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## PHARMACOGNOSTICAL STUDIES ON THE STEM BARK OF *CAESALPINIA BONDOC* (L.) ROXB.

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**ABSTRACT: Background:** *Caesalpinia bonduc* (L.) Roxb. belonging to family Caesalpiniaceae, commonly known as ‘Kuberaksha’, is a large scandant shrub. The stem bark is useful as febrifuge, emmenagogue, anthelmintic, germicidal and anti-pyretic. **Aim:** The present study was conceived to standardize the stem bark of *Caesalpinia bonduc* to determine correct identity and purity of the drug have been undertaken besides in detection of adulteration. **Materials and Methods:** Pharmacognostical studies include (study of morphological characters), microscopical characters and powder microscopy and physicochemical parameters along with phytochemical, histochemical studies and fluorescence behavior were studied. **Results:** Transverse section of stem bark revealed the presence of outer most cork interspersed with stone cells, inner phelloderm cells with rhombic crystals and biseriate medullary rays. Tangential Longitudinal Section (T.L.S) revealed presence of uni to biseriate medullary rays, mostly biserate. Physicochemical standards showed that foreign organic matter (Nil), loss on drying (11.07%), total ash (5.45%), acid insoluble ash (0.45%), water soluble ash (1.45%), petroleum ether extractive (0.45%), chloroform (1.65%), ethanol (6.5%), methanol (7.55%), water (11.65%). Preliminary phytochemical screening of various extracts showed the presence of alkaloids, phenolic compounds and tannins, phytosterols, flavonoids, terpenoids and carbohydrates (were found be major components), besides histochemical studies and fluorescence behavior is given. **Conclusion:** Established Pharmacognostical characters, physico-chemical and phytochemical parameters of *Caesalpinia bonduc* stem bark will serve as standards of drug in the crude form and also would help in distinguish the drug from its adulterants or other closely related species. The study will provide referential information for correct identification of the crude drug.

**INTRODUCTION:** *Caesalpinia bonduc* (L.) Roxb. belonging to family Caesalpiniaceae, in Ayurveda it is known as Putikaranja, is medicinally important plant predominantly distributed in tropical and subtropical regions of Asia<sup>1</sup> (up to 1000 m in the Himalayas also in the plains, waste

lands and coastal areas widely distributed all over the world specially in India, Sri Lanka, Burma and Andaman-Nicobar Islands). Description: It is Large scandant or scrambling shrubs; branches armed with recurved prickles. Leaves bipinnate, pinnae 6-11 pairs, leaflets stalked, flowers yellow, or fragrant, in axillary and terminal racemes.

Pods oblong, densely armed with sharp wiry prickles, dehiscent, seeds 1 or 2, globose or ovoid, grey. It is locally known as Nata karanja (Hindi), gachha (Telugu) and possess a lot of applications in folk medicines. Review of literature: Seed and bark extracts have been used as Anthelmintic, anti-

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cancer, anti-malarial, anti-rheumatic and anti-pyretic agents <sup>2, 3</sup>. The traditional practitioners residing in the vicinity of Western Ghats of Karnataka are using the leaves and stem bark to cure jaundice and liver disorders <sup>4</sup>. In Ayurveda it is used to treat febrifuge and emmenagogue, anthelmintic, germicidal, anti-pyretic, anthelmintic and styptic <sup>5</sup>, in homoeopathy it is used for treating febrifuge, emmenagogue, anthelmintic, tonic, styptic, depression, intermittent fever, headache, worm infestation, chronic fever and malaria <sup>6</sup>. Stem bark is reported as febrifuge, anthelmintic, antimalarial, hypoglycemic, antipyretic, anti-inflammatory and antirheumatic <sup>7</sup>. Some of the chemical compounds found in stem barks of *Caesalpinia bonduc* are Hematoxylin, Stereocholenol - A, 6<sup>1</sup>-O-acetylloganic acid, 4<sup>1</sup>-O-acetylloganic acid, 2 - O -  $\beta$  - D - glucosyloxy - 4 - methoxybenzenecarboxylic acid, Ethacrynic acid <sup>8</sup>.

