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Prathapa Reddy M
M.Sc. (Chemistry), Lab Tech. (Chemistry), Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Shantha TR
Ph.D. (Botany), Research Officer (Botany), Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Naveen Kumar SP
M.Sc. (Botany), Rsearch Scholar (Botany), Dept. of Botany, Bangalore University, Bangalore, Karnataka, India

Rama Rao V
Ph.D. (Botany), Research Officer (Botany), Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Shiddamallayya N
Ph.D. (Botany), Research Officer (Botany), Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Amit Kumar Dixit
Ph.D. (Bio-Chemistry), Assistant Director, in Charge Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Correspondence
Prathapa Reddy M
M.Sc. (Chemistry), Lab Tech. (Chemistry), Regional Ayurveda Research Institute for Metabolic Disorders, Ashoka Pillar, Jayanagar, Bangalore, Karnataka, India

Pharmacognostical studies on fruits of *Babbula- Acacia nilotica* (L.) Delile

Prathapa Reddy M, Shantha TR, Naveen Kumar SP, Rama Rao V, Shiddamallayya N and Amit Kumar Dixit

Abstract
Babbula is an important traditional medicinal plant in Ayurveda and Unani systems of medicine. All parts of the plant are useful as medicine/therapeutic agents to cure various ailments. Its bark is useful in cough, bronchitis, diarrhoea, dysentery, biliousness, burning sensation, piles, leucoderma, urinary discharges, ascites etc. Levaes are useful in bronchitis, piles, liver tonic, healing of fractures, eye diseases and the fruit is astringent to the bowls, cures biliousness. The gum cures biliousness, leprosy, liver tonic, urinary discharges, vaginal and uterine discharges, healing of fractures, sour throat, lung troubles etc. The flowers are a powerful tonic and good for insanity. Fruits are useful dysentery and in ophthalmia. Though all parts of the plant are used for curing in different ailments many works have carried out on gum, bark, seeds and leaves. But Pharmacognostical work has not been carried out on pods/fruits. Hence, we have carried out macro microscopiacal, powder studies, physico-chemical and preliminary phytochemical studies on the fruits of *Acacia nilotica*. The microscopic studies revealed the presence of elongated macrosclerides, abundant small starch grains and oil globules, uni seriate short tuffted trichomes, small rounded stone cells, lumen filled with brown content of tannin and thick walled parenchymatous cells of mesocarp region. The preliminary phytochemical analysis showed the presence of carbohydrates, proteins, saponins, phenols, steroids and tannins.

Keywords: *Acacia nilotica*, *Babbula*, pharmacognosy, pods, seeds, Ayurveda

1. Introduction
Babbula is equivalent to *Acacia nilotica* (L.) Delile, belonging to the family *Fabaceae* and sub-family *Mimosaceae* is a moderate-sized spiny evergreen tree with a short trunk, a spreading crown and feathery foliage, found throughout drier parts of India. Bark dark brown to almost black, longitudinally fissured or deeply cracked; leaves 2.5-5.0 cm. long, bipinnate with spinescent stipules, pinnules narrowly oblong; flowers golden-yellow, fragrant, crowded in long-stalked globose heads, 1.5 cm in diam, forming axillary clusters of 2-5 heads. Pods stalked, 7.5 to 15 cm long and 1.3 to 1.6 cm width, monoliform, compressed, constricted at the sutures between the seeds, densely and persistently grey downy. Each pod contains 8-12 seeds. The fruit is dry, acrid, sweet; cooling, astringent to the bowels; cures kapha and biliousness. It is an important weed tree, indigenous to the plains of Andhra Pradesh and Maharashtra, found many drier parts of India and pakistan up to an altitude of 900meters. Flowering occurs during rainy season, but sometimes extended up to December-January, Fruiting is usually from April to June. The pods are readily eaten by sheep, goats and cattle and the seeds are disseminated by them [1-3].

