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INVESTIGATION OF MICROSCOPICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF PLANTS WITH ANTI-DIABETIC ACTIVITY

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ABSTRACT: Herbal medicines are gaining more and more attention all over the world, due to their long historical clinical usage and comparatively less side effects. Standardization of herbal drugs and their quality control is needed with proper integration of modern scientific techniques and traditional knowledge. The analysis and quality control of herbal medicines are moving towards an integrative and comprehensive direction, in order to better address the inherent holistic nature of herbal medicines. The techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics. The present study highlights the pharmacognostical studies including parameters such as powder analysis by microscopical evaluation, and study of physicochemical parameters i.e. proximate analysis and preliminary phytochemical screening of seed kernel of *Caesalpinia bonducella* Linn. and fruits and roots of *Coccinia indica* Wight & Arn. which are used as antidiabetic agents. The present study, thus, will help to provide important information with respect to the proper identification of the plant for utilization in different herbal products.

INTRODUCTION: Diabetes mellitus is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion or insulin action which results in impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins¹. In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease^{2,3}.

Medicinal plants have been used as a source of medicine since times immemorial. Herbal medicine is still the mainstay of health care in several developing countries.

The efficacy and safety of herbal products therefore rely on the quality and proper identification of the raw material or the original plant source.

One major obstacle that might impair the potential use of traditional medicine as medicine of choice is the lack of standardization. Adulterations and substitutions are common in raw material trade of medicinal plants.

Unintentional adulterations also exist in herbal raw material trade due to various reasons such as confusion in vernacular names between indigenous systems of medicine and local dialects, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology and/or aroma or careless collection⁴.

To avoid this, accurate authentication it is very important to prevent the adulteration of target plant with other plant species.

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Standardization of medicinal plant products is the prime need of the current time. The techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics⁵.

Caesalpinia bonducella Linn. commonly known as Fever nut⁶ and locally known as 'Sagargotha'⁷ is a shrub widely distributed throughout the coastal region of India. The tribal people of India use it for controlling blood sugar. The seeds are reported to possess anti-diabetic or hypoglycaemic activity⁸.

Coccinia indica Wight & Arn. commonly known as Ivy gourd⁹ and locally known as 'Tondali'⁹ grows abundantly and wildly all over India and is used as a vegetable. Indigenous people use various parts of the plant to get relief from diabetes mellitus⁹.

In the literature¹⁰, microscopy study of seeds *Caesalpinia bonducella* Linn. has been reported but not specifically on the seed kernel part.

In the literature¹¹, microscopy study of aerial parts of *Coccinia indica* Wight & Arn. has been reported. A systematic pharmacognostic study of fruits has not been reported. There is no report of any study of the pharmacognostic parameters of roots of *Coccinia indica* Wight & Arn.

Hence in the present study an effort has been made to establish the pharmacognostical parameters of seed kernel powder of *Caesalpinia bonducella* L. and fruit and root powder of *Coccinia indica* W & A.

MATERIALS AND METHODS:

Materials: The first step in standardization of herbal drugs is the correct identification of plant, macroscopic and microscopic characters. The seeds of *Caesalpinia bonducella* L. (CBS) and fruits of *Coccinia indica* W & A. (CIF) and roots of *Coccinia indica* W & A. (CIR) were collected from Keshav Shrushti, Mumbai, India. The herbaria of *Caesalpinia bonducella* L. and *Coccinia indica* W & A. were prepared and authenticated from Botanical Survey of India, Pune with Voucher no. SHSHACAB1 and SHSHACOG2 dated: 10/11/2010.

A duplicate copy of herbarium is preserved in Chemistry Department of Ramnarain Ruia College.

Iodine S, Phloroglucinol S, Sudan III, Ruthenium Red stain (used for microscopy), used were procured from Lobachemie, Glycerine (90% purified), Safranin stain (used for microscopy), (AR Grade) used procured from Merck.

