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Development and Quality Control Methods for *Saraswatarishtam* - An Approach to Standardization

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ABSTRACT

Saraswatarishta is a well-known Ayurvedic fermented formulation extensively used as a nervine tonic, memory enhancer, and rejuvenating agent. Owing to the increasing demand for herbal medicines, standardization and quality assessment of marketed Ayurvedic formulations have become essential to ensure their safety, efficacy, and consistency. The present study was undertaken to evaluate and compare different marketed formulations of Saraswatarishta using modern analytical techniques. The study involved phytochemical screening, physicochemical evaluation, microbial analysis, chromatographic profiling, and spectroscopic characterization of marketed samples. Preliminary phytochemical investigation revealed the presence of important bioactive constituents such as alkaloids, tannins, carbohydrates, steroids, and saponins. Physicochemical parameters including total ash, acid-insoluble ash, water-soluble ash, sulphated ash, and extractive values were determined according to standard pharmacopoeial procedures. The alcohol content of the formulations was estimated and found to be within the acceptable range for Ayurvedic fermented preparations. Microbiological evaluation confirmed the absence of pathogenic microorganisms including Escherichia coli, Staphylococcus aureus, yeasts, and moulds, indicating the microbial safety of the formulations. Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FT-IR) were employed to establish characteristic chemical fingerprints and confirm the presence of major phytoconstituents. Comparative analysis demonstrated only minor variations among the marketed products, which may be attributed to differences in raw materials and manufacturing practices. The findings of the study indicate that the evaluated Saraswatarishta formulations possess acceptable quality, safety, and phytochemical integrity. The application of modern analytical tools provides a scientific basis for the standardization and quality control of Ayurvedic formulations, thereby promoting their reliability, therapeutic effectiveness, and global acceptance.

Keywords
Saraswatarishta,
Ayurveda,
TLC, FT-IR
Microbial Analysis
Herbal Formulation,

Introduction

Ayurveda, one of the world's oldest traditional systems of medicine, has been practiced in India for more than 5,000 years and continues to play a significant role in preventive and therapeutic healthcare. The system emphasizes maintaining the equilibrium of body, mind, and spirit through appropriate diet, lifestyle modifications, and herbal medicines. Among the numerous Ayurvedic dosage forms, Arishta preparations are unique self-generated hydroalcoholic fermented formulations that possess enhanced stability, improved extraction of phytoconstituents, prolonged shelf life, and superior therapeutic efficacy owing to the presence of naturally produced alcohol generated during fermentation (1,2). Saraswatarishta is a classical Ayurvedic fermented formulation extensively prescribed as a Medhya Rasayana (nootropic rejuvenator) for improving memory, cognition, speech disorders, anxiety, epilepsy, insomnia, and other neurological disorders. The formulation primarily contains Centella asiatica (Brahmi), together with several medicinal plants including Asparagus racemosus (Shatavari), Withania somnifera (Ashwagandha), Tinospora cordifolia (Guduchi), Terminalia chebula (Haritaki), Elettaria cardamomum (Ela), Piper longum (Pippali), and Woodfordia fruticosa (Dhataki), each contributing synergistically to the neuroprotective, antioxidant, adaptogenic, anti-inflammatory, and immunomodulatory properties of the formulation (3–5).

The therapeutic efficacy of Saraswatarishta is largely attributed to its diverse phytochemical constituents, including triterpenoids, flavonoids, tannins, phenolic compounds, alkaloids, glycosides, saponins, and volatile oils. Among these,

triterpenoid saponins such as asiaticoside from Centella asiatica, withanolides from Withania somnifera, and diterpenoid lactones from Tinospora cordifolia have been reported to possess significant antioxidant, neuroprotective, and cognitive-enhancing activities. Furthermore, the natural fermentation process facilitates the extraction and preservation of these bioactive constituents while improving their bioavailability (6–8).

Despite its widespread clinical use, considerable variability exists among commercially available Saraswatarishta formulations due to differences in raw material quality, geographical origin of medicinal plants, processing techniques, fermentation conditions, storage practices, and manufacturing standards. Such variations may influence phytochemical composition, alcohol content, physicochemical characteristics, microbial quality, and ultimately the therapeutic effectiveness of the product. Therefore, systematic quality assessment and standardization of marketed formulations are essential to ensure product consistency, safety, efficacy, and regulatory compliance (9–11).

