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ANALYTICAL EVALUATION OF BHALLATAKA SHODHANA USING TWO METHODS AND THEIR SIGNIFICANCE.

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ABSTRACT:

Introduction: *Bhallataka* (*Semecarpus anacardium* Linn.) is first classified in the text named Rasopnishata under *Upavisha* group and is also listed in Schedule E1 drug in the Drugs and Cosmetics Rules 1945. Due to its toxic properties, *Bhallataka* should only be used after undergoing proper purification. Various classical texts outline different methods for purifying *Bhallataka* to ensure its safe and effective use in medical treatments. **Methods and Materials:**In this study, two methods for the purification of *Bhallataka* fruits were explored. Sample 1 was *Ashuddha Bhallataka*, while sample 2 was purified *Bhallataka* fruits using coconut water, as described in the Rasa Tarangini and sample 3 underwent purification with cow urine, cow milk, and brick powder, following the Rasamrita method. A comparative analytical study was conducted on all three samples, evaluating various parameters such as organoleptic characteristics, physicochemical properties, phytochemical composition, total polyphenol, and GC-MS (Gas Chromatography-Mass Spectrometry) analysis. **Result:** Three samples of *Bhallataka* were analyzed for their physicochemical and phytochemical properties. The total polyphenol content was highest in sample 1 (3.41 %), followed by sample 2 (2.70 %) and sample 3 (2.57 %), indicating a decrease in phenolic compounds after purification. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, and glycosides in all samples, with variations in the intensity of certain compounds. GC-MS analysis identified 20 distinct compounds in each sample, with sample 1 exhibiting 5 spectra, sample 2 showing 4 spectra, and sample 3 displaying 5 spectra, each with varying retention times. **Discussion:** Purification significantly impacts *Bhallataka's* properties. Sample 1, the unpurified form, had the highest total polyphenol content, while sample 2 and sample 3, after purification, showed lower levels of phenols. GC-MS analysis revealed distinct chemical profiles for each sample, confirming that purification alters the composition of *Bhallataka*.

Keywords: *Bhallataka, Shodhana*, Physico-chemical Analysis, Phytochemical Analysis, GC-MS

INTRODUCTION:

Bhallataka (Semecarpus anacardium Linn.) was first classified in the text named Rasopnishata (9-11th century) under *Upavisha* group [1] and also listed in Schedule E1 drug in the Drugs and Cosmetics Rules 1945. It is one of such drugs, widely described in almost all Ayurvedic classics as *Rasayana* and Used for various therapeutic purposes such as *Kushtha, Arsha, Krimi, Prameha,* etc. It has *Laghu, Tikshna Guna* and *Ushna Veerya* [2] which is quite like poison. The oil in the fruit is responsible for the irritation [3]. *Bhallataka* fruits contain 90 % Anacardic acid and 10 % of Cardol. Other chemical constituents are bhilawanol (Naidu et al., 1925) [4], semecarpol [5] and anacardol [6]. Recent studies reported that bhilawanols are known as urushiols and anacardic acids was closely related to urushiol. There are many documented cases of accidental topical poisoning of *Bhallataka*.[7] Hence to avoid these complications, *Bhallataka* should always be used as medicine after doing proper purification. *Shodhana* was a specialized purification process in Ayurveda used to detoxify medicinal plants known as *Upavisha* to remove or neutralize harmful properties. This practice highlights the critical role of purification before use in Ayurvedic medicine as an ingredient to ensure that the final products are safe and effective for use.

Different principles for *Bhallataka Shodhana* like *Swedana* (boiling), *Nimmajan* (soaking) and *Gharshana* (rubbing) have been mentioned in Ayurvedic classics by using various *Shodhana* media such as *Ishtika Churna* (brick powder), *Narikela Udaka* (coconut water), *Gomutra* (cow's urine), *Godugdha* (cow's milk), *Dadhi* (curd) and *Ushna Jala* (warm water). Some research works have been conducted comparing different methods of *Bhallataka Shodhana* and their analytical evaluation. *Gomutra* (cow's urine) is noted for its antimicrobial and antibacterial properties.[8] Cow's milk is suggested as an antidote for blisters caused by *Bhallataka*.[9] Brick powder has absorbent properties that help to absorb the irritating oil in the fruit. If *Bhallataka* is purified with cow's urine, cow's milk, and brick powder, toxic urushiol is converted into less toxic anacardol due to the decarboxylation of the oil.[10] The analytical results demonstrate the chemical changes that occur during the purification process, but it is not evident which method should be preferred for the purification of *Bhallataka*. So, it is necessary to determine the appropriate method of *Bhallataka Shodhana* which provides the drug's quality, safety and efficacy.

