Phytopharmacopoeial specifications of *Garcinia indica* fruit rinds

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ABSTRACT

Introduction: Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Herbal medicines are the products of therapeutic experiences, of generations of practising physicians of indigenous systems of medicine for over hundreds of years. *Garcinia indica* Choisy Syn *Brindonia indica*, commonly known as kokum and belonging to Guttiferae family, is a popular plant native to certain regions of India. The plant has long been used in traditional Ayurvedic medicine for its magical effects in curing various diseases. **Objective:** To present a systematic investigation of phytopharmacopoeial standards for the fruit rinds of *Garcinia indica* by performing pharmacognostical parameters. **Material and Methods:** Fresh fruit rinds sample and dried power of the fruit rinds were studied macromorphologically and cytomorphologically along with its detail physicochemical and phytochemical investigation. Other WHO recommended parameters for standardizations were also investigated. **Results:** Macroscopic studies showed that fruit rind shape - round, oblong or oval with pointed tips and, were crowned by the four parted stalkless stigma with reddish black colour, aromatic odour and sour taste. The detailed microscopy revealed presence of parts of pericarp, oleo resin cells etc. the other physicochemical parameters were stands within the standardized range. **Conclusion:** The standardization parameters provide referential information for correct identification of the plant material and will also be useful in preparation of monographs on these plants.

Keywords: *Garcinia indica,* phytochemical characterization, phytopharmacopoeial standards, standardization.

INTRODUCTION

Medicinal herbs are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. The Indian flora offers a variety of plants having medicinal properties. These plants can be exploited to find out effective alternative to synthetic drugs.[1] However, a key obstacle, which hindered the acceptance of the alternative medicines, is the lack of documentation and stringent control. Therefore, there is a need for documentation and stringent quality control of research work carried out on traditional medicines. With this backdrop it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. Correct identification and

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quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics.[2] Herbal drugs or medicinal plants, their extracts and their isolated compound(s) have demonstrated spectrum of biological activities. These have been used and continued to be used as medicine in folklore or food supplement for various disorders. One such plant, *Garcinia indica* Choisy Syn *Brindonia indica*, commonly known as kokum and belonging to Guttiferae family, is a popular plant native to certain regions of India. The plant has since long been used in traditional Ayurvedic medicine for its magical effects to cure various diseases. In the traditional Indian system of medicine like Ayurveda and in various folk systems of medicine, the fruit rinds, seeds and leaves are used to treat various inflammatory ailments, rheumatic pain and bowel complaints. Chemically, it contains tannin, organic acids like (−)-hydroxycitric acid, hydroxycitric acid, lactone and citric acid; the anthocyanins, cyanidin-3-glucoside and cyanidin-3-sambubioside; and the polyisoprenylated phenolics, garcinol and isogarcinol. Preclinical studies have shown that kokum and/or some of its phytochemicals possess antibacterial, antifungal, anti-ulcerogenic, cardioprotective, anticancer, chemopreventive, free radical scavenging, antioxidant and anti-obesity effects.[3] But the pharmacognostical and phytochemical standardization of the fruit rinds were hitherto not reported. In view of the importance of this plant, the systematic investigation of phytopharmacopoeial standards for the species has been undertaken. The present study deals with pharmacopial standardization parameters for the fruit rinds of *Garcinia indica*. Emphasis is being laid on the areas of the most recent interest and those which have not been presented in earlier reports. The information will be used for further identification and preparation of plant monograph and will assist in standardization for quality, purity and sample identification.

MATERIALS AND METHODS

Collection of Samples

The fruit rinds of *Garcinia indica* was collected from the local area in Goa. Their identity and authentication was done by Department of Pharmacognosy, Marathwada Mitra Mandal's College of Pharmacy, Pune, by correlating their macromorphological characters with those given in literatures. The remaining fruit rind samples were dried in shade. Coarse powder (60 #) of dried fruit rinds of plants was stored for the microscopical study and phytochemical investigations.