Modern analytical techniques have become indispensable for establishing the quality profile of Ayurvedic formulations. Physicochemical parameters such as ash values, extractive values, pH, and alcohol content provide valuable information regarding purity and manufacturing quality. Preliminary phytochemical screening enables the identification of major classes of bioactive compounds responsible for therapeutic activity, whereas microbial limit tests ensure microbiological safety. In addition, chromatographic fingerprinting techniques such as Thin Layer Chromatography (TLC) provide rapid and reliable authentication of herbal formulations by generating characteristic chemical profiles that can be used for routine quality control (12–15).

Although several investigations have reported the pharmacological activities of the individual medicinal plants present in Saraswatarishta, comprehensive comparative studies evaluating the quality attributes of different marketed formulations remain limited. Scientific validation of marketed Ayurvedic preparations is essential for strengthening evidence-based traditional medicine and promoting their acceptance in global healthcare systems.

Therefore, the present study was undertaken to evaluate and compare two commercially available marketed formulations of Saraswatarishta using physicochemical characterization, qualitative phytochemical screening, alcohol estimation, microbial limit testing, and thin layer chromatographic fingerprinting. The findings of this investigation are expected to provide valuable information regarding the quality, safety, and standardization of marketed Saraswatarishta formulations and contribute to the development of reliable quality control protocols for classical Ayurvedic medicines.

Materials and Methods

Chemicals and Reagents

All chemicals and analytical reagents used during the study were of analytical reagent (AR) grade. Methanol, ethanol, petroleum ether, chloroform, benzene, glacial acetic acid, hydrochloric acid, sulphuric acid, silica gel G, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, Fehling's solution, Ferric chloride, Liebermann–Burchard reagent, and other chemicals required for phytochemical analysis were procured from certified laboratory suppliers. Microbiological media including MacConkey agar, Baird–Parker agar, Sabouraud Dextrose Agar (SDA), Nutrient Agar, Corn Meal Agar and Casein Soybean Digest Agar were used for microbial evaluation.

Procurement of Samples

Two commercially available marketed formulations of Saraswatarishta manufactured by Baidyanath and Vyas Pharmaceuticals were procured from licensed Ayurvedic medical stores. The formulations were stored at room temperature in their original containers until analysis. The ingredients of Saraswatarishta were verified according to the Ayurvedic Formulary of India.

Organoleptic Evaluation

The marketed formulations were evaluated for their organoleptic characteristics including appearance, colour, odour, taste and clarity. Solubility studies were carried out in distilled water, methanol, ethanol, petroleum ether, benzene and chloroform. The pH of each formulation was determined using a calibrated digital pH meter at room temperature.

Physicochemical Evaluation

Physicochemical parameters were determined according to standard procedures prescribed in the Ayurvedic Pharmacopoeia of India and Indian Pharmacopoeia.

Determination of Total Ash

Three grams of accurately weighed sample were transferred into a previously ignited silica crucible and incinerated gradually by increasing the temperature to 450°C until carbon-free ash was obtained. After cooling in a desiccator, the crucible was weighed and the percentage of total ash was calculated.

Determination of Acid-Insoluble Ash

The total ash obtained was boiled with 25 mL of dilute hydrochloric acid for 5 min. The insoluble residue was collected on an ashless filter paper, washed thoroughly with hot distilled water, ignited to constant weight and expressed as acid-insoluble ash.

Determination of Water-Soluble Ash

The total ash was boiled with 25 mL distilled water for 5 min. The insoluble matter was filtered, washed, ignited and weighed. The difference between total ash and insoluble ash represented the water-soluble ash.

Determination of Sulphated Ash

The total ash was treated with concentrated sulphuric acid followed by ignition at 600 ± 25°C until complete oxidation occurred. The process was repeated until constant weight was achieved.

