Research Article

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QUANTITATIVE PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF THE FRUIT OF *SEMECARPUS ANACARDIUM* LINN.

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ABSTRACT

Bhallataka (*Semecarpus anacardium* Linn.) is a significant medicinal plant in Ayurveda, valued for its therapeutic properties despite its toxic nature. This study aims to standardize the fruits of Bhallataka through macroscopic, microscopic, physicochemical, phytochemical, and GC-MS analyses to ensure their purity and quality. The macroscopic and microscopic characteristics confirmed the fruit's identical features, while physicochemical analysis revealed a pH of 5.63, a total ash content of 3.50 %, and an alcohol-soluble extractive value of 31.89 %. Phytochemical screening identified alkaloids, glycosides, flavonoids, and carbohydrates, with a total polyphenol content of 3.41 %. Gas Chromatography Mass Spectrometry analysis detected 20 chemical compounds, with Pyridine-3-carboxylic acid, 1 [(bicyclo [4.1.0] heptane 7 carbonyl) amino 3] being the most abundant (79.31 %) as the predominant component. The study provides a comprehensive standardization protocol for Bhallataka fruits, which can be used to ensure the quality of the plant material used in various formulations.

Keywords: Marking nut, Pharmacogenetic evaluation, Preliminary analysis, GC-MS

INTRODUCTION

Bhallataka (*Semecarpus anacardium* Linn.), known as marking nut, is an important medicinal plant in Ayurvedic medicine. It is known for its potent therapeutic effects and is classified under the Upavisha (semi-poisonous) Varga (group) due to its toxic properties. Despite its poisonous properties, it is utilised as a Rasayana (rejuvenation) drug. Acharya Charaka has been mentioned in various formulations such as Bhallataka Sarpi (ghee), Ksheera (milk), Kshaudra (honey), Guda (jaggery), Yusha (soup), Taila (oil) and Lavana (salt) in the Rasayana chapter.[1] It is widely used in various diseases like Arsha, Kushtha (skin disease), Aanaha (flatulence), Krimi (worm), Jvara (fever) and Shwitra (leucoderma).[2]

There are abundant references of Bhallataka found in classical texts in different dosage forms like Svarasa (fresh juice), Kalka (paste), Kashaya (decoction), Churna (powder), Lepa (external application) and other forms. It is also used in many formulations such as Chincha Bhallataka Vati, Amritabhallataka Avaleha, Bhallatakasava, Surana Vataka, Sanjeevani Vati, Narsinha Churna and many more. It has unique collection and preservation methods mentioned in Charaka Samhita[3], Ashtanga Samgraha[4] and Ashtanga Hridya[5].

According to Charaka Samhita and Ashtanga Sangrah its fruit should be collected during the month of Shuchi-Shakra (May-June) and used in the month of Saha-Sahasya (November-December). Collecting Bhallataka during its optimal season allows it to retain higher concentrations of active compounds, which enhance its therapeutic properties.

The major chemical constituents found in Bhallataka are Bhilawanol, fixed oil, Anacardic acid and phenolic compounds[6], and cis and trans isomers of urushiol (3-pentadactyl-8′catechol)[7]. Bhallataka has many pharmacological activities viz. anti-inflammatory activity[8], anti-oxidant activity[9], hypoglycaemic activity, antioxidative, antihyperglycemic activity[10] and many more. Since Bhallataka is used in numerous formulations for various treatments and exhibits many therapeutic effects, its standardization is necessary. Standardization validates a drug's identity and assesses its quality and purity a critical necessity in contemporary times.[11]

This study aims to authenticate its identity and quality through macroscopic and microscopic analysis, physicochemical analysis, phytochemical screening, and GC-MS analysis for medicinal use. GC-MS is specifically employed for analysing compounds such as esters, fatty acids, alcohols, aldehydes, and terpenes.

MATERIALS AND METHODS

Sample collection

The fruits of Bhallataka were procured from the Government Ayurved Pharmacy, Vadodara, Gujarat. Bhallataka was identified at the Pharmacogenetic Laboratory of the Upgraded Department of Dravyaguna, Government Ayurved College, Vadodara, Gujarat.

