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An Analytical Study to Determine the Safety of *Brahmi Avleha*

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ABSTRACT

Introduction: *Brahmi Avleha* was prepared as per the Ayurvedic classics indicated in *Manovaha Srotas Vikara*. *Brahmi Avleha* reference was taken from *Kashyapa Samhita* and prepared in *Nagarjuna Rasashala* of PGIA, Jodhpur. There is need of time to assess the quality control and standardization of the mentioned formulations.
Material and Method: Above formulations has been evaluated on the basis of organoleptic characters like colour, appearance and odour, Physio-chemical like Alcohol soluble extractive, Loss on drying at 105°C/Moisture Content, pH value, Total Ash, Water Soluble extractive, etc and Finger printing by Thin Layer Chromatography (TLC).
Result and Discussion: The analytical values of the formulation was under the normal values, which indicates that the formulation are standardized, safe and effective in the management of *Manovaha Srotas Vikara*.

Keywords: Ayurvedic formulation, *Avleha, Taila,* Analytical Study

INTRODUCTION

An analytical study in the context of Ayurvedic formulations involves examining the composition, quality, and safety of the traditional medicines using modern scientific techniques. The aim is to bridge the gap between ancient knowledge and contemporary standards, ensuring that the formulations are both effective and safe for use. To conduct an analytical study of an Ayurvedic formulation, several steps can be followed to ensure the quality, safety, and efficacy of the formulation like Raw material collection, Preparation of formulation, organoleptic evaluation, phytochemical analysis, physiochemical analysis, heavy metal studies and documentation and reporting[1]. Analytical study of *Brahmi Avleha* was carried out under the clinical trial title "Clinical study to evaluate the efficacy of *Brahmi Avleha* and *Brahmi Taila Pratimarsha Nasya* in the management of Attention deficit hyperactive disorder in children" registered on CTRI with registration no: CTRI/2023/06/053855. *Brahmi Avleha*, described in the *'Kashyapa Samhita*' under the '*Lehadhyaya'* 18th chapter, was taken for trial[2]. This *Avleha* primarily consists of *Medhya Rasayana* herbs are taken in equal amount, which is considered the preferred drug for central nervous system stimulation in Ayurveda.

Table No 1. Showing ingredients of *Brahmi Avleha*:

S. No	Ingredients	Latin name	Part used	Quantity
1	*Brahmi*	*Bacopa monnieri* (L.) Wettst.	Whole plant	1 Part
2	*Mandukparni*	*Centella asiatica* Linn.	Whole plant	1 Part
3	*Haritaki*	*Terminalia chebula* Retz.	Fruit	1 Part
4	*Vibhitak*	*Terminalia belerica*	Fruit	1 Part
5	*Aamlaki*	*Emblica officinalis*	Fruit	1 Part
6	*Chitrak*	*Plumbago zylenica* Linn.	Root	1 Part
7	*Vacha*	*Acorus calamus* Linn.	*Bhumi kanda*	1 Part
8	*Shatpushpa*	*Anethum sowa*	Seeds	1 Part
9	*Shatavari*	*Asparagus racemosus*	Root	1 Part
10	*Danti*	*Baliospermum montanum* (Willd.)	Root	1 Part
11	*Nagbala*	*Sida veronicoefolia*	Root	1 Part
12	*Nishoth*	Operculina terpenthum R. Br.	Root	1 Part

Method of Preparation

Herbs of *Brahmi Avleha* was procured from local market which was identified by pharmacologist. After proper identification, all ingredient were clean & dried. After that, *Brahmi Avleha* was prepared under aseptic condition in pharmacy attached to University of Post Graduate Institute of Ayurveda for Research & Studies, Jodhpur under the supervision of competent authority. During preparation of drug, all related SOP's was strictly followed. After the preparation of medicine, drug was stored in airtight container and labelled with the date of manufacturing and drug liscence number. Dose of *Brahmi Avleha* decided as per Young Formula (Adult dose of *Churna* was considered during the calculation of dose as per *Acharya Sharangdhara*)

Objectives

- To analyse the physical, organoleptic character, physiochemical parameters of *Brahmi Avleha* formulation prepared by the classical method.
- To standardize the parameters of *Brahmi Avleha* formulation
- To validate the safety and efficacy of formulation in children.

MATERIAL AND METHOD

Parameters Studied in *Brahmi Avleha* was taken from Ayurveda Pharmacopoeia of India and published by the Government of India, Department of Ayurveda, Yoga — Naturopathy, Unani, Siddha & Homeopathy (AYUSH), New Delhi, served as the basic for the parameters used in the numerous investigations.

