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Evaluation of Ayurvedic formulation for Pharmacognostic parameters, Phytochemical screening, and acute toxicity

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ABSTRACT

In this age, there is an urgent need to meticulously examine the present indisputable conclusion on traditional herbal medicines, which are used for a favourable outcome for various maladies. Therefore the current investigation aimed to formulate traditional Ayurvedic medicine *Amrtabhallataka Ghrta*. (ABG) and evaluate it for pharmacognostic, phytochemistry and acute toxicity on Wistar rats. The organoleptic and microscopical features of *Semecarpus anacardium* were studied. Further, *Bhallantaka* nut was evaluated for phytochemical constituents. After the *Shodhana* process, *Amrtabhallataka Ghrita* was prepared. *Ghrita* was analysed for its organoleptic, physicochemical features and screened its acute toxicity in Wistar rats. The progress in analytical methodologies could serve as a specific basis for examination in herbal drug technology, thereby, aid the Ayurvedic industry to lay down quality standards and parameters, for the establishment of therapeutic efficacy, safety and purity of herbal drugs.

Keywords: *Semecarpus anacardium, Ghrita* Standardization, Herbal ghee, Ayurvedic medicine Acute Toxicity

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INTRODUCTION

Ayurvedic medicine is an ancient system of health care that is native to the Indian subcontinent. It is presently in daily use by millions of people in India, Nepal, Sri Lanka, China, Tibet, and Pakistan. It is now in practice for health care in European countries. The main motive of Ayurveda is *"Swasthasya Syasthya Rakshanam, Aaturashcha Vikar Prashamanam",* "and Preservation to the health of a healthy person and treating ailments with breaking causative factors of pathogenesis".[1,2]

The word Semecarpus is derived from "simeion" in Greek means marking/tracing and "carpus" in Greek means nut. Hence, it is popularly known as marking nut. Semecarpus anacardium (SA) L. F. (Anacardiaceae) is a deciduous tree distributed in the sub-Himalayan tract and hotter parts of India. [3] It is commonly known as *'Ballataka'* or *'Bhilwa'* in Indian system of medicine. Like the closely related cashew, the fruit (Figure 1) is composed of two parts, a reddish-orange accessory fruit and a black drupe that grows at the end. The nut (Figure 2) is about 25 millimetres long, ovoid and smooth lustrous black. The accessory fruit is edible and sweet when ripe, but the black fruit is toxic and produces a severe allergic reaction if it is consumed or its resin comes in contact with the skin. The seed inside the black fruit, known as "Godambi", is edible when properly prepared.[3,4] Traditionally *Ballataka* used to treat brain diseases, improve memory, as a rejuvenating drug and also used for neurological disorders. In Siddha system of medicine, S. anacardium has been reported to have anti-arthritic, immunomodulatory, anti-inflammatory and neurological properties.[4] Further, in ancient time it was also used for a non-medicinal purpose like marking of cloth, hair dye etc. Several studies have already validated it's purported Ayurvedic claims and has demonstrated several therapeutic properties, like anti-atherogenic[3], anti-inflammatory[5],

antioxidant[6], antimicrobial[7], CNS stimulant, anticarcinogenic, hair growth promoter and hypoglycaemic, [8,9] activities. However, studies also have reported the cytotoxic effect of SA and toxic at dose-dependent levels in animal studies.[10] Processing of medicinal ingredients used in Ayurveda to detoxify them is known as shodana. It not only reduces the toxic effects but also enhances the therapeutic effect of the drugs at times imparts additional qualities.[11,12]

Standardisation of herbal formulations is essential to assess the quality of drugs, based on the concentration of their active principles, physical, chemical, phytochemical, standardisation, and pharmacological evaluation. The quality assessment of herbal formulations is of paramount importance to justify their acceptability in the modern system of medicine. India needs to explore the medicinally important plants. If the herbal products are scientifically evaluated and analysed using sophisticated modern techniques, can the therapeutic efficacy of traditional formulations be validated.[13]

World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in natural health care programmes because these drugs are easily available at low cost, safe and people have faith in them. WHO has in several resolutions has emphasised the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. [14,15]

Till date, however, little work has been carried out on the Ayurvedic formulation of this plant. The present study was undertaken to standardise the formulation as per Ayurvedic Pharmacopoeial standards and evaluate its acute toxicity potential.

