

# Investigation on Chemical Changes Occur During Purification (Shodhana) of Bhallataka by HPTLC

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**Abstract**—Ayurveda is a highly progressed and organized system of life and health science based on its own unique original concepts and fundamental principle. Bhallataka is one of the most powerful and fast acting Ayurvedic herb. Bhallataka mentioned under the list of Poisonous substances. Ayurveda advocates Ballataka after shodhana. Bhallataka must be purified before administrating to patients. There are different purification methods (Shodhana) mentioned in Ayurveda. The change that takes place during the Shodhana process can be explored by modern analytical methods. Among the modern Analytical tools HPTLC is a powerful analytical method.<sup>9, 11</sup> Shodhana process certainly makes changes in the chemical composition of the Bhallataka, So that the toxic and allergic components either eliminated or their quantitative presence is reduced. Bhallataka extract (Before and after purification) subjected to HPTLC study for measuring its quantitative presence in all the extracts, it reveals that after purification quantitatively it has been lost from extract. The results stated here certainly indicate the variation of chemical profile of Bhallataka.

**Keywords**—Bhallataka, Shodhana, HPTLC.

## I. INTRODUCTION

Ayurveda is a highly progressed and organized system of life and health science based on its own unique original concepts and fundamental principle. In Ayurveda almost all medicinal preparations are derived from plants. Herbs and plants are not only effective or valuable for their active ingredients but also for their minerals, vitamins, volatile oils, glycosides, alkaloids, acids, alcohols, esters etc.<sup>1</sup> Ayurveda emphasises selection of genuine and quality drugs for therapeutic uses. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides economical, effective and their easy availability,<sup>2</sup> so to ensure quality of medicinal plant products by using modern techniques is become a need.<sup>2</sup>

In Ayurveda and siddha medicinal systems most of the preparations are based on plants, Bhallataka is one of the most powerful and fast acting Ayurvedic herb.<sup>3</sup> Bhallataka is identified botanically as *Semecarpus anacardium* Linn. comes under the family Anacardiaceae. It is distributed in the sub Himalayan region, tropical and central part of India.<sup>1, 3, 5</sup> It has been freely used all over India from centuries. In Ayurveda it is also known as 'Ardha-Vaidya'.<sup>3</sup> Bhallataka means sharp like spear. Bhallataka or marking nut is known to world since ancient times<sup>4</sup> where fruits, gum, nut, stems, oil is used for their medicinal properties<sup>3, 4</sup> such as alleviates skin diseases, fever, asthma, also acts as antitumour, antiallergic, antineoplastic, cardiac depressant, antioxidant, analgesic, anticancer. Maharashi Charaka has categorized Bhallataka as an appetizer, accumulation breaking herb, anti-diuretic and anti dermatosis, it is drug of choice in the treatment of piles of vata and kapha types.<sup>1</sup> It is classified in Ayurveda under the category of toxic plants,<sup>4</sup> it contain chemical constituents such as phenols minerals vitamins, flavonoids, fixed oils, bilwanoals, semecarpol, semecarpetine, urushenol, anacardol, oleic acid, linoleic acid stearic acid. The fruits contain tarry oil

which causes contact dermatitis. They are always associated with several side effects, if used unpurified.

It is stated that, Bhallataka is one of the Upavishadravya (Semi poisonous drug).<sup>6</sup> Upavisha are the group of drugs which were less toxic in nature and not so lethal but produce certain toxic symptoms on consumption or administration. The symptoms produced in the body due to Upavisha are less toxic, less severe, usually not life threatening and their toxicity can be controlled by therapeutic measures.<sup>7</sup> Bhallataka mentioned under the list of Poisonous substances under the Ayurvedic including Siddha, Unani Systems of Medicines, Ayurveda advocates Ballataka after shodhana. Bhallataka must be purified before administrating to patients.

There are different purification methods (Shodhana) mentioned in Ayurveda, The Shodhana process described in various Ayurvedic Classics is not simply a process of separation or detoxification rather it increases the therapeutic efficacy of the drug and also the changes takes place in the purified drugs, which may be beneficial for therapeutic purposes,<sup>7</sup> Shodhana is a process of separation by which physical and chemical impurities get separated from the substances by treatment with various drugs.<sup>7</sup> Various Media are used for purification process (Shodhana) specific media is used for Shodhana of particular substances. The media used for Shodhana also play a crucial role in either breaking down or transforming the toxic chemical constituents in to their relatively nontoxic derivatives. The change that takes place during the Shodhana process can be explored by modern analytical methods. Among the modern Analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks.<sup>9, 11</sup>

## II. OBJECTIVES

- i. To explore various purification methods of Bhallataka mentioned in Traditional system of medicine.