Powder Characteristics: The plant parts seed kernel powder of *Caesalpinia bonducella* L. and fruit and root powder of *Coccinia indica* W & A. were washed with water to remove soil particles, dried in the shade, and finely powdered. The powder was passed through the 85 mesh sieve and stored in an airtight container at room temperature (28 ± 2°C) and used for further analysis.

The powders of both the plants were examined microscopically using the compound microscope LABOMED LX 300, fitted with 3.5 Mega pixel camera, with software PixelPro using 10X, 45X, 100X magnifying lenses.

The seed kernel powder of *Caesalpinia bonducella* L. and fruit and root powder of *Coccinia indica* W & A. were macerated and mounted on glass slide using glycerine, covered with cover slip and viewed under microscope. Each powder was also stained with Iodine S, Phloroglucinol S, Sudan III, Ruthenium Red stain and examined under the microscope at desired magnification.

Preliminary Phytochemical Screening¹²: The preliminary Phytochemical screening of the above mentioned plant parts was carried out qualitatively by adding suitable reagents to the plant powder. The tests carried out are given in **Table 1**.

The results of preliminary phyto-chemical analysis are shown in **Table 2**.

Physicochemical analysis¹³: The physico-chemical analysis includes number of parameters such as physical state, colour, taste, percentage of loss on drying, total ash content, ash value (water and acid soluble or insoluble ash), and water and alcohol soluble extractives.

TABLE 1: TESTS FOR QUALITATIVE SCREENING OF PHYTOCHEMICALS

Phytochemical	Test	Observation
Tannins	0.2 g plant material + 10 mL distilled water. Filter. 2 mL of filtrate + 2mL FeCl ₃	Blue or black precipitate
Alkaloids	0.2 g plant material + 10 mL methanol. Filter. 2 mL filtrate + 1% HCl + 6 drops of Dragendroff's reagent	Brown or red or orange or creamish precipitate
Terpenoids	2.0 mL of methanolic filtrate + 2 mL acetic anhydride + 1 mL of conc. H ₂ SO ₄	Blue or green ring
Flavonoids	0.2 g plant material + 10 mL of ethanol. Filter. 2 mL of filtrate + conc. HCl + magnesium ribbon	Pink, tomato or red colour
Cardiac glycosides	2mL aqueous filtrate + 1 mL glacial acetic acid + 1 drop FeCl ₃ + 1 mL of conc. H ₂ SO ₄	Green or blue ring
Saponins	0.5 mL of methanolic filtrate + 5 mL of distilled water	Persistent frothing on shaking
Steroids	0.2 g plant material + 10 mL chloroform. Filter. 2 mL of filtrate + 2mL of acetic anhydride + 1 mL of conc. H ₂ SO ₄	Green or blue ring

- Total ash:** About 2.0 g of dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed and transferred to two different silica crucibles and were ignited with a flame of bunsen burner, for about 1 hour. The ignition was completed by keeping in a muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was formed. The silica crucibles were then cooled in a desiccator and weighed to a constant weight. The results obtained are given in **Table 3**.
- Acid insoluble ash:** About 2.0 g of the dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed and transferred to three different silica crucibles and were ignited with a Bunsen burner, for about 1 hour. The silica crucibles were then kept in a muffle furnace at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was formed. After cooling, each ash was taken in beaker (capacity 50 mL) and to it 25 mL of dilute hydrochloric acid (2N HCl) was added, and crucibles were kept covered and heated on a water bath, for 10 minutes. They were allowed to cool, and contents were filtered through Whatman filter paper no. 41 (E. Merck, Mumbai India). The residues were then washed with water, till washings were free from chloride. The filter papers along with the residues of respective plant powders were placed in three silica crucibles and ignited in a muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, for 1 hour.

The crucibles were cooled and weighed to a constant weight. The percentage of acid insoluble ash was then calculated for dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. The results obtained are given in **Table 3**.

- Water soluble ash:** About 2.0 g of the dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed and transferred to three separate silica crucibles and were ignited with a Bunsen burner, for about 1 hour. The crucibles were then kept in a muffle furnace at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was obtained. After cooling, each ash was taken in beaker (capacity 50 mL) and to it, 25 mL of distilled water was added, and all the beakers were kept covered and heated on a water bath, for 10 min. They were allowed to cool, and contents were filtered through Whatman filter paper no. 41 (E. Merck, Mumbai India).