The herbarium voucher specimen HS13227 of *Semecarpus anacardium* (family: Anacardiaceae) was collected in India on October 2, 2000, and is identified with the national identity IC260121 at the National Herbarium of Cultivated Plants.

Macroscopical evaluation: Bhallataka Fruit was subjected to macroscopical studies which comprised organoleptic features like colour, odour, taste and appearance.

Powder microscopy: For the powder microscopic study, drugs were powdered. The powder was spread on glass slides and observed under a microscope at different magnifications. Microphotographs were taken by using a digital microscope.

Physicochemical parameters: Physicochemical parameters are essential for assessing the purity of crude drugs. These parameters provide valuable information about the composition and quality of the drug, helping to ensure its effectiveness and safety. In this study, physicochemical parameters including loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive values were evaluated using the standard methods outlined in the Ayurvedic Pharmacopoeia of India.[12]

Phytochemical parameters:[13]

Qualitative test for various functional groups
The techniques employed to isolate active substances are termed extraction methods. Crude extracts obtained from such processes can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups, which play a very important role in the expression of biological activity.

Total polyphenol (%)
The most significant components of the *Semecarpus anacardium* Linn. oil are phenolic compounds. Two main phenolic compounds are bhilawanol A (monoenepentadecyl catechol I) and bhilawanol B. Vesicant reactions of Bhallataka possibly due to these phenolic compounds.[14]

Sophisticated advanced analytical technique

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.

Preparation of sample: Take 10 mg of the sample and dilute 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.

Instrument specification:
Instrument specification for GCMS was mentioned in Table 1.

RESULT AND DISCUSSION

The findings from the macroscopic evaluation, organoleptic characteristics, physicochemical parameters, primary phytochemical parameters, and GC-MS analysis of Bhallataka are detailed below.

Macroscopical evaluation: Botanical distribution: Fruit laterally flattened, drupaceous, dark brown, nut 2.5-3 cm long, obliquely ovoid, smooth, shining with residual receptacle.[15] Figure 1 showing dried and powdered fruits of *Semecarpus anacardium* Linn.

Organoleptic characters: The organoleptic characters of Bhallataka are mentioned in Table 2.

Powder microscopy: The powder of Bhallataka is dark brown, has a distinctive odour and a rough texture. Powder microscopy of Bhallataka consists of Prismatic crystals of calcium oxalate, Secretory trichomes, Crystal, non-glandular trichome, Fragment of epicarp cell, Fixed oil globules, fragment of Testa, Parenchymatous cell of mesocarp, Fragment of vessels, Epidermal trichomes and Pitted vessel. The findings from this study compliance API standard for Bhallataka[16] fruit, which indicated its identification. Figures of powder microscopy mentioned in Figure 2.

Physicochemical parameters: Physicochemical parameters of Bhallataka are shown in Table 3.

The Bhallataka sample has a pH of 5.63, with a loss on drying of 5.199 % (%w/w). The total ash content is 3.50 %, while the acid-insoluble ash constitutes 0.130 %. The water-soluble extractive is 10.88 %, and the alcohol-soluble extractive is 31.89 %.

Phytochemical parameters: Qualitative tests for various functional groups and total polyphenol (%) of Bhallataka are shown in Table 4 and 5 respectively.

The qualitative tests for functional groups revealed the presence of alkaloids, glycosides, flavonoids, and carbohydrates in the samples. However, tannins, steroids, proteins, and starch were not detected.

In the research conducted by Rajakrishnan *et al.,* the screening of Bhallataka shows that the choice of extraction medium significantly influences the detection of various compounds. The findings indicate that methanol extract of *Semecarpus anacardium* Linn. revealed the presence of sugars, flavonoids, steroids, saponins and tannins. Sugars were also seen in petroleum ether, chloroform and ethyl acetate extracts while steroids were in ethyl acetate and chloroform extracts. The petroleum ether extract also contains quinones.