Macromorphology

The entire fruit rinds of *Garcinia indica* and the powder was evaluated for their sensory profile by observing their colour, odour and taste along with some extra macroscopical characters as per standard WHO guidelines.[4–6]

Cytomorphology

The transverse sections of the fruit rinds were taken, cleared with clearing agent and mounted in glycerine water. Microscopy of dried fruit rind powder was studied for evaluating various parts present in the given fruit rind powder. The detail cytomorphological characters were observed under digital microscope (MOTIC-B1) and organ detection was reported according to the prescribed method.[7,8]

Microchemical Testing

For detection of cell wall composition and cell contents, the transverse sections of fruit rinds and powders were treated with different but specific staining reagents and observed under digital microscope (MOTIC-B1). The cell wall composition, cell contents and tissue detection was reported separately.[7,8]

Physicochemical Evaluation

Evaluation of crude drug ensures the identity of a drug and determines the quality and purity of drugs. The main reason behind the need forthe evaluation of crude drugs is biochemical variation in the drug, effect of treatment and storage of drugs and adulteration and substitutions.[9] Phytopharmacopoeial specification for the plant materials should be developed to enable the quality control chemists to verify and approve the materials.[10] The various physicochemical parameters were viz. ash values, extractive values and loss on drying. Determinations of these physicochemical constants were done as per the procedures mentioned in accordance with the WHO guidelines.[11,12]

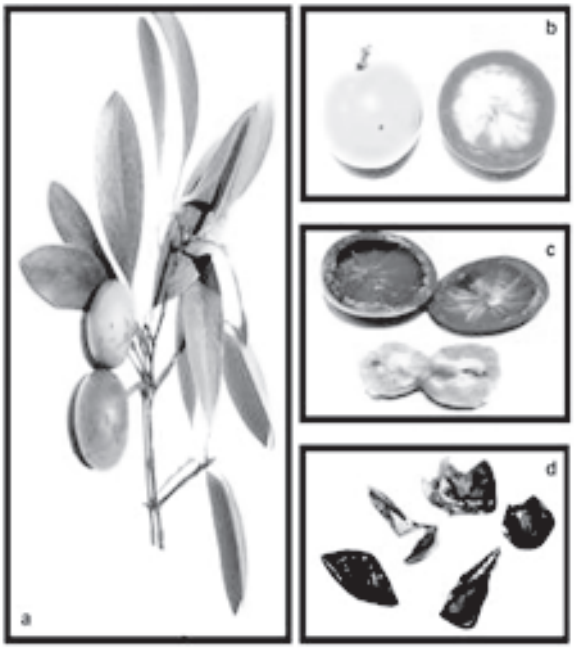
Preliminary Phytochemical Screening

The chemical evaluation includes qualitative chemical tests which are used for identification of various phytoconstituents present in the powered crude drug.[13,14]

RESULTS

Macrmorphological Description

Kokum tree is dioecious and grows up to a height of 12 to 20 m. The fruits were round, oblong or oval with pointed tips and, were crowned by the four parted stalkless stigma. The morphological details of the plants and fruit rinds are observed in Figure 1. The organoleptic evaluation of the fruit rinds and fruit rind powder revealed that the fruit rinds were dark red and the powder was reddish black in colour, with aromatic odour and sour taste. The results of morphological characters are mentioned in Table 1.



a. Plant with leaves and ripe Fruits
b. Raw fruits and ripe fruit with the inner seed arranged like in orange
c. Raw fruit rind and seeds
d. Dried rinds

Figure 1. Macrmorphological description.

Table 1. Macrmorphologcal description.

Sr.No.	Characters	Observation
Organoleptic characters		
1.	Colour	Dark red
2.	Odour	Aromatic
3.	Taste	Sour
Quantitative macromorphology		
4.	Size	2.5 – 3.7 cm in diameter
Macroscopical features		
5.	Shape	Globose or Spherical
6.	Type	Simple (Dry fruit- Dehiscent)

Cytomorphological Description

The outline of transverse section showed normal shape. Figure 2 revealed the transverse section of the pericarp, which was divided into three layers. The outermost was compactly arranged quadrangular or polygonal layer of cells is commonly called epicarp. The middle loosely arrange reticulated thin walled cell layer, is called mesocarp. Beneath the mesocarp, narrow elongated cells were arranged commonly called endocarp. The oleoresineous cells were also observed in a distributed manner within the mesocarp region which was stained by Sudan Red-III while the other starch grains were also observed in discrete manner which were stained by the N/50 Iodine solution. Figure 3 revealed the presence of epicarp region which also contributes to the attachment of a fruit with the tree through the region called as dessipiment. Figure 4 contributes to the presence of the oleoresin cells which were distributed throughout the mesocarp region. In

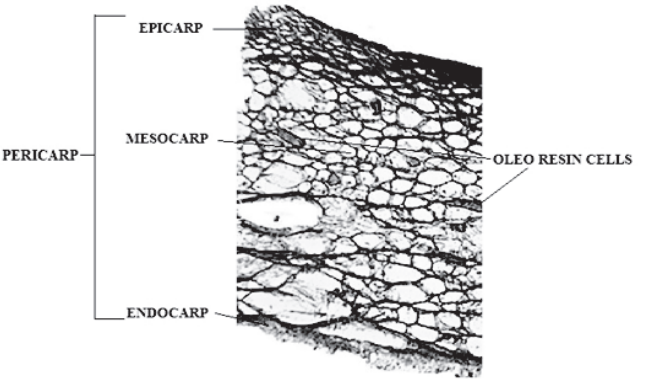


Figure 2. Cytomorphological description.