Stem barks extracted and tested with different solvents were found to possess anthelmintic properties proved by analysing the mechanism of paralyzing and killing when tested against earthworms <sup>9</sup>. Chloroform extracts of *C. bonduc* leaves, stem barks and isolated constituents were reported to have anti-bacterial effects. The stem barks extract exposing a minimum inhibitory concentration of 2 mg/ml against *B. subtilis* and *P. aeruginosa* <sup>10</sup>. From the study conducted <sup>11</sup> for analyzing the anti-inflammatory and anti-cancer property of *Caesalpinia* stem barks having high levels of phenolics and flavonoids found to be influencing such inhibitory activities. Objectives: standardization of quality parameters in stem bark of *Caesalpinia bonduc* (L.) Roxb., identification and differentiation of raw drug from closely related or allied species like *Caesalpinia sappan* L., *Caesalpinia coriaria* (Jacq.) Willd., *Caesalpinia decapetala* (Roth) Alston and *Caesalpinia crista* L.

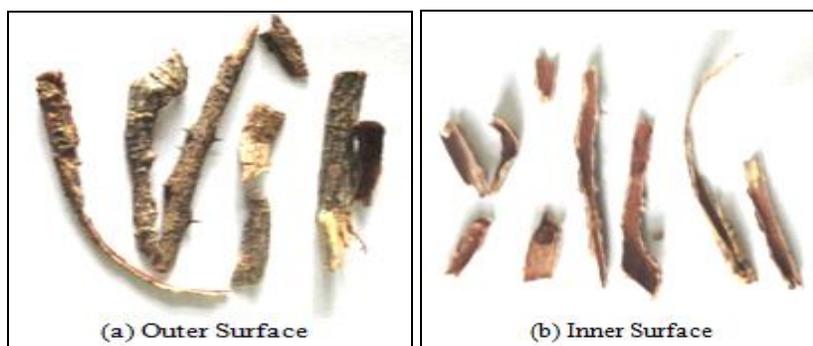
## MATERIALS AND METHODS:

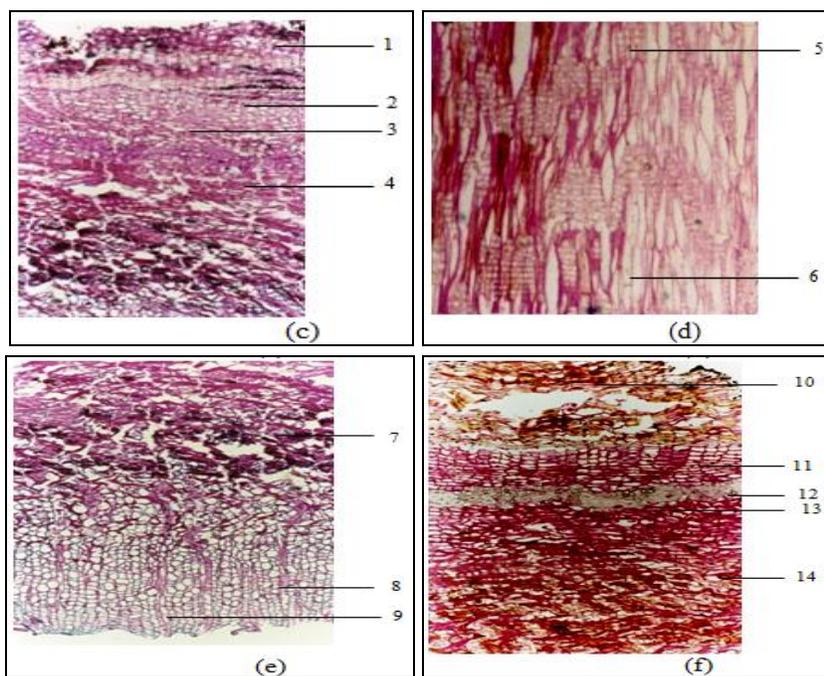
**Pharmacognostical Studies:** The fresh stem bark material of *Caesalpinia bonduc* was collected from Osmania University campus, Hyderabad and identified. The voucher specimens of the plant were deposited in Herbarium Hyderabadensis (HY), Osmania University, Hyderabad. The material was preserved in glycerin and alcohol mixture (1:1) for softening. Sections of stem bark were cut in transverse and longitudinal planes, 8-12  $\mu$ m thickness on Leica RM 2155 microtome. The sections were stained with crystal violet and basic fuchsin and mounted in Canada balsam. portion of fresh stem bark was macerated and examined for various elements. Dried stem bark was ground to fine powder. A small amount of powder boiled in water, stained in safranin and mounted in glycerin and observed for microscopical characters. The photomicrographs were taken with Olympus ch-2 microscope <sup>12</sup>.