In this study, purification of *Bhallataka* was carried out as per the reference of Rasatarangini [11] and AFI [12]. This study aims to develop the Standard Manufacturing Process (SMP) of *Bhallataka Shodhana* and to evaluate the changes occurring in the quality *Bhallataka* after purification through an analytical testing comparison of sample 1 (*Ashuddha Bhallataka*), sample 2 (Rasatarangini 2nd method) and sample 3 (method mentioned in AFI) was conducted to assess the effects of purification on the quality and safety of *Bhallataka*.

MATERIALS AND METHODS:

Procurement and authentication of raw materials

The procurement of *Bhallataka* fruits, Cow's milk, fresh coconut and cow's urine from

Vdadodara, Gujarat. *Bhallataka* fruits were identified at the Pharmacognostical laboratory of the upgraded department of Dravyaguna, Government Ayurved College, Vadodara, Gujarat.

Selection of *Bhallataka* fruits for *Shodhana*

As per the acceptable qualities of *Bhallataka* fruit mentioned into Ayurvedic classics [13], *Bhallataka* fruits were first dropped in a beaker containing water and fruits that sunk in water were collected and used for the *Shodhana* process. *Bhallataka* fruits are rich in oil, which contains volatile compounds and they should float on water due to their low specific gravity. However, even substances with lower specific gravity can sink if they are densely packed. In the case of *Bhallataka* fruits, the oil is densely packed within the fruit's outer walls, leaving little to no empty cells. This increased density causes them to sink, even if their size appears similar to that of unacceptable fruits, which float. Thus, the interplay of molecular weight, density, and the amount of occupied space is crucial in determining whether a substance floats or sinks.[14]

Preparation of sample

Bhallataka Shodhana was done at the pharmaceutical laboratory of the upgraded department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat.

***Bhallataka Shodhana* by *Narikelodaka* (R.T 2nd method) (SBN) (Sample 2)**

Ashudhha Bhallataka (AB) fruits (150 g) were taken and the cap was removed with the help of a cutter. It was kept in cotton cloth and tied so, that *Pottali* was prepared. S.S. vessel was taken and two small holes were made at the neck of the vessel opposite to each other. A rod was inserted in these holes to hang the *Pottali* of the drug inside the liquid in such a way that it did not touch the bottom of the vessel. Liquid media was filled up to the neck of the vessel and *Pottali* was immersed properly into it. The whole apparatus (*Dola Yantra*) was put on the gas stove and 85℃ - 90℃ temperature of liquid media was maintained for *Swedana* of *Ashodhita Bhallataka* for 3 hours. After completion of *Swedana*, *Pottali* was removed and the material was washed with lukewarm water. Then *Shodhita Bhallataka* was weighed and stored in an airtight container.

***Bhallataka Shodhana* by *Gomutra, Godugdha* and *Ishtika Churna* (AFI method) (SBGGI) (Sample 3)**

The cap of the *Ashuddha Bhallataka* fruits (150 g) were removed with a cutter. The *Bhallataka* fruits were put in a stainless-steel vessel, and cow's urine was added. Each day, the cow's urine was replaced with fresh cow's urine. This process was repeated for a total of seven days. On the eighth day, the fruits were removed, rinsed with lukewarm water, and transferred to a new vessel where cow's milk was added. Each subsequent day, the cow's milk was replaced with fresh cow's milk This procedure was repeated daily for the next 7 days. On the fifteenth day, the fruits were removed, washed with lukewarm water, and allowed to dry thoroughly. They were then immersed in brick powder for three days. After this, the fruits were taken out, rinsed with lukewarm water, and dried again. Then *Shodhita Bhallataka* was weighed and stored in an airtight container.

GC-MS (Gas Chromatography-Mass Spectrometry)

Gas chromatography-mass spectrometry (GC-MS) is the synergistic combination of two powerful analytic techniques. The gas chromatograph separates the components of a mixture in time, and the mass spectrometer provides information that aids in the structural identification of each component.

Sample preparation:

10 mg of the sample was taken and diluted it with 10 ml of methanol. Then, it was subjected to extraction in a nitrogen evaporator and two microliter solutions were injected into the column.

Retention time (RT) is the amount of time a compound spends on the chromatography column after it has been injected. Retention time data provided by the GC forms a way of identifying the chemical properties of the component. The area will be based on the numbers of components taken by the mass spectrometer detector at the point of retention.

OBSERVATION

Observations and results of *Shodhana* were shown in tables no.1 and 2.

