were detected in the developed TLC of the raw seed extract with an R_f value range of 0.16-0.9. In the case of 366 nm, four spots were detected with an R_f value in the range of 0.12-0.89, whereas the derivatized sample recorded eight major spots with an R_f value in the range of 0.02-0.80. But the derivatized sample in visible light detected five major spots with an R_f value range of 0.05-0.80 (Fig. 5).

**Fig 5:** TLC profile of the seed extract of *C. bonduc*

Discussion

The essential things about herbal medicines are purity, safety, potency, and efficacy for consumers. Therefore, standardization and quality control of the herbal drugs and raw materials are always mandatory before formulations. Herbal medicines are becoming more popular especially in developed countries. Still, one of the significant barriers to their acceptance is the lack of a standard quality control profile before and after its formulations. For evaluating medicinal plants, correct identification, quality control, and the establishment of precise pharmacognostic values are needed. According to the WHO, the first steps towards determining the correct identity of medicinal plants are macroscopical and microscopical evaluation reports and

degree of purity and this must be completed before the formulation of any herbal medicine [5, 20]. In this study, we have extensively studied macroscopical and microscopical parameters of *C. bonduc* with special reference to the seed.

Adulteration or substitution is simply the replacement of the unique plant material with another plant material or the intentional addition of any foreign substance to increase the weight or effectiveness of the product or to reduce its cost. The quality and quantity of medicinal plants chemical constituents determine their therapeutic efficacy. The misapplication of any herbal medicine or natural products begins with incorrect identification. The most common mistake is to use the same common vernacular name for two or more completely different species [21]. Pharmacognostic research of medicinal plants can answer all of these issues. Thus it is critical to establish pharmacognostic standards for medicinal plants that are employed in a variety of medications. The branch of study of medications derived from natural sources, primarily plants, is known as "Pharmacognosy". It primarily focuses on the standardization, authenticity, and research of natural medicines. The majority of pharmacognosy research has focused on identifying and authenticating commonly used traditional medicinal herbs using morphological, phytochemical, and physicochemical studies. In recent years, the significance of pharmacognosy especially in the field of herbal medicine has been extensively recognized.

In contrast to taxonomic documentation of the medicinal plants, the pharmacognostic analysis includes the detection of various adulterations in dry powder form. This is critical because the plant loses its morphological identity and becomes more susceptible to adulteration once dried and ground into powder. Pharmacognostic studies ensure the correct identity of the medicinal plants and lay down standardization parameters that will surely help preventing various forms of adulteration [22]. Therefore, pharmacognostic research will aid in the validation of medicinal plants and the consistent eminence of herbal goods, resulting in the safety and efficacy of natural products, especially those made from medicinal plants. The pharmacognostic standardization parameters thus play an essential role in solving all the issues mentioned above. Hence, we have conscientiously studied the pharmacognostic properties of the *C. bonduc* raw seed.

Analysis of foreign matter, loss on drying (LOD), ash values, extractive values, and heavy metal levels are essential physicochemical criteria for the standardization and quality assurance of plant-based medications. Because plant materials used for formulations must be free from contamination, the foreign matter analysis of powdered medications can be used to assess the purity of herbal drugs [20]. Here our sample is devoid of any foreign materials and thus it indicates its purity. LOD is a commonly used test method for determining the moisture content of raw material, especially in powdered forms. It should be noted that the level of moisture content should be minimal in raw materials, which is absolutely important to discourage the growth of bacteria, yeast, or fungi during storage [22]. In our sample, the LOD is only 8.83%, which suggests that the shelf life for *C. bonduc* appears to be longer as it comprises a lower amount of moisture, which will discourage the growth of unwanted microorganisms that may cause spoilage. Ash values are usually used to find out the quality and purity of the raw materials. The ash values specify the presence of various impurities such as carbonate, oxalate, silicate, etc. The inorganic compounds present in drugs are usually estimated through the values of water-soluble ash.

The acid insoluble ash mainly comprises silica and shows contamination with earthy material [23]. The ash content of 3.26% in our study clearly reveals that the seed is rich in minerals. When extracted with a certain solvent, the amount of active components in a given amount of plant sample is determined by estimating extractive values. The extraction of any plant sample with a particular solvent yields a solution comprising diverse phytochemicals in varying concentrations. The composition of these phytochemicals will depend upon the nature of the plant materials and the solvent used for extraction. It also specifies whether or not the crude drug has been exhausted [23, 24]. The extractive values in our study suggest that our sample gratifies purity values. Moreover, the extractive values also clearly indicated that our sample is rich in polar compounds as well as non-polar compounds.

The High-Performance Thin Layer Chromatography (HPTLC) method has become a powerful tool for qualitative and quantitative phytochemical investigation of herbal medications and formulations over the last two decades. This comprises the TLC fingerprint profile and estimation of various chemical markers. The HPTLC profile of the seed extract was noted with several spots with different R_f values when viewed at short (254 nm) and long (366 nm) UV light, which confirms the presence of various types of metabolites in the crude extract of *C. bonduc* seeds. The occurrence of multiple bands with different colors and R_f values indicated the presence of various types of chemical metabolites in the seed extract of *C. bonduc*. As a result, TLC analysis of the herbal raw materials can provide standard fingerprints that can be used as a reference standard for drug standardisation and quality control.

Conclusion

In the current global scenario, the essential requirement of traditional medicine systems is the standardisation of crude drugs and formulations. The pharmacognosy study of *C. bonduc* was carried out to ensure its correct identity and standardisation. This study used macroscopy, microscopic and powder microscopical evaluations to identify various anatomical markers along with other pharmacognostic evaluations. From our investigation, it is possible to conclude that any pharmacognostic character that differs from the data reported in the current study can be regarded as an adulteration or substitution of *C. bonduc*.

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