ORIGINAL ARTICLE

Quality control of *Brahmi Ghrita* (medicated ghee preparation)

Prem Shankar Pandey, K. Ramachandra Reddy, Priya Kumari

Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Abstract

Background: According to Ayurvedic literature, *Ghrita* is highly recommended for the management of psychiatric problems since it acts on the brain and has a lipophilic activity, making it well-known that it can pass the blood-brain barrier. In traditional Ayurvedic medicine, *Brahmi Ghrita*, a polyherbal formulation is recommended for management of various psychological disorders such as *Alakshmi* (Inauspicious) *Unmada* (Insanity), and *Apasmara* (Epilepsy). Drug quality is a basic necessity in this day and age, and analytical testing can help combat impurities or subpar pharmaceuticals. **Methodology:** Physicochemical analysis, including moisture content, acid value, saponification value, iodine value, and refractive index, was conducted in accordance with Ayurvedic pharmacopeia and the World Health Organization guidelines. **Results:** Studyproduces analytical data for *Brahmi Ghrita*. **Conclusion:** By employing a wide array of testing procedures, manufacturers are able to ensure compliance with regulatory requirements and supply consumers with a better product that effectively delivers *Brahmi's* therapeutic benefits. In conclusion, analytical tool can be applied to quality control, contaminant detection, chemical identification, research and development, of *Ghrita kalpana*.

Key words: Aflatoxins, analytical, *Brahmi Ghrita*, liquid chromatography-mass spectrometry, microbial content, physico chemical

INTRODUCTION

A polyherbal combination "*Brahmi Ghrita*" used for treating psychiatric problems is a well-known Ayurvedic traditional medicine. Based on research, most psychiatric disorders can benefit from the widespread internal and external use of *Brahmi Ghrita*. In addition to *Ghrita* formulation, the medication's lipophilic nature suggests that they will affect the blood-brain barrier more strongly.[1] The word "*Brahmi*" comes from the name of the mythological creator of the Hindu pantheon, "*Brahma*." Since the brain is the center of creative activity in the human body, *Brahmi* refers to substances that promote brain health. The Charak Samhita has the earliest direct mention of *Brahmi's* ability to improve memory. *Brahmi* is recommended as a treatment for mental disorders (retardation) that result in insanity.

Nowadays, a drug's traits are more crucial in the treatment of illness. A high-quality medicine is more valuable in today's world. To produce pharmaceuticals of high quality, we must choose raw materials of high quality and follow good manufacturing, storage, and other procedures. Sometimes, even after doing all of these procedures, the properties of that specific medication are unknown until analysis. Many analytical tests with standard values are mentioned to overcome this. A prepared medication can be regarded as high grade if it passes certain tests.[2] These tests encompass both qualitative and quantitative analyses of its constituents, contaminants, and physical characteristics. Analytical tests evaluate the active substances, but they also look for possible pollutants including pesticides, heavy metals, and microbiological infections. To make sure that heavy metal analysis is in accordance with the acceptable limits established by regulatory bodies, methods such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry (MS) are usually used. To find and measure any pesticide residues in the formulation, pesticide residue

Address for correspondence:
K. Ramachandra Reddy, Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.
E-mail: krcreddy@bhu.ac.in

Received: 05-05-2024
Revised: 17-06-2024
Accepted: 24-06-2024

analysis uses techniques such as liquid chromatography-tandem MS and gas chromatography-MS.[3]

In addition, *Brahmi Ghrita* overall appearance and organoleptic qualities are evaluated based on physical attributes such as color, odor, texture, and pH. Regarding the formulation's stability, uniformity, and acceptability for consumer usage, these metrics offer important information. These qualities shed light on the stability, uniformity, and acceptance of the formulation by the consumer.[4]

To accomplish the goal, for the 1st time we carried out an analytical examination of *Brahmi Ghrita* prepared by this method.

METHODOLOGY

Methodology of *Brahmi Ghrita*

The formulation of *Brahmi Ghrita* selected for the present thesis work to investigate Ayurveda medicine (*Brahmi Ghrita*) efficacy in dementia this work has been undertaken to evaluate scientifically for the management of dementia.

