Validation of Phytochemical Profile and Quality Control of *Bhallatakadi Modaka*

Sarojani Kuchanur[1*], Avnish Pathak[2] Archana Muraleedharan[3]

1. Ph.D Scholar, Department of Shalyatantra, SGT University, Gurgaon- 122505, India.
2. Professor & HOD, Department of Shalyatantra, SGT University, Gurgaon- 122505, India.
3. Assistant professor, Department of Shalyatantra, GS Ayurveda medical college & hospital, UP- 245304, India.

[1] sarojanish@gmail.com
[2] avnish_fims@sgtuniversity.org
[3] archanamurali3333@gmail.com

Corresponding Author
Sarojani Kuchanur, Ph.D Scholar, Department of Shalyatantra, SGT University, Gurgaon- 122505, India. **Contact no.**- 7011940769.

Abstract

In Ayurvedic text, for management of bleeding piles, *Bhallatakadi Modaka* is one of the formulations, improving digestion, promoting proper bowel movements, and purifying the blood and reduce inflammation. *Bhallataka* has been found to contain bioactive compounds such as Bhilawanols, flavonoids, and phenolic acids, which contribute to its anti-inflammatory, antioxidant, and digestive properties. The integration of stringent quality control measures in the production of herbal formulations is essential for ensuring safety, efficacy, and compliance. By investing in robust quality control systems, manufacturers can contribute to the overall integrity of the herbal industry, ultimately benefiting consumers and supporting the future of herbal medicine. The present study was undertaken to revalidate phytochemical profiling assisted with fingerprinting for ensuring quality assurance of *Bhallatakadi modaka*. Key bioactive constituents such as alkaloids, tannins, phenols, terpenoids, and phytosterols, are confirmed through HPTLC and other phytochemical tests.

Keywords: *Bhallataka, Haritaki,* HPTLC, Analytical evaluation, *Raktarsh,* Haemorrhoids.

Introduction

Herbal formulations have been used for centuries in traditional medicine systems around the world. With the increasing popularity of herbal products in modern healthcare, the importance of quality control has become paramount. Ensuring the safety, efficacy, and consistency of these products is essential for both consumer health and regulatory compliance.

In Ayurvedic text, for management of bleeding piles, *Bhallatakadi Modaka* is one of the formulations[1], improving digestion, promoting proper bowel movements, and purifying the blood and reduce inflammation. *Bhallataka* has been found to contain bioactive compounds such as Bhilawanols, flavonoids, and phenolic acids, which contribute to its anti-inflammatory, antioxidant, and digestive properties. Its ability to stimulate digestive enzymes makes it effective for improving metabolism, preventing the build-up of *Ama* (toxins), and treating conditions related to poor digestion. In modern perspective S*emecarpus anacardium* possesses strong anti-inflammatory properties, which are crucial in reducing the swelling and pain associated with haemorrhoids. The anti-inflammatory action of *Bhallataka* helps reduce the inflammation in the rectal veins, thereby preventing further damage and promoting faster healing.

The integration of stringent quality control measures in the production of herbal formulations is essential for ensuring safety, efficacy, and compliance. As the herbal market continues to grow, the need for high-quality products becomes increasingly important. By investing in robust quality control systems, manufacturers can contribute to the overall integrity of the herbal industry, ultimately benefiting consumers and supporting the future of herbal medicine. Implementing these practices not only protects consumers but also enhances the credibility and sustainability of herbal products in the global market.

In line with this, the World Health Organization has also highlighted the importance of quality assurance for traditional medicines[2]. Therefore, present study has been undertaken to revalidate phytochemical profiling assisted with fingerprinting for ensuring quality assurance of *Bhallatakadi modaka* has been undertaken.

Material& Methods

Procurements of Plant material

Formulation composition has been detailed at Table 1. *Shuddha Bhallataka* was procured from Jwala Ayurveda GMP certified company, Aligarh. Other crude drugs used in preparation were purchased from the Kari baowli, local Market, New Delhi. The drug was authentified by Department of *Dravyaguna*, GS Ayurveda Medical College and Hospital, Piklhuwa,UP (GSAMC&H/PO/2024/0132).