The entire pod contains 12-19% tannin and after removal of seeds the pods contain 19-27% tannin. On dry basis the pods yielded 15.8% crude protein [3]. The seeds contain high percentage of proteins and fatty acids of various lipid classes and can be used as animal feed [4]. Pods contains many polyphenols like Gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, gallolylated flaven-3, 4-diol and robidandiol (7, 3', 4, 5'-tetrahydroxyflavan-3, 4-diol). The pods are used to some extent by local tanners for drenching or bating. The seeds of babul are eaten roasted or raw in times of acute scarcity. Analysis of the seeds gave 26.4% crude protein, 2.7% crude fibre, calcium, phosphorus, iron, niacin, ascorbic acid and thiamine. The essential amino acids like histidine, lysine, methionnine, cystine, phenylalanine, tyrosine, leucine, isoleucine, valine and threonine are present in seed crude protein [1]. Pods Methanol Extract showed Antihypertensive and Antispasmodic Activities in experimental rats [5]. Pods Aqueous methanolic extract showed a decrease in blood glucose levels and decrease the diabetes induced rise in lipid levels and decreases the risk of atheromatous disease in alloxan induced diabetic rabbits [6].

1.1 Therepeutic uses in Ayurveda: Pods are one of the major constituent in an Ayurvedic formulation namely *madhumehari*, which is useful for treating *Prameha* (Incresed frequency and tubidity of urine), *madhumeha* (Diabetis mellitus), *Iksumeha* (glycosuria) and *lalameha* (albuminurea) [7]. In *Udarashoola* (Colic), the powder of babbula fruit is slightly heated within an earthen saucer and then taken with boiled water to relieve pain. In *Snayuka roga* (Guinea worm) the paste of *babbula* seeds is applied locally. As per Ayurveda the leaves are useful in diarrhoea, Obesity, eye-diseases, syphilis and in Ascites [8].
Dose: 3-6 gm in powder form [3].

1.2 Pharmacodynamics as per Ayurveda: ***Rasa*** (taste)**:** *Kashaya* (astringent); *Guna* (quality): Guru (heavy), *Ruksha* (creates dryness); *Veerya* (potency): *Sheeta* (conserves energy during metabolism & digestion); *Vipaka* (digestive effect): *Katu* (pungent); *Karma* (action)-Grāhī, lekhana; Doshaghnata (effect on doshas)**:** Kaphahara (Alleviator of kapha) [9].

1.3 Ethnobotanical Studies: The fruits are found to be useful in diarrhoea, dysentery and diabetes [10]. The pods are used for impotency, urino-genital disorders [11, 12] and in dry cough [13]. The decoction of pods is said to be beneficial in Urinological diseases [14].
It is known as follows in different regional language in India: Sanskrit: Bavari; Assami: Babala; Bengali: Babla; English: Babula tree, Indian gum Arabic tree; Gujarathi: Baval, Kaloabaval; Hindi: Babula, Babura, Kikar; Kannada: Karijali, Karigobli, Pulai jail; Kashmiri: Sak; Malayalam: Velutha, Karuvelam; Marati: Babhul, Babhula; Oriya: Babula, Babala; Punjabi: Kikkar; Tamil: Karuvelam, Karuvel; Telugu: Nalla tumma, Thumma [1-3].

2. Materials and Mthods

The fruits of *Babbula* were collected from Kudligi forest range, Bellary district, Karnataka, authenticated by Survey of medicinal plant unit (Herbarium reference no. RRCBI-1171), Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. The fruits were shade dried, some are pulverized by mechanical grinder to get coarse powder and stored in a closed vessel, to carryout microscopical studies, powder studies, physico-chemical and preliminary phytochemical analysis as per the standard protocols. Macroscopical, microscopical and powder studies were carried out as per the standard procedures [15, 16].

2.1 Hydro-Alcoholic Extract: 50g of coarse powder was taken in an extractor and added 50 per cent aqueous alcohol, about 3 times the quantity of raw material and refluxed for 3-4 hours. Filtered the extract through a whatmann no.1 filter paper. The same was extracted three times more and concentrated to syrupy consistency and dried by using a rotary evaporator at a reduced temperature [17].

2.2 Water Extract: 50g of coarse powder was taken in an extractor and added distilled water, about 3 times the quantity of raw material and heated at a medium temperature for 3-4 hours. Filtered the extract through a whatmann no.1 filter paper. The same was extracted three times more and combined filtrates were concentrated to syrupy consistency and dried by using a rotary evaporator at a reduced temperature [17].