**Physico-Chemical Studies:** Stem bark was dried in under shade; the dried material was mechanically powdered. These powdered materials were used for physico-chemical parameters viz., Moisture content, foreign organic matter, total ash, water soluble ash, acid insoluble ash values and extractive values were determined as per standard procedure <sup>13,14</sup>. Histochemical studies, phytochemical constituents and Fluorescence studies have been carried out by standard methods <sup>15-22</sup>.

## RESULTS

**Macroscopic:** From the microscopic figures, the stem bark pieces were observed as they were thin, double or compound quilled; 1-2 mm thick, scaly; externally blackish brown with intermittent creamish brown patches; inner surface was smooth, pale brown; fracture uneven, splintery (Plate-1A & 1B).





**PLATE 1: CAESALPINIA BONDOC (LINN.) ROXB. STEM BARK.** (a,b) Dried Stem bark, (c) TS of stem bark showing cork and phelloderm, (d). TLS of stem bark, (e) TS of stem bark showing secondary phloem, (f) TS of stem bark showing cork enlarged. 1= Cork , 2= phellogen , 3=Phelloderm , 4= Stone Cells , 5= Medullary rays , 6= Phloem fibers , 7= Obliterated tissue , 8= Medullary rays , 9=Sieve elements, 10=Tannin , 11=Cork, 12=Rhombic crystals, 13=Stone cells , 14= Phelloderm

**Microscopic:** In the transverse section, the stem bark shows, outer most cork 40-45 layered; cells rectangular, tangentially elongated, measuring 17.5 - 70 µm (31.5) long and 3.5 - 21µm (7) wide; walls slightly thick, contents slightly with golden yellow coloured, interspersed with stone cells, isolated or in groups, mostly rectangular, measuring 21 - 42 µm (35) long and 7 - 17.5 µm (10.5) wide; walls thick, contents scanty. Phellogen is 1-2 layered. Phelloderm is separated into outer and inner zones, outer 9-12 layered, cells rectangular or tangentially long, measuring 21-108.5 µm (49.5) long and 7-24.5 µm (17.5) wide; interspersed with stone cells in bands, in between outer and inner zones phelloderm cells are large, banded stone cells are irregular, mostly isodiametric, measuring 10.5 – 35µm (21) in diameter; measuring 10.5 - 38.5µm (35) in diameter; walls thick, lignified, lumen small with ramified pits. Inner phelloderm is multilayered, cells varying shape and size, mostly polygonal, measuring 17.5 - 73.5 µm ( 28) long and 10.5 - 21µm (17.5) wide; walls slightly thick,

contents dense with golden yellow or reddish contents and rhombic crystals; inner phelloderm is interspersed with cortical fibers.