Preparation of *Bhallatakadi modaka*

Bhallatakadi Modaka (BM) was prepared at GMP certified Sharmaa Healthcare Pvt Ltd, New Delhi, according to the standardized procedure detailed in the Ayurvedic Formulary of India.[3] *Haritaki* and *Tila* seeds were ground and pass through sieve no. #85. *Guda* was pounded in stony mortar and pestle followed by addition of Shuddha *Bhallataka*, *Haritaki* and *Tila* seed powders. Trituration was done till homogenous blend was obtained. The mixture was rolled into pills and stored in an air tight container and subject to analytical tests.

Table 1: Ingredients of *Bhallatakadi Modaka*

Sr. No.	Name of Drug	Botanical Name/ English Name	Part used	Parts
1	*Shuddha Bhallataka*	*Samecarpus anacardium*	Fruit	1 Parts
2	*Haritaki*	*Terminalia chebula*	Pericarp	1 Parts

3	*Tila*	*Sesamum indicum*	Seed	1 Parts
4	*Guda*	Jaggery	-	6 Parts

Analytical Evaluation

Organoleptic evaluation encompassing Texture (*Rupa*) Taste (*Rasa*) Odour (*Gandha*) of *Bhallatakadi Modaka* were evaluated. Physicochemical analysis consisted of determination of Ash value[4], Extractive Value (Water soluble extractive[5] and Alcohol soluble extractive[6], pH[7], Reducing sugar[8], Non-reducing sugar, Friability, Disintegration time[9]. All tests were performed in a triplicate manner.

High performance thin layer chromatography (HPTLC): Extraction of Polar and nonpolar components were carried out to ensure comprehensive chromatography profiling.

Sample preparations:

(A) 10 g of crushed BM was weighed and refluxed with 75 ml of methanol for 30 minutes. The solution was filtered to remove insoluble matter. Filtrate was concentrated to 10 ml under reduced pressure. Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) v/v/v/v was taken as mobile phase [10].

(B) 10 g of crushed *Bhallatakadi Modaka* was heated with 75 ml of n-hexane in a water bath for 30 minutes followed by filtration and concentration the filtrate to 10 ml. Toluene: Ethyl acetate (7:3) v/v was taken as mobile phase [10].

Chromatographic conditions: Sample was applied through CAMAG Linomat V" 5_230245" S/N230245 (1.00.13) with Syringe size of 100 micro litre, Band width was fixed at 8.00 mm. Filtering System used was Whatman filter paper No. 1. TLC plates silica gel 60 F 254 of size 20×10 cm, manufactured by E. MERCK KGaA was used for band application, Development mode was CAMAG TLC Twin Trough Chamber 10×10 cm with Chamber saturation time of 30 minutes. CAMAG TLC Plate Heater was used for drying of plates. Visualizations were performed at 254, 366 nm and after derivatization with anisaldehyde-sulphuric acid reagent was scanned on 540 nm through CAMAG TLC Scanner_230698 (2.01.02) at specifications as follows:

Slit Dimensions- 6.00 × 3.00 mm, micro, Scanning speed- 20nm/s, Lamp- Deuterium (D2) and Mercury (Hg), Measurement type- Remission and Measurement mode: Absorption.

Result:

Results of organoleptic, physicochemical and phytochemical evaluation of *Bhallatakadi modaka* are depicted in Table 1, 2, 3 respectively.

Table 1: Organoleptic evaluation of *Bhallatakadi Modaka*

Parameters	Observations	API Part II, Vol.I
Colour	Blackish brown	Black
Odour	Characteristic	Characteristic
Taste	Astringent	Astringent
Texture	Smooth	Rough

Table 2: Physicochemical evaluation of *Bhallatakadi Modaka*

Sr. No.	Parameters	Average of 3 values	API Part II, Vol.I
1.	**pH (5% Aq. Solution)**	4.5	4-4.5