Figure 1: *S. anacardium* fruits

Figure 2: Bhallataka Nuts

MATERIALS AND METHODS

Plant material

Marking Nut fruit was purchased from local market in Ramnagar, Uttrakhand (latitude = 29.394764, longitude = 79.126503 and elevation = 345 m) in summer of 2016. It was authenticated by Dr Ashok Kumar, Department of Botany, School of Sciences, IFTM University. The herbal drug was cleaned of physical impurities and dried in the sun for ten days. Later the dried fruit was removed and nuts so obtained were cleaned and stored in an airtight container.

Chemicals

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), and SD Fine-Chem. Ltd. (Mumbai, India). Fresh cow's ghee (clarified butter) of *Gopal Ji* brand and Amul cow's milk were obtained from the local market. All the chemicals used in this study were of analytical grade.

Pharmacognostic evaluation:

Different organoleptic (sensory) parameters of a drug such as colour, odour, taste and texture were evaluated by the sense organs and recorded.[16]

Microscopic studies: Marking nut fruit was studied for histological characters; *S. anacardium* nut was powdered (**Figure 3**) and it was observed under the compound microscope. Briefly, the drug was boiled and treated with chloral hydrate. Standard microtome techniques were followed for anatomical investigation. Transverse sections were prepared and stained with the reagent. [17,18] Photomicrographs were taken using a digital camera.

Figure 3: *S. anacardium* nut powder

Preparation of plant extracts

The crude extracts of SA nut were prepared by cold maceration technique. The mixture was concentrated in a rotary evaporator. The resulting *S. anacardium* nuts extract (SNE) were evaluated for phytoconstituents.

Preliminary Phytochemical Study

For the identification of various phytochemical constituents, the SNE extract was subjected to qualitative tests as per the standard procedure. [19,20]

Physicochemical evaluation of S. anacardium nut:

The parameters studied were a loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, petroleum ether soluble extractive value, as per standard protocols.[21]

In-house preparation of *Amrtabhallataka Ghrita* (ABG)

Ripe fruit was coarsely abraded, washed in hot water and dried in the shade. The nuts were collected and coarsely powdered. Powdered nuts (about two hundred gms) were suspended in one litre of double distilled water and heated at 50oC, till the quantity was reduced to about one third the original volume. The decoction was allowed to cool and further about two hundred militres of milk was added to it. The resulting mixture was further heated at 45oC until the quantity reduced to one-fourth and then stained. *Shodhana* of *Bhallataka* was carried out in cow's milk and followed by water for seven days each. Further, it was mixed with clarified butter at 100oC, and the resulting formulation was filtered. *Amritabhallataka Ghrita* (ABG)was prepared as per AFI reference 22. It was subjected to modern parameters of evaluation as well as organoleptic characters.

Physico-Chemical Tests:

ABG was subjected to physicochemical tests like organoleptic, acid value, refractive index, specific gravity, rancidity, saponification value, iodine value, and peroxide value. The tests were conducted as per standard procedure. [21]

Selection and Procurement of animals

Experimental animals

For acute oral toxicology assessment, (total of Fifteen), age (6 weeks) and weight (129.1 to 140.2 gm) female Wister rats (WR) pathogen-free rats were used for an acute oral toxicity test. The toxicity study was put into effect to fulfil in- toto OECD test guideline-423.23 All animals were kept in the wire-bottomed cages at 25 ± 3o C temperature, 50–60% humidity, and a 12h light-dark cycle. Rats were acclimatised to the laboratory environment for seven days ahead of the experiments. They were granted free to passage rat pellet diet (Lipton India Ltd, Mumbai, India) and water ad libitum. The bedding materials of the enclosure were changed every day. All the experimental trial was carried out in conformity with the CPCSEA guidelines. The study design was approved by the Institutional Animal Ethics Committee (IAEC) of IFTM University.

Acute toxicity

Animals were randomly divided in the group (n=3), a control group (Group A0) and dose levels (Groups A1, A2 and A3 of 500, 1000 and 2000 mg ABG / kg body weight respectively). ABG was given once by oral gavage at a weight of 10 mL/kg body weight. The control animals (A0) have been treated with distilled water in the same volume. Animals were attended for three hours after dose administration changes in behavior. The rats were weighed, and visual observations for mortality, behavioral pattern (salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain, and any signs of illness were conducted once daily during the subsequent period of 14 days.

Statistical analysis

The results were expressed as mean ± SEM (n = 5). Data were analyzed using Student's t-test, and results were considered significant when $p < 0.05$.

