

SELECTION OF BHALLATAKA (*Semecarpus anacardium* Linn.) FRUITS FOR THERAPEUTIC USES – A SCIENTIFIC APPROACH

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Abstract:

Ayurveda emphasizes selection of genuine and quality drugs for therapeutic uses. Selection criteria of the individual herbal drugs differ from drug to drug. In ancient times, Special parameters were followed to fix the grade of the drugs. It is reported that Bhallataka (*Semecarpus anacardium* Linn.) fruits which sink in water, should be selected for the therapeutic uses. The reason behind this logic is not explored scientifically. An attempt has been made to find out the reason for the selection of the sunken Bhallataka fruits pharmacognostically and phytochemically following standard parameters. Macroscopically, the floated fruits were elongated instead of heart shape, laterally flattened, obliquely ovoid and some ruptured. The Rf values were 0.09, 0.17, 0.31, 0.52, 0.66 and 0.06, 0.24, 0.22, 0.60, 0.66 for sunken and floated respectively. Though in both the samples 5 spots were observed the difference in Rf value proves the difference of poly phenols in sunken and floated Bhallataka fruits.

Keywords: Bhallataka; *Semecarpus anacardium*; Floated; Sunken.

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INTRODUCTION

In dravyavinishchaya (Drug evaluation), pharmacognostical parameters act as an important tool. In recent years, the plant material and herbal remedies, derived from them, represent a sustained proportion and has gained place in the health management throughout the globe. Herbal drugs have served the human society from time immemorial in curing various ailments. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.^{[1][2]} Thus the World Health assembly has emphasized the need to ensure the quality of medicinal plant products by using modern techniques and applying suitable standards.^[3]

Ayurveda emphasizes selection of genuine and quality drugs for therapeutic uses. Selection criteria of the individual herbal drugs differ from drug to drug. In ancient times, special parameters were followed to fix the grade of the drugs. It is reported that Bhallataka (*Semecarpus anacardium* Linn.) fruits which sink in water, should be selected for the therapeutic uses.^{[4][5]} The reason behind this logic is not explored scientifically.

Though the drug Bhallataka is well known and used since long, the pharmacognostical characters of Bhallataka fruit particularly transverse section (T.S.) of Bhallataka fruit (*Semecarpus anacardium* Linn.) is not mentioned in Ayurvedic pharmacopeia.^[6] The pharmacognostical study of bhallataka fruits was carried out and reported recently.^[7] To find out the scientific reason, a comparative pharmacognostical and phytochemical study including TLC (Thin Layer Chromatography) of floated and sunken Bhallataka fruits were carried out.

MATERIAL AND METHODS

Matured fruits of *Semecarpus anacardium* Linn. (Anacardiaceae), were collected from the trees growing wildly in Jalna (19°50'N 75°53' E 19.83° N 75.88°E), Maharashtra, India, in the last week of December 2009. The fresh and dried fruits were authenticated and voucher specimen was preserved in the Pharmacognosy department, I.P.G.T. & R.A., GAU, Jamnagar. (Vide no. 6010/2009)

The fruits which sunk in water were separated from the floated fruits and both sunken and floated fruits were dried under shade. (Figure 1)

Procedure:

500 g of raw Bhallataka fruit was taken and put into the glass jar (3 L capacity) containing water. It was stirred well with stainless steel ladle. The fruits which were floated on water were separated by using stainless steel ladle with filter and the fruits which sunk in water were collected separately in a tray. The collected fruits were dried under shade properly and stored in zipped polythene bags for further studies. The same procedures were repeated for three times to standardize the method. (Figure 1) To remove the moisture in it, the Bhallataka fruits were dried properly.

Macroscopical evaluation:

Various parameters of the plant material, such as size, shape and colour of the fruit were recorded.^{[8][9]}

Microscopic evaluation:

Transverse section (T.S.), longitudinal section (L.S.) and powder microscopy of sunken and floated Bhallataka fruits were studied with and without staining. Thin free hand sections of fruit was made and mounted with coconut oil. Powder obtained by scrapping the fruit.

Stained with Phloroglucinol and Conc. HCl and Sudan III for the observation oil globules.^{[10][9]} Microphotographs were taken using Carl Zeiss Binocular Microscope attached with camera.