2.	**Total ash value (%)**	4%	NMT 2.5%
3.	**Acid insoluble ash (%)**	0.14%	NMT 0.25%
4.	**Water soluble extractive**	77.86%	NLT 75%
5.	**Alcohol soluble extractive**	17.84%	NLT 65%
6.	**Friability**	0.06%	--
7.	**Disintegration Time (min.)**	49 min	--
8.	**Reducing Sugar (mg/mL) %**	23.6%	23-24%
9.	**Non- reducing Sugar (mg/mL) %**	57.2%	56-58%

Table 3: Phytochemical evaluation of *Bhallatakadi Modaka*

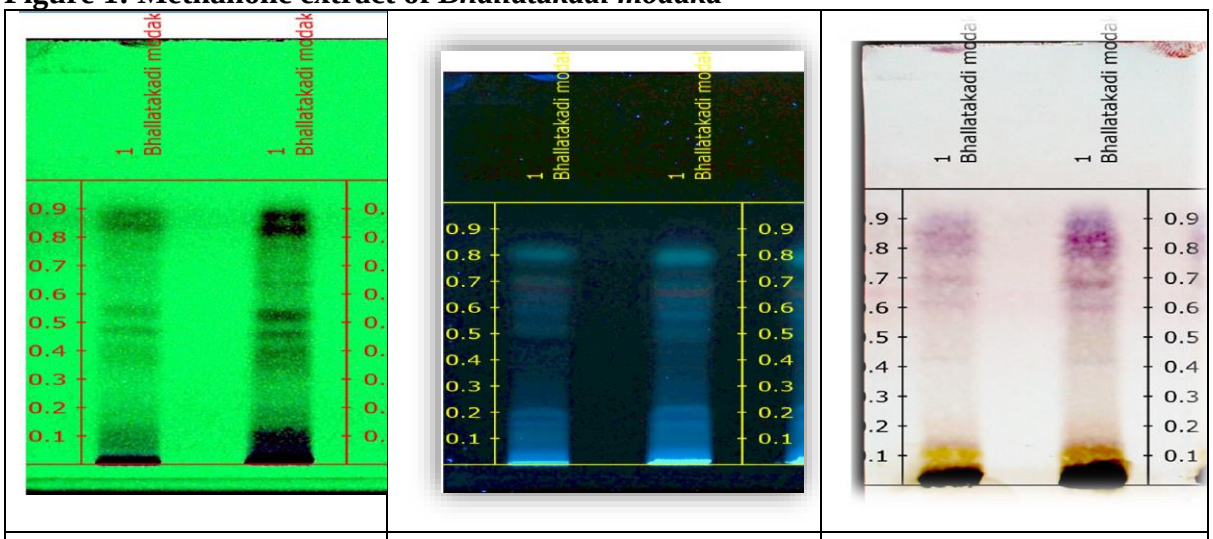
S. No.	Properties	Tests	Bhallatakadi Modaka
1.	Alkaloid	Mayer's test	+
		Hager's test	-
		Wagner's test	+
		Dragondraff's test	+
2.	Carbohydrates	Molish Test	+
3.	Phytosteriods	Salkowaski test	+
4.	Phenols	Ferric chloride test	+
		Lead acetate test	+
5.	Tannins	Ferric chloride test	+
6.	Terpenoids	Copper acetate	+

R_f values observed in HPTLC fingerprinting of methanolic and n-hexane extract have been presented in Table 5 & 6. Band development have been shown in Figure 1, 2& 3 respectively.

Table 5: Observations of HPTLC profile of methanolic extract *Bhallatakadi Modaka*

Sample	254 nm	366 nm	540 nm
Bhallatakadi Modaka	0.46, 0.53,0.83	0.11, 0.19, 0.79	0.14, 0.43, 0.62, 0.70, 0.85