Ingredients

Serial no	Name in ayurveda	Scientific name	Parts used	Parts
1.	*Brahmi*	*Bacopa monnieri*	(W. P.)	1/7 part
2.	*Vaca*	*Acorus calamus*	(Rt.)	1/7 part
3.	*Mundi*	*Sphaeranthus indicus*	(Lf.)	1/7 part
4.	*Nilotpala (Kamala)*	*Nelumbo nucifera*	(Flw.)	1/7 part
5.	*Kustha*	*Saussurealappa*	(Rt.)	1/7 part
6.	*Saindhavalavana*	[Rock salt]	….	1/7 part
7.	*Pippali*	*Piper longum*	(Frt.)	1/7 part
8.	*Brahmi*	*Bacopa monnieri*	(W. P.) Swairasa	Q. S. (for *Bhavana*)
9.	*Ghrita*	[Cow's ghee]	……	4 part
10.	*Brahmi*	*Bacopa monnieri*	(W. P.) Swairasa	16 part

Method of Preparation

Equivalent amounts of the aforementioned medications Nos. 1 through 7 are converted into *Kalka* (paste) form by performing *Bhavana* with *Brahmi swarasa* for a week. Following this, *Ghrita* and *Brahmi swarasa* are combined, and *Ghrita* is prepared in accordance with *Ghritapaka* protocol.[5]

Methodology of Physicochemical Analytical Test

To conduct refractive index, specific gravity, acid value, peroxide value, saponification value, iodine value, congealing point, and identification (ID) test for mineral Oil of *Ghrita* standard protocols mentioned in Ayurvedic Pharmacopeia of India were used.[6]

High-performance Thin-layer Chromatography (HPTLC) of *Brahmi ghrita* for Aflatoxins

Standard Solution: Standard solution of Aflatoxin B1 (1 µg/mL), B2 (0.3 µg/mL), G1 (1 µg/mL), and G2 (0.3 µg/mL) was used as standard solutions for HPTLC quantification.

Preparation of test solution

Weigh accurately 5 g sample in 250-mL conical flask. Add 100 mL methanol and mix well. Sonicate the solution for 15 min. Add 40 mL water in the same solution and sonicate further for 10 min. Keep the solution at room temperature for 10 min. Filter the solution with Whatman filter paper no.1. Take 250 mL separating funnel; add 20 mL of obtained filtrate, 20 mL 10% sodium chloride solution and 20 mL petroleum ether. Mix well and allow standing for phase separation. Discard the petroleum ether layer. Take the aqueous phase and add 25 mL dichloromethane solution. Collect the dichloromethane layer. Repeat this process 3 times. Collect all dichloromethane layers in one beaker. Keep the sodium sulfate on what man filter paper no. 1 to remove the access amount of moisture. Filter the dichloromethane layer, and evaporate on the water bath, and reconstitute in 0.5–2 mL of methanol. Filter the solution with a syringe filter of 0.45 µm. Use the test solution thus obtained for HPTLC quantification.

Preparation of spray reagent (1% light liquid paraffin [LLP] in Hexane): 1 mL LLP mixed with 100 mL Hexane.

Chromatographic Conditions

Application mode: CAMAG Linomat 5 – applicator

Filtering system: Whatman filter paper No. 1

Stationary phase: MERCK – TLC/HPTLC Silica gel 60 F254 on Aluminum sheets

Application (Y axis) start position: 10 mm

Development end position: 80 mm from plate base

Sample application volume: 10 µL

Standard application volume: 7.5 µL

Distance between tracks: 20 mm

Development mode: CAMAG TLC Twin trough chamber

Chamber saturation time: 30 min

Mobile phase: Chloroform: Acetone: Water (14: 2: 0.03 v/v)

Pre-chromatographic washing: Pre-chromatographic derivatization was done by Diethyl ether

Visualization: @ 366 nm (after derivatization)

Spray reagent: 1% LLP in Hexane

Derivatization mode: CAMAG – Dip tank for about 1 min

Drying mode, Temp. and Time: TLC plate heater preheated at 100 ± 5°C for 3 min.

RESULTS

Refractive Index

The refractive index of 1.465 suggests clarity and purity in *Brahmi Ghrita*, indicating minimal impurities or contaminants affecting its optical properties [Table 1].

Specific Gravity

The specific gravity of 0.920 indicates the density of *Brahmi Ghrita* relative to water, which is lower than the density of water. This characteristic suggests that *Brahmi Ghrita* floats on water due to its lower density [Table 1].

Acid Value

With an acid value of 1.41, *Brahmi Ghrita* exhibits a low level of free fatty acids. This low acidity is favorable, indicating stability and quality in the preparation, which is crucial for its therapeutic effectiveness and shelf life [Table 1].