Analytical methods:

The organoleptical characters like colour and texture of the samples were observed properly.^{[11][9]}

Preparation of extract:

The coarse powder of the fruit was subjected for extraction in methanol and water for 18 hrs and the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yield were calculated. The extracts were used for preliminary phyto-chemical screening with a set of various chemical tests viz., Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics; foam test for saponin glycosides.

The phyto-chemical test of sunken and floated Bhallataka fruits was carried out by standard procedure.^[12]

Physico-chemical analysis:

Loss on drying, ash value, acid insoluble ash, water soluble extractive, methanol soluble extractive of sunken and floated Bhallataka fruits were determined.^[13]

TLC (Thin Layer Chromatography):

TLC was done by the standard methods.^[9] Mobile phase: The solvent system for poly phenols (Toluene : Ethylacetate 9.3 : 0.7) and the solvent system for fats and oils (Benzene : Ethylacetate 6:1) were selected to perform the TLC.^[14]

OBSERVATION AND RESULTS

Macroscopic / Organoleptic characters of floated Bhallataka fruits:

Majority of the fruits were laterally flattened, obliquely ovoid, 2.5-3 cm long, blackish brown in colour and smooth, shining with residual receptacle. Some fruits were elongated, brownish in colour and with ruptured pericarp. Cotyledon was brown or brownish white in colour and 0.7 -1.0 cm long and spoiled by worm infestation. (Table 2) (Figure 2)

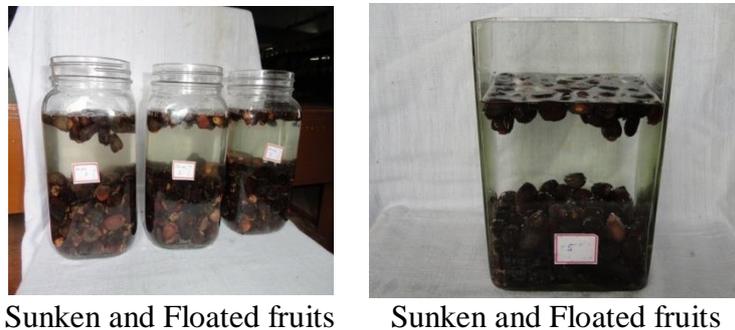
Microscopic characters of floated Bhallataka (T.S):

The fruit is divided into three layers i.e. epicarp, mesocarp and endocarp. There was no difference observed in the epicarp layer. In mesocarp, the lysigenous cavities are bigger in size, the number of papillae and oil globules were less in floated one when compared to sunken fruits. The cotyledons were brownish in colour along with proteins and fixed oil. The brownish colour may be due to the infection of the cotyledon. These are the differences observed in floated Bhallataka when compared with sunken Bhallataka microscopically. (Figure 3)

Powder Microscopic characters of floated Bhallataka:

Diagnostic characters like inner and outer layer of endocarp, trichome, fibres, fixed oil, oil globule, fragment of cotyledon was observed in floated Bhallataka. There was no much difference observed in floated Bhallataka powder microscopy when compared to sunken fruits.

Figure 1: Pharmaceutical procedure of selection sunken Bhallataka fruits



Sunken and Floated fruits

Sunken and Floated fruits

Figure 2: Macroscopical differentiation of sunken and floated Bhallataka fruits



Sunken and Floated fruits



T.S of floated fruits

Powder microscopic characters of floated Bhallataka treated with sudan III:

Oil globule was observed clearly when the powder was treated with sudan 3 + Ethyl alcohol.

Macroscopic characters of sunken Bhallataka fruits:^[17]

Fruit drupaceous, laterally flattened, obliquely ovoid, 2.5 - 3 cm long, blackish brown, smooth, shining with residual receptacle. Cotyledon is white, 1 - 1.5 cm long. (Figure 2)

Microscopic characters of sunken Bhallataka through TS:

The fruit is divided into three layers i.e. epicarp, mesocarp and endocarp.

Epicarp: Epicarp shows epidermis consisting of single layer of elongated cells arranged radially. Epidermis covered with a thin layer of cuticle. **Mesocarp:** Epicarp is followed by mesocarp, consisting of a big zone of parenchymatous cells arranged in numerous layers approximately 40 to 50 layers. Just below the epidermal cells few of layers of parenchymatous cells are big where as few layers of parenchymatous cells below are smaller in size. These are then differentiated into big cavities called lysigenous cavities. The lysigenous cavities are seen with a number of papillae and oil globules. **Endocarp:** Endocarp is differentiated into two layers. The outer layer is small and the inner layer is big. Both layers have same sort of cells. The innermost endocarp layer is formed by very elongated cells arranged radially in thick manner. Below this are the cotyledons, seen with proteins and fixed oil.