**TABLE 1: ORGANO-LEPTIC CHARACTERS OF STEM BARK OF CAESALPINIA BONDOC (L.) ROXB.**

Character	Observation
Colour	Pale reddish brown
Touch	Coarse
Odour	Disagreeable
Taste	Bitter

**TABLE 2: PHYSICO-CHEMICAL STANDARDS OF STEM BARK OF CAESALPINIA BONDOC (L.) ROXB.**

Parameter	Standards or content (% by weight)
Loss on drying% w/w	11.07%
Foreign organic matter % w/w	Nil
Total ash % w/w	5.45%
Acid insoluble ash % w/w	0.45%
Water soluble ash % w/w	1.45%
Pet. Ether % w/w	0.45%
CHCl <sub>3</sub> % w/w	1.65%
EtOH % w/w	6.25%
MeOH % w/w	7.55%
Water % w/w	11.65%

**TABLE 3: HISTOCHEMICAL COLOUR REACTIONS OF STEM BARK OF CAESALPINIA BONDOC (L.) ROXB.**

Reagents	Constituents	Colour	Result
Pinch of Phloroglucinol + Dil. HCl	Lignin	Magenta (or) pink	Present
Aqueous ferric chloride solution	Tannins	Black	Present
Iodine solution	Starch	Blue	Present
Sudan-III	Oil globules	–	–
Ruthenium red solution	Mucilage	–	–

**TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING IN STEM BARK OF CAESALPINIA BONDUC (L.) ROXB.**

S. no.	Test for (or) Constituents	Water extract	Pet. ether extract	Chloroform extract	Ethanollic extract	Methanolic extract
1	Alkaloids	-	+	+	-	-
2	Phenolic compounds & Tannins	+	-	-	+	+
3	Phytosterols	-	-	+	+	+
4	Fixed oils and Fats	-	-	-	-	-
5	Saponins	+	-	-	-	-
6	Flavanoids	-	-	-	+	+
7	Aminoacids	-	-	-	-	-
8	Terpenoids	-	-	+	+	+
9	Carbohydrates	+	-	-	+	+

“+” indicates presence of constituents “-” indicates absence of constituents.

**TABLE 5: FLUORESCENCE BEHAVIOUR OF STEM BARK OF CAESALPINIA BONDUC (L.) ROXB.**

S. no.	Powder+Reagents Used	Light source	colour
1	Powder as such	Visible	Brown
		Short UV(254 nm)	Brown
		Long UV (366 nm)	No
2	Powder + 50% HNO <sub>3</sub>	Visible	Light green
		Short UV(254 nm)	Green
		Long UV (366 nm)	Leaf green
3	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Visible	Pae green
		Short UV(254 nm)	Light green
		Long UV (366 nm)	Light green
4	Powder + 50% KOH	Visible	Brown
		Short UV(254 nm)	Brown
		Long UV (366 nm)	No
5	Powder + 1N HCl	VISIBLE	Light orange
		Short UV(254 nm)	Misty vale
		Long UV (366 nm)	English elms
6	Powder + 1N NaOH IN Ethanol	Visible	Thar desert
		Short UV(254 nm)	Leaf green
		Long UV (366 nm)	Milk toffee
7	Powder + 1N NaOH IN methanol	Visible	Casablanca
		Short UV(254 nm)	Leaf green
		Long UV (366 nm)	English elms
8	Powder + Pet.ether	VISIBLE	Milky white
		Short UV(254 nm)	White
		Long UV (366 nm)	White
9	Powder + Methanol	Visible	Thar desert
		Short UV(254 nm)	Light green
		Long UV (366 nm)	Sandal wood
10	Powder + Acetone	Visible	Sporty yellow
		Short UV(254 nm)	Leaf green
		Long UV (366 nm)	Wild yellow