Extractive Values

Alcohol-soluble, water-soluble and petroleum ether-soluble extractive values were determined by macerating accurately weighed samples with respective solvents. The filtrates were evaporated to dryness and the percentage extractive values were calculated with reference to the air-dried sample.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the marketed Saraswatarishta formulations was performed according to the standard methods described by Kokate and Trease & Evans. The

formulations were qualitatively evaluated for the presence of major phytoconstituents, including alkaloids, carbohydrates, glycosides, steroids, triterpenoids, saponins, tannins, flavonoids, and fixed oils and fats, using their respective standard chemical tests. The appearance of characteristic colour changes or precipitates was considered indicative of the presence of the corresponding phytochemical constituents.

Determination of Alcohol Content

The alcohol content of the marketed formulations was estimated by the distillation method described in the Ayurvedic Pharmacopoeia. Twenty-five millilitres of each sample were diluted with distilled water and distilled. The distillate was collected, adjusted to a definite volume, and the specific gravity was measured at 25°C. Alcohol percentage (v/v) was calculated using standard alcohol tables.

Microbial Limit Test

Microbiological evaluation was carried out according to WHO guidelines for herbal medicines.

Detection of Escherichia coli

Ten grams of each formulation were inoculated into lactose broth and incubated at 37°C. Serial dilutions were plated on Mac Conkey agar and incubated for 24 h. Typical colonies were confirmed using biochemical tests.

Detection of Staphylococcus aureus

Samples were inoculated on Baird–Parker agar and incubated at 37°C for 48 h. Suspected colonies were confirmed by coagulase and DNase tests.

Detection of Yeasts and Moulds

Samples were cultured on Sabouraud Dextrose Agar and Corn Meal Agar and incubated under appropriate conditions. Colony morphology was recorded and compared with pharmacopeial standards. Total aerobic microbial count was also determined to assess the microbiological quality of the formulations.

Thin Layer Chromatography (TLC)

Thin layer chromatographic fingerprinting was performed using silica gel G-coated TLC plates as the stationary phase. Samples were dissolved in methanol and applied as bands using capillary tubes. The mobile phase consisted of chloroform:glacial acetic acid: methanol: water (60:32:12:8). After chromatographic development, the plates were dried and visualized under UV light at 254 nm and 366 nm. The retention factor (Rf) values were calculated and compared between marketed formulations to establish their chemical fingerprint.

Statistical Analysis

All experimental determinations were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Comparative analysis between the marketed formulations was carried out using descriptive statistical methods. Differences were considered significant at $p < 0.05$ wherever applicable.

Results and Discussion

Organoleptic Evaluation

Both marketed Saraswatarishta formulations exhibited similar organoleptic characteristics. The formulations were dark brown in colour with a characteristic aromatic alcoholic odour and a sweet taste. Both samples were freely soluble in water, methanol, and ethanol but insoluble in petroleum ether, chloroform, and benzene. The pH values of the Baidyanath and Vyas formulations were 3.86 and 3.88, respectively, indicating an acidic environment produced during natural fermentation, which contributes to the stability and preservation of the formulation.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis demonstrated the presence of alkaloids, steroids, tannins, carbohydrates, and saponins in both marketed formulations, whereas amino acids, flavonoids, and fixed oils and fats were absent (Table 1). These phytoconstituents are known to possess antioxidant, adaptogenic, anti-inflammatory, and neuroprotective activities, which support the traditional therapeutic use of Saraswatarishta.

Table 1. Preliminary phytochemical screening of marketed Saraswatarishta formulations

Phytochemical	Standard	Baidyanath	Vyas
Alkaloids	+	+	+
Steroids	+	+	+
Amino acids	–	–	–
Tannins	+	+	+
Carbohydrates	+	+	+
Saponins	+	+	+
Flavonoids	–	–	–
Fixed oils & fats	–	–	–

The phytochemical profile obtained agrees with previous reports indicating that fermented Ayurvedic formulations contain abundant tannins, steroidal compounds and saponins, which contribute to their pharmacological activities. Fermentation enhances extraction efficiency and improves the availability of these phytoconstituents.

Physicochemical Evaluation: Physicochemical analysis is an important quality control parameter for herbal formulations. The marketed formulations complied with acceptable quality standards with respect to ash values and extractive values (Table 2). The absence of foreign matter, sand, silica and heavy metals further confirmed the purity of the formulations.