Table 1: Results of physicochemical analysis

Sr. No.	Parameters	Results	Limit as per API
1.	Refractive index	1.465	NA
2.	Specific gravity	0.920	NA
3.	Acid value	1.41	NA
4.	Peroxide value	9.09	NA
5.	Saponification value	233.03	NA
6.	Iodine value	33.39	NA
7.	Congealing point	35°C	NA
8.	ID test for mineral oil	Negative	NA

API: Ayurvedic pharmacopeia of India, ND: Not detected, g/mL: Gram per milliliter, ppm: Parts per million, cfu/gm: Colony forming unit per gram

Peroxide Value

The peroxide value of 9.09 indicates the extent of oxidation in *Brahmi Ghrita*. While this value is within acceptable limits, it suggests some degree of oxidation, which may influence the oil's shelf life and nutritional content [Table 1].

Saponification Value

The saponification value of 233.03 indicates the average molecular weight of the fatty acids present in *Brahmi Ghrita*. This value is useful for determining the purity and composition of the oil [Table 1].

Iodine Value

The iodine value of 33.39 reflects the degree of unsaturation of the fatty acids present in *Brahmi Ghrita*. This value is important for understanding the oil's stability, as well as its potential for oxidation and rancidity [Table 1].

Congealing Point

The congealing point of 35°C indicates the temperature at which *Brahmi Ghrita* solidifies. This characteristic is significant for handling and processing the oil, particularly in colder climates where solidification may occur at lower temperatures [Table 1].

ID Test for Mineral Oil

The negative result for the ID test indicates the absence of mineral oil in *Brahmi Ghrita*, confirming its authenticity and adherence to quality standards [Table 1].

Total Microbial Plate Count

The total microbial plate count for *Brahmi Ghrita* was found to be 2228 colony-forming units per gram (cfu/g), not exceeding the specified limit of not more than 10^5 cfu/g. This indicates a lower microbial load than recommended [Table 2].

Total Yeast and Mold Count

The total yeast and mold count in *Brahmi Ghrita* was found to be absent, meeting the specified limit of not more than 10^3 cfu/g. This absence suggests good microbial control and hygienic practices during production and storage [Table 2].

Staphylococcus aureus

S. aureus was found to be absent in *Brahmi Ghrita*, meeting the safety standards. The absence of this bacterium is crucial

Table 2: Results of microbial content in *Brahmi Ghrita*

Microbiological analysis			
1.	Total microbial plate count	2228 cfu/g	NMT 10^5cfu/g
2.	Total yeast and mold count	Absent	NMT 10^3cfu/g
3.	*Staphylococcus aureus*	Absent	Absent
4.	*Salmonella* spp.	Absent	Absent
5.	*Pseudomonas aeruginosa*	Absent	Absent
6.	S. *Escherichia coli*	Absent	Absent

API: Ayurvedic pharmacopeia of India, ND: Not detected, g/mL: Gram per milliliter, ppm: Parts per million, cfu/gm: Colony forming unit per gram

as it can cause food poisoning and other infections if present [Table 2].

Salmonella spp.

Salmonella species were not detected in *Brahmi Ghrita*, indicating compliance with safety standards. The absence of *Salmonella* is essential as it can cause severe gastrointestinal illness if ingested [Table 2].

Pseudomonas aeruginosa

P. aeruginosa was not detected in *Brahmi Ghrita*, aligning with safety guidelines. Its absence is critical as it can lead to infections, particularly in individuals with compromised immune systems [Table 2].

Escherichia coli

E. coli was absent in *Brahmi Ghrita*, meeting safety requirements. The absence of *E. coli* is crucial as certain strains can cause severe foodborne illnesses [Table 2].

Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2 were all found to be absent in *Brahmi Ghrita*, according to the aflatoxins analysis [Table 3; Figures 1 and 2].

DISCUSSION

Similar to other Ayurvedic formulations, *Brahmi Ghrita* derives its medicinal properties from particular botanical constituents. Analytical testing verifies the existence and quantity of these active ingredients, guaranteeing that the purity and efficacy of each batch remain constant.[7] In herbal formulations, contaminants such as pesticides, heavy metals, and microbiological infections can present major health hazards. To determine and measure these impurities and guarantee that *Brahmi Ghrita* is safe to consume, analytical testing is used.[8] Global regulatory agencies set strict standards for the potency and security of herbal remedies. To prove compliance with these restrictions and enable the lawful distribution and sale of *Brahmi Ghrita* in both domestic and international markets, analytical data are necessary.[4]

Analytical testing provides important information about the chemical composition and properties of *Brahmi Ghrita*. By optimizing the formulation for increased stability, shelf-life, and bioavailability, manufacturers can increase the product's overall effectiveness. Regular testing produces analytical data that can also be used to guide ongoing research and development projects that try to improve the method or develop new uses for *Brahmi Ghrita* in the medical field. Ayurvedic medicine is constantly improving and innovating due to this iterative approach.