- ii. To study the chemical changes occur during purification process of Bhallataka.
- iii. To provide a scientific background to the traditional knowledge

### III. MATERIALS AND METHODS

#### PART 1

##### Collection of Plant Material and Authentication

The fresh nuts of Bhallataka (*Semecarpus anacardium* Linn.) were collected from Manakarnika Aushadhalaya (Center for Quality Ayurvedic Medicines) Pune (Maharashtra). The Authentication of nuts was done from Department of Botany of Yashwantrao Chawan College of Sciences Satara (Maharashtra). The nuts were sundried for 3 days and cleaned by removing foreign matter and stored in air tight container.

##### Extraction of Bhallataka<sup>14</sup>

Crude nut extract was prepared by soxhlet extraction method. The shade dried nuts of plant were crushed in moter pestle and 20 gm powder of Bhallataka nuts were extracted with 400 ml methanol. This process of extraction continues till the solvent in siphon tube of an extractor is become colourless (about 8 days). The excess solvent was evaporated to dryness. The extract left over which is oily and black in colour and stored in sterile container.



Fig. 1. Extraction of Bhallataka before purification.

#### PART 2

##### Preparation of methanolic extract after Shodhana process of Bhallataka<sup>14</sup>.

Purification of Bhallataka was carried out by three methods which were mentioned in Ayurvedic books. The purification was done by using cow's urine, brick powder and coconut water as purification media. Following are the procedures which were used for purification of Bhallataka.

##### Method 1- Purification by using Gomutra.<sup>18</sup>

200gm of Bhallataka were taken that Bhatllataka was kept in a pottali which was made up of 3-4 folds of cotton cloth and that pottali was placed in dolyayantra. The vessel (Dolyayantra) which was filled up with solvent gomutra (cow urine) and boiled for 3 hours (one prahara). Then it was washed with hot water and cut in two pieces vertically. Kernel of fruit was removed and fruits were powdered for further use.



Fig. 2. Bhallataka kept in dolyayantra with solvent cow urine.



Fig. 3. Dolyayantra heating for one prahar.

##### Method 2 –Purification by using Brick powder.<sup>19</sup>

200 gm of Bhallataka was cut and then it was kept inishtikachurna (brick powder) for 7 days in a pottali. After 7 days the pottali was rubbed by hand by applying moderate pressure. Then wash the nuts with hot water, and the nuts were boiled in godugdha (cow milk).



Fig. 4. Bhallataka kept in brick powder.



Fig. 5. Pottali kept for 7 days.



Fig. 6. After 7 day nuts were boiled in cow milk.

**Method 3- Purification by using Coconut water.<sup>18</sup>**

200 gm of Bhallataka nuts were cut and kept in pottali, that pottali was placed in a Dolyayantra containing green coconut water. This Dolyayantra was heated for three hours (one prahar) to getshuddhaBhallataka.



Fig. 7. Bhallataka kept in dolyayantra with solvent coconut water.

**Dolyayantra (Swing Appratus)<sup>20</sup>**

Dolyayantra is used for the purpose of swedana. it is an earthen pot and two holes are made on both sides of the neck of vessel in which a rod is put. The vessel is filled half with

the required liquid. And the drug (to be processed) kept in pottali (cloth pouch) is bound with strings to the rod, so that pottali may stay swinging in the liquid. Then the vessel is subjected for slow heating. This apparatus is known as dolyayantra that is swing apparatus.



Fig. 8. Dolyayantra.

**Preparation of Extract After purification of Bhallataka**

After carrying out purification (Shodhana) process by using 3 different methods, the Bhallataka nuts so obtained were subjected to extraction with soxhlet apparatus. The extraction was carried out separately for each method purified Bhallataka



Fig. 9. Extracts of Bhallataka before and after purification.

The Fraction of column chromatography of all the extracts of Bhallataka before and after purification (sample BP, G,B, C) were further analysed by HPTLC analysis

**High Performance Thin Layer Chromatography of Eluent/Fraction from raw and Purified Bhallataka<sup>9,10</sup>**

HPTLC analysis of all the extracts of Bhallataka viz. (BP, G, B, C) was carried out. CAMAG was used for the analysis. All the extracts of Bhllataka were spotted in the form of band of width of 6 mm with space between bands of 8 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany)

using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 6 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using n-hexane:ethylacetate:methanol:galceial acetic acid (6:3.5:1:0.5 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 8 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 250 nm operated by WINCATS software version 1.4.2.

**Preparation of Sample Solutions:**

All samples were diluted with methanol and 2 µl volume was applied

**IV. RESULTS**

**HPTLC analysis**

The results from HPTLC scanned at wavelength 250 nm for extract of before and after purification of Bhallataka.

