IMPACT OF AYURVEDIC SHODHANA (PURIFICATORY PROCEDURES) ON BHALLATAKA FRUITS (SEMECARPUS ANACARDIUM LINN.) BY MEASURING THE ANACARDOL CONTENT

Ilanchezhian R1*, Acharya R N2, Roshy Joseph C3, Shukla V J4

1Associate Professor, Dept. of Dravyaguna, A.L.N.Rao Memorial Ayurvedic Medical College & PG centre, Koppa – 577 126, Karnataka.
2Associate Professor, Dept. of Dravyaguna, Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar - 361 008, Gujarat.
3Lecturer, Dept. of Rasashastra & Bhaishajya Kalpana, Govt. Ayurveda Medical College, Nagercoil – 629 001, Tamil Nadu.
4Head, Pharmaceutical chemistry laboratory, Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar - 361 008, Gujarat.
*Corresponding Author: E-Mail: ayurilan@yahoo.com

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ABSTRACT

Bhallataka (Semecarpus anacardium Linn.) is reported under upavisha dravya (semi poisonous drugs), in classical Ayurvedic pharmacopoeias. It is advocated that shodhana (Purificatory procedures) of the fruits should be carried out before its internal administration. Though there are different shodhana methods mentioned in Ayurveda, Ayurvedic Pharmacopoeia of India (API) recommends only one method for the shodhana of Bhallataka fruits. In this study, cow’s urine, cow’s milk and brick powder, were used as media. The impact of shodhana was evaluated by pharmaceautical, physico-chemical and chromatographical parameters. Rf values of methanolic extract of processed bhallataka fruits shows the difference when compared to the raw bhallataka fruits, this clearly proves the chemical changes during shodhana. Increased level of anacardol was observed in Shodhita (processed) fruits in comparison to the raw fruits.

KEYWORDS: Shodhana; Bhallataka; Semecarpus anacardium; Anacardol, Ayurvedic Pharmacopoeia of India

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INTRODUCTION

Bhallataka (Semecarpus anacardium Linn.; Anacardiaceae) fruit is one of the upavisha dravya (semi poisonous drugs). Its importance and utility is increasing day by day because of its therapeutic significance in many diseases including cancer. Though the fruits of Bhallataka has many therapeutic values, pharmacies are scared to use this drug because of its irritant vesicating nature. The fruit contains tarry oil which causes contact dermatitis. Medically it is named as Urushiol Induced Contact Dermatitis because the chemical Urushiol is responsible for the dermatitis. If this vesicant nature is removed, the drug could be a good source for pharmaceutical industries in manufacturing many formulations containing Bhallataka as an ingredient.

Ayurveda advocates bhallataka after shodhana (purificatory procedures). Though there are different shodhana methods mentioned in Ayurveda, the shodhana method mentioned in the text Rasamrutam was adopted and quoted in API (Ayurvedic Pharmacopoeia of India). The procedure is soaking the fruits in cow’s urine, cow’s milk and rubbing it in brick powder. Shodhana is the purificatory measure used in Ayurveda to purify toxic medicinal plants (upavisha dravyas), by various pharmaceutical procedures like soaking, rubbing and washing etc. with specific medias like gomutra (cow’s urine), godugdha (cow’s milk) etc. Physico-chemical changes and reduction of the toxic chemicals from the poisonous plants like strychnine, brucine in kupilu and scopolamine in dhattura are reported already. Recent studies proved the changes of Rf values in shodhita samples of bhallataka when compared to raw bhallataka. To prove the impact of the Ayurvedic shodhana methods, recommended by API, in this research work, an attempt has been made to analyse the raw and shodhita (purified) samples pharmacologically and analytically including HPTLC.

MATERIALS AND METHODS

Collection & Selection of drug:

Matured fruits of Semecarpus anacardium Linn., were collected from the trees growing wildly in Jalna (19°50′N 75°53′ E 19.83° N 75.88°E), Maharashtra, India. The fruits were authenticated and voucher specimen was preserved in the department (Vide no. 6010/2009). The fruits were sun dried for 10 days. Then the fruits were cleaned by removing the false fruit and stored in a container. Bhallataka fruits, which sunk in water, were recommended for therapeutic purposes. So fruits which sunk in water were collected and dried properly and used for research purpose. The dried fruits were mixed thoroughly and sample was selected randomly.

Collection of the media:

Fresh cow’s urine was collected from the Goshala (cow shed) early in the morning. Amul brand cow’s milk was used for the shodhana. Ishtika choorna (brick powder) was collected from the local area.

Equipments for Shodhana:

Stainless steel vessel (10 litre capacity), Stainless steel spatula (30 cm long), Stainless steel filter, thick cotton cloth were used for processing of the nuts.

Pharmaceutical study:

200 g of sunken Bhallataka fruits, in water, were randomly taken. The thalamus portion of the fruits was removed with the help of a steel cutter. Then it was taken in a vessel containing gomutra (cow’s urine) and kept for seven days. Every day the fruits were taken out of the media and washed with water and fresh gomutra was used. On eighth day Bhallataka was washed and shifted to the vessel containing godugdha (cow’s milk) and kept for seven days. Each day it was washed with water and fresh Godugha was added. On 15th day the samples were taken out of the media and washed with water then shifted to a bag
containing brick powder and rubbed thoroughly. It was allowed for three days in the bag containing brick powder. On 18th day it was washed thoroughly with hot water to remove the brick powder in the sample. Later, the samples were dried properly to remove the moisture and stored in air tight glass container for further studies.