Table No.1: Showing result of *Bhallataka Swedana* by Narikelodaka (Sample 2)

Sr. No.	Parameters		Results
1.	Total days taken for *Bhallataka Shodhana*		02
2.	Initial quantity of *Ashodhita Bhallataka* (g)		150
3.	Weight of *Ashodhita Bhallataka* after removing cap (g)		118
4.	Weight of cap of *Bhallataka* (g)		32
5.	Quantity of *Narikela Udaka* before *Shodhana* (ml)		600
6.	Quantity of *Narikela Udaka* during Shodhana (ml)		506.66
7.	Remaining quantity of *Narikela Udaka* after *Shodhana* (ml)		450
8.	Total quantity of *Narikela Udaka* required for *Shodhana* (ml)		1106.66
9.	Weight of *Swedita Bhallataka* (g)		170.66
10.	Weight of *Bhallataka* after washing (g)		167.33
11.	Final Weight of *Shodhita Bhallataka*	g	110.66
		%	73.77
12.	Total loss	g	39.33
		%	26.21
13.	Reason of loss		Due to removal of cap and reduction of oil content

Table No.2: Showing result of *Bhallataka Swedana* by *Gomutra, Godugdha* and *Ishtika Churna* (Sample 3)

Sr. No.	Parameters		Results
1.	Total days taken for *Bhallataka Shodhana*		21
2.	Initial quantity of *Ashodhita Bhallataka* (g)		150
3.	Weight of *Ashodhita Bhallataka* after removing cap (g)		127
4.	Weight of cap of *Bhallataka* (g)		23
5.	Total quantity of *Gomutra* required for *Bhallataka Shodhana* (ml)		1400
6.	Total quantity of *Godugdha* required for *Bhallataka Shodhana* (ml)		1750
7.	Total quantity of *Ishtika Churna* required for *Bhallataka Shodhana* (g)		450
8.	Weight of *Bhallataka* after 7 days *Nimajjana* in *Gomutra* (g)		187
9.	Weight of *Gomutra Shodhita Bhallataka* after washing (g)		185
10.	Weight of *Bhallataka* after 7 days *Nimajjana* in *Godugdha* (g)		191
11.	Weight of *Godugdha Shodhita Bhallataka* after washing (g)		187
12.	Weight of *Godugdha Shodhita Bhallataka* after drying (g)		145
13.	Weight of *Ishtika Churna Shodhita Bhallataka* (g)		151
14.	Weight of *Ishtika Churna Shodhita Bhallataka* after washing (g)		147
15.	Final Weight of *Shodhita Bhallataka*	g	107
		%	71.33
16.	Total loss	g	43
		%	28.66
17.	Reason of loss		Due to removal of cap and reduction of oil content

Analytical study

The findings from the organoleptic characteristics, physicochemical parameters, primary phytochemical analysis, and GC-MS analysis of *Bhallataka*, before and after purification are outlined below.

A) Organoleptic characteristics

Organoleptic characteristics of sample 1, sample 2, sample 3 and the liquid media used for the purification process are mentioned in table no.3.

Table No.3: Showing organoleptic characteristics of sample 1, sample 2 and sample 3

Sr. No.	Ingredients	Colour	Texture	Odour	Appearance
1	Sample 1	Brownish-black	Hard	odourless	Oval shape
2	Sample 2	Black	Hard and rough	odourless	Oval shape

3	Sample 3	Black	Hard and rough	odourless	Oval shape

B) Physico-chemical parameters [15]

Physico-chemical parameters including loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive values, using the standard methods outlined in the Ayurvedic Pharmacopoeia of India.

Physico-chemical parameters of sample 1, sample 2 and sample 3 are mentioned in table no.5 and primary physico-chemical parameters such as colour, pH and solid content of liquid media used for purification are mentioned in table no.4.

Table No.4: Details of colour, pH and solid content of liquid media used for purification

Liquid media	Colour		pH		Solid content	
	Before *Shodhana*	**After *Shodhana***	**Before *Shodhana***	**After *Shodhana***	**Before *Shodhana***	**After *Shodhana***
Coconut water	Clear liquid	Brown	5.33	4.23 ↓	6.71	11.78 ↑
Cow's urine	Yellow	Brown	7.30	7.10 ↓	4.87	5.17↑
Cow's milk	white	Light blackish	6.50	5.15 ↓	12.21	13.94 ↑

(↓- decrease ↑ -increase)

C) **Phytochemical parameters [**16]

Qualitative tests for various functional groups and total polyphenol (%) of sample1, sample 2, and sample 3 are shown in table no.5 and 6 respectively.