Figure 3: Microscopical characters of floated Bhallataka fruits



Fig - 3.1a



Fig - 3.1b

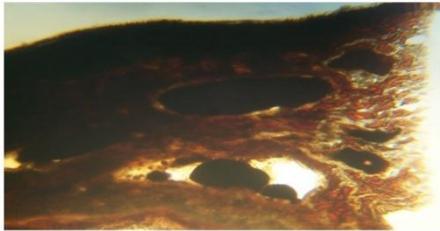


Fig - 3.1c

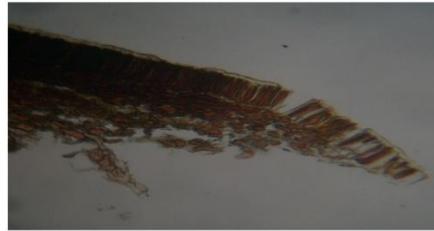


Fig - 3.1d



Fig - 3.1e

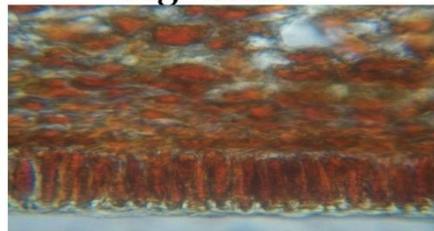


Fig - 3.1f



Fig - 3.1g

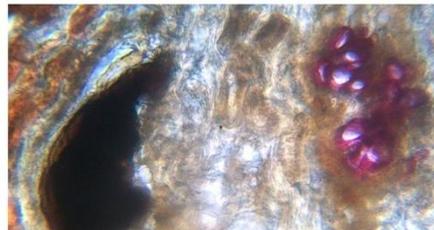


Fig - 3.1h



Fig - 3.1i



Fig - 3.1j

3.1a. Macroscopy of floating fruit; 3.1b. T.S. of fruit 3.5x; 3.1c. Mesocarp cells with fixed oil 10x; 3.1d. Cuticle with mesocarp 10x; 3.1e. Fragment of endocarp cells 10x; 3.1f. Epicarp and mesocarp cells 45x; 3.1g. Fibre 10x; 3.1h. Vascular bundle and fixed oil 45x (lignified); 3.1i. Fragment of cotyledon with oil content 10x (Sudan III treated); 3.1j. Oil globule 45x (Sudan III treated)

Microscopic characters of sunken Bhallataka through LS:

L.S microscopical studies were also carried out and the observed characters were compared with API.^[19] The pericarp was differentiated into epicarp, mesocarp and endocarp layers. Epidermis is covered with thin cuticle. Fixed oil was observed in epidermal cells. Followed by the epidermal cells are closely arranged mesocarp layer; it is broad zone, 30-40 layers thick, composed of parenchymatous cells. Below that are wide lysigenous cavities with numerous papillae. Endocarp consists of two distinct layers, i.e. inner and outer layer of endocarp. Innermost layer is elongated radially, highly thickened but the outer layer is shorter and thinner than innermost layer. Cotyledons below to the endocarp are with proteins and fixed oil.

Powder microscopic characters of sunken Bhallataka:

The diagnostic characters like fragment of epidermis with cuticle, epidermal cells, trichome, and fragment of cotyledon filled with oil globule, starch grains with hilum, endosperm cells, fragment of inner and outer layer of endocarp, oil content are observed in powder microscopy. The characters which are present in mesocarp layers like stone cells, crystals, aleurone grains, papillae, oil globule, fixed oil and starch grains with hilum was observed in powder. Stained fruit powder showed the characters like stone cells, papillae, mesocarpal cells, pitted vessel, annular vessel, fibre, and stone cells, oil globule, fragment of inner and outer layer of endocarp. (Table 1)

Powder microscopic characters of sunken Bhallataka treated with sudan III:

Oil globule was observed clearly when the powder was treated with sudan 3 + Ethyl alcohol.

Histochemical analysis of sunken and floated Bhallataka fruits

Histochemical test showed the presence of the lignified cells, starch grains, Calcium oxalate crystals and tannin content. (Table 3)

When the powder was treated with Phloroglucinol+ Conc. HCl. solution red mejanya colour was observed in sunken Bhallataka as well as all the floated samples. It showed the presence of lignified cells.