Analytical Study of *Brahmi Avleha*

Place of work

Cultivator Phyto Lab Pvt. Ltd. Sonamukhi Nagar, Sangaria Fanta, Jodhpur. Sample Registration No. – CPL/O/24/09/01482/1. Sample sent to Lab Date and start of analysis-13/09/2023 and completed on18/09/2023 in 6 days.

Analytical study was done under the following headings-

1. Organoleptic Characters
2. Physio-chemical Parameters
3. Chromatographic fingerprint – TLC

Organoleptic characters:

Organoleptic characters refer to the sensory properties of a substance, which are evaluated using the senses (sight, smell, taste, touch, and hearing). These characters help in assessing the quality and authenticity of the product. These characters include appearance, odour, taste, texture, etc.

Table No 2 Showing organoleptic characters of *Brahmi Avleha*

S. No.	Organoleptic characters	*Brahmi Avleha*
1.	Appearance	Powder
2.	Color	Greenish Yellow
3.	Odour	pleasant

Physiochemical Parameters

Physicochemical parameters refer to the physical and chemical characteristics of Ayurvedic formulations. These parameters are crucial for assessing the quality,

consistency, and stability of the formulations. Parameters include Alcohol soluble extractive, Moisture content, pH value, Water soluble extractive values, Total ash, etc.

Alcohol Soluble extractive

5grams of the sample taken in the glass-stoppered flask with 100 ml of 90% ethanol (alcohol) after that the mixture was frequently shaken for the first 6 hours and then allow it to stand for 18 hours. Filtration of the mixture rapidly to prevent loss of solvent and transfer 25 ml of the filtrate to a pre-weighed evaporating dish after that the solvent was evaporated on a water bath. The residue was dried at 105°C to a constant weight. The residue was weighted and the percentage of alcohol-soluble extractive was calculated using the formula[3]:

Percentage of alcohol-soluble extractive= (Weight of residue / Weight of sample) ×100

Loss on Drying/ Moisture content

Firstly the Weight of a clean, dry, and pre-weighed glass-stoppered shallow weighing bottle was taken after that about 1-2 grams of the sample taken in bottle. Bottle with the sample kept in a drying oven set at 105° and dried to a constant weight, typically for 3 hours. After drying, the bottle removed from the oven and the bottle was closed with the stopper and allow it to cool to room temperature in a desiccator. Weigh was measured of the bottle with the dried sample and calculated with the below formula[4].

LOD (%) = (Initial weight−Final weight / Initial weight) ×100

pH Value

To determine the pH value of an Ayurvedic powder, 1 gram of the Ayurvedic powder dissolved in 100 ml of distilled water to prepare a 1% w/v solution with proper stir. The pH meter was calibrated using standard buffer solutions (usually pH 4.0, 7.0, and 9.0) to ensure accurate readings. The electrode of the pH meter was rinsed with distilled water and gently dried. The electrode was immersed into the prepared solution, after stabilization the pH value displayed on pH meter[5].

Total Ash Value

Total Ash value of formulation was calculated as follows: Accurately about 2 grams of the sample was taken in previously ignited and tarred silica crucible and heated to 500-600°C until the material appears white, indicating the absence of carbon. The crucible was cooled in the desiccator and weighted. Ash value was calculated with the below formula. (Same ph value reference)

Total Ash (%) = (Weight of ash / Weight of sample) ×100

Water Soluble Extractive

About 5 grams of the sample taken in glass-stoppered flask with 100 ml of chloroform water after that the mixture was frequently shaken for 6 hours and then allow to stand for 18 hours. Filtration of the mixture rapidly to prevent loss of solvent and transfer 25 ml of the filtrate to a pre-weighed evaporating dish after that the solvent was evaporated on a water bath. The residue was dried at 105°C to a constant weight. The residue was weighted and the percentage of alcohol-soluble extractive was calculated using the formula[6].

Percentage of water-soluble extractive= (Weight of residue / Weight of sample)×100

Table No 3 Showing physiochemical parameters assessed in *Brahmi Avleha*

S. No	Test Parameter(s)	Unit	Result	Test Method	Normal values
Discipline: Chemical			**Product Group: AYUSH Products**		
Physio-Chemical					
1	Alcohol soluble extractive	%	18.45	API Part II Vol III: 2010	6 – 33 %
2	Loss on drying at 105°C/Moisture Content	%	7.60	API Part II Vol III: 2010	3 – 7 %
3	pH value	-	4.87	API Part II Vol III: 2010	4.0 – 7.0
4	Total Ash	%	9.64	API Part II Vol III: 2010	5 – 10 %
5	Water Soluble extractive	%	29.89	API Part II Vol III: 2010	12.60 – 33 %

Finger Printing of *Brahmi Avleha* by Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a widely used analytical technique in the assessment of physiochemical analysis. TLC is particularly useful for the standardization and quality control of Ayurvedic formulations, as it allows for the identification of multiple active constituents simultaneously. It is a relatively simple, cost-effective, and reliable method for ensuring the consistency and efficacy of Ayurvedic products.