Secondary phloem is scanty consists of phloem parenchyma, sieve tubes accompanied with companion cells and medullary rays. Phloem parenchyma cells isodiametric, 10.5 - 38.5µm (35) in diameter; walls thick, contents slightly dense with light red or bluish colour matter and resinous mass. Medullary rays straight, uni to biseriate, 8-10 cells high, ray cells rectangular, radially elongated, measuring 21 - 52.5 µm (42) long and 7 - 21µm (17.5) wide; walls thin, contents scanty

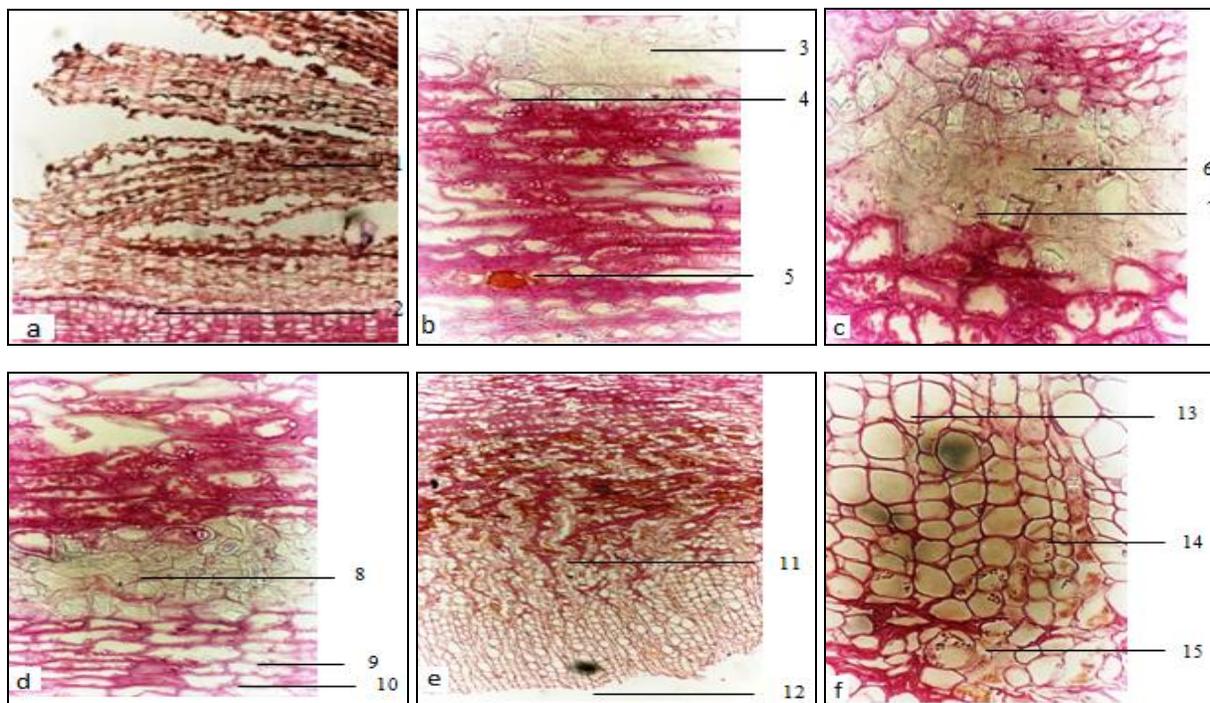
In tangential longitudinal section structure of the medullary rays, phloem fibres and sieve elements were seen.

Medullary rays are straight and exclusively uni to biseriate. The rays cells are rectangular, thin walled, compact, contents are scanty. The rays are uniformly homocellular consisting with only one type cells. [Plate-1 (c, d, e & f) and Plate 2 (a, b, c, d, e & f)].

