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Evaluation of the Anti-aging Potential of *Bhallataka Rasayana* via High-Performance Thin Layer Chromatography (HPTLC)

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Abstract

Bhallataka (*Semecarpus anacardium* Linn., Family: Anacardiaceae) is a potent medicinal plant widely used in the Ayurvedic system of medicine for its *Rasayana* (rejuvenative) and *Vata-Kapha* pacifying properties. However, owing to the presence of corrosive phenolic lipids like urushiol and anacardic acids in its pericarp, it is classified as an *Upavisha* (semi-poisonous drug) in the *Drugs and Cosmetics Act* (1940), necessitating rigorous *Shodhana* (purification) prior to therapeutic application. The formulation *Bhallataka Rasayana* is a traditional herbo-mineral preparation designed to harness the therapeutic efficacy of the fruit while mitigating its toxicity.

This study aimed to standardize *Bhallataka Rasayana* through organoleptic, physicochemical, and phytochemical evaluation, specifically utilizing High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting to establish a quality control profile. The study also sought to validate the efficacy of a specific two-stage purification protocol involving *Ishtika Churna* (brick powder) and *Narikelodaka* (coconut water).

Raw *Bhallataka* fruits were procured and subjected to *Shodhana*. The initial phase involved adsorption of toxic oils using brick powder for 10 days, followed by *Swedana* (boiling) in coconut water for 3 hours. The purified fruits were then processed into *Bhallataka Rasayana* by boiling with cow's milk (*Go Dugdha*) and water for 22 hours, followed by the addition of cow's ghee (*Go Ghrita*) and sugar (*Sharkara*) (17% w/w). The finished product underwent physicochemical analysis for pH, ash values, and extractive values.

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Methanolic extracts were analyzed via HPTLC using Toluene:Ethyl Acetate (7:3 v/v) as the mobile phase.

The optimization study indicated that 17% w/w sugar concentration yielded the ideal organoleptic consistency. Physicochemical parameters were within standard limits, ensuring purity and safety. HPTLC fingerprinting revealed a distinct phytochemical profile with significant bands at Rf 0.25–0.28 (corresponding to anacardic acids, representing ~59–69% of the composition) and Rf 0.77–0.81 (lipid-bound complexes). The presence of supportive flavonoid bands (Rf 0.42–0.44) confirmed the retention of antioxidant principles. The chromatographic profile demonstrated that the purification and cooking processes successfully modified the toxic lipid fractions while preserving the bioactive core responsible for therapeutic action.

The HPTLC and physicochemical standards developed in this study provide a robust framework for the quality assurance of *Bhallataka Rasayana*. The data confirms that the traditional *Shodhana* and *Paka* processes effectively transform the toxic *Semecarpus anacardium* into a safe, lipid-enriched therapeutic agent, validating its classical use as a rejuvenator and immunomodulator. These findings serve as a reference for future regulatory compliance and clinical applications.

Key words : Anti-aging, Bhallataka Rasayana, HPTLC, Inshtika Churna, Narikelodaka.

The fruit of Bhallataka (*Semecarpus anacardium* L., Anacardiaceae) has been widely utilized in indigenous medical systems like Ayurveda, which are common in the Indian subcontinent. It is an extremely potent medication with strong therapeutic properties. Bhallataka is a drug of choice in the treatment of Vata and Kapha predominant disease conditions.[1] One It is a common component of many Ayurvedic remedies and is recommended for numerous illnesses.[2,17] The Drugs and Cosmetics Act (India) 1940 lists the fruit of Bhallataka as a toxic herbal substance.[6] Only after Shodhana (purificatory methods) can it be given internally. It is well known to have Rasayana (rejuvenating) properties in addition to anti-inflammatory, antibacterial, antioxidant, analgesic, anticancer, antihelminthic, and atherogenic effects.

Standardization and quality assurance of Ayurvedic medications are also important concerns in current times. In light of this, pharmacological, physicochemical, phytochemical, and nutritional evaluation criteria will be used to standardize Bhallataka Rasayana.

Procurement of Raw materials : The raw fruits of *Bhallataka* (*Semecarpus anacardium* Linn.) were collected from a personal cultivation farm located in (Malegaon/

warangi). Fresh Cow's milk (*Go Dugdha*) was procured from a *Gaushala* situated in the vicinity of the institute. The other ingredients required for the preparation, specifically Sugar (*Sharkara*) and Cow's Ghee (*Go Ghrita*), were purchased as standard market samples (Amul Brand) from the local market of Vadodara. Raw material were authenticated by Pharmacognocist at the Institute.