When the powder was treated with FeCl₃ solution solution, dark blue to black colour was observed in sunken Bhallataka as well as all the floated samples. It showed the presence of tannin content in the samples.

When the sample treated with Sudan III, oil globules were clearly observed in red colour.

Physico-chemical analysis:

Qualitative tests reveal the presence of tannins, phenolic compounds and flavanoids in both the sunken and floated aqueous extract samples and the presence of steroids in both the samples of sunken and floated fruits methanolic extracts. (Table 4)

Significant changes were observed in the physico-chemical parameters of sunken and floated Bhallataka fruits. (Table 5)

T.L.C of Floated and Sunken Raw Bhallataka:

Differences in R_f value was observed in the methanol extractives by using the mobile phase Toluene : ethyl acetate of sunken and floated fruits. (Table 6)

Table 1: Powder characters of sunken raw Bhallataka

Unstained	Stained with Phloroglucinol + Conc. Hcl
Fragment of Epidermis with cuticle	
Epidermal cells	Stone cells
Trichome	Stone cells covered with oil content
Fragment of cotyledon filled with oil globule	Papillae lignified
Endosperm cells	Papillae base
Fragment of inner and outer layer of endocarp	Simple fibre
oil content	Mesocarp cells
Stone cells	Pitted vessel
Crystals	Annular vessel
Aleurone grains	Lignified Fibre
Oil globule	Oil globule
Papillae	Fragment of inner and outer layer of endocarp
Fixed oil	

Table 2: Macroscopical characters of floated and sunken Bhallataka

Macroscopical Characters	Raw Bhallataka	
	Floated	Sunken
Colour	Brownish colour fruits	Blackish brown
Shape	Elongated instead of heart shape, laterally flattened, obliquely ovoid, Some elongated, ruptured	Heart shaped, laterally flattened, obliquely ovoid
Size	2.5-3 cm long, some varies 2.5-4cm	2.5-3 cm long
Nature	Wrinkled, ruptured	Smooth, shining with residual receptacle
Cotyledon	Brown, brownish white instead of white. Small in size.	White in colour and in size of 1-1.5 cm long.

Table 3: Histochemical analysis of floated and sunken Bhallataka fruits

Reagents	Observation	Characteristics	Float	Sunk
Phloroglucinol + Conc. HCl	Red – mejanta	Lignified cells	+	+
Iodine solution	Blue	Starch grains	+	+
Phloroglucinol + Conc. HCl	Dissolved	Calcium oxalate crystals	+	+
FeCl ₃ solution	Dark blue to black	Tannin content	+	+
Sudan III	Red	Oil globule	+	+

Table 4: Qualitative tests of sunken and floated Bhallataka fruits

Parameters	Aqueous extract		Methanol extract	
	Floated	Sunken	Floated	Sunken
Tannins	+	+	-	-
Alkaloids	-	-	-	-
Steroid	-	-	+	+
Phenolic compounds	+	+	-	-
Flavonoids	+	+	-	-

‘+’ = Presence of the compounds ‘-’ = Absence of the compounds

Table 5: Average physico-chemical parameters of floated and sunken Bhallataka fruits

Parameters	Raw Bhallataka	
	Floated	Sunken
Loss on drying 110°C (% w/w)	6.20	6.29
Ash Value (% w/w)	1.85	1.94
Methanol soluble extractive (% w/w)	41.57	36.00
Water soluble extractive (% w/w)	10.54	09.87

Table 6: Rf values of Floated and Sunken Bhallataka fruits

Sl.No.	Mobile phase	Sunken (T1)	Floated (T2)
1.	Toluene:Ethylacetate 9.3:0.7	0.09, 0.17, 0.31, 0.52, 0.66	0.06, 0.24, 0.22, 0.60, 0.66
2.	Benzene:Ethylacetate 6:1	0.09, 0.18, 0.32, 0.54, 0.72, 0.81	0.09, 0.18, 0.32, 0.54, 0.72, 0.81