Method of TLC

To perform Thin Layer Chromatography (TLC) on *Avleha*, these step were followed:
About 1 gram of the *Avleha,* the sample was mixed with a suitable solvent (e.g., methanol or ethanol) by shaking it for 30 minutes and the extract was filtered and concentrate in to a small volume.

A pre-coated silica gel TLC plate was taken; a baseline was drawn about 1 cm from the bottom of the plate using a pencil. Small spots of the concentrated extract was applied on the baseline using a capillary tube or micropipette and dried.
A suitable mobile phase (solvent system) was prepared in a TLC chamber (e.g., a mixture of chloroform and methanol) and after that TLC plate placed in the chamber, ensuring the baseline is above the solvent level. The chamber was covered and allow the solvent to rise up the plate by capillary action until it reaches about 1 cm from the top. The plate was removed from the chamber and allowed to dry. Visualize the spots under UV light or by spraying with a suitable detecting reagent (e.g., iodine vapor or anisaldehyde-sulfuric acid). The distance traveled by each spot and the solvent front was measured. The Rf value for each spot calculated by using the formula[6]:

Rf= Distance traveled by the compound / Distance traveled by the solvent front

Table No 4 Showing TLC values of *Brahmi Avleha*

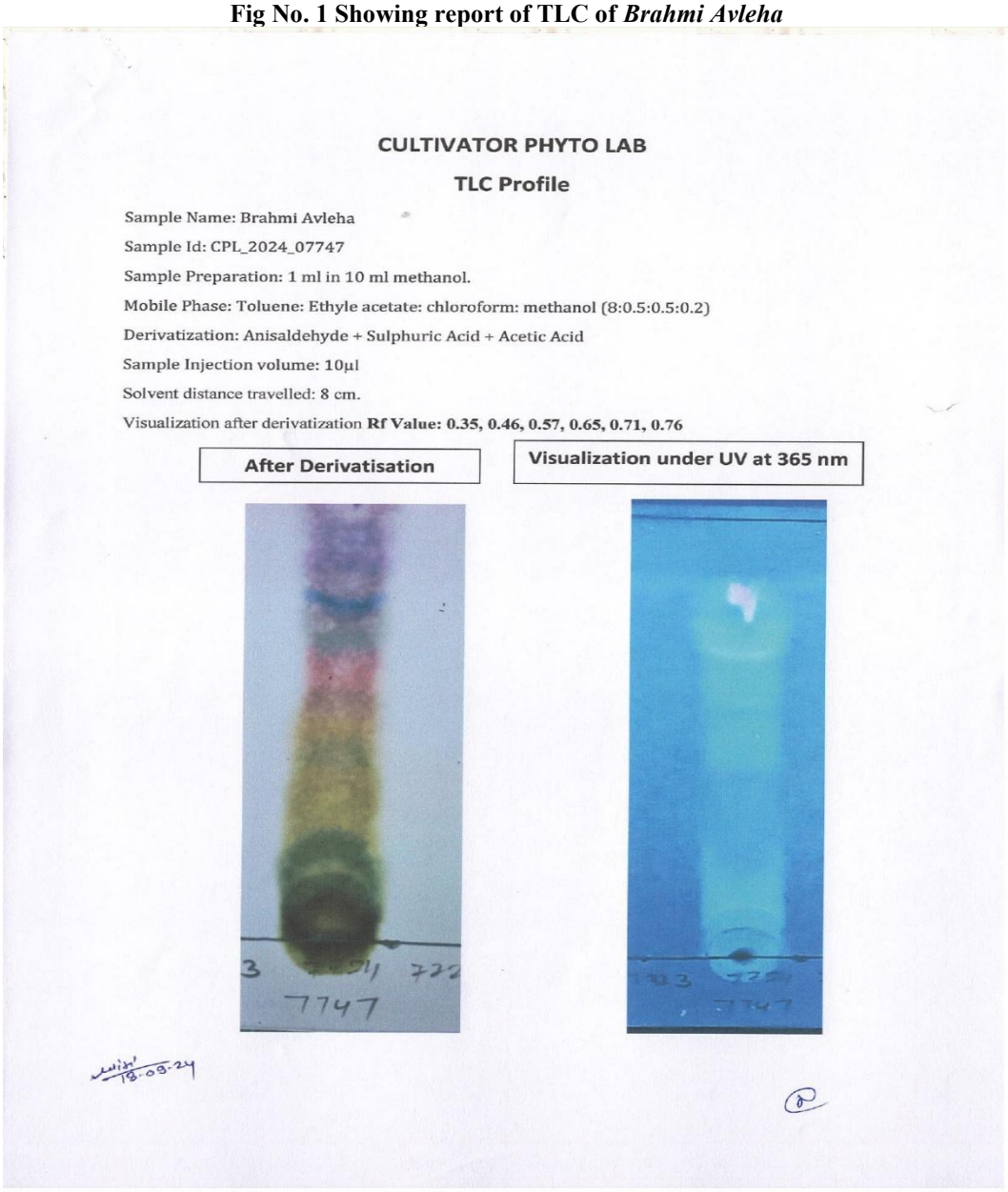
	Test Parameter(s)	Unit	Rf values	Test Method
Discipline: Chemical			Product Group: AYUSH Products	
Physio-Chemical				
Brahmi Avleha	Thin-Layer Chromatography (TLC)	-	0.35, 0.46, 0.57, 0.65, 0.71, 0.76	API Part II Vol IV: 2017