Refractive Index and Specific Gravity

Determining the refractive index is a crucial component of *Ghrita* production quality control procedures. *Ghrita's* refractive index acts as a distinguishing feature that aids in its ID and separation from other oils or lipid-based materials. It helps to confirm the authenticity and purity of *Ghrita* compositions. The refractive index of *Ghrita* reflects its purity, showcasing its clarity and consistency, ensuring optimal quality. A refractive index of 1.465 and a specific gravity of 0.920 are indicative of the optical clarity and density of *Brahmi Ghrita*, respectively. These values are crucial in determining the purity and concentration of the ghee, essential for its efficacy and dosage accuracy in Ayurvedic formulations.[9]

Acid Value

The computation of the acid value yields crucial details regarding the amount of free fatty acids in *Ghrita*. It measures how much alkali is needed to neutralize free fatty acids, indicating how much lipids are broken down by oxidation and hydrolysis. Increased quantities of free fatty acids are indicated by elevated acid readings in *Ghrita*, which may be the consequence of oxidative rancidity or lipid hydrolysis. The monitoring of acid levels aids in determining the degree of lipid degradation and the emergence of unpleasant odors and flavors, all of which can negatively impact the oil's acceptability and quality. The low acid value of *Ghrita* signifies minimal free fatty acids, ensuring stability and longer shelf life, maintaining its therapeutic potency. The acid value of 1.41 suggests a low level of free fatty acids in *Brahmi Ghrita*. This is desirable as it indicates minimal rancidity and good quality control during preparation, ensuring the preservation of its therapeutic.

Peroxide Value

Determining the peroxide value is the main way to measure oxidative rancidity in *Ghrita*. It gauges the amount of peroxides

Table 3: Results of aflatoxins in *Brahmi Ghrita*

Parameter	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Sample
Weight	NA	NA	NA	NA	5.002 gm
Rf	0.81	0.77	0.72	0.66	....
AUC	11542	6151.8	8523.6	6880.2	....
Aflatoxins	....	....	....	....	Absent

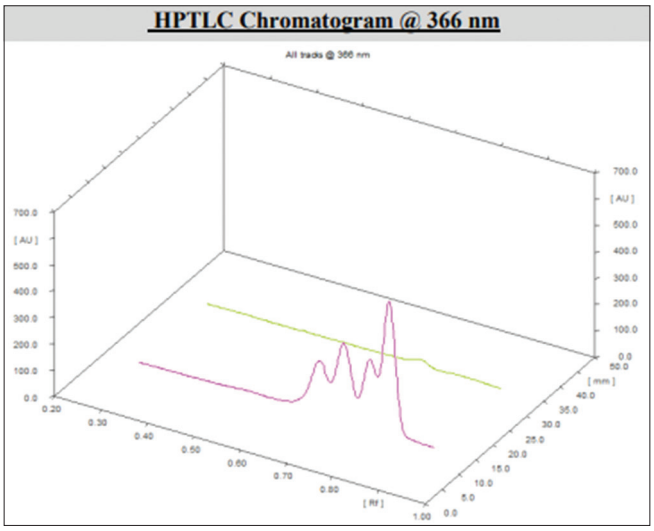

Figure 1: Chromatogram of *Brahmi Ghrita* for aflatoxins

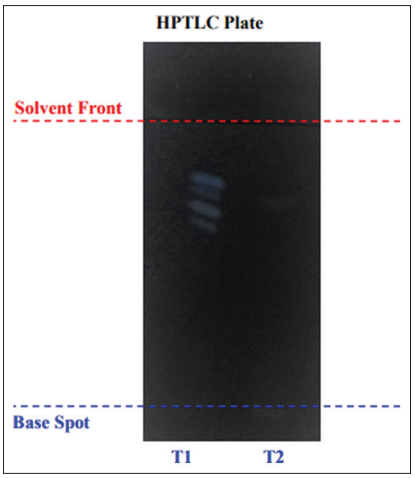

Figure 2: High-performance thin-layer chromatography plate Track T1: Aflatoxin standard (B1, B2, G1, and G2); Track T2: *Brahmi Ghrita*

produced when lipids oxidize, which indicates how much the oil has deteriorated due to oxidation. *Ghrita's* high fat content makes it prone to oxidative breakdown. Keeping a tab on the peroxide value aids in determining the quality of *Ghrita* items; lower values indicate less oxidation and more vitality. With a less peroxide value, *Ghrita* demonstrates excellent oxidative stability, preserving its nutritive components and medicinal properties. The peroxide value of 9.09 indicates the extent of oxidation in *Brahmi Ghrita*. While this value is within acceptable limits, oxidative processes might affect the shelf life and stability of the ghee, necessitating proper storage conditions.[10]