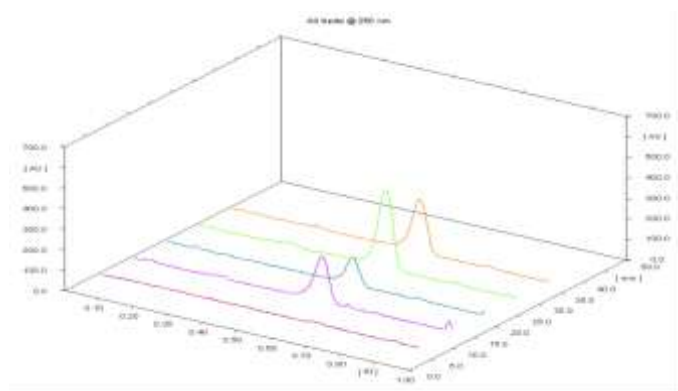


Fig. 10. 3d graph of G, B, BP and C.

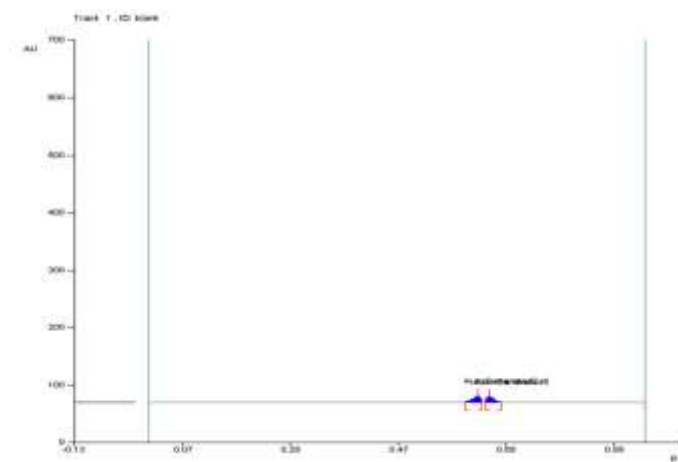


Fig. 11. Blank.

**HPTLC of extract of Bhallataka before purification**

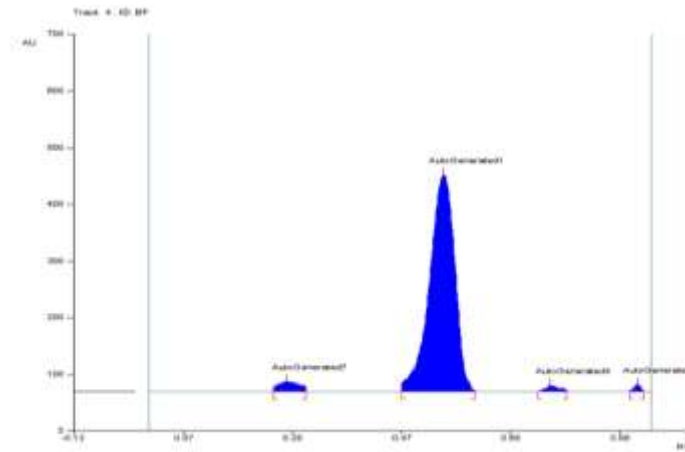


Fig. 12. HPTLC of sample BP.

**HPTLC of different extracts of Bhallataka after purification**

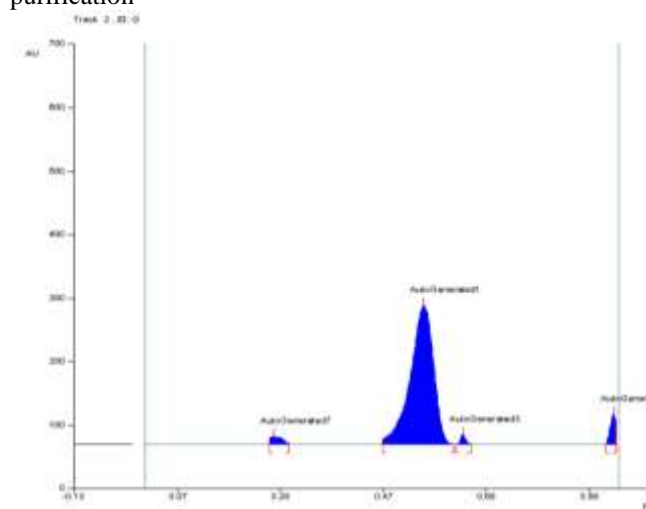


Fig. 13. HPTLC of sample GUV.