The filter paper along with the residues of each plant powder were placed in different crucibles and ignited in a muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, for 1 hour. The crucibles were cooled and weighed to a constant weight to obtain water insoluble ash. The percentage of water soluble ash for each powder was then calculated by subtracting water insoluble ash from total ash. The results obtained are given in **Table 3**.

4. **Moisture content:** The Karl Fischer test is used to determine the moisture content in the substances. The moisture in the reaction vessel in which the analysis is to be carried out needs to be neutralized before any analysis. The reaction vessel was filled with approximately 25 mL of methanol. The autotitrator was filled with pyridine-free Karl Fischer reagent and then added to the vessel. Addition of Karl Fischer reagent is stopped once methanol in reaction vessel is moisture free, and the amount of Karl Fischer reagent in “mL”, which is consumed to neutralize the traces of moisture in the vessel, is displayed by the instrument. After this neutralization step, the samples moisture content to be determined was then added to the reaction vessel.

Accurately weighed, about 100 mg of dried seed kernel powder of *Caesalpinia bonducella* Linn. was transferred to the reaction vessel. Titration with Karl Fischer reagent was carried out as described above. The same above procedure was repeated to determine the moisture content of fruits and roots of *Coccinia indica* W & A. The results obtained are given in **Table 3**.

5. **Loss on Drying:** About 5.0 g of dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed, in previously dried, wide mouthed flat weighing bottles. The bottles were then placed in an air oven, maintained at $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$, for 2 hours. The bottles were then removed, covered and placed in a desiccator. The respective bottles were weighed after cooling to room temperature and were reheated until two consecutive weightings do not differ by more than 5 mg. The results obtained are given in **Table 3**.
6. **Alcohol soluble extractive:** About 1.0 g of dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed in three separate stopper

conical flasks. To each flask, 10.0 mL of ethanol was added, it was then shaken at 80 rpm, on a conical flask shaker at room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$), for 6 hr and allowed to stand for 18 hrs. The contents of each flask were then filtered through Whatmann No.1 filter paper in separate pre-weighed dry beakers and each filtrate was evaporated to dryness on a water bath. Each dried residue was then weighed and the percentage extractive values were calculated. . The results obtained are given in **Table 3**.

7. **Water soluble extractives:** About dried seed kernel powder of *Caesalpinia bonducella* Linn., fruit powder and root powder of *Coccinia indica* Wight & Arn. were accurately weighed in three separate stopper conical flasks. To each flask, 10.0 mL of water was added it was then shaken at 80 rpm, on a conical flask shaker at room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$), for 6 hr and allowed to stand for 18 hrs. The contents of each flask were then filtered through Whatmann No.1 filter paper in separate pre-weighed dry beakers and each filtrate was evaporated to dryness on a water bath. Each dried residue was then weighed and the percentage extractive values were calculated. . The results obtained are given in **Table 3**.

RESULTS AND DISCUSSION:

Microscopical evaluation:

1. **Seed kernel of *Caesalpinia bonducella* Linn.:** The most important character observed in seed kernel powder of *Caesalpinia bonducella* Linn. were stone cells (**Figure 1a**) which were rectangular to oval in shape with pitted and lignified cell walls. Another important character observed was the presence of simple and compound starch grains (**Figure 1b**), which were spherical in shape with concentric rings.

The powder also showed presence of fragmented oil cells (**Figure 1c**) and parenchyma cells containing starch grains (**Figure 1d**).