Table 2. Comparative physicochemical characteristics of marketed *Saraswatarishta* formulations

Parameter	*Standard*	*Baidyanath*	*Vyas*
Total ash (% w/w)	*8.0*	*1.10*	*1.02*
Acid-insoluble ash (% w/w)	*1.0*	*0.54*	*0.52*
Water-insoluble ash (% w/w)	*1.2*	*0.40*	*0.42*
Sulphated ash (% w/w)	*0.98*	*0.35*	*0.37*
Alcohol-soluble extractive (% w/w)	*40.1*	*30.2*	*31.5*
Water-soluble extractive (% w/w)	*39.5*	*28.5*	*27.5*
Petroleum ether extractive (% w/w)	*20.5*	*15.8*	*15.15*

The ash values observed indicate minimal inorganic contamination and confirm the purity of the formulations. Water- and alcohol-soluble extractive values revealed efficient extraction of polar phytoconstituents during fermentation. Minor differences between the marketed brands may be attributed to variations in raw materials, fermentation conditions, storage, and manufacturing practices.

Determination of Alcohol Content

Natural fermentation generated alcohol in both formulations. The alcohol content of Baidyanath and Vyas samples was found to be 9.2% v/v and 9.5% v/v, respectively. The alcohol content observed was within the pharmaceutically acceptable range for Ayurvedic fermented preparations. Self-generated alcohol acts as a natural preservative and enhances the extraction, solubility and bioavailability of phytoconstituents.

Microbial Evaluation

Microbial limit testing confirmed the absence of Escherichia coli, Staphylococcus aureus, yeasts and moulds in both marketed formulations, demonstrating satisfactory microbiological quality.

Table 3. Microbial quality evaluation of marketed Saraswatarishta formulations

Microorganism	***Baidyanath***	***Vyas***
Escherichia coli	Absent	Absent
Staphylococcus aureus	Absent	Absent
Yeasts	Absent	Absent
Moulds	Absent	Absent

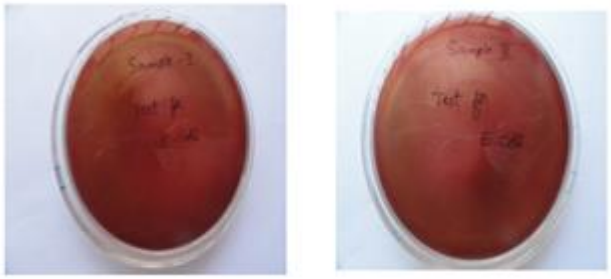

Figure 1. Microbial evaluation showing absence of *E. coli*.

Figure 2. Microbial evaluation showing absence of *Staphylococcus aureus*

Figure 3. Microbial evaluation showing absence of yeasts and moulds.

The absence of pathogenic microorganisms indicates that both formulations comply with microbiological quality requirements. The acidic pH and naturally generated alcohol produced during fermentation are likely to contribute to microbial inhibition and improved product stability.

Thin Layer Chromatographic Fingerprinting

Thin layer chromatography produced distinct chromatographic profiles for both formulations. The calculated Rf values were **0.87** for the Baidyanath formulation and **0.89** for the Vyas formulation, indicating similar phytochemical composition.

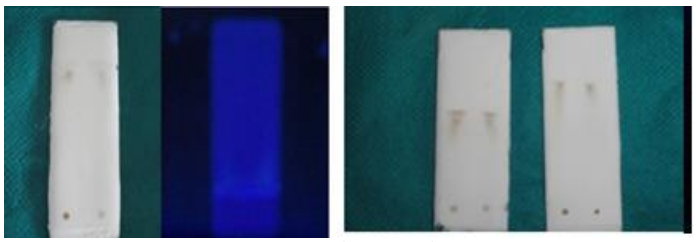

Figure 4. Determination of R_f value for centilla asiatica

TLC fingerprint of standard, Baidyanath and Vyas Saraswatarishta formulations. The close similarity in Rf values suggests comparable chemical fingerprints between the marketed formulations. Slight variations in spot intensity may result from differences in raw material quality and fermentation processes. TLC fingerprinting provides a rapid and economical technique for authentication and routine quality control of Ayurvedic fermented formulations.