Table No.5: Qualitative test for various functional groups of *Bhallataka*

Sr. No.	Qualitative test for various functional groups	Performed test	Sample 1	Sample 2	Sample 3
1	Alkaloids	Dragendroff's test	+++	+++	+++
2	Glycoside	Molish test	++	++	-
3	Flavonoids	Shinoda test	+++	++	+++
4	Tannins	Ferric Chloride Test	-	-	-
5	Steroid	Salkowski test	-	-	-
6	Terpenoids	Salkowski test	-	+	+
7	Saponin	Foam test	-	-	-
8	Carbohydrate	Molish test	++	+	++
9	Protein	Barbiturate test	-	-	-
10	Starch	Iodine test	-	-	-

("+, ++, +++" indicate Present in increasing order, "-" indicates absent)

Table. No.6: Total polyphenol (%) of *Bhallataka*

Sr. No.	Name of sample	Total polyphenol (%)
1	Sample 1	3.41
2	Sample 2	2.70
3	Sample 3	2.57

D) Sophisticated advanced analytical technique

GC-MS (Gas Chromatography-Mass Spectrometry)

Results of GC-MS analysis of sample 1, sample 2, and sample 3 are shown in table no.7.

Table. No.7: Results of GC-MS analysis

Sr. No.	Name of sample	Number of compounds	Number of spectra	Number of chemicals
1	Sample 1	5	1	20
2	Sample 2	11	4	20 (each spectra)
3	Sample 3	11	5	20 (each spectra)

In mass spectrum intensity which covers more area has a higher percentage of that compound in the given sample for GC. Here in sample 1, 20 chemicals were observed in one spectra with 36.50 min running time, while in sample 2, 20 chemicals were observed in 4 spectra with 36.75 min running time and in sample 3, 20 chemicals were observed in 5 spectra with 35.48 min running time**.**

Retention time (RT), number of components and area of percentage covered by sample 1 are shown in table no.8.

Table No.8: Retention time, number of compounds and area of percentage in sample 1

Sr. No.	Retention time	Number of compounds	Area	% Area
	23.652	1	38.463	11.75435028
	27.173	2	3626031	2.316407496
	30.419	3	8730253	5.577123717
	34.091	4	1627287.875	1.039555876
	38.463	5	124153376	79.31256263

79.31256263 % area was covered by component 5, name of chemicals are identified in this area are mentioned in table no. 9.

Table No.9: Name of chemicals present in 38.463 cm spectra

Sr. No.	38.463 cm spectra
1	4-nitrophenyl bicyclo [4.1.0] heptane-7-carboxylate
2	Bicyclo [4.1.0] heptane-7-carboxylic acid, 3,5 -dinitrophenyl ester
3	Bicyclo [2.2.2] octane, 1-methyl-4-(methyl sulfonyl)-
4	1,4-hexadiene, 2,3,4,5-tetramethyl-

5	Pyridine-3-carboxylic acid, 1- [(bicyclo [4.1.0] heptane-7 carbonyl)amino-
6	P-menth-3-en-9-ol
7	6-oxo-6h-pyran-3-carboxylic acid, n'- (bicyclo [4.1.0] heptane-7-carbonyl)
8	1h-indene, 5-decyloctahydro-
9	1-(1-ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethenone
10	1h-indene, 5-decyloctahydro-
11	2(1h)-pentalenone, hexahydro-4-1odo-
12	3-fluorobenzoic acid, undec-2-enyl ester
13	1h-indene, 5,5'-(1,10-decanediyl) bis [octa hydro-
14	Trans-1,3,3-trimethylbicyclo [3.1.0] hexane-1- carboxaldehyde
15	4-fluorobenzoic acid. Oct-3-en-2-yl ester
16	2-cyclohexen-1-one, 5-bromo-4,4-dimethyl-
17	Hydrazine, n-(bicyclo[4.1.0]hepten-7-yl)carbonyl-n'-(3 pyridyl carbonyl
18	Cyclopentene, 1,2,3,4,5-pentamethyl-
19	Cyclopentene, 1,2,3,3,4-pentamethyl-
20	3-methyl-5-(1,4,4-trimethyl cyclo hex-2-enyl) pentan-1 ol

Retention time, number of compounds and area of percentage covered by sample 2 are mentioned below in table no. 10.

Table No. 10: Retention time, number of compounds and area of percentage in sample 2

Sr. No.	Retention time	Number of compounds	Area	% Area
1.	15.058	1	2928969	0.144838349
2.	19.995	2	92359616	4.567209243
3.	21.226	3	3617979	0.178910089
4.	21.286	4	9526015	0.471064147
5.	21.826	5	721382528	35.6725709
6.	21.966	6	84144888	4.160988611
7.	23.437	7	1956029.625	0.096726221
8.	23.652	8	8713211	0.430870759
9.	24.617	9	4713433.5	0.233080625
10.	24.652	10	4617597	0.228341483
11.	25.343	11	2185161.5	0.108056857

4.567209243 %, 35.6725709 % and 4.160988611 % areas were covered by compound 2, 5 and 6 respectively, name of the chemicals are identified in this area are mentioned below in table no. 11.