2.3 Thin-layer chromatography: Thin layer chromatography was carried out on a precoated silica gel 60_{254} plate. For powder sample, test solution was prepared by taking 2.0 g of the test material in 25 ml of methanol and heated on a water bath for 10 minutes, cooled and filtered. For extracts 10 mg of the test extract was taken in 10 ml of methanol. Standard solution was prepared by dissolving 10 mg of Gallic acid standard in 10 ml of methanol. Few drops of test and standard solutions applied on a TLC plate as bands and developed the plate to a distance of 8 cm from the line of application. Dried the plate in air and examined at 254 nm light, recorded the R_f values and sprayed with spraying reagent. Heated the plate after sparing at 110 ^{0}C for about 5 minutes and recorded the R_f values [17].

2.4 Physicochemical analysis: Analysis of physico-chemical parameters such as total ash, acid-insoluble ash, loss on drying at 105 ^{0}C, pH of aqueous solution, water and Hydro-alcoholic extracts were carried out for powder drug. The water and water-alcoholic extracts were dried and subjected to various tests like total ash, acid-insoluble ash, pH of aqueous solution and total soluble solids as per the latest Ayurvedic Pharmacopoeia of India protocols [16, 17].

2.5 Preliminary phytochemical analysis: The powdered drug was extracted with water, alcohol and petroleum ether and chloroform for carrying out different preliminary phytochemical tests for alkaloids, carbohydrates, proteins, phenols, tannins, saponins, starch, flavonoids and steroids etc. by using standard testing protocols [18, 19].

3. Results

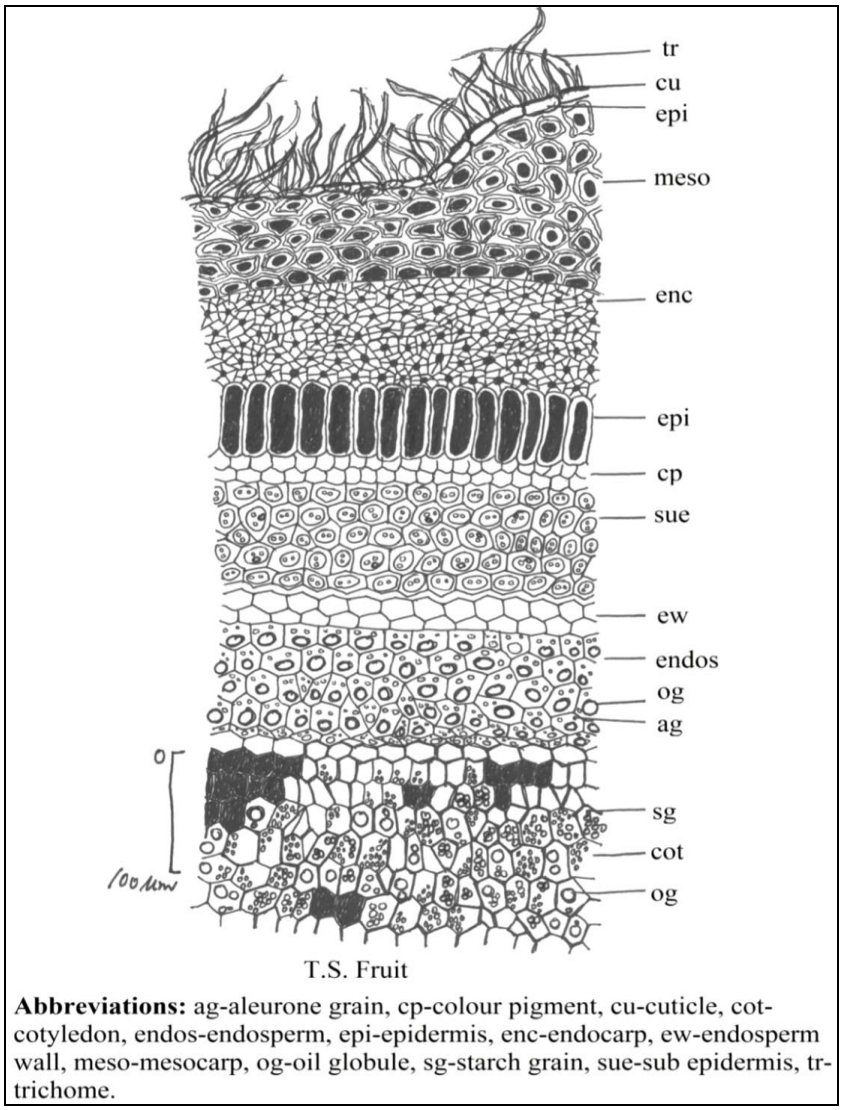
3.1 Macroscopic: Pods variable, indehiscent dark brown to grey, flat, straight or slightly curved, glabrous to velvety compressed but rather thick, seeds 6-16 per pod, lying transeversely to long axis of pod. Pods when young with reddish hairs, becoming dark blackish when mature, deeply constricted between each seed they do not split open, but break up transversely on the ground in to single seeded segments. Pods 7.5 to 15.0 cms in length and contracted between the circular seeds. Seeds deep blackish-brown, sub circular, compressed, areole 6-7 mm long, and 4.5 - 5 mm wide [Figure-1 & 2].