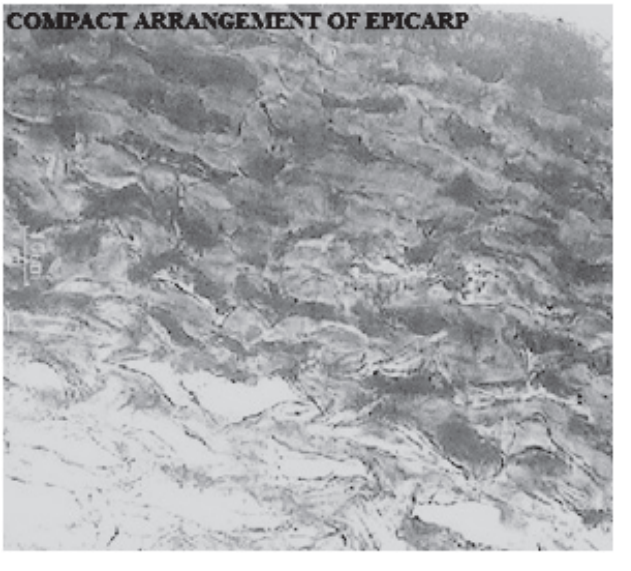


Figure 3. Compact arrangement of Epicarp.

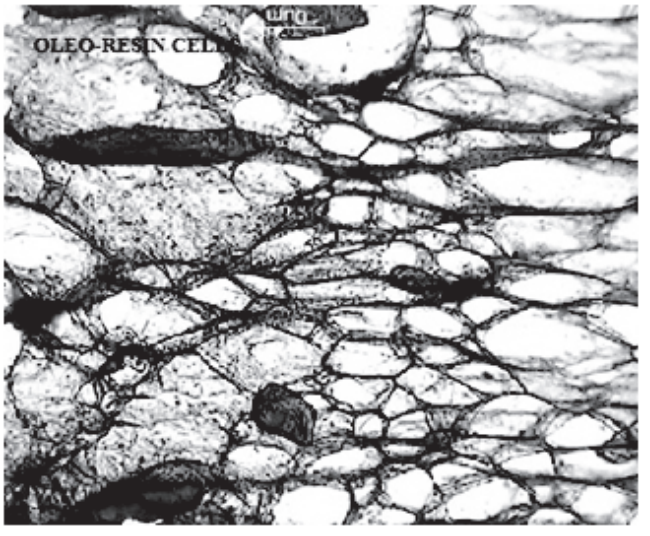


Figure 4. Oleoresin cells within Mesocarp.

powder microscopy Figure 5 showed the presence of fragments of epicarps which were compactly arranged cells. Figure 6 showed the presence of elongated lignified fibers which were stained by mixture of phloroglucinol solution and sulphuric acid in equal proportion the location of these fibers was found to be embedded within mesocarp region.

Microchemical Testing

Characterization of the cell wall components and cell contents were done with the help of microchemical testing

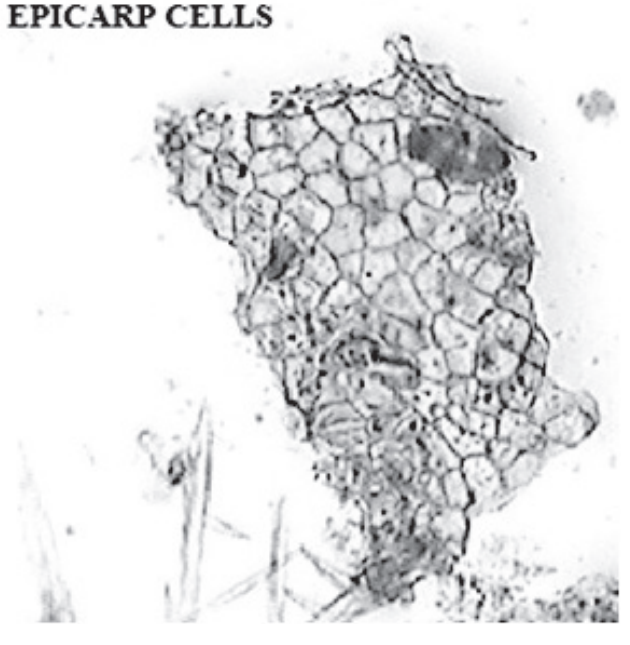


Figure 5. Powder Microscopy (Epicarp cells).