Table No. 11: Name of chemicals in present four different spectra

Sr. No.	20.000 cm	21.836 cm	21.971 cm	26.183 cm
1	n- hexadecanoic acid	oleyl alcohol	octadecanoic acid	trans-1,3,3-trimethylbicyclo[3.1.0]hexane-1-carboxaldehyde
2	n- hexadecanoic acid	Trifluoroacetate	octadecanoic acid	2-(2-ethyl-1,3- dimethyl-cyclopent-2-enyl)-propan-2-ol
3	n-hexadecanoic acid	palmitoleic acid	octadecanoic acid	3-methyl-5-(1,4,4-trimethylcyclohex-2-enyl)pentan-1-ol
4	pentadecanoic acid	cis-10-heptadecenoic acid	octadecanoic acid	1,2 dimethyl -4-oxycyclohex-2 enecarboxalde
	eicosanoic acid	9-octadecen-1-ol, (z)-	eicosanoic acid	ethanone, 1-[2- methyl-5-(1-methylethenyl)cyclopentyl]-, (1.alpha 2.alp
6	octadecanoic acid	1,19-eicosadiene	octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	2-cyclohexen-1-one, 4-ethyl-3,4-dimethyl-
7	tridecanoic acid	13-octadecenal, (z)-	pentadecanoic acid	2-ethyl-1,3-dimethyl cyclo pent-2-enecarboxylic acid
8	octadecanoic acid	13-octadecenal, (z)	n- hexadecanoic acid	2,4,6-trimethyl-2-(4- methyl-pent-3enyl)-2h-pyran
9	tetradecanoic acid	1,19-eicosadiene	tetradecanoic acid	trans, cis-1,8-dimethylspiro [4.5] decae
10	octadecanoic acid	(z)-tetradec-11-en-1- yl 2,2,3,3,4,4,4-heptafluorobutanoate	octadecanoic acid	trans, trans-1,8-dimethylspiro[4.5] decane

11	tridecanoic acid	e-9-tetradecenoic acid cyclododecanemethanol	heptadecanoic acid	3-cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-
12	dodecanoic acid	(e)-tetradec-11-en-1-yl 2,2,3,3,4,4,4-heptafluorobutanoate	tridecanoic acid	1-allyloxy-4- methoxy-benzene
13	octadecanoic acid	trans-9-octadecenoic acid, pentyl ester	pentadecanoc acid	5,9-undecadien-2-ol, 2,6,10-trimethyl-
14	pentadecanoic acid	(z)-tetradec-11-en-1-yl 2,2,3,3,3-pentafluoropropanoa te	n- hexadecanoic acid	cyclohexene, 1,5,5- trimethyl-6- acetylmethyl-
15	pentadecanoic acid	(e)-tetradec-11-en-1-yl 2,2,3,3,3-pentafluoropopanoate	eicosanoic acid	4-n-dodecylresorcinol
16	heptadecanoic acid	eicosen-1-ol, cis-9-	docosanoic acid	cis, trans-1,9-dimethylspiro[5.5]un decane
17	dodecanoic acid	cis-5-dodecenoic acid	nonadecanoic acid	trans, trans- and trans, cis-1,8-dimethylspiro[5.5]un decane
18	tridecanoic acid	9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e)-	pentadecanoi c acid	2-allyloxyanisole
19	tetradecanoic acid	oxacyclopentadecan- 2-one	tetradecanoic acid	1ar,3s,3as,7r,7ar,7bs) -(-)-1a,2,3,3a,4,5,6,7,7a,7b-decahydro- 1,1,7,7a-tetram
20	nonadecanoic acid	1,21-docosadiene	tridecanoic acid	1,2-Dipentylcyclopropene

RT, number of compounds and area of percentage covered by sample 3 are mentioned in below table no.12.

Table No.12: Retention time, number of compounds and area of percentage in sample 3

Sr. No.	Retention time	Number of compounds	Area	% Area
1.	17.774	1	2485010.75	0.428299479
2.	19.08	2	5377156.5	0.926769967

3.	19.885	3	26937250	4.642720419
4.	21.591	4	68853136	11.86705623
5.	21.776	5	12525398	2.158792047
6.	24.482	6	4636017.5	0.799032311
7.	24.522	7	4763026.5	0.820922714
8.	24.657	8	3577155.75	0.616534132
9.	25.948	9	270774656	46.66887022
10.	25.993	10	174164592	30.01781947
11.	26.403	11	6110610	1.053183002

4.642720419 %, 11.86705623 %, 2.158792047 %, 53.58834709%, 46.66887022 % and 30.01781947 % area were covered by compound 3, 4, 5, 9 and 10 respectively, name of chemicals are identified in this area are shown in table no. 13.