GC MS analysis (Gas Chromatography-Mass Spectrometry): Gas Chromatography-Mass Spectrometry (GC-MS) plays a key role in the analysis of unknown components of plant origin. Interpretation on mass-spectrum GC–MS was conducted using the database of the National Institute of Standard and Technology (NIST). Identification of the unknown components was carried out by matching their recorded spectra with the data bank mass spectra of the NIST library. Retention time is the amount of time a compound spends on the chromatography column after it has been injected. Retention time (RT) 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. RT, components name and area of percentage covered by the Bhallataka sample are mentioned Table 6.

Components 1, 2, 3, and 4 are present in smaller amounts, with area percentages ranging from 1.04 % to 11.75 %. Component 5 is the most abundant, 79.31256263 % area was covered by component 5, which indicates its predominant presence in the sample. The names of the Chemicals identified in this area are mentioned in Table 7.

Graphical Peak patterns obtained from GC-MS of Bhallataka are presented in Graph 1 (GCMS profiling of Bhallataka) and 2 (Graphical peak at spectra 38.463 cm).

The compound with the highest percentage area (79.31 %) at a retention time of 38.463 minutes is particularly dominant in the

sample. Here in the Bhallataka sample, 20 chemicals were observed in one spectra with a 36.50 min running time.

The choice of extraction media significantly impacts the results obtained from GC-MS analysis for Bhallataka fruits. According to research by Rajakrishnan *et al.*, the diethyl ether extract exhibited 11 distinct peaks, with n-Nonadecanol-1 (3.67%) and 9-Octadecanoic acid (27.84%) being identified as the predominant compounds.[17]

10 kg of *Semecarpus anacardium* Linn. fruits were separated into two groups based on a sinking test in water. The sunken fruits were considered acceptable (AF), and the floating fruits were deemed unacceptable (UF). The quantities obtained were 5866 g (60.30%) for AF and 3861 g (39.70%) for UF. Oil extraction was performed using the Indian System of Medicine (ISM) method. The oils from acceptable fruits (SAO-1) and unacceptable fruits (SAO-2) were analysed using GC-MS, revealing 44 and 32 phytoconstituents, respectively. SAO-1 contained fewer toxic compounds than SAO-2. Some compounds in both oils, like Pentadecenyl, Benzenediol, and Acridine, exhibited toxic potential. Common compounds, such as Dioxabori, Thiophen, and Borinic acid, showed antimicrobial activity. Certain compounds in SAO-1, including Imidazolo and Pyrrole, require further study for their pharmacological properties. SAO-2 showed higher levels of toxic compounds like Benzenedimethanol and Naphthalene, suggesting it is more poisonous than SAO-1. These findings support the traditional method of selecting high-quality fruits based on the water test.[18]

Table 1: Instrument specification for GCMS

Model	Auto system XL with Turbo mass
Make	Perkin Elmer
Column use	Elite-5MS (30 meters x 0.250mm x0.250um)
Carrier Gas	Helium
Flow rate	1ml/min
Injector Temp	260˚C
Oven Temp	75 ˚C hold for 5 minutes Rate 10 ˚C per minute up to 280 ˚C hold for 10 minutes
El source Temp	220 ˚C
Scan range	20 to 610 amu
Injection volume	2 microliters

Table 2: Organoleptic characteristics Bhallataka

Ingredients	Colour	Odour	Texture	Appearance
Bhallataka fruit	Brownish-black	Odourless	Hard	Oval shape

Table 3: Physicochemical Analysis of the Bhallataka

Parameters	Bhallataka
pH	5.63
Loss on drying (%w/w)	5.199
Total Ash (%)	3.50
Acid insoluble Ash (%)	0.130
Water soluble extractive (%)	10.88
Alcohol soluble extractive (%)	31.89

Table 4: Qualitative test for various functional groups of Bhallataka

Qualitative test for various functional groups	Performed test	Ballataka
Alkaloids	Dragendorff's test	+++
Glycoside	Molisch test	++
Flavonoids	Shinoda test	+++
Tannins	Ferric Chloride Test	-
Steroid	Salkowski test	-
Terpenoids	Salkowski test	-
Saponin	Foam test	-
Carbohydrate	Molisch test	++
Protein	Barbiturate test	-
Starch	Iodine test	-

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

Table 5: Total polyphenol (%) of Bhallataka

Name of Sample	Total polyphenol (%)
Bhallataka fruit	3.41

Table 6: Peak details of Bhallataka

Retention time	Number of components	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

Table 7: Name of components 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-(methylsulfonyl)-
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)-ethanone
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 [octahydro-
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 pyridylcarbonyl
18	Cyclopentene, 1,2,3,4,5-pentamethyl-
19	Cyclopentene, 1,2,3,3,4-pentamethyl-
20	3-methyl-5-(1,4,4-trimethylcyclohex-2-enyl) pentan-1 ol

Figure 1: Dried and powdered fruits of *Semecarpus anacardium* Linn.