Preparation of Bhallataka Rasayana : Examination of Prashasta-Aprashasta Bhallataka Phala

According to traditional standards, acceptable Bhallataka fruits were selected.[15] Bhallataka fruits were meticulously cleaned of physical contaminants like dust, sand, false fruits, and other tree herbs. These fruits were submerged in drinkable water that was stored in a stainless steel container. After dipping the Bhallataka fruits, care was taken to ensure that no water flowed out. For thirty minutes, the vessel was not disturbed. The Bhallataka fruits that floated on the water's surface were then deliberately removed and stored separately on butter paper to dry. The Bhallataka fruits that had settled at the bottom of the jar were also taken out, placed on butter paper, and let to dry in the sun for two days. In order to prepare Bhallataka Rasayana and perform Shodhana, Bhallataka fruits were submerged in water. Tables 1 and 2 show the outcomes of Ashuddha Bhallataka Phala impurity and *Prashasta-Aprashasta Bhallataka Phala.*

Table-1. Results obtained after removal of physical impurities from Ashuddha Bhallataka Phala

Initial weight of Ashuddha Bhallataka (g)	Weight of Ashuddha Bhallataka after removal of physical impurities (g)
3000g	2986

Table-2. Results obtained after examination of Prashasta-Aprashasta Bhallataka Phala

Weight of Ashuddha Bhallataka (g)	Weight of Prashasta Bhallataka (g)	Weight of Aprashasta Bhallataka (g)	% Yield of Prashasta Bhallataka
2986	2140	846	71.67

Shodhana (Purification) of Bhallataka Fruits :

The purification of *Bhallataka* (*Semecarpus anacardium* Linn.) was carried out in two sequential stages to ensure the removal of toxic oily secretions and to enhance biological efficacy.

Ishtika Churna Shodhana (Purification with Brick Powder) : Red bricks were hammered and subjected to sieving to obtain *Ishtika Churna* (brick powder) of uniform particle size to facilitate optimal absorption. The raw *Bhallataka* fruits were gently pressed in a *Khalwa Yantra* (mortar) to facilitate the exudation of oil. The fruits were then mixed with the prepared brick powder, tied into a *Pottali* (bolus), and rubbed mechanically for 15 minutes. The fruits

remained buried in the brick powder for a period of 10 days to allow for the absorption of the oil (*Bhallataka Taila*). After 10 days, the *Pottali* was rubbed again for 15 minutes. The fruits were then separated from the brick powder and washed twice with warm water to remove any adhered brick particles. Finally, the washed fruits were dried in sunlight for one day.

Narikelodaka Shodhana (Purification with Coconut Water) *:* Following the initial processing, a second purification method was performed using *Narikelodaka* (Coconut water). A total of 2100 g of the processed *Bhallataka* fruits were subjected to *Swedana* (boiling) in 4 litter of *Narikelodaka*. The boiling process was carried out on a low flame (*Mandagni*) for a duration of 1.5 *Yama* (approximately 3 hours). Upon completion, the fruits were separated from the liquid media and allowed to dry overnight.

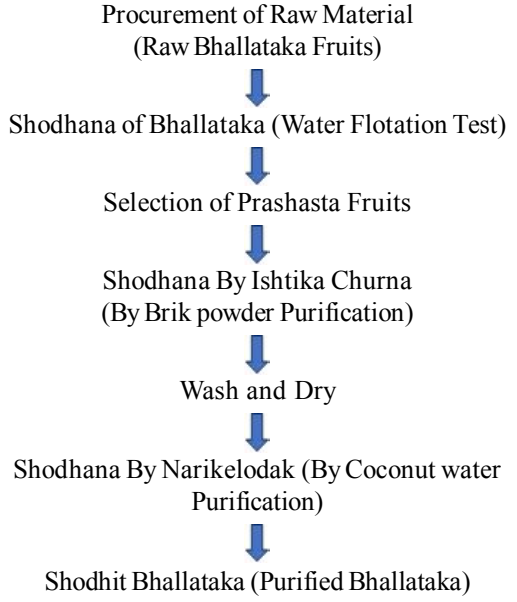
Preparation of Bhallataka Rasayana:

The preparation was initiated by mixing 2100 g of purified *Bhallataka* fruits with 12 liters of *Go Dugdha* (Cow's milk) and 70 liters of water. This mixture was subjected to boiling for a duration of 22 hours until it reduced to a decoction of 27 liters. The decoction was then filtered, and 5 kg of *Go Ghrita* (Cow's Ghee) was added to it. The mixture was subjected to heating on a low flame (*Mandagni*), with the temperature maintained strictly between 90°C and 100°C. The cooking process (*Paka*) continued for approximately 24 hours until the moisture evaporated and the formulation achieved the desired consistency. Heating was discontinued upon the observation of classical *Avaleha Siddhi Lakshanas* (confirmatory signs of perfection), specifically *Darvi Pralepa* (sticking of the mass to the ladle), *Apsumajjanam* (sinking of a drop in water), *Patitastu Na Sheeryate* (stability of the drop without spreading), and *Rasa Gandha Varnotpatti* (attainment of characteristic taste, odor, and color). The entire pharmaceutical process took approximately **46 hours** to complete.

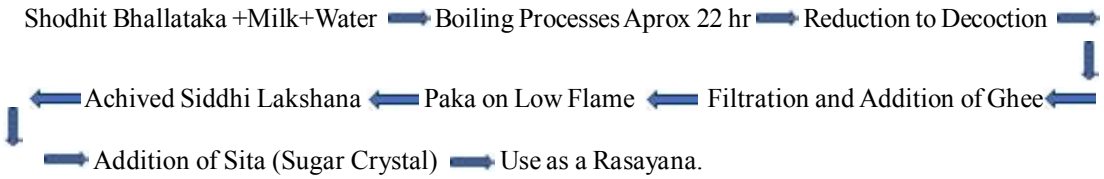
Optimization of Sugar concentration (Pilot study) :

A pilot study was conducted to achieve the desired level of palatability and consistency. A trial batch of 100 g of the formulation base was prepared, to which varying quantities of sugar were added. It was observed that the addition of **17 g of sugar per 100 g of the formulation** yielded the optimal organoleptic characteristics and consistency. This standardized ratio (17% w/w) was subsequently adopted for the final large-scale production.

Phase 1 Selection and Purification



Phase 2 Preparation of Rasayana



Preliminary analytical profile :

The physicochemical evaluation of the prepared *Shodhita Bhallataka Rasayana* was conducted in accordance with the standards prescribed by the Ayurvedic Pharmacopoeia of India (API). The samples were analyzed at the analytical laboratory for relevant physicochemical parameters, including Loss on Drying (LOD) at 105°C [10], Total Ash value [9], Water-soluble extractive value[10], Alcohol-soluble extractive value[8], and pH value[7]. Additionally, a qualitative phytochemical analysis was performed at the institute to identify the presence of various functional groups in the finished product. To ensure the quality and standardization of the formulation, High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting was implemented. The chromatographic profile of *Bhallataka Rasayana* was established to evaluate the presence of active constituents and to serve as a reference for quality control.

High-Performance Thin-Layer Chromatography (HPTLC)[18]:

HPTLC was used to analyze the Bhallataka rasayana methanolic extract in order to assess its phytochemical profile.

Preparation of Test solution :

Table-3. Chromatographic conditions for HPTLC of *Bhallataka Rasayana*

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 (S/N: 280008) Applicator
Filtering System	Simple filter
Stationary Phase	MERCK - HPTLC Silica gel 60 F_{254} on Aluminium sheets
Application (Y axis) Start Position	8.0 mm
Development End Position	80 mm from the plate base
Sample Application Volume	30 µL
Distance Between Tracks	22.4 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	20 minutes
Mobile Phase (MP)	Toluene: Ethyl acetate (7:3 v/v)
Visualization	@ 254 nm and @366 nm
Drying Mode, Temp. & Time	At room temperature for 5 minutes

Macroscopic Analysis : The Bhallataka Rasayana are brownish. It has a sweet taste and pungent odor.

Organoleptic evaluation: The organoleptic analysis is as follows (Rasayana).

Table-4. Organoleptic characteristics of Bhallataka Rasayana

Sr.no.	Parameters	Result
1	**Odour**	Pleasant
2	**Colour**	Chocolate colour
3	**Taste**	Sweet
4	**Consistency**	Jam like

Physio-chemical Analysis :

The observations of various physiochemical analyses are mentioned below. (Table 5).