The same shodhana procedure was repeated thrice to standardize the procedure pharmaceutically.

**Physico-chemical evaluation:**

Physico-chemical evaluations like moisture content, ash values, acid insoluble ash, alcohol soluble extractive and water soluble extractive values were determined. The determinations were performed in triplicate and results are expressed as mean. The percentage w/w values were calculated with reference to the air-dried drug.\(^{10,11}\)

**Preliminary phytochemical screening:**

The coarse powder of the fruit was subjected for extraction in methanol and water for 18 h and the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yield were calculated. The extracts were used for preliminary phytochemical 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. These parameters were carried out by following the standard procedure.\(^{12}\)

**Equipments used for HPTLC**

Pre coated silica gel GF 60254 aluminium plates as 5 mm bands, 5 mm apart and 1 cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. The mobile phase used was Benzene: Ethyl acetate \((6 : 1)\). The plates were developed in Camag twin trough chamber \((20 \times 10 \text{ cm}^2)\) and spots were detected in short U.V. \((254 \text{ nm})\), Long U.V \((366 \text{ nm})\). Camag Scanner II \((\text{Ver. 3.14})\) and Cats soft ware \((\text{Ver. 3.17})\) were used for documentation.

**Preparation of raw and shodhita sample solutions:**

Methanol extractives were prepared by standard method. The concentrated methanol extracts were used as test solutions. 10 mg of extract was accurately weighed and dissolved in methanol in standard flask and final volumes were adjusted to 10 ml with methanol \((1 \mu g/\mu l)\). 5 µl of each test solutions were spotted along with 5 µl standard solutions of anacardol. The plates were developed in mobile phase of Benzene : Ethyl acetate \((6 : 1 \text{ v/v})\) and scanned at 254 nm.

**Application of sample:**

The sample solutions 5µg was applied on TLC plates \((10 \text{ cm} \times 10 \text{ cm})\), precoated with silica gel as 5 mm, 5 mm apart and 1 cm from the edge of the plates by using CAMAG Linomat V sample applicator. The mobile phase used was Benzene : Ethyl acetate \((6 : 1)\). The plates were developed in Camag twin trough chamber \((20 \times 10 \text{ cm}^2)\) up to a distance of 77 mm at a temperature of \(30 \pm 2^\circ\text{C}\). and spots were detected in short U.V. \((254 \text{ nm})\), Long U.V \((366 \text{ nm})\). Camag Scanner II \((\text{Ver. 3.14})\) and Cats soft ware \((\text{Ver. 3.17})\) were used for documentation.

**RESULTS**

**Pharmaceutical study:**

200 g of samples were taken for Shodhana. Three batches of Shodhana were carried out. The average loss of the sample after shodhana was 17.32%. \((\text{Table 1}; \text{Figure 1})\)

**Analytical study:**

In analytical study the parameters like physico-chemical analysis, qualitative tests of raw and shodhita Bhallataka was carried out and systematically presented in Table 2 and Table 3 respectively. Chromatography studies
were carried out and the Rf values observed under 254 nm has been presented in Table 4. Anacardol in raw *bhallataka* was 47.51% and 50.62% in processed. (Figure 2)

Table 1: Pharmaceutical study of *Bhallataka* fruit:

<table>
<thead>
<tr>
<th>Batch</th>
<th>Weight of the <em>Bhallataka</em> fruit (g)</th>
<th>% of the <em>Bhallataka</em></th>
<th>Average loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Obtained</td>
<td>Loss/ gain</td>
</tr>
<tr>
<td>SBMDI – 1</td>
<td>200</td>
<td>155.6</td>
<td>44.4</td>
</tr>
<tr>
<td>SBMDI – 2</td>
<td>200</td>
<td>167.7</td>
<td>32.3</td>
</tr>
<tr>
<td>SBMDI – 3</td>
<td>200</td>
<td>172.8</td>
<td>27.2</td>
</tr>
</tbody>
</table>

Table 2: Showing physico-chemical parameters of raw and *shodhita Bhallataka* fruit:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw</th>
<th>Shodhita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying 110°C (% w/w)</td>
<td>6.68</td>
<td>8.14</td>
</tr>
<tr>
<td>Ash Value (% w/w)</td>
<td>2.68</td>
<td>5.83</td>
</tr>
<tr>
<td>Acid insoluble ash (% w/w)</td>
<td>–</td>
<td>3.34</td>
</tr>
<tr>
<td>Methanol soluble extractive (% w/w)</td>
<td>35.40</td>
<td>28.71</td>
</tr>
<tr>
<td>Water soluble extractive (% w/w)</td>
<td>6.99</td>
<td>4.23</td>
</tr>
</tbody>
</table>