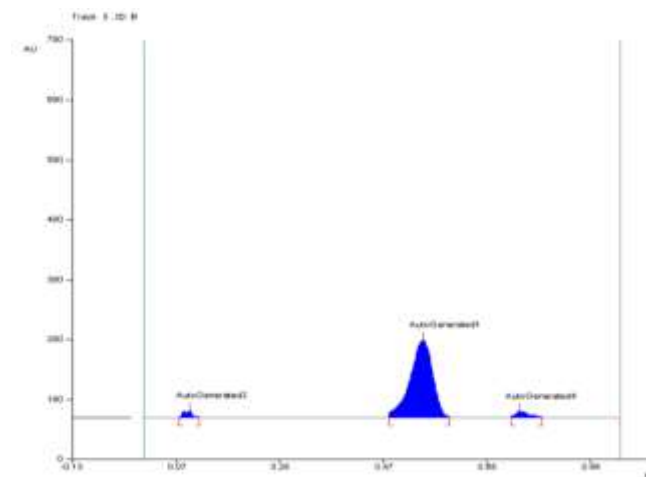


Fig. 14. HPTLC of sample B.



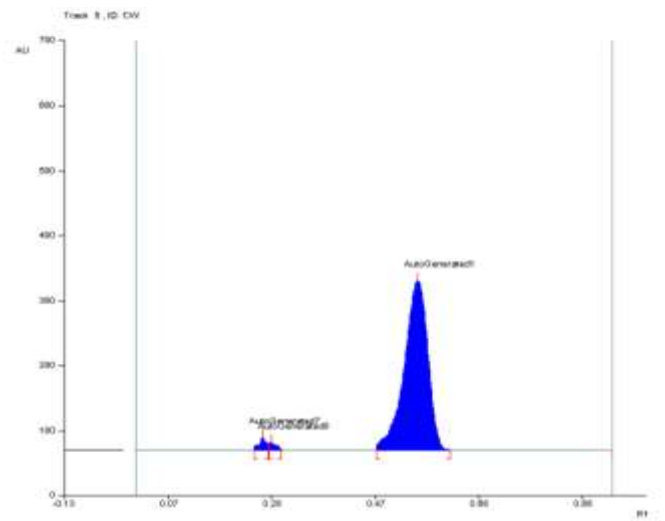


Fig. 15. HPTLC of sample C.

TABLE I. Rf values of Bhallataka before and after purification.

Sample	Rf Values	Area
BP	0.55	15676.6
G	0.56	8731.8
B	0.55	4978.2
C	0.56	10588.7

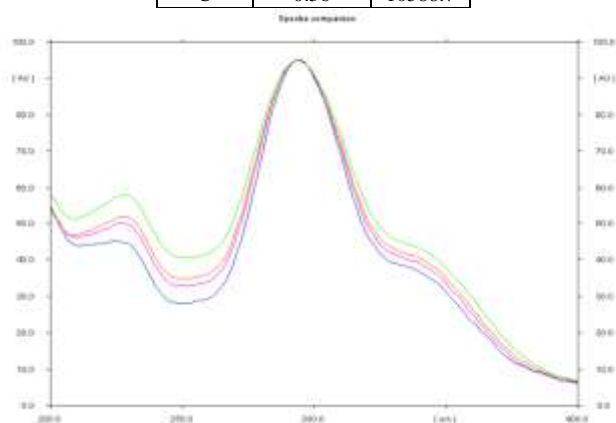


Fig. 16. Overlaid UV spectrum of component at Rf 0.56 of B, G, BP, C.



Fig. 17. Developed plate at visible light Fig. 18. Developed plate at 254 nm



Fig. 19. Developed plate at 366 nm.

### V. CONCLUSION

Bhallataka is very important Ayurvedic drug and used for various kind of health issues since a long period. The drug is also known for its toxic effect on skin and allergy produced by it. The drug should be used with certain precautions to get optimum benefits from it.

Shodhana is a very important process before the actual use of Bhallataka in the medicine. Shodhana process has been carried out by using different medias which are mentioned in Ayurvedic texts. Shodhana process certainly makes changes in the chemical composition of the Bhallataka, So that the toxic and allergic components either eliminated or their quantitative presence is reduced.

The results stated here certainly indicate the variation of chemical profile of Bhallataka. Bhallataka extract (Before and after purification) subjected to HPTLC study for measuring its quantitative presence in all the extracts, it reveals that after purification quantitatively it has been lost from extract. The area under curve shows clear difference in the quantity of unknown component of Bhallataka.

The study also reveals that, Brick powder could be best media for Shodhana process of Bhallataka. This fact could be confirmed by the significant results obtained by Brick powder method compared with other methods.

In the nut shell, Bhallataloosesit's chemicals during shodhana process and further study required to check the quantitative determination of each component before and after Shodhana process.

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