Saponification Value

Determining the saponification value gives important details regarding *Ghrita's* fatty acid composition. It measures how much potassium hydroxide (or sodium hydroxide) is needed to saponify the fats and oils in *Ghrita*, which is indicative of the fatty acids' average molecular weight. In *Ghrita*, lipid quality is indicated by the saponification value. By comparing the experimental saponification value with a standard reference, it is possible to evaluate the oil's authenticity and purity. Variations in lipid composition values or adulteration may be indicated by deviations from the expected saponification value. The saponification value of *Ghrita* indicates its purity and quality, reflecting the precise formulation process and adherence to standards. A saponification value of 233.03 reflects the average molecular weight of the fatty acids present in *Brahmi Ghrita*. This value provides insights into the composition and purity of the ghee, crucial for its medicinal applications.

The previous study on *Brahmi Ghrita* prepared by different ingredients has shown its saponification value to be in the range of 227–230, which is found to be more than that of plain *Ghrita* (199.15). On explaining the reason of saponification value, the study has stated that fats containing long-chain fatty acids have low saponification value, while due to the presence of short-chain fatty acids (SCFAs), they have a high saponification value. As *Brahmi Ghrita* prepared in this study has an even more higher saponification value, so it indicates the presence of more SCFAs. SCFAs are readily absorbed; a greater increase in SCFA production and potentially a greater delivery of SCFAs, specifically butyrate, to the distal colon, may result in a protective effect and improve the colonic and systemic health and it is easily absorbed and digested.

Iodine Value

The iodine value of *Ghrita* suggests its lipid composition, highlighting its suitability for specific therapeutic applications and absorption characteristics. The oxidative stability of *Ghrita* can also be determined by analyzing the iodine value. More oxidation sensitivity is indicated by higher iodine levels, and this might eventually cause rancidity and oil degradation. Lower iodine levels, on the other hand, are associated with

improved oxidative stability and extended shelf life. The iodine value of 33.39 denotes the degree of unsaturation of fatty acids in *Brahmi Ghrita*. This value influences the ghee's stability, resistance to oxidation, and suitability for various therapeutic uses.

Congealing Point

While developing *Ghrita*-based dosage forms, understanding the congealing point is crucial. *Ghrita's* solidification temperature is determined by its congealing point, which allows pharmaceutical experts to create dosage forms with the appropriate stability profiles and release characteristics. *Ghrita's* congealing point denotes its consistency and solidification temperature, ensuring ease of handling and administration in various formulations. The congealing point of 35°C indicates the temperature at which *Brahmi Ghrita* solidifies. This property is significant for its handling and processing, ensuring consistency and ease of use in different formulations

ID Test for Mineral Oil

Mineral oil may have a number of negative consequences and health issues in products meant for internal or externally usage, including: Potential carcinogenicity (Mineral oil is derived from petroleum, and some varieties of it can include recognized or suspected carcinogens called polycyclic aromatic hydrocarbons, or PAHs. Long-term exposure to PAHs has been associated with a higher risk of cancer, possibly including other types of cancer as well as skin cancer.) Skin irritation and allergic reactions (Mineral oil can produce a barrier on the skin and clog pores, which can worsen pre-existing skin disorders like dermatitis or eczema or cause acne. Furthermore, some people may experience hypersensitivity or allergic reactions to mineral oil, which can cause skin irritation, redness, swelling, and gastrointestinal disturbances (Intentional or inadvertent ingestion of mineral oil can cause gastrointestinal distress, including nausea, vomiting, diarrhea, and abdominal discomfort. By lubricating the intestines, mineral oil can function as a laxative; however, overindulgence in it may cause dehydration or interfere with the body's ability to absorb vital nutrients.) and respiratory issues (Breathing difficulties, coughing episodes, or respiratory irritation can result from inhaling mineral oil vapors or aerosols, especially in workplaces like factories. Lipid pneumonia or pulmonary inflammation can result from prolonged exposure to mineral oil droplets.)