DISCUSSION

The Organoleptic characters of Brahmi Avleha is mentioned in Table 2. The Physio-chemical parameters of formulation is detailed in Table 3 along with normal values. TLC photo documentation of Methanolic fraction of formulation is shown in Fig.1 and Rf values is detailed in Table 4. The physiochemical standards would serve as preliminary test for the standardization of the formulation. Tests such as Alcohol soluble extractive, Moisture content, pH value, Water soluble extractive values, Total ash, results of TLC photo documentation, the unique Rf values, obtained under UV at 365 nm wavelength can be used as fingerprint to

identify the polyherbal formulation of *Brahmi Avleha*.

Fig No. 1 Showing report of TLC of *Brahmi Avleha*



Organoleptic characters of *Brahmi Avleha* were normal, *Avleha* was in powdered form, having pleasant odour and normal colour *Avleha* was greenish yellow. Physio chemical factors of *Brahmi Avleha* like pH were measured to prevent stomach discomfort, and the moisture content was measured to identify any weight gain brought on by moisture absorption. It was discovered that the value obtained fell within the acceptable range. A rise in ash value denotes contamination, substitution, and adulteration since the process oxidises product components. After full combustion, the total Ash value indicates the total quantity of inorganic material, and the acid insoluble ash value indicates silicate impurities that may have developed as a result of improperly washing crude pharmaceuticals. It was discovered that the ash readings were within the acceptable ranges. Monitoring the moisture content over time helps in determining the shelf life of the formulation. Keeping the moisture content within

acceptable limits ensures that the product remains stable and effective throughout its intended shelf life. High moisture content can promote the growth of bacteria, yeast, and moulds. By controlling the moisture content, manufacturers can ensure the microbial safety of the formulation. Specific gravity provides information about the density of the formulation, which can affect its stability and storage. For example, oils with higher specific gravity may be more viscous and require different handling and storage conditions. Thin layer chromatography (TLC) was done under the visualization of UV rays at 365 nm in which 1 ml solution was mixed with 10 ml ethanol and the distance travelled by solvent is 10 cm with values of 0.35, 0.46, 0.57, 0.65, 0.71, 0.76 which lies under normal range.

CONCLUSION

Only a small number of Ayurvedic formulas have been standardised to date, despite the use of contemporary technologies. Enough information was obtained for accurate identification using the present standardisation process. The development of analytical methods can be used as a specialised tool in the study of herbal drugs, enabling producers to establish quality requirements and standards in order to apply for regulatory bodies' marketing approval for the medicinal effectiveness, safety, and shelf life of herbal medications. It goes without saying that the goal of standardising medicinal herbs is to guarantee their effectiveness. Thus, it is crucial to preserve the quality of these plant-based goods. The results of Brahmi Avleha and Brahmi Taila analysis revealed specific identities which will be useful in preparation and identification of the formulation. The method of preparation of Brahmi Avleha and Brahmi Taila and analytical data mentioned in Table No. 4-6 are important findings for evaluation of quality control parameters for polyherbal ayurvedic formulations.

Declaration by Authors

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