Table 1 : Phytochemical Analysis of *S.anacardium nut* extract (SNE)

S. No.	Phytochemical	Result
1.	Acidic compounds	+
2.	Amino acid	++
3.	Alkaloids	+
4.	Coumarin glycosides	-
5.	Flavanoids & phenols	+++
6.	Fats & Oils	+
7.	Glycosides	+
8. .	Protein	+
9.	Saponin	+
10.	Steroids	++
11.	Tannin	++

RESULTS

Pharmacognostic evaluation

Powder Microscopy

The images and detailed descriptions of powder microscopy of *Bhallataka* seeds are shown in Figures A-I. Several diagnostic characters of S. anacardium nut were noticed in Powder Microscopy. The notable features are the existence of pieces of little, thin-walled epidermal cells, elongated parenchyma cells, and oil globules in parenchymatous cells. Other characteristic features observed included not only, compressed stone cells with the narrow lumen, annular xylem vessels but also lignified fibers.

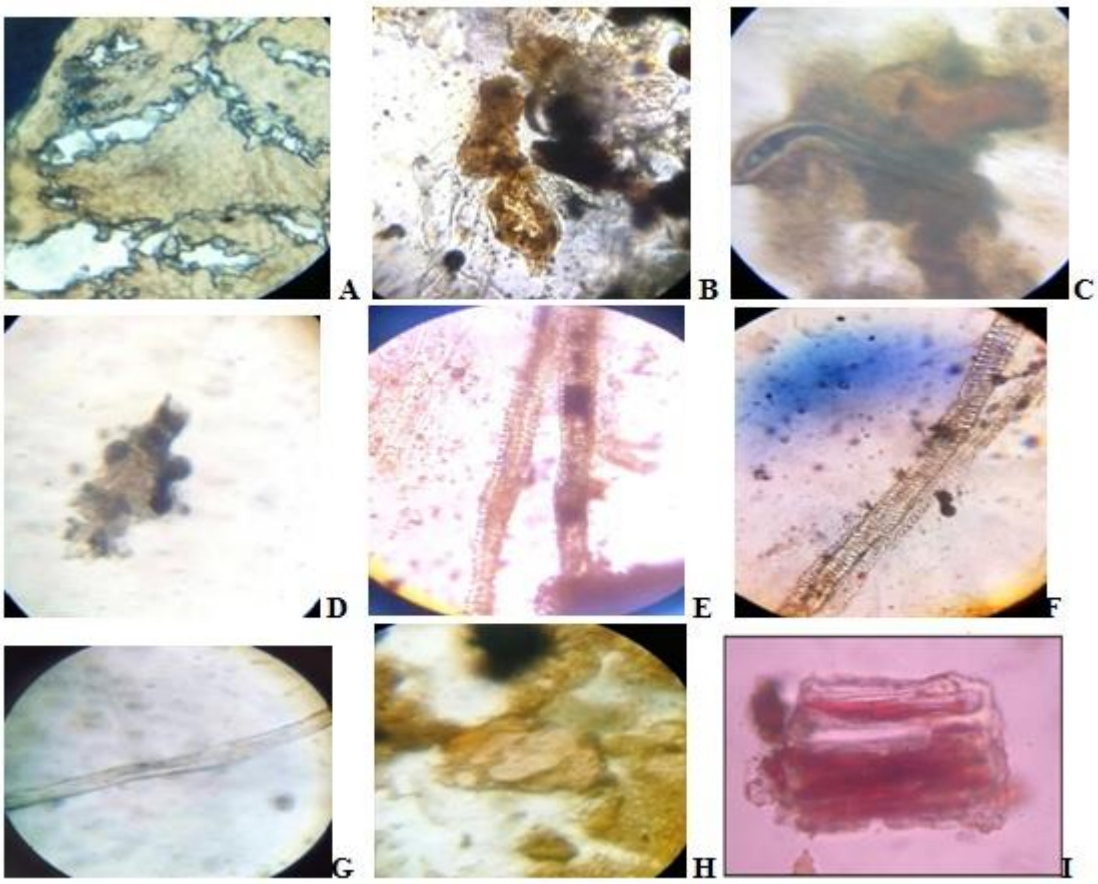

Figure A- I Power Microscopy of *Semecarpus anacardium* nuts.

A-Endosperm cells with crystals, **B**- stone cells **C**- papillae **D**- mesocarp cells, **E**-pitted vessels, **F**- annular vessel, **G**- lignified fiber, **H**-oil globule & **I**- parenchyma cells with reddish pigment.