Table 3: Showing qualitative tests of raw and *shodhita bhallataka* fruit:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aqueous extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steroid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

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

Table 4 - Showing Rf values of raw and *shodhita Bhallataka* fruit at 254 nm

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extractive</th>
<th>Solvent system</th>
<th>Viewing reagent</th>
<th>Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Raw</td>
</tr>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>Benzene:Ethyl acetate</td>
<td>Iodine vapour</td>
<td>0.17, 0.28, 0.37, 0.42, 0.50</td>
</tr>
</tbody>
</table>
Fig – 1a. Thalamus removed Bhallataka fruits soaked in cow’s urine; Fig – 1b. Bhallataka fruits soaked in cow’s milk; Fig – 1c. Fruits mixed with ishtika choorna; Fig – 1d. Bhallataka tied in pottali; Fig – 1e. Fruits separated from ishtika choorna and washed with hot water; Fig – 1f. Fruits washed thrice; Fig – 1g. Drying of shodhita fruits; Fig – 1h. Dried Shodhita fruits stored in airtight glass container.
T1 – Raw Bhallataka; T2 – Shodhita Bhallataka; T3 - Anacardol
Fig – 2a. Viewed under long UV; Fig – 2b. Viewed under short UV; Fig – 2c. Viewed after iodine vapour spray; Fig – 2d. Densitograph; Fig – 2e. Calibration curve
DISCUSSION

Urushiol induced contact dermatitis is a clinical condition caused by the contact of urushiol on skin. Urushiol is present in most of the species in Anacardiaceae, and one of the major chemical constituent of Bhallataka. Bhallataka is a drug commonly known for its blister causing nature. In Ayurvedic literature, the synonym Sopha hetu, Spota hetu, agnika are given to this drug based on its blister causing nature. The oil in the fruit is responsible for the irritation. The bhilatako fruit contains 90% Anacardic acid and 10% of Cardol. Other chemical constituents are bhilawanol (Naidu et al., 1925), semecarpol and anacardol. Recent studies reported that bhilawanols are known as urushiols. Anacardic acids are closely related to urushiol. Another study reported that the corrosive juice from the pericarp of the fruit is found to contain catechol, fixed oil and anacardol (C₁₅H₁₃O₃.COOH) to which the corrosive properties of the juice are due to two phenolic acids C₁₆H₁₅O₃.COOH and C₁₄H₁₃O₃.COOH. Three batches of bhallataka were processed by the shodhana method mentioned in API. Weight loss was observed in all the three samples (Table 1). This loss may be due to the reduction of the oil content in the fruits. The media gomutra (cow’s urine) is reported for its antimicrobial, antibacterial etc. Cow’s milk is recommended as one of the antidote for bhallatako blisters. Brick powder is having adsorbing property; by which it absorbs irritant oil in the fruit. The methanol soluble extractive was 35.40% w/w in raw Bhallatako and 28.71% w/w in shodhita bhallatako. The difference between raw and shodhita is 6.69% w/w. It reveals that after shodhana the methanol soluble extractives are reducing. The ash value of processed nut was more when compared to raw. Ash value of raw bhallatako and shodhita was 2.68% w/w and 5.83% w/w respectively. The increase in ash value may be due to the residue of the brick powder. The ash value represents the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of the drug. Qualitative test showed no variation in raw and shodhita sample. In HPTLC study, the RF values were observed under 254 nm. In 254 nm, raw Bhallatako showed five spots at the rf 0.17, 0.28, 0.37, 0.42, 0.50 but the shodhita bhallatako showed only three spots at the rf 0.17, 0.30, 0.47. The chemical anacardol was compared with both raw and shodhita sample. The anacardol was present in both the samples but the quantity was increased in shodhita sample. Research studies reported that S. anacardium fruit contains 90% of oxy acid i.e. anacardic acid and 10% of cardol. The corrosive juice from the pericarp of the fruit found to contain catechol, fixed oil and anacardol (C₁₈H₁₅O₃.COOH) to which the corrosive properties of the juice are due to two phenolic acids C₁₆H₁₅O₃.COOH and C₁₄H₁₃O₃.COOH. In Bhallatako bhilawanols and anacardic acids are the main chemical constituent responsible for the blisters. Bhilawanol is known as Urushiol and the anacardic acids are closely related to Urushiol. Due to the decorboxylation of the oil, the anacardic acid gets converted into less toxic anacardol. Decorboxylation process may start right from cutting the fruit itself and will be catalyzed by giving heat/fire treatment. The increased level of anacardol in the shoditha bhallatako may be due to the decorboxylation of the anacardic acid in the fruits. More percentage of oil might have got reduced by soaking the fruits in the gomutra and godugda. The brick powder is having the adsorbing nature, so some percentage of oil may be absorbed by the brick powder. There are probable chances that some chemical changes might have taken place due to the various Medias like gomutra, godugda etc used for its purification. Further studies should be carried out to find out the chemical interactions between the media and the bhallatako fruits during shodhana procedure.

CONCLUSION

Shodhana (purificatory procedure) increases the anacardol level in shodhita bhallatako fruit samples. More percentage of
the anacardol was due to the conversion of toxic urushiol into Anacardol.

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