Figure 6. Powder Microscopy (Lignified fibers).

which revealed the presence of various types of cells and also characterization of these cells. It also signifies the tissues present in the fruit rind powder. The details of these microchemical testings are reported in Table 2.

Physicochemical Evaluation

Table 3 narrates the results of the physicochemical constants of powdered fruit rinds which lie within the limit; this signifies that the quality and purity of raw material was good enough. The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So the moisture content of a drug should be determined and controlled. The result of the moisture

Table 2. Microchemical testing.

Sr. No.	Staining Reagents	Observations	Characteristics
1.	Phlorglucinol + conc. HCL	Pink	Lignified fibers
2.	Sudan red III	Red	Oil globules
3.	Iodine solution	Blue	Starch present

Table 3. Physico-chemical constants.

Sr. No.	Test	Result (%w/w)
1.	Moisture content	8.8
2.	Ash value:	
	Total ash	17.5
	Water Soluble ash	1.5
	Acid Insoluble ash	0.5
	Sulphated ash	14.3
3.	Extractive value:	
	Water soluble	60
	Alcohol soluble	75

content was found to be 8.80% which signifies that the drug was properly dried. The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. Different type of ash values are used for detection of crude drugs like, total ash, acid insoluble ash, water soluble ash and sulphated ash. The values were 17.5% for total ash, 1.5% for water soluble ash, 0.5% for acid insoluble ash and 14.3% for sulphated ash. The extracts obtained by exhausting crude drugs with different solvents are approximate measures of their chemical constituents. Extractive values of the crude drug were found to be 60% w/w for water soluble and 75% w/w for alcohol soluble extractive value.

Preliminary Phytochemical Screening

The powder drug was subjected to preliminary phytochemical screening for the presences of type of phytoconstituents. The powder was found to contain cardiac glycosides, saponin, flavonoids, tannins, citric acid and ascorbic acid. The results of the preliminary phytochemical screening are expressed in Table 4.

DISCUSSION

To ensure reproducible quality of herbal products, proper control of starting material is almost essential. Thus, in recent years there has been an emphasis on the standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostic studies is still more reliable, accurate and in expensive. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and purity and should be carried out before any tests are undertaken. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried fruits, typical tongue sensitizing aromatic taste and aromatic and characteristic odour of fruits which are useful diagnostic characters. Microscopic evaluation is indispensable in the initial identification of herbs, as well as identifying small fragments of crude or powdered herbs, and in detection of adulterants as well as identifying the plant by characteristic tissue features. Every plant possesses a characteristic tissue structure, which can be demonstrated through study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents and media.[9] From macromorphology and cytomorphology, the identification of *Garcinia indica* was confirmed which showed the special characters of Guttiferae family. The preparation of crude drug from the harvested drug plants involves cleaning or garbling to remove soil or other extraneous materials followed by drying which plays a very important role in the quality as well as purity of the material. The objectives of drying fresh material are, to aid in their preservation, to 'fix' their constituents, i.e., to check enzymatic or hydrolytic reaction that might alter the chemical composition of the drug, to facilitate subsequent comminution (grinding into a powder) and to ascertain their weight and bulk. Insufficient drying favours the spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only is the ultimate dryness of the drug important, equally important is the rate at which the moisture is removed and the condition under which it is removed. If the rate is too slow, much spoilage may occur before the drying process is completed.[15] The limited moisture content of drug signifies that the drug was properly dried and the rate of drying was also good enough. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash,' which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand, siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards. The ash content of the crude drug signifies that the sample of crude drug was of good quality without any adulterant or substitution. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The colour of the extract sometimes may roughly indicate the physical and chemical features of constituents present and it was found that the crude drug contains more amount of polar constituents as compare to non

Table 4. Preliminary phytochemical screening.

Sr. No.	Parameters	Observations
1.	Cardiac glycosides	+
2.	Flavonoids	+
3.	Tannin	+
4.	Saponins	+
5.	Citric acid	+
6.	Ascorbic acid	+

polar with the highest proportion of colouring pigments. The plant material was subjected to preliminary photochemical screening by various chemical tests for qualitative detection of various chemical constituents where it indicates the presence of saponins, flavanoids and tannins and this data was useful for selection of solvent for extraction purpose.[16]

CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy, physiochemical and phytochemical parameters. As there is no record on pharmacognostical work on fruit rinds of *Garcinia indica*, the present work was undertaken to produce some phytopharmacopoecial standards. Here the information collected was useful for further pharmacological and therapeutic evaluation of plant material.

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