Table No. 13: Name of chemicals present in five different spectra

Sr. No.	19.890 cm	21.596 cm	21.781 cm	25.948 cm	25.998 cm
1	n-hexadecanoic acid	9-octadecenoic acid	9-octadecenoic acid	2-methyl-4-trimethylsilylbu t-1-en-3-yne	trons-1,3.3 trimetallicyco[3.1.0]hexane 1-carboxaldehye
2	n-hexadecanoic acid	cis-vaccenic acid	cis-vaccenic acid	2-(2-ethyl-1,3- dimethyl-cyclopent-2- enyl)-propan-2-ol	4-fluorobenzoic acid. oct-3-en-2- yl ester
3	n- hexadecan oicacid	cis-11-eicosenoic acid	cis-11-eicosenoic acid	1-benzoxirene, 5a- [3-oxo-1- butenyl]perhydr o-2-hydroxy-1a,5,5-trimeth	bicyclo[2 2.21octane, 1- methyl-4-(methylsulfonyl)
4	pentadecanoic acid	cis-10-nonadecenoic acid	cis-10-nonadecenoic acid	cyclopent-2- ene-1-carboxylic acid, 2,3-dimethyl-1- ethyl-, ethyl ester	2-(2-ethyl-1,3- dimethyl-cyclopent-2- enyl)-propan-2- ol
5	eicosanoic acid	trans-13-octadecenoic acid	trans-13-octadecenoic acid	4,4'-bi-4h-pyran. 2,2',4,4,6,6'-hexamethyl-	pyridine-3- carboxylic acid 1-[(bicyclo[4.1.0] heptane-7-carbonyl) amio]

6	octadecanoic acid	palmitoleic acid	palmitoleic acid	4,4'-isopropylidened icyclohexanol	3-fluorobenzoic acid, undec-2- enyl ester
7	octadecanoic acid	9-hexadecenoic acid	9-hexadecenoic acid	bicyclo[2.2.1]he ptan-2-one, 4-bromo-1,7,7-trimethyl-	3-fluorobenzoic acid, oct-3-en-2- yl ester
8	tridecanoic acid	cis-10-heptadecenoi acid	cis-10-heptadecenoic acid	4-n-Dodecylresorci ol	6-oxo-6h-pyran-3-carboxylic acid, n'-(bicyclo[4.1.0] heptane-7-carbonyl)
9	tetradecanoic acid	6-octadecenoic acid, (z)-	6-octadecenoic acid, (z)-	trans-2-(1- hydroxycyclohexyl)-furan	bicyclo[4.1.0]heptane-7-carboxylic acid, 3,5-dinitrophenyl ester
10	octadecanoic acid	hexadecenoic acid, z-11-	hexadecenoic acid, z-11-	3-buten-2-one, 4-(2,5,5-trimethyl-3-oxatricyclo[5.1.0.0(2,4)]oc-4-yl), [1.al	3-fluorobenzoic acid, 2-bromo- 4-fluorophenyl ester
11	tridecanoic acid	e-9-tetradecenoic acid	e-9-tetradecenoic acid	1h-indene, 5-decyloctahydro-	4-nitrophenyl bicyclo[4.1.0]he ptane-7-carboxylate
12	dodecanoic acid	z-11-tetradecenoic acid	z-11-tetradecenoic acid	3-heptyne, 5,5- diethyl-	1h-indene, 5-decyloctahydro-
13	pentadecanoic acid	myristoleic acid	myristoleic acid	bicyclo[4.1.0]heptane-7-carboxylic acid, 3,5-dinitrophenyleter	1-(1-ethyl-2,3- dimethyl-cyclopent-2- enyl)-ethanone
14	pentadecanoic acid	trans-9-octadecenoic acid, pentyl ester	trans-9-octadecenoic acid, pentyl ester	1,4-benzenediol, 2- octadecyl-	4-fluorobenzoic acid, undec-2- enyl ester
15	octadecanoic acid	z-7-tetradecenoic acid	z-7-tetradecenoic acid	4-pyrimidinamine , 2,6-dimethyl-	2(1h)-pentalenone, hexahydro-4-iodo-
16	heptadecanoic acid	9-octadecen oic acid (z)-, 2,3-	9-octadecenoic acid (z)-, 2,3-	1,4,4-trimethylcycloh ex-2-enecarboxylic acid	trans-2-(1- hydroxycycloexyl)-furan