Figure 1: Methanolic extract of *Bhallatakadi modaka*

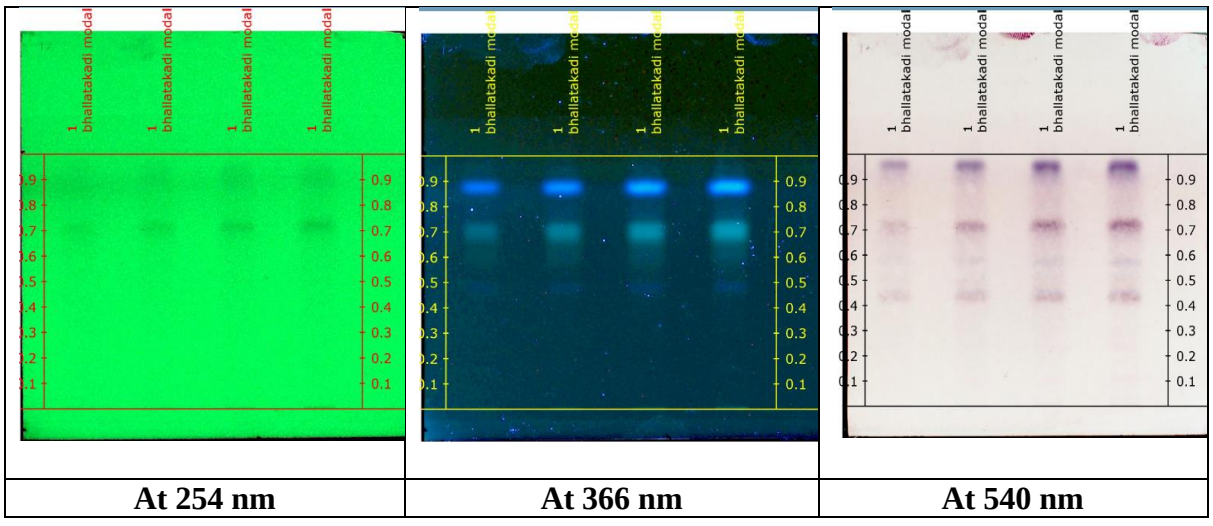


At 254 nm	At 366 nm	At 540 nm

Table 6: Observations of HPTLC profile of n-hexane extract *Bhallatakadi Modaka*

Sample	254 nm	366 nm	540 nm
Bhallatakadi Modaka	0.71, 0.88	0.70, 0.79, 0.87	0.47, 0.59, 0.71, 0.95

Figure 2: n-hexane extract of *Bhallatakadi Modaka*



At 254 nm	At 366 nm	At 540 nm

Discussion

Organoleptic parameters such as color, odour, taste, and texture offer an initial assessment of the formulation's authenticity and quality. The blackish-brown color differs slightly from the API's expected black, which may result from variations in raw materials or processing techniques. However, the characteristic odour and astringent taste, indicating consistency in its therapeutic profile. The smooth texture, while differing from the rough texture mentioned in API standards, could suggest modifications in preparation that may enhance patient compliance without compromising efficacy. Physicochemical evaluation, pH of *BM* (4.5) found slightly acidic, essential for stability and preservation. This pH level also ensures compatibility with the body's tissues, particularly in treating