Fig 1: Fresh fruits on habitat

Fig 2: Dried fruits and seeds

3.2 Microscopic: T.S. of the fruit shows the pericarp layer where exocarp consists of epidermis covered by thin cuticle and cells of the epidermis elongate to form tufted uniseriate short to elongated trichomes. The mesocarp made up of 6 to 8 layers of thick walled parenchymatous layer filled with brownish content of tannin. Endocarp consists of several layers of stone cell layers with small lumen filled with brownish content of tannin.

The seed of Fabaceae differentiates from an ovule with 2 integuments. The inner of the integuments disappears during the ontogeny of the seed, where as outer integument differentiates in to palisade layer of sclereids. (Macrosclereid) with unevenly thickened walls. One to two palisade layers occur in the hilum region. The epidermis made up of palisade layer is followed by 1 to 2 layers of pigment cells followed by many layers of sub epidermal layers of cells which are thick walled, orange red coloured with crystals of oxalate salts. The sub epidermis is followed by 1to 2 layers of endosperm wall and many layers of endosperm loaded with oil and aleurone grains. Endosperm region is followed by cotyledon region made up of many layers of compactly arranged thin walled parenchymatous cells, heavily loaded with small starch protein (aleurone grains) grains and oil (lipid globules) [Figure-3].



T.S. Fruit

Abbreviations: ag-aleurone grain, cp-colour pigment, cu-cuticle, cot-cotyledon, endos-endosperm, epi-epidermis, enc-endocarp, ew-endosperm wall, meso-mesocarp, og-oil globule, sg-starch grain, sue-sub epidermis, tr-trichome.

Fig 3: Fruit microscopy

3.3 Powder analysis: Powder light black in colour, shows different fragments of tissues like abundant unicellular trichomes, tufts of trichomes, palisade cells in single and in groups, groups of stone cells which are rounded with small lumen, epidermal cells with trichomes and thick walled parenchymatous cells (Mesocarp region) and fragments of cotyledon portion with parenchymatous cells filled with starch grains [Figure-4].