Preliminary Phytochemical Analysis

Many secondary metabolites were found in phytochemical studies of crude extract (Table 1). It was observed that extracts of Ballantaka nut contained a significant concentration of secondary metabolites like Saponins, Flavonoids, Glycosides, Phytosterol, Tannins. Recent studies on phenolic compounds found in fruits and vegetables have attracted a good deal of curiosity thanks to their potential as antioxidants.[24]

Physicochemical evaluation of Marking Nut:

The S. anacardium nut was evaluated for physicochemical parameters like a loss on drying, total ash, acid-insoluble ash, alcohol, and water-soluble extractive values, petroleum ether soluble extractive value, and the results are presented in Table 2.

Table 2: Physicochemical evaluation of *S. anacardium* nuts[21]

Parameter	%Value (Mean ± SEM)	Observation
Foreign matter	0.63±0.06	✓
Total ash	3.34±0.92	✓
Acid-insoluble ash	0.45±0.61	✓
Water soluble ash	1.30±0.80	NA
Alcohol Soluble Extractive	13.73±1.25	✓
Water Soluble Extractive	5.88±2.40	✓
Pet ether (60-80^{0}C) soluble extractive value	1.24±0.09	NA

√: Within standard limits , NA : Not applicable

In-house preparation of *Amrtabhallataka Ghrita* (ABG).

The *Ghrita* (Figure 4)was prepared as per the conventional technique mentioned in AFI22 and stored in a well-closed glass container until further studies.

Figure 4: Prepared *Amrtabhallataka Ghrita* (ABG)

Organoleptic characters of the ABG

The traditional formulation was evaluated for organoleptic characters, and the results are tabulated din in Table 3.

Table 3: Organoleptic characters of the ABG

S. No.	Parameter	Observation
1	**Colour**	Brownish black
2	**Consistency**	Moderately hard
3	**Odor**	Odorless Characteristic
4	**Taste**	Sweet with Pungent after taste
5	**Texture**	Sticky
6	**Appearance**	Semi solid, viscous coarse

Analytical evaluation of *Amrtabhallataka Ghrita*

The Ayurvedic formulation prepared was evaluated as per the modern techniques, and it is presented in Table 4. All the parameters are in a normal range.

Table 4: Analytical evaluation *Amrtabhallantaka Ghrita*

S. No.	Parameters	Results
1	Acid value	7.8
2	Iodine value	68
3	Peroxide value	2.2
4	Refractive Index	1.478
5	Saponification value	337.72
6	Rancidity test	Fat didn't oxidize

Acute Toxicity Profile:

Safety evaluation of plant extracts used in folk medicine has generated considerable interest in the scientific community in an attempt to identify those that can potentially cause toxicity to humans.[19] In this context, the objective of the present study was to investigate the acute toxicity of *Amrtabhallataka Ghrita*. Toxic signs and the severity, onset, progression, and reversibility of the signs have been observed and recorded about dose and time. The animals were observed continuously for 14days after dosing. Neither mortality nor any abnormal clinical signs were observed in rats treated at the dose level of 2000 mg kg-1 body weight in the studies.

Discussion: To observe and interpret the changes that might occur during the formulation process and present it in the present-day scenario this study was planned. It was found that microscopic and phytochemical along the physicochemical evaluation of S. anacardium nut was similar to the previously reported values.[25] The Ayurvedic formulation was also evaluated for several parameters, to validate its utility as a formulation under modern testing. Briefly, the formulation was found to comply with the standards. Saponification value is more than the normal range, and it indicates lower molecular saturated fatty acids. Higher the iodine value, the less stable will be Ghrita and the more susceptible it is to oxidation and free radical production. High iodine value Ghritas are prone to oxidation and polymerization, and the sample becomes rancid, which decreases the shelf life and stability of the product. If the acid value is more, then chances of photo-oxidation and rancidity are more. The obtained values of these tests were found within normal limits in *Amrtabhallataka Ghrita*, which indicate good quality of the product. Besides, no rancidity was found in the finished product. Further, acute toxicity studies confirmed the non-toxic effect on Wistar rats.

CONCLUSION

These findings suggest that ABG is comparatively safe formulation and does not possess acute untoward effects. The observations and results, acquired from this study may be used as the standard in the further quality control research of various Ayurvedic semi-solid formulations. Nevertheless, data collected during this study is insufficient to reach a definite conclusion regarding ABG efficacy, since its purported use was needs to Be Further Validated By Pre-Clinical Trials For Therapeutic Efficacy.

Conflict of Interest: We declare that we have no conflict of interest.

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