Figure 4: T.L.C of Floated and Sunken methanolic extract of Bhallataka fruits

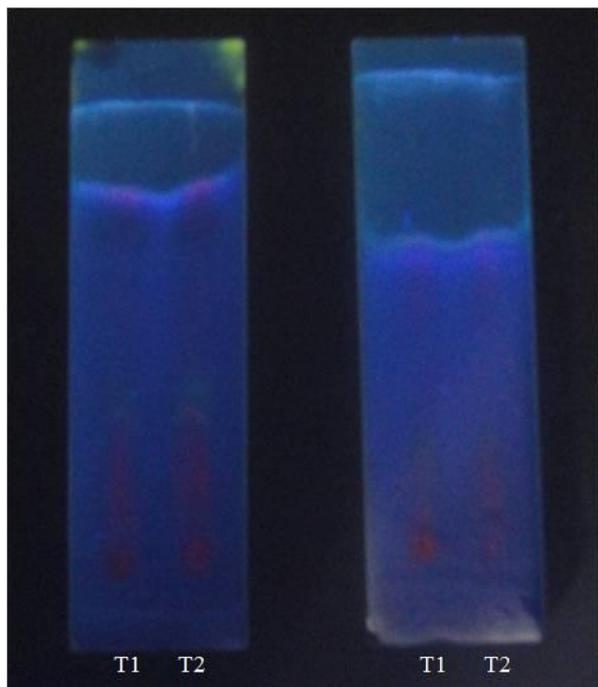


Fig. 4.1

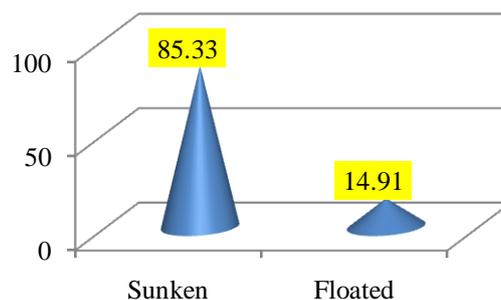
Fig. 4.2

T1 – Sunken Bhallataka fruit methanol extractive; T2 – Floated Bhallataka fruit methanol extractive; Fig.4.1. Mobile phase of Toluene : Ethyl acetate; Fig 4.2. Mobile phase of Benzene : Ethyl acetate

DISCUSSION

According to Ayurveda, the Bhallataka fruits which sink in water should be used for treatment purposes.^[4] In the present study, the experiment was repeated for three times to standardize the method. (Figure 1) There was marked difference observed between the sunken and floated Bhallataka fruits by weight, colour, shape, texture etc. (Table 2) The result showed 14.91% of floated and 85.33% of sunken Bhallataka. (Graph 1) The change in shape, irregular and big sized lysigenous cavities may be the reason for the floating nature of the fruits over the water.

Graph 1: Average weight of sunken and floated Bhallataka fruits



Physico-chemically differences were observed in sunken and floated fruits. Methanol soluble and water soluble extractive was more in floated Bhallataka fruits than the sunken one. (Table 5) The differences may be due to some chemical variations in between the sunken and floated fruits. TLC was carried out by using two solvent system for poly phenols [Toluene:Ethylacetate (9.3:0.7)] and fats, oils [Benzene:Ethylacetate (6:1)].^[14] Differences in Rf value was observed in the methanol extractives by using the mobile phase Toluene : ethyl acetate of sunken and floated fruits. The Rf values were 0.09, 0.17, 0.31, 0.52, 0.66 and 0.06, 0.24, 0.22, 0.60, 0.66 for sunken and floated respectively. Though in both the samples 5 spots were observed the difference in Rf value proves the difference of poly phenols in sunken and floated Bhallataka fruits. But there was no change of Rf values observed by using the Benzene:Ethyl acetate which is for fat and oils. (Table 6)

Qualitative tests with aqueous extracts showed the presence of tannins, phenolic compounds and flavanoids. With methanolic extract the presence of steroids was observed. The physico-chemical parameters of sunken and floated samples showed no much remarkable difference except in the methanol soluble

extractive. It was 36.00 (% w/w) and 41.57 (% w/w) for sunken and floated fruits respectively.

CONCLUSION

Floated Bhallataka fruits can be differentiated from sunken ones by its ruptured and elongated shape, smaller size, and presence of wide space in the mesocarp layer and brown or brownish white colour of the cotyledon. Differences in Rf values especially for poly phenols and the physico-chemical parameters proved the chemical changes in sunken and floated Bhallataka fruits. This may be the reason that Sunken Bhallataka fruits were selected for therapeutic uses in the system of Ayurveda. Further studies have to be carried out on specific changes in the chemical profile of sunken and floated Bhallataka fruits.

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