A negative result for the ID test confirms the absence of mineral oil in *Brahmi Ghrita*, ensuring its authenticity and adherence to quality standards set by Ayurvedic pharmacopeia.[6] These interpretations provide valuable insights into the quality, purity, and suitability of *Brahmi Ghrita* for therapeutic and medicinal purposes in Ayurveda.

Total Microbial Plate Count

Elevated total microbial plate count values may suggest issues with hygiene during production, processing, or storage. High microbial counts can lead to product spoilage and compromise product quality and safety.

Total Yeast and Mould Count

The absence of yeast and mold in *Brahmi Ghrita*, meeting the specified limit of not more than 10^3 cfu/g, indicates good microbial control and hygienic practices during production and storage. The presence of yeast and mold can lead to product spoilage and affect sensory attributes. Moreover, some strains may produce harmful toxins.

Presence of Specific Pathogens

S. aureus, *Salmonella* spp., *P. aeruginosa*, and *E. coli* were all found to be absent in *Brahmi Ghrita*. The absence of these pathogens aligns with safety standards and ensures the microbiological safety of the product (European Pharmacopeia, 10th Edition). The presence of these pathogens could lead to foodborne illnesses and infections, making their absence critical for consumer health and safety.

Aflatoxins

Aflatoxins are potent carcinogens so, when exposed over time, can have a fatal effect on the immune system, damage the liver, and lead to other serious health problems. The analysis for aflatoxins in *Brahmi Ghrita* revealed the absence of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2, with all values reported as absent. The presence of aflatoxins in food products offers significant health hazards because they are extremely toxic substances generated by certain molds, mainly *Aspergillus flavus* and *Aspergillus parasiticus*.

CONCLUSION

Ensuring the efficacy, safety, and purity of *Brahmi Ghrita* is largely dependent on analytical testing. Utilizing an extensive range of tests, producers can guarantee adherence to legal specifications and offer customers a superior product that efficiently provides *Brahmi's* medicinal advantages. This study has generated the physicochemical analysis data, including moisture content, acid value, specific gravity, acid value, peroxide value, congealing point, ID test for mineral oil, saponification value, iodine value, and refractive index data for *Brahmi Ghrita*. Furthermore, the study shows the absence of microbial content and aflatoxins in *Brahmi Ghrita*.

REFERENCES

1. Manu P, Shetty SK, Savitha HP. Critical review on effect of Brahmi Ghrita in psychiatric disorders. Int J Res Ayurveda Pharm 2017;8:16-8.
2. Hosur AF, Mamatha KV, Kupati VB. Physico-chemical analysis of Tangashree Ghrita. J Ayurveda Integr Med Sci 2017;2:86-8.
3. Ji C, Xiao L, Wang X, Hua MZ, Wu Y, Wang Y, *et al*. Simultaneous determination of 147 pesticide residues in traditional Chinese medicines by GC-MS/MS. ACS Omega 2023;8:28663-73.
4. Bansal G, Suthar N, Kaur J, Jain A. Stability testing of herbal drugs: Challenges, regulatory compliance and perspectives. Phyther Res 2016;30:1046-58.
5. Reddy KR, Chandana H, Vinay K. Efficacious formulations and important Ayurvedic therapeutic procedures of Vaidya Chintamani -An Ayurvedic great treatise of South India. Int J Green Pharm 2023;17:52.
6. Anonymous. The Ayurvedic Pharmacopoeia Of India. Vol. 2, Part II. New Delhi: Department of ISM and H, Ministry of Health and Family Welfare; 2008. p. 170-4, 199-202.
7. Reddy RC, Deo YK. Standardization of Brahmi Ghrita with special reference to its pharmaceutical study. Int J Ayurvedic Med 2012;3:16-21.
8. Tripathy V, Saha A, Kumar J. Detection of pesticides in popular medicinal herbs: A modified QuEChERS and gas chromatography-mass spectrometry based approach. J Food Sci Technol 2017;54:458-68.
9. Kumari R, Kotecha M. A review on the standardization of herbal medicines. Int J Pharma Sci Res 2016;7:97-106.
10. Pal RS, Mishra A. Standardization of Dhatryadi Ghrita: A herbal ghee based ayurvedic medicinal preparation. Open Med J 2018;5:47-55.

Source of Support: Nil. **Conflicts of Interest:** None declared.