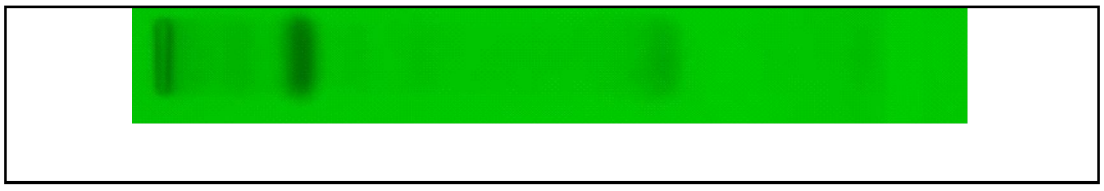
Table-5. Physio-Chemical Parameters of *Bhallataka Rasayana*

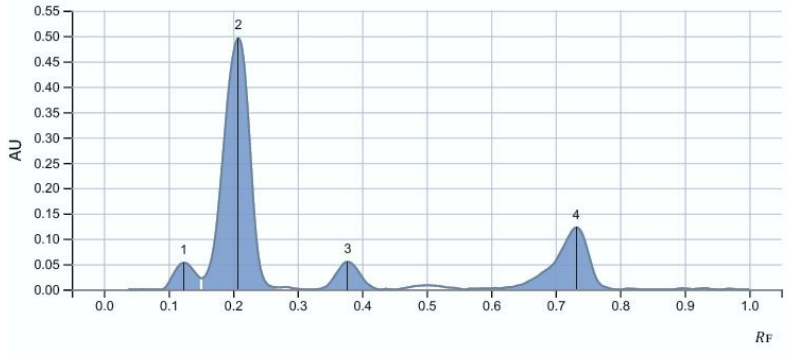
Sr.no.	Parameters	Result
1	PH	7.2
2	Loss on drying at 110 c (%w/w)	15%
3	Total Ash (%w/w)	8%
4	Test for heavy metals	Not found
5	Sugar	21%
6	Fat	11.12%
7	Rencidity	Absent
8	Organic impurities	Not found
9	Assessfor phytochemicals	Flavonoids, Alkaloids, Glycosides, Carbohydrates-Present

HPTLC Analysis :

The HPTLC Chromatograph of *Bhallataka Rasayana* developed at a wavelength of 254nm with the volume of 15.0 µL, 20.0 µL, and 30.0 µL is depicted in figure 1, 2, and 3 respectively, it showing R_f value Chromatograph fingerprint, peak height, and percentage area. By comparing the results (Fig. 1-3) at Pubchem and chemical substances were then identified is mentioned in Table-6 along with their Bioactivity (Source: PubChem, Google Scholar).

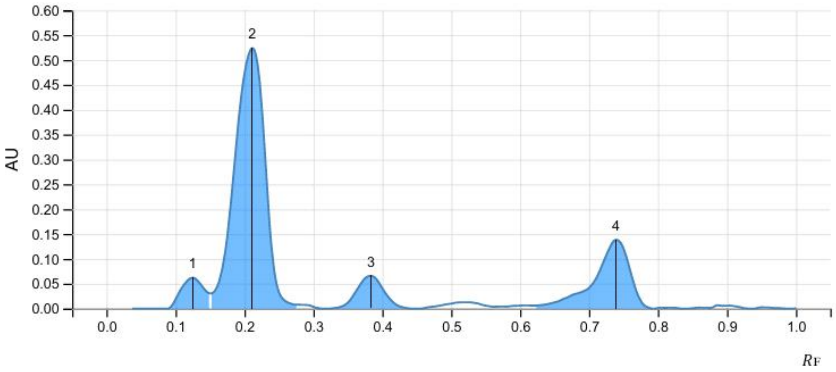
Figure 1: HPTLC Chromatograph of *Bhallataka Rasayana* @254 nm and 15 .0 µL volume (A=Fingerprint, B= Peak height, C= R_f Value and Area percentage)