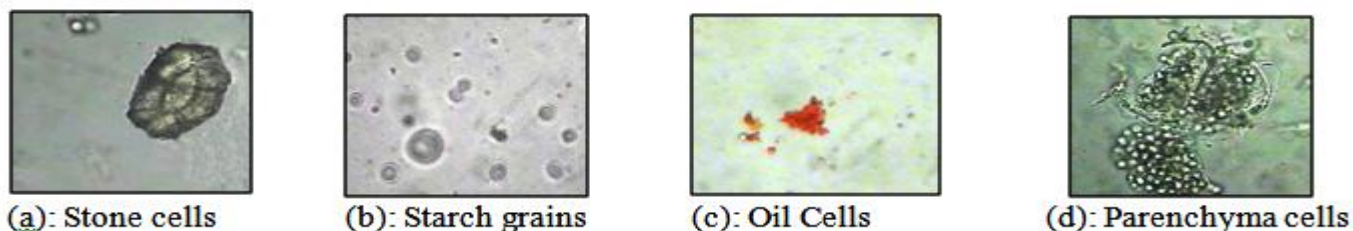


FIGURE 1: POWDER CHARACTERISTICS OF SEED KERNEL OF *CAESALPINIA BONDUCELLA* LINN.

2. **Fruit powder of *Coccinia indica* Wight & Arn.:** The fruit powder of *Coccinia indica* Wight & Arn. showed characteristic presence of parenchyma cells of placenta (**Figure 2a**), Columnar parenchyma cells (**Figure 2b**). The epicarp cells (**Figure 2c**) which were observed are quadrangular to polygonal in shape with smooth cuticle. Fragmented oil cells (**Figure 2d**) were observed.

Simple starch grains (**Figure 2e**) present in the powdered plant material were sub spherical or ovoid in shape and slightly flattened. Hilum occurred as a small point towards narrower end

of the starch grain and was surrounded by eccentric striations.

Fibers (**Figure 2f**) observed were fairly large, thin walled with narrow lumen and lignified tapering ends. Trichomes (**Figure 2g**) observed were short, conical, unicellular dagger shaped with bulbous base.

Bundles of xylem vessels (**Figure 2h**) and a single xylem vessel with spiral thickening of lignin (**Figure 2i**) were observed. These microscopical characters of the fruit can serve as diagnostic parameters.