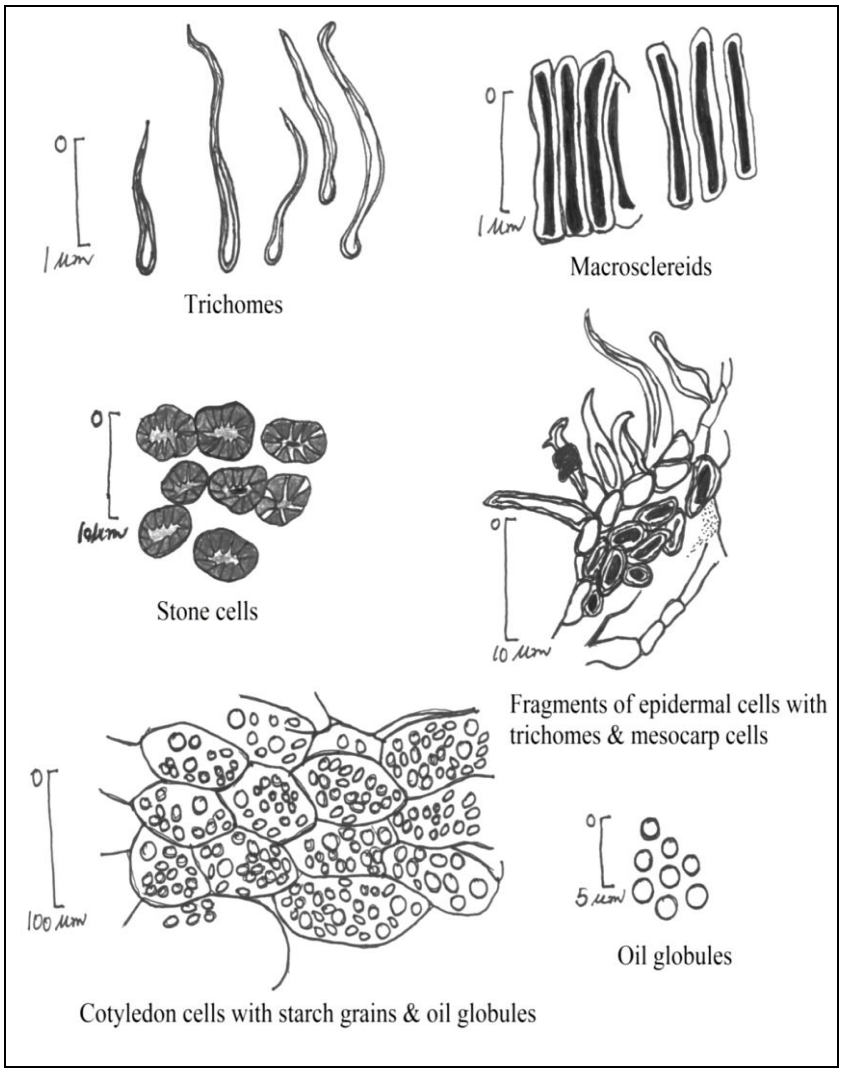


Fig 4: Powder study

3.4 Diagnostic Characters

- Presence of elongated macrosclereids.
- Presence of abundant small starch grains and oil globules.
- Presence of uniseriate short trichomes.
- Presence of small, rounded stone cells with small lumen filled with brown content of tannin.

3.5 Physicochemical analysis

Physico-chemical parameters such as total ash, acid-insoluble ash, loss on drying at 105 ^{0}C, water soluble and water-alcohol soluble extractive values were carried out for fruits powder sample and results were given in table-1. The water and water-alcoholic extracts were dried and subjected to various tests like total ash, acid-insoluble ash, pH of aqueous solution and total soluble solids as per the latest Ayurvedic Pharmacopoeia of India protocols and results were given in table-2.

Table 1: Physicochemical parameters *A. nilotica*-Fruit powder

S. No.	Name of the parameter	Values
1.	Description	Light black coarse powder
2.	pH (5% w/v aq. solution)	4.20
3.	Loss on drying at 105 ^{0}C	5.69% w/w
4.	Total ash	4.43% w/w
5.	Acid-insoluble ash	0.049% w/w
6.	Water-soluble extractive	27.28% w/w
7.	Alcohol-soluble extractive	21.94% w/w
8.	Water-Alcohol extractive	25.0% w/w

Table 2: Physicochemical parameters of *A. nilotica* - Fruit extracts

S. No.	Name of the parameter	Values	
		hydro-alcoholic extract	water extract
	pH (5% w/v aq. solution)	3.30	3.30
	Loss on drying at 105 ^{0}C	3.68% w/w	1.14% w/w
	Total ash	2.09% w/w	6.34% w/w
	Acid-insoluble ash	0.27% w/w	0.06% w/w
	Total soluble solids	97.30% w/w	98.0% w/w

3.6 Phytochemical analysis

Preliminary phytochemical analysis have been carried out for detecting the presence of alkaloids, carbohydrates, proteins, phenols, tannins, saponins, starch, flavonoids and steroids etc. and the results were given in table-3.