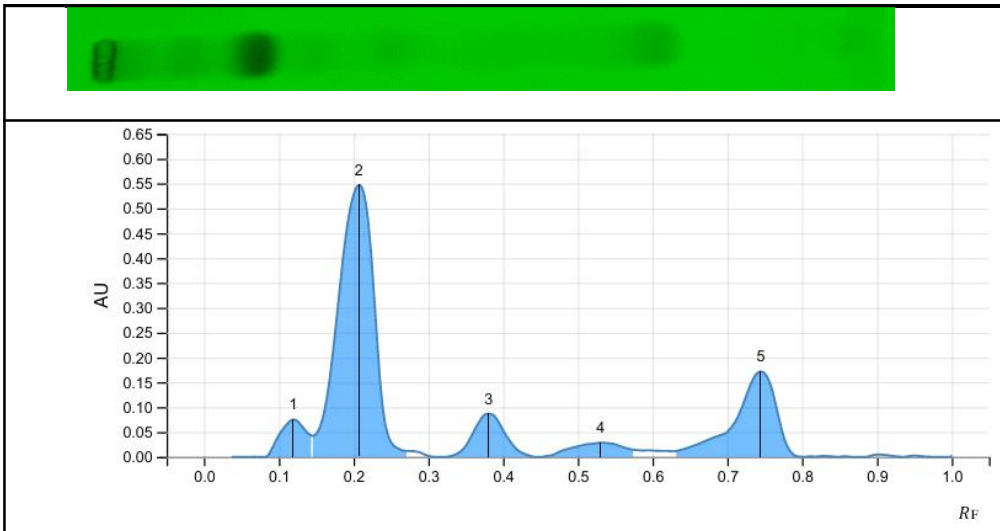
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.089	0.0000	0.124	0.0530	7.30	0.150	0.0226	0.00197	5.65
2	0.151	0.0223	0.208	0.4956	68.30	0.271	0.0040	0.02324	66.53
3	0.317	0.0001	0.378	0.0546	7.53	0.428	0.0001	0.00232	6.63
4	0.613	0.0023	0.732	0.1225	16.88	0.796	0.0000	0.00740	21.19

Figure 2. HPTLC Chromatograph of *Bhallataka Rasayana* @254 nm and 20.0 µL volume (A=Fingerprint, B= Peak height, C= R_f Value and Area percentage)



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.089	0.0000	0.125	0.0621	7.86	0.150	0.0305	0.00239	5.92
2	0.151	0.0304	0.211	0.5237	66.28	0.283	0.0076	0.02676	66.24
3	0.321	0.0000	0.383	0.0659	8.34	0.444	0.0000	0.00300	7.42
4	0.618	0.0061	0.739	0.1384	17.51	0.793	0.0000	0.00825	20.43

Figure 3. HPTLC Chromatograph of *Bhallataka Rasayana* @254 nm and 30.0 µL volume (A=Fingerprint, B= Peak height, C= R_f Value and Area percentage)



Peak	Start		Max			End		Area	
#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.082	0.0000	0.119	0.0751	8.26	0.144	0.0426	0.00303	5.94
2	0.146	0.0425	0.207	0.5466	60.15	0.274	0.0119	0.03024	59.24
3	0.322	0.0000	0.381	0.0868	9.55	0.444	0.0000	0.00424	8.31
4	0.451	0.0000	0.531	0.0287	3.16	0.586	0.0127	0.00243	4.76
5	0.628	0.0111	0.744	0.1716	18.88	0.801	0.0000	0.01110	21.75

Interpretation and Discussion :

Table-6. Substance name and their Bioactivity based on R_f Value@254 nm and 15.0 µL volume, 20.0 µLvolume, 30.0 µLvolume

R_f Range	Probable Phytoche-micalClass	Likely Plant Source(s)	Probable Pharmaco-logical Role	Area % Relevance	Citations
0.14–0.15	Low-molecular-weight phenolics, simple acids, amino acid derivatives	Minor polar components of Bhallataka, milk-derived small molecules	Mild antioxidant and detoxification support;may assist in early -phase liver protection	~5.7–5.9% → Minor but consistent peak, indicates background polar compounds	(5–16)
			Major anti-inflammatory,		