Discussion

The present investigation demonstrated that both marketed Saraswatarishta formulations possessed comparable physicochemical characteristics, phytochemical composition, alcohol content, microbial safety and chromatographic fingerprints. The observed variations were minimal and remained within acceptable quality limits. These findings indicate that both products maintain satisfactory quality and are suitable for therapeutic use. Furthermore, the study highlights the importance of integrating modern analytical techniques, including physicochemical evaluation, microbial limit testing and chromatographic fingerprinting, for the standardization and quality assurance of classical Ayurvedic formulations.

Summary

The present study was conducted to evaluate and compare the quality of two commercially available marketed formulations of Saraswatarishta, a classical Ayurvedic fermented preparation widely used as a Medhya Rasayana for improving memory, cognition, and neurological health. Owing to the increasing global acceptance of herbal medicines, scientific standardization and quality assessment of traditional formulations have become essential to ensure their safety, efficacy, and batch-to-batch consistency. The marketed formulations manufactured by Baidyanath and Vyas Pharmaceuticals were subjected to comprehensive quality evaluation using physicochemical, phytochemical, microbiological, and chromatographic techniques. Organoleptic examination revealed that both formulations possessed similar characteristics, including dark brown colour, characteristic aromatic odour, sweet taste, and acidic pH, which are typical features of fermented Ayurvedic preparations. Preliminary phytochemical screening confirmed the presence of important secondary metabolites, including alkaloids, steroids, tannins, carbohydrates, and saponins, whereas amino acids, flavonoids, and fixed oils were absent. These phytoconstituents are known to contribute to the antioxidant, adaptogenic, neuroprotective, immunomodulatory properties of Saraswatarishta. Physicochemical evaluation demonstrated that both formulations complied with acceptable quality parameters with respect to total ash, acid-insoluble ash, water-insoluble ash, sulphated ash, and extractive values. Minor

variations observed between the marketed formulations could be attributed to differences in the source of raw materials, manufacturing processes, and fermentation conditions. Alcohol estimation showed that the Baidyanath and Vyas formulations contained 9.2% v/v and 9.5% v/v alcohol, respectively, indicating successful natural fermentation. The self-generated alcohol not only serves as a natural preservative but also facilitates the extraction and stability of bioactive phytoconstituents. Microbial limit testing confirmed the absence of Escherichia coli, Staphylococcus aureus, yeasts, and moulds in both formulations, demonstrating their microbiological safety and compliance with acceptable quality standards. Thin Layer Chromatography produced characteristic chromatographic fingerprints with Rf values of 0.87 and 0.89 for Baidyanath and Vyas formulations, respectively, confirming comparable phytochemical composition and authenticity. Overall, the study generated scientific evidence supporting the quality, safety, and consistency of the marketed Saraswatarishta formulations and demonstrated the importance of integrating modern analytical techniques for the standardization of classical Ayurvedic medicines.

Conclusion

The present investigation successfully evaluated and compared two commercially available Saraswatarishta formulations using modern quality assessment techniques. The findings demonstrated that both formulations possessed acceptable organoleptic characteristics, satisfactory physicochemical properties, comparable phytochemical profiles, appropriate alcohol content, and were free from pathogenic microbial contamination. The phytochemical screening confirmed the presence of therapeutically important secondary metabolites, while physicochemical analysis indicated compliance with pharmacopeial quality standards. The naturally generated alcohol content was found to be within the acceptable range for Ayurvedic fermented formulations, suggesting efficient fermentation and adequate product stability. Furthermore, TLC fingerprinting established characteristic chromatographic profiles that can serve as reliable markers for routine quality control and authentication. Although minor variations were observed between the marketed brands, these differences were not significant enough to affect the overall quality of the formulations and are likely attributable to natural variability in raw materials and manufacturing practices. The present study highlights the importance of applying modern analytical tools, including physicochemical evaluation, phytochemical screening, microbial limit testing, and chromatographic fingerprinting, for the scientific validation and standardization of Ayurvedic formulations. Such quality control approaches are essential to ensure product consistency, therapeutic reliability, regulatory compliance, and wider global acceptance of traditional herbal medicines. Future investigations employing advanced analytical techniques such as HPTLC, HPLC, LC–MS/MS, GC–MS, and pharmacological as well as clinical studies are recommended to further characterize the bioactive constituents and establish the therapeutic efficacy and quality standards of Saraswatarishta.

Conflict of Interests

The authors declare no conflict of interest

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AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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