conditions like Rakta Arsha (bleeding piles), where excessive acidity could worsen inflammation. The total ash value of 4% indicates the presence of inorganic compounds like minerals or impurities. This value exceeds the API limit (not more than 2.5%), which may be due to the nature of raw materials used for preparing the formulation. Acid insoluble ash measures the siliceous matter (like sand or soil) present in the formulation. The value of 0.14% falls well within the permissible limits, ensuring that the product is relatively free from soil and other silicate impurities. This is crucial for maintaining the formulation's purity, as excessive amounts of insoluble ash could indicate poor manufacturing practices or contamination during processing. Water soluble extractive indicates the presence of water-soluble compounds such as sugars, glycosides, and other polar constituents that are essential for the formulation's therapeutic properties. The value of 77.86% slightly exceeds the minimum limit of 75%, ensuring that the formulation has an adequate concentration of active ingredients. High water-soluble content is particularly relevant for effective absorption and therapeutic action, especially in the treatment of bleeding piles where water-soluble components can help reduce inflammation and improve circulation. The low alcohol soluble extractive value (17.84%) compared to the API standard (not less than 65%) raises concerns about the extraction efficiency of non-polar compounds such as alkaloids, flavonoids, and terpenoids. These compounds are crucial for the formulation's efficacy, particularly in providing anti-inflammatory and antioxidant effects. Although, the percentage of extraction was found less, however, phytochemical screening indicated towards their presence in the formulation. Reducing sugars such as glucose are involved in Maillard reactions and can influence the taste and shelf life of the formulation. The value of 23.6% is within the API range, ensuring sweetness for patient compliance and energy supply. In the context of hemorrhoids, reducing sugars can act as mild laxatives, softening stools and preventing strain during defecation, which is crucial for managing piles. Non-reducing sugars like sucrose contribute to the overall carbohydrate content without participating in Maillard reactions. At 57.2%, the non-reducing sugar content is optimal for stability, flavour, and energy release. These sugars can help maintain the energy balance in patients with bleeding piles, where a smooth digestive process is critical. Friability indicates the mechanical strength of the formulation, ensuring it can withstand handling, transportation, and storage without breaking. A friability of 0.06% is extremely low, which is favourable, as it suggests that the BM have good physical stability. Disintegration time 49 min. reflects how quickly the formulation breaks down in the body. A disintegration time of 49 minutes is slightly longer than typical, which could affect the release of active ingredients. However, slower disintegration may be desired for prolonged action, particularly in the gastrointestinal tract, providing sustained relief for conditions like *Rakta Arsha*. The phytochemical analysis of BM reveals the presence of alkaloids, phenols, tannins, terpenoids, and phytosterols, which contribute to its pharmacological significance in treating *Rakta Arsha*. Alkaloids provide anti-inflammatory and analgesic effects, reducing pain and swelling. Tannins and Phytosterols exhibit astringent and hemostatic properties, controlling bleeding and promoting tissue healing. Phenols and terpenoids offer antioxidant and anti-inflammatory activities, protecting tissues from oxidative stress and enhancing vascular health.

HPTLC is an efficient, rapid, and straightforward method for both qualitative and quantitative analysis of compounds. It is utilized to identify and quantify active constituents and impurities in drugs or compounds. When used with the methanol extract of samples, HPTLC demonstrates the maximum separation of constituents in a solvent system consisting of Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2% v/v). The apparent number of active principles present can be determined based on the number of spots observed. In the current investigation, three Rf (retention factor) values (0.46, 0.53,0.83 ± 0.02) were observed at a wavelength of 254 nm. At 366 nm three Rf values (0.11, 0.19, 0.79± 0.02) were observed and at 540nm 5 Rf values (0.14, 0.43, 0.62, 0.70, 0.85) were observed. These finding suggests 0.11 (blue spot) indicates the presence of flavonoids, known for their anti-inflammatory and venotonic properties which improves venous circulation and preventing venous dilation. These actions are particularly beneficial in controlling the inflammation and bleeding in *Rakta Arsha*., 0.46 Likely another flavonoid, contributing to the anti-inflammatory and blood vessel stabilizing effects, 0.53 (light brown spot) indicates the presence of tannins, which act as natural astringents, aiding in tightening tissues and reducing bleeding (Fig. 1). When used with the n-hexane extract of samples, HPTLC demonstrates the maximum separation of constituents in a solvent system consisting of Toluene: ethyl acetate (7:3: v/v). The apparent number of active principles present can be

determined based on the number of spots observed. In the current investigation, at 254nm wavelength two Rf (0.71, 0.88) at wavelength 366 nm three Rf (0.70, 0.79, 0.87) and at 540 nm four Rf (0.47, 0.59, 0.71, 0.95) were observed. 0.47 (purple spot) likely represents terpenoids**,** which are known for their ability to reduce inflammation and improve venous tone.0.59 (dark blue spot) may be corresponds to a lipophilic flavonoid or polyphenolic compound, contributing to anti-inflammatory and antioxidant actions. The antioxidant properties of flavonoids and gallic acid protect the rectal tissues from oxidative stress and free radical damage. By reducing oxidative damage, these compounds prevent further damage of the rectal veins and tissues [11]. 0.70 (purple spot) indicates the presence of alkaloids or terpenoids, which help reduce venous congestion and provide pain relief (Fig. 2). The HPTLC analysis of *Bhallatakadi Modaka* provides valuable insights into its chemical profile and potential therapeutic effects in managing *Rakta Arsha* (bleeding piles)**.** The presence of flavonoids, tannins, terpenoids, and alkaloids confirms its anti-inflammatory, haemostatic, astringent, venotonic**,** and antioxidant properties [12]. These pharmacological actions address the core symptoms of bleeding piles by reducing inflammation, controlling bleeding, promoting tissue healing, and providing pain relief.