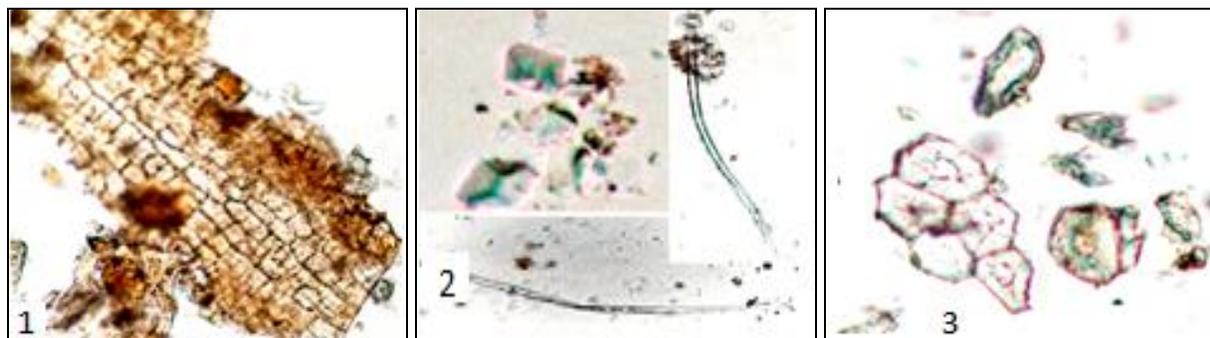
**TABLE 6: POWDER TREATED WITH DIFFERENT CHEMICAL REAGENTS OF STEM BARK OF CAESALPINIA BONDUC (L.) ROXB.**

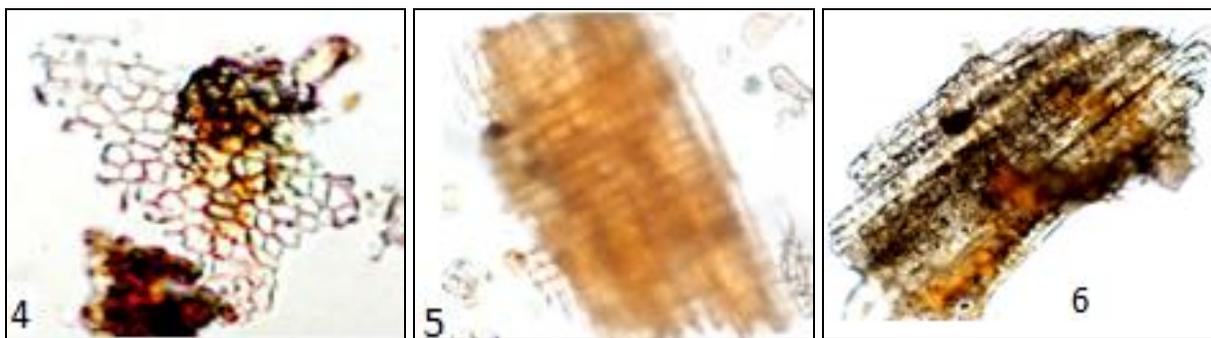
S. no.	Powder + Reagent	Colour
1	Powder as such	Pale brown
2	Powder + con.Sulfuric acid	Pink
3	Powder + Con.HCl	Pale brown
4	Powder + Con. Nitric acid	Pale yellowish with fumes
5	Powder + Acetic acid	Pale brown
6	Powder + 10% NaOH	Black
7	Powder + Ammonia	Dark brown
8	Powder + Acetone	Light brown
9	Powder + Ethyl acetate	Colourless
10	Powder + 1N HCl	Colourless

**Powdered Microscopy:** On microscopic examination of stem bark powder shows cork consisting of polygonal cells in surface and rectangular cells in sectional view; prismatic crystals of calcium oxalates; lignified stone cells, isolated or grouped fibers; crystal fibers, radially cut medullary rays; abundant starch grains. Plate-3(1, 2, 3, 4, 5 & 6).