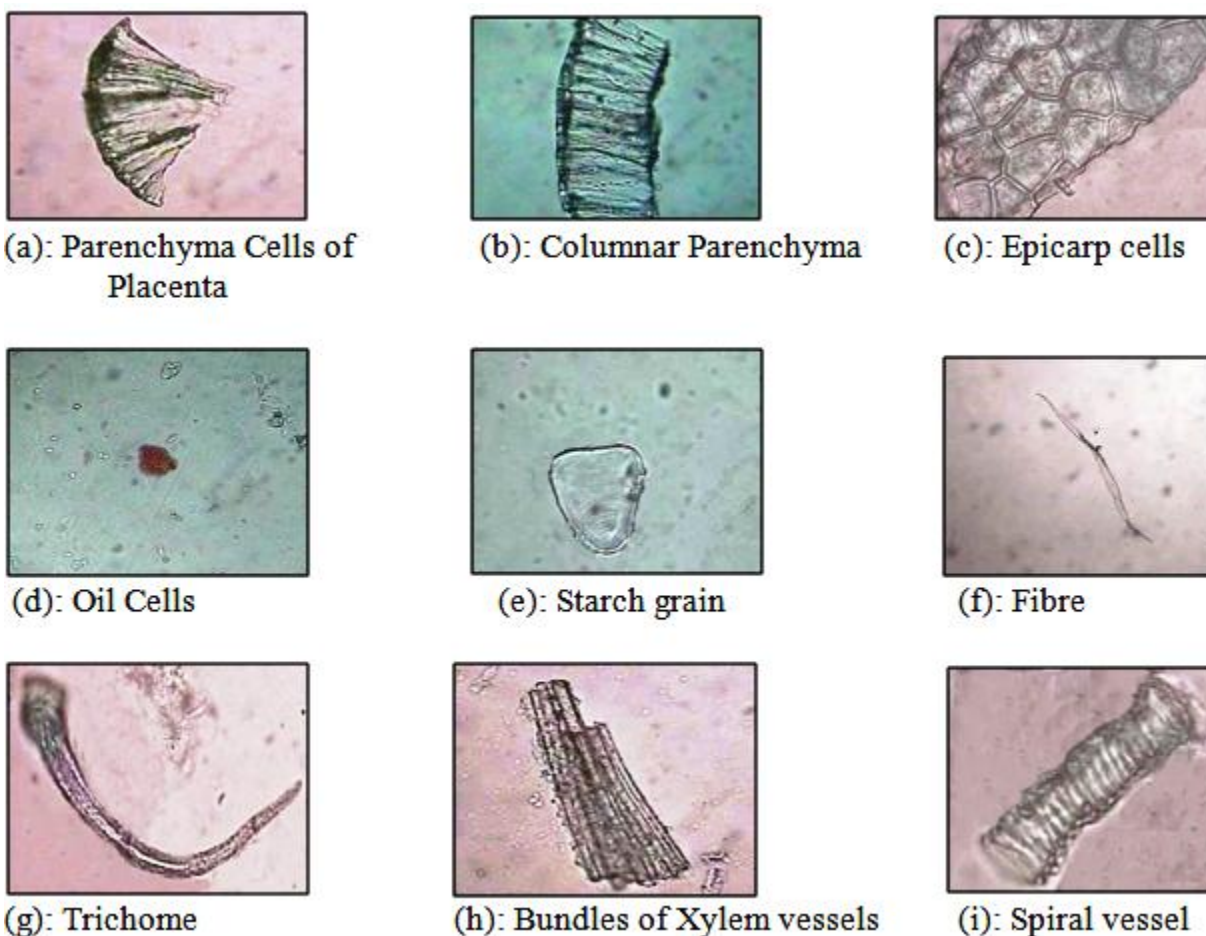


FIGURE 2: POWDER CHARACTERISTICS OF FRUITS OF *COCCINIA INDICA* WIGHT & ARN.

3. **Root powder of *Coccinia indica* Wight & Arn.:** The distinguishing character observed in the root powder of *Coccinia indica* Wight & Arn. was presence of raphides (Figure 3a) which are needle like calcium oxalate crystals, slender, long, pointed at ends and asterosclerides (Figure 3b) which are isodiametric with lignified cells. It showed presence of stone cell with lignified cell walls (Figure 3c). The root powder also showed presence of simple starch grains (Figure 3d) which were ovoid in shape and slightly

flattened. Hilum occurred as a small point towards narrower end of the starch grain and surrounded by eccentric striations. It also showed presence of compound starch grains (Figure 3e) which were polyhedral in shape, with sharp angles, without any striations. Group of xylem vessels (Figure 3f) and single xylem vessel (Figure 3g) were also observed. Multicellular uniseriate trichomes (Figure 3h) were observed. Fibers (Figure 3i) observed were fairly large with narrow lumen and lignified tapering ends.