Conclusion

Bhallatakadi Modaka provides a robust validation of its therapeutic efficacy, particularly in managing *Rakta Arsha*. The presence of key bioactive constituents such as alkaloids, tannins, phenols, terpenoids, and phytosterols, confirmed through HPTLC and other phytochemical tests, aligns with its traditional usage for anti-inflammatory, hemostatic, and tissue-repair properties. Variation in ash value and alcohol soluble extractive values in comparison to API underscores the importance of conduction quality analysis for addressing the variations at the levels of raw drug and manufacturing. HPTLC fingerprinting was attempted for the first time for this formulation. Ayurvedic standards and modern scientific protocols ensures both the safety and efficacy of this classical medicine, reinforcing its role in contemporary clinical practice. Further studies should focus to enhance bioavailability, pharmacological studies to elucidate the mechanisms of action and clinical trials assessing its effectiveness.

Reference

1 Shastri Ambikadutta. Bhaishajya Ratnavali of Vd. Rajeshwar Datta, 15th ed.; Chaukhamba Sanskrit Sansthana; Varanasi, Chapter 09/59, Arsharogachikitsa, 2013, Pg. 303.

2 WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Retrived from: https://www.who.int/publications/i/item/9789241594448

[3] The Ayurvedic Formulary of India, Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH, 2000, Part – I, 3:21, Pg.147.

4 The Ayurvedic Pharmacopoeia of India. 1st Edition, New Delhi Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH) 2006; Part II, Vol- I, Appendix 2.2.8., pp 159.

5 Ministry of Health and Family Welfare, Department of AYUSHA, Government of India. *The Ayurvedic Pharmacopoeia of India.* New Delhi: Ministry of Health and Family Welfare, Department of AYUSHA, Government of India; 1999. p. 213. Part 1. Appendix 2, 2.2.7.

6 Ministry of Health and Family Welfare, Department of AYUSHA, Government of India. *The Ayurvedic Pharmacopoeia of India.* New Delhi: Ministry of Health and Family Welfare, Department of AYUSHA, Government of India; 1999. p. 213. Part 1. Appendix 2, 2.2.6

7 Ministry of Health and Family Welfare, Department of AYUSHA, Government of India. *The Ayurvedic Pharmacopoeia of India.* New Delhi: Ministry of Health and Family Welfare, Department of AYUSHA, Government of India; 1999. p. 213. Part 1. Appendix 2, 3.3.

8 The Ayurvedic Pharmacopoeia of India, Part II, Volume I, First edition, Ministry of AYUSH, Government of India, New Delhi, 2007; Appendix-3(3.10):73.

9 *The Ayurvedic Pharmacopia of India.* 1st ed. Vol. 3. New Delhi: Ministry of Health and Family Welfare, Goverment of India; 2010. Anonymus; p. 214. ((318), 216 (319)). Part-2, Appendix-3.

10 Anonymous. The Ayurvedic Pharmacopoeia of India. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy (AYUSH). 1st edition. New Delhi: The Controller of Publication; 2006. Part II, Vol- I, p. 6.

11 Câmara JS, Perestrelo R, Ferreira R, Berenguer CV, Pereira JAM, Castilho PC. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. *Molecules*. 2024; 29(16):3861

12 Hadidi M, Liñán-Atero R, Tarahi M, Christodoulou MC, Aghababaei F. The Potential Health Benefits of Gallic Acid: Therapeutic and Food Applications. *Antioxidants*. 2024; 13(8):1001.