		dihydroxy propyl ester	dihydroxypro ply ester		
17	dodecanoic acid	(e)-13-docosenoic acid	(e)-13-docosenoic acid	zinc, bis (2,2- dimethyl-3(cis)- (1-methylprop- 2-enyl)-cyclopropyl)-	3-heptyne, 5,5- diethyl-
18	tridecanoc acid	9-octadecenal, (z)-	9-octadecenal, (z)-	1h-indene, 5-decyloctahydro	2-fluorobenzoic acid, undec-2- enyl ester
19	tetradecanoic acid	cis-9-hexadecenal	cis-9-hexadecenal	acetic acid, 1- acetyl-4-methyl-4-(5- methylfuran-2-yl) pentyl ester	1h-indene, 5,5'-(1,10-Decanediyl) bis [octahydro-
20	-	-	-	-	cyclopentene, 1,2,3,4,5-pentamethyl-
21	nonadecanoc acid	e-11-hexadecenal	e-11-hexadecenal	(1ar-(1aalpha, 4abeta,8 as (*)))- 4a,8,8-trimethyloctahydrobenzo(c)cyo	-

Sample 1 contains distinct compounds such as 4-Nitrophenyl Bicyclo [4.1.0] Heptane-7-Carboxylate and 1H-Indene, 5-Decyloctahydro-, which are not found in the other samples, giving it a unique chemical profile. Similarly, sample 2 is characterized by compounds like Bicycl [4.1.0] Heptane-7-Carboxylic Acid, 3,5-Dinitrophenyl Ester and 2-Methyl-4-Trimethylsilylbut-1-en-3-yne and sample 3 features unique components such as 1-Benzoxirene, 5a-[3-oxo-1-butenyl] perhydro-2-hydroxy- and Myristoleic acid, which are absent in samples 1 and 2.

Some compounds were found to be common in all three samples, with the majority being fatty acids mentioned with their biological activity in shown in table no.14.

Table No. 14: Chemical compounds and their biological activities of different samples of *Bhallataka*

Sr.No.	Name of compound	Nature of compound	Biological activities
1	Octadecanoic acid	Fatty acid	Antibacterial, antifungal and Antitumor17
2	n-hexadecanoic acid	Fatty acid	Anti-inflammatory18, anti- androgenic, antifibrinolytic, antioxidant, antipsychotic,

			hemolytic, hypocholesterolemic, 5-Alpha reductase inhibitor19
3	Pentadecanoic acid	Fatty acid	Anti-bacterial, anti-fungal20
4	Heptadecanoic acid	Fatty acid	Act against skin cancer protein21
5	Tridecanoic acid	Fatty acid	Anti- enteric efficacy22
6	Tetradecanoic acid	Fatty acid	Anti-virulence property23
7	Palmitoleic acid	Fatty acid	Beta cell apoptosis by glucose or saturated fatty acid24
8	Eicosanoic acid	Fatty acid	Anti-fungal25
9	Dodecanoic acid	Fatty acid	Use in cardiovascular disease26
10	Phenol, 3-pentadecyl	Fatty acid	Anti-inflammatory, anti-oxidant27

DISCUSSION

Acharya Vagbhatta (6th -7th century) was the first who describe *Bhallataka Shodhana* in *Ishtika Churna* (brick powder) by using the *Gharshana* (rubbing) principle.[28] After that purification method of *Bhallataka* has been mentioned in many Ayurvedic classics by using various purification media such as brick powder, coconut water, cows' urine, cow's milk, buffalo dung and cow's ghee using different purification principles such as boiling, socking and rubbing. As per the criteria given in Ayurvedic texts for the selection of *Bhallatak fruits*, those that sink in water are chosen for purification process. Numerous research studies on the *Shodhana* of *Bhallataka* have highlighted the chemical changes occur through the *Shodhana* methods outlined in classical texts.Among these, the method described in *Rasamrita*, which is included in the Ayurvedic Formulary of India (AFI), is preferred for its superior quality and the *Shodhana* method found in *Rasa Tarangini* (2nd method) was widely utilized, However, no study has yet compared this method with the 2nd method described in Rasatarangini. So, in this study, *Shodhana* of *Bhallataka* was carried out as per the reference of Rasatarangini [29] and AFI [30]. A pilot batch was carried out to get knowledge about the required quantity of liquid media, temperature pattern of flam and liquid media, and changes in *Bhallataka* after *Shodhana* including yield percentage for the entire *Shodhana* procedure.