**PLATE 2: CAESALPINIA BONDUC (LINN.) ROXB. STEM BARK.** (a) TS of stem bark showing cork enlarged, (b) TS of stem bark showing starch grains, (c,d) TS of stem bark showing stone cells enlarged, (e) TS of stem bark showing secondary phloem enlarged, (f) TS of stem bark showing medullary rays enlarged, 1= Stratified cork layers, 2= Phellogen, 3= Rhombic crystals, 4= Phloem fibers, 5= Starch grains, 6= Stone cells, 7= Prismatic crystal, 8= Stone cells, 9= Starch grains, 10= Phloem parenchyma, 11= Obliterated tissue, 12= Medullary rays, 13= Sieve elements, 14= Medullary rays, 15= Starch grains.





**PLATE-3: POWDERED ELEMENTS OF CAESALPINIA BONDOC (L.) ROXB.** 1= Parenchyma , 2= Crystals and fibers, 3 =Stone cells, 4 =Cork, 5 =Medullary ray, 6= Crystal fibers.

**Organoleptic Characters:** By observing the organoleptic properties the colour of the bark was found to be Pale reddish brown and was coarser in touch, bitter in taste with a disagreeable odour **Table 2.**

**DISCUSSION:** Earlier studies conducted in *Caesalpinia bonduc (L.) Roxb.* stem barks lack microscopic and physic-chemical analysis. Pharmacognostic characters of herbal drugs play an important role since particular- macroscopic features are unique for each plants. Macro and microscopic evaluation is simple and cost effective. The microscopic and macroscopic studies of the herb should be the first and fundamental step to authenticate the botanical source. Stem bark pieces are thin, double quilled or compound quilled. External bark blackish brown and scaly while inner surface is smooth. Fracture is splintery. Cork in T.S consists of rectangular cells with golden yellowish content and interspersed with stone cells. Phelloderm is wide interspersed with many bands of stone cells.

Secondary phloem is scanty with phloem parenchyma cells encrusted with resinous masses. Medullary rays are biseriate with rectangular, radially long cells. The Powder microscopic features and organoleptic characters presented (loc.cit.) are of diagnostic significance.

Physicochemical parameters revealed absence of foreign organic matter indicating purity of drug. Standards of loss on drying 11.07% is indicating to avoid the microbial contamination, total ash, acid insoluble ash, water soluble ash values found to be 5.45%, 0.45%, 1.45% respectively and extractive values in various solvents are ( Petroleum ether extractive value 0.45%, chloroform extractive value 1.65% , ethanol extractive value 6.25%,

methanol extractive value 7.55% , water extractive value 11.65%) in acceptable range. Phytochemicals have been used for the treatment and prevention of various health ailments from time immemorial. The results of preliminary phytochemical screening of various extracts reveals presence of alkaloids, phenolic compounds and tannins, phytosterols, flavonoids, terpenoids and carbohydrates helps in establishing genuinity and efficacy of the drug. The preliminary phytochemical screening shows that *Caesalpinia bonduc* stem bark contains different chemical constituents which indicate the presence of both polar and non-polar compounds in the drug **Table 1-7.**

From the result of this study it has been found that the dried whole plant of *Caesalpinia bonduc* stem bark can be easily identified macro-microscopically and phytochemically. The study sets specific morpho-anatomical and preliminary phytochemical studies, histochemical colour reactions, powder treated with different chemical reactions and fluorescence behavior of stem bark which are helpful in standardization and efficacy of the drug.

**CONCLUSION:** The present study on pharmacognostical characters of *Caesalpinia bonduc L.* stem bark provides useful information in regards to its correct identity and helps to differentiate from the closely related other species of *Caesalpinia* genus. Standardization of herbal medicine is a key importance in establishing its correct identity, safety, efficacy and purity of drugs besides checking of the adulteration as well. Botanical authentication, physic-chemical and phytochemical parameters will give an idea about the quality of drug. The macroscopic, microscopic, Physico-chemical and phytochemical analysis are the confirmatory test for standardization and

quality control, which may be helpful in authentication of crude drug and checking the adulteration of raw materials as well.

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**CONFLICT OF INTEREST:** Nil

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