0.25–0.28	Anacardic acids, cardanols, cardols (major phenolic lipid class of Bhallataka)	Bhallataka (Semecarpus anacardium) pericarp oil	immunomodulatory, analgesic and antioxidant activity; documented inhibition of COX, LOX, NF-κB and cytokines; supports detoxification and modulates immunity	~59–69% (dominant peak) → Represents primary active constituents of Bhallataka Ghrita	[5,14,3–13]
0.42–0.44	Flavonoids, flavanones, catechol-like phenolics	Bhallataka nut kernel flavonoids	Additional antioxidant activity, scavenging of free radicals, hepatoprotection	~6–8% → Moderate supportive role; enhancessynergy with anacardic acids	[14,13]
0.77–0.80	High-molecular-weight phenolic lipids, semi- polar long-chain compounds	Bhallataka oil fractions, heated/lipid-bound derivatives, ghee-bound phenolics	Anti-inflammatory and tissue-support functions; related to anti-arthritic and immunomodulatory actions	~17–21% in 10–20 µL → Second most important pharmacological band	[5,3,13]
0.80–0.81 (seen only at 30 µL)	Lipophilic phenolics + gheeassociated lipid complexes	Ghee (Go-ghrita) + Bhallataka lipid complexes	Delivery of lipid-soluble actives, improves bioavailability,supports deep tissue penetration	21.75% at 30µL → becomes major at higher concentration, reflects gheemediated solubilization	[16,4]
0.99–1.00	Long-chain triglycerides, fatty acids (oleic, linoleic, palmitic)	Ghee, Bhallataka kernel oil	Provide energy, support absorption of lipophilic actives	Low (2.02%, only at 10 µL) → minor but expected lipid tail	[16,4]

Discussion :

HPTLC and physicochemical testing ensure Ayurvedic medicine quality by verifying authenticity, purity, and potency. These methods detect contaminants, heavy metals, and pesticides, safeguarding consumer health. By standardising formulations, they support clinical research, enhance therapeutic efficacy, and advance evidence-based Ayurveda for better patient care. Analysis of different formulations mentioned in *Ayurvedic* treatises is the need of the day.

The primary rejuvenating action of Bhallatak Rasayan can be attributed to its dominant phytochemical constituents—**anacardic acids, cardanols, and other phenolic lipids**—which form the major peak in the HPTLC fingerprint. These compounds are known for their potent **anti-inflammatory, antioxidant, and immunomodulatory** properties, which collectively protect tissues from wear, oxidative stress, and early degeneration. By reducing inflammatory mediators and enhancing cellular repair mechanisms, these bioactive molecules help maintain the functional integrity of body tissues (dhatus), thereby promoting **longevity, vitality, and rasayana effects**. Their strong biological activity aligns with classical descriptions of Bhallataka as a powerful agent for revitalization when properly purified and processed.

Alongside the primary phenolic lipids, Bhallatak Rasayan contains **flavonoids, flavanones, and catechol-like phenolics**, which contribute significantly to its supportive antioxidant network. These secondary constituents act as **free radical scavengers**, reducing oxidative burden and preventing cellular injury. By preserving mitochondrial function and protecting lipids, proteins, and DNA from oxidative damage, these compounds enhance the body's resilience to stress and promote **healthy cellular turnover**, which is essential for rejuvenation. Their presence supports and amplifies the effects of the primary rejuvenating molecules, creating a synergistic phytochemical profile that strengthens overall biological restoration.

Bhallatak Rasayan's bioavailability and deep-tissue distribution are greatly enhanced by the presence of ghee-bound lipophilic complexes and long-chain lipid fractions. Lipophilic compounds improve fat-soluble phytochemicals' solubility, absorption, and systemic distribution, allowing them to enter deeper tissues (srotas) and have a longer-lasting therapeutic effect. Targeted delivery is also supported by the traditional Yogavāhi feature of ghee without sacrificing power. Rasayana effects including increased strength, vitality, cellular nourishment, and total systemic rejuvenation are maximized by this greater penetration, which guarantees that rejuvenating substances reach cellular and subcellular levels where regeneration is most needed.

The presence of well-known bioactive derivatives of Semecarpus anacardium, namely anacardic acids and enhanced by flavonoids, high-molecular-weight phenolics, and ghee-bound lipid complexes, is confirmed by the HPTLC profile of Bhallatak Rasayan. When taken as a whole, these components offer a solid mechanistic foundation for the revitalizing benefits seen in healthy people. When processed properly, the phytochemical distribution confirms Bhallataka's promise as

a scientifically based rejuvenative therapy and validates the traditional Rasayana beliefs.

Conflict of Interest :

There is no conflict of interest, according to the authors.

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