A total 150 g of *Ashodhita Bhallataka* fruits were taken for purification in both sample preparation. Changes were observed in colour, pH and total solid content in liquid media after purification (Table no.6). Change in colour of liquid media may be due to the infusion of *Bhallataka* oil in *Shodhana* media. Due to the presence of anacardic acid, the pH of the liquid media was decreased.[31] Total solid content of liquid media was increased due to impurities of *Bhallataka*. In sample 2 and sample 3, the yields obtained were 79.33 % and 71.33 %, respectively. An average 1106.66 ml coconut water was used in the preparation of sample 2, while the average 1400

ml of cow's urine, 1750 ml of cow's milk and 450 g of brick powder were used in the AFI *Shodhana* method. In sample 2 average 73.77 % yields and 26.21 % loss were obtained during the purification. In sample 3, an average 74.22% yields and 25.77 % loss were obtained during purification. There were no significant differences in yield percentage between the two purification methods. The average loss of the sample after undergoing *Shodhana* with the SBGGI method was 17.32 % (Ilanchezhian R, 2012).[32] This loss might be attributed to a decrease in the oil content of the fruits. *Ashuddha Bhallatahka* was brownish black with a smooth surface that turned black with rough surface after purification. Coconut water, cow's urine and cow's milk turned brown after purification.

LOD values of samples 1, 2 and 3 were 5.199 %, 3.50 % and 8.36 % respectively. Ash value represents the inorganic part of the plant. The ash values (% w/w) were 3.50 %, 2.33 % and 3.53 % in samples 1, 2 and 3 respectively. The acid insoluble ash refers to the portion of the total ash that is insoluble in diluted hydrochloric acid. Acid insoluble ash value indicates siliceous impurities.[33] An average Acid insoluble Ash value of samples 1, 2 and 3 were 0.130 %, 0.2039 % and 3.34 %, respectively. The water-soluble extractive value is an essential factor in crude drug assessment. Less extractive value indicates adulteration.[34] An average water-soluble extractive value of samples 1, 2 and 3 were 10.88 %, 9.1 % and 8.13 % respectively. The alcohol soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates adulteration.[35] An average alcohol soluble extractive value of samples 1, 2 and 3 were 31.89 %, 32.33 % and 26.44 %. pH of samples 1, 2 and 3 were 5.63, 5.91 and 6.05 respectively. Here pH value of sample 1 was 5.63. It indicates the acidic nature of it. According to results, alkaloids, carbohydrates and flavonoids are present in all samples. Terpenoids are present in coconut water36 and cow's urine.[37] So due to this reason, terpenoids are present sample 2 and 3.

The most significant components of the S. anacardium oil are phenolic compounds. Two main phenolic compounds are bhilawanol A (monoenepentadecyl catechol I), bhilawanol B. Vesicant reactions of *Bhallataka* possibly due to these phenolic compounds.[38] Comparative quantitative estimation of polyphenol in all samples shows that extraction of polyphenol content was less in the *Shodhita* sample which indicates the impact of the purification procedure (Table no.8). Some research studies reported that S. anacardium fruit contains 90% anacardic acid and 10% of cardol. The corrosive juice from the pericarp of the fruit was found to contain catechol, fixed oil and anacardol (C8H1303.COOH) to which the corrosive properties of the juice are due to two phenolic acids C16H1503.COOH and C14H1303.COOH. In *Bhallataka* 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 anacardol level was 47.51 % before Purification and increased to 50.62 % afterward using the SBGGI method for *Shodhana* (Ilanchezhian R, 2012).[39]

Analysis of sample 1, suggested the presence of 20 chemicals. While in samples 2 and 3 combinedly showed the presence of more than 100 chemicals. Chemicals that are present in sample 1 are absent after the purification procedure and the addition of new chemicals was observed. It may be due to the purification procedure. 10 compounds are found commonly in all the samples. Some compounds were present before the purification like urushiol (1, 2-benzenediol, 3-(8, 11, 14-pentadecatrienyl). After purification, some compounds present in *Bhallataka* are structured like anacardol (m-pentadecyl-m-pentadecylphenol).[40] It indicated that Purification helps in the conversion of toxic urushiol into nontoxic anacardol.

CONCLUSION

Bhallataka has been used for a long time of history for medicinal purposes. It is used after Purification. The percentage of polyphenol content of sample 1 (3.41 %), sample 2 (2.70 %) and s ample 3 (2.57 %) showed significant differences which concluded that the purification procedure affected the active principle of *Bhallataka*. GC-MS analysis suggested that the chemical components present in Sample 1 were absent after the purification procedure and the new chemical components were present which are mostly fatty acid components.

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