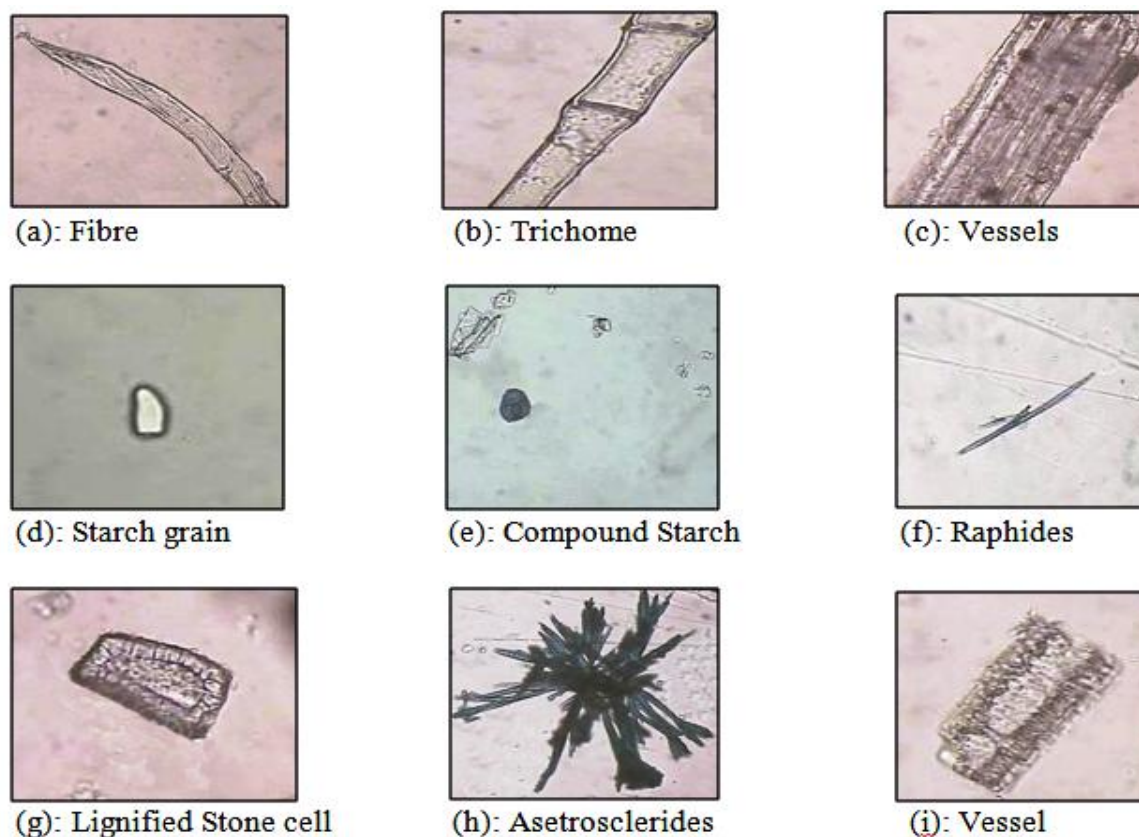


FIGURE 3: POWDER CHARACTERISTICS OF ROOTS OF *COCCINIA INDICA* WIGHT & ARN.

Preliminary Phytochemical Screening:

Preliminary phytochemical analysis indicated presence of saponins, tannins, alkaloids, terpenoids and flavonoids which were found in all the three

plant powders whereas cardiac glycosides and steroids were found only in seed kernel powder of *Caesalpinia bonducella* Linn.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF POWDERED PARTS OF *CAESALPINI. BONDUCELLA* LINN. AND *COCCINIA INDICA* W&A.

Phytochemicals	Dried Seed kernel powder of <i>C. bonducella</i> L.	Dried Fruit powder of <i>C.indica</i> W&A.	Dried Root powder of <i>C .indica</i> W&A.
Tannins	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Cardiac Glycosides	+	-	-
Flavonoids	+	+	+
Steroids	+	-	-

(+): Present (-): Absent

Proximate analysis: The values of moisture content, ash content, water soluble extractive, methanol soluble extractive, acid insoluble ash, water insoluble ash, water soluble and alcohol soluble extractive values are given in **Table 3**.

TABLE 3: PHYSICO-CHEMICAL PARAMETERS OF POWDERED PARTS OF CAESALPINIA BONDUCELLA LINN. AND COCCINIA INDICA WIGHT & ARN.

Parameter	Dried Seed kernel powder of <i>C. bonducella</i> L.	Dried Fruit powder of <i>C. indica</i> W&A.	Dried Root powder of <i>C. indica</i> W&A.
% Total ash	4.06	10.27	8.48
% Acid insoluble ash	0.45	0.96	1.08
% Water soluble ash	1.39	2.41	5.79
% Moisture Content	4.77	7.33	5.12
% Loss on drying	3.00	7.78	8.43
% Water Soluble extractive	8.24	6.86	9.10
% Alcohol Soluble extractive	20.01	30.85	37.03

CONCLUSION: The methods carried out in the present research work, like powder analysis, proximate analysis and phytochemical screening will serve as standard reference for identification and distinguishing characteristics of seed kernel powder of *Caesalpinia bonducella* L. and fruit and root powder of *Coccinia indica* W & A., its substitutes and adulterants. The characters established for the plant powder could be employed as quality control standards for evaluating its identity and can be used for routine analysis.

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