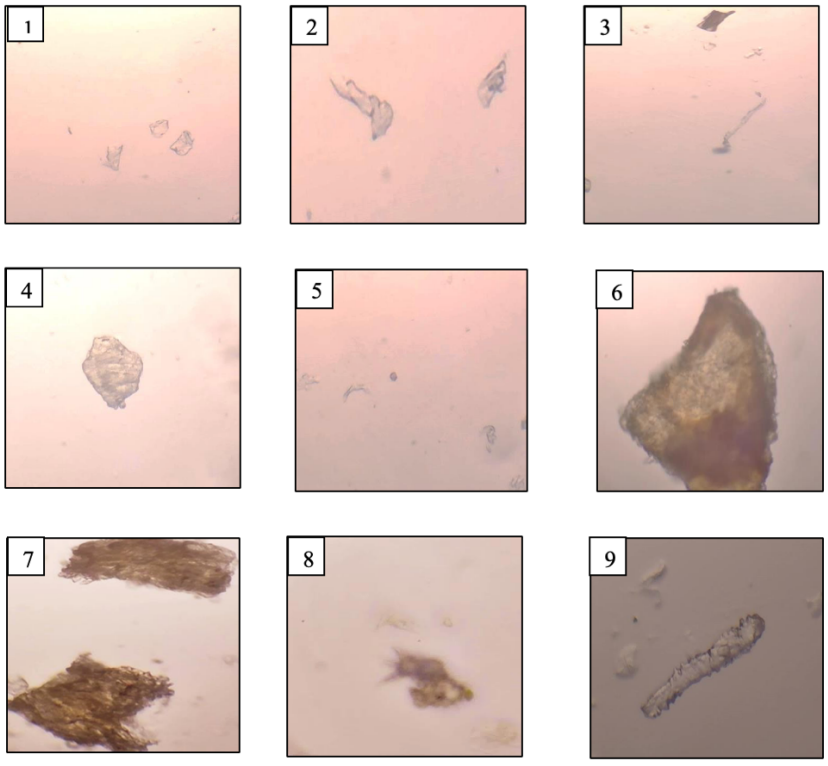
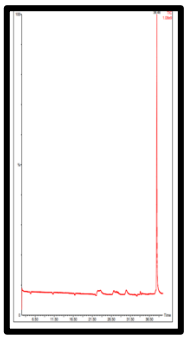
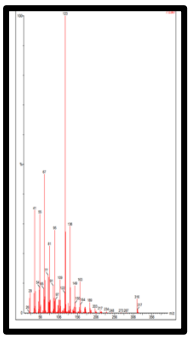


Figure 2: Powder microscopy of Bhallataka fruit powder
(1) Prismatic crystals of calcium oxalate; (2) Secretory trichomes; (3) Crystal, non-granular trichome; (4) Fragment of epicarp; (5) Fixed oil globule; (6) Fragment of vessel; (7) Parenchymatous cell of mesocarp;(8) Epidermal trichome; (9) Pitted vessel



Graph 1: GC-MS profiling of Bhallataka



Graph 2: Graphical peak at spectra 38.463 cm

CONCLUSION

A systematic approach in pharmacogenetic and phytochemical studies is essential for verifying the identity, purity, and quality of medicinal plants. In this study of Bhallataka (*Semecarpus anacardium* Linn.), comprehensive analyses—including powder microscopy, physicochemical testing, preliminary phytochemical screening, and GC-MS, have successfully characterized its bioactive compounds. These findings support its therapeutic potential and emphasize the need for standardization. The parameters evaluated according to standard norms in this pharmacogenetic and phytochemical study of Bhallataka are expected to offer valuable insights for future research.

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