Table 3: Preliminary Phytochemical tests for different extracts of *A. nilotica*-Fruit

Natural product group	Test for natural products	Presence (+)/Absence (-)
Alkaloids	(a) Dragendorff's test	-
	(b) Hager's test	-
	(c) Mayers's test	-
	(d) Wagner's test	-
Carbohydrates	(a) Anthrone test	++
	(b) Benedict's test	++
	(c) Fehling's test	++
	(d) Molisch's test	++
Flavonoids	--	+
Phenols	(a) Ferric chloride test	+
	(b) Lead acetate test	
Proteins	(c) Biuret's test	+++
	(d) Millon's test	+++
Saponins	--	++
Steroids	Salkowski reaction	+
Tannins	(a) Ferric chloride test	+++
	(b) Lead acetate test	+++

3.7 Thin-layer chromatography: Thin layer chromatography was carried out on a precoated silica gel 60_{254} plate using Gallic acid as a reference standard. Few drops of test and standard solutions applied on a TLC plate as bands. Developed the plate to a distance of 8 cm from the line of application by using *Toluene: ethyl acetate: methanol: formic acid* (3:5:2:0.1) as Mobile phase (solvent system). Dried the plate in air and examined under 254 nm, recorded the Rf values. The plates were sprayed with Anisaldehyde- *Sulphuric acid* reagent. Heated the plate at 110 ^{0}C for about 5 minutes and recorded the Rf values. The chromatogram obtained with test solution shows a band at R_f~0.75 corresponding to that of Gallic acid. The chromatogram obtained with powder test solution shows major R_f values under UV-254nm ~ 0.325, 0.625, 0.75, 0.80 and after spraying at ~ 0.075, 0.19, 0.325, 0.625, 0.75. The hydro-alcoholic extract test solution shows major R_f values under UV-254nm at ~ 0.25, 0.275, 0.50, 0.66, 0.75 and after spraying at ~ 0.075, 0.25, 0.50, 0.625, 0.75, 0.90. Water extract test solution shows major R_f values under UV-254nm at ~ 0.325, 0.625, 0.75 and after spraying at ~ 0.075, 0.19, 0.325, 0.625, 0.75 [Figure-5].

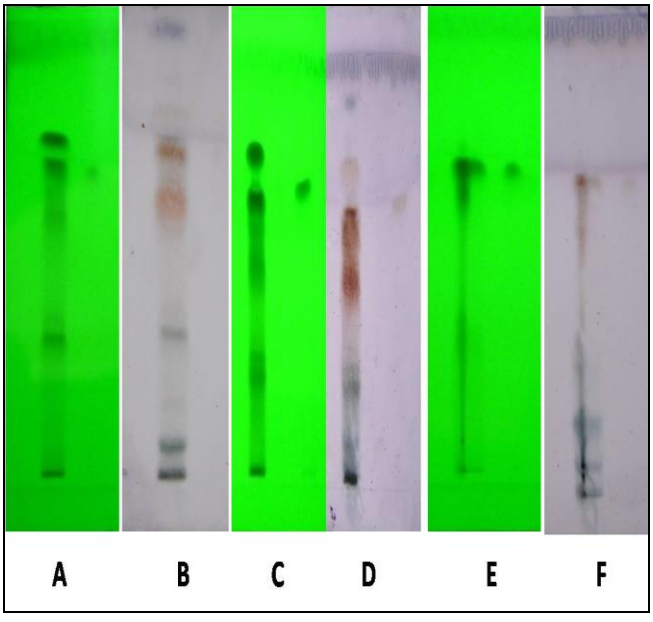


Fig 5: Thin Layer Chromatography of A. *nilotica*-Pods: A, B-Powder extract UV-254nm & after spraying; C, D-Hydro-alcoholic extract UV-254nm & after spraying; E, F-Water extractuv-254 & after spraying

4. Conclusion

Though all parts of *Babbula* are useful for treating different ailments. As per our knowledge pharmacognostical work on pods have not been carried out. This study helps students, researchers and Ayurvedic physicians for correct identification of crude drug both in fresh, dried and in powder form. Further studies are needed for revalidation of the fruits, which will be more beneficial for researchers, Ayurvedic physicians and students.

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