Research Article

Physicochemical Analysis in Standardization of Siddha Herbal Drug *Aswaganthi Ilagam* (AGL)

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ABSTRACT

Aswaganthi Ilagam (AGL), a traditional siddha medicine noted for its usage in pregnant women and children. Standardizing these formulations is essential for ensuring their efficacy and safety in scientific contexts. This study aims to perform a comprehensive physicochemical analysis of AGL to establish its quality parameters and standardize its preparation. Raw materials for AGL were sourced and authenticated. The formulation was prepared according to literature evidence. Physicochemical evaluations included assessments of loss on drying, total ash, acid-insoluble ash, water-soluble and alcohol-soluble extractives, pH, and solubility profiles. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) analyses were conducted to identify and quantify the phytoconstituents. AGL exhibited a dark brown, semi-solid consistency with a sweet taste. The physicochemical analysis revealed a loss on drying of 11.41%, total ash content of 3.967%, and acid-insoluble ash of 0.026%. Water-soluble and alcohol-soluble extractives were found to be 12.64% and 7.96%, respectively, with a pH of 5.88. Chromatographic analyses identified nine prominent peaks with Rf values ranging from 0.04 to 0.82, indicating the presence of multiple phytoconstituents. The physicochemical and chromatographic profiles of AGL provide a foundational standardization framework, confirming its quality and supporting its traditional use.

Keywords: *Aswaganthi Ilagam* (AGL), Standardization, Physicochemical Analysis, Siddha medicine, Chromatography, Quality Parameters.

INTRODUCTION

Fossil records indicate that humans have been using plants for medicinal purposes since at least the Middle Palaeolithic era, around 60,000 years ago. Traditional healing encompasses the methods and knowledge passed down through generations of indigenous cultures, aimed at enhancing health, preventing and controlling disease, and ensuring the overall physical, mental, social, and spiritual well-being of individuals and their communities[1,2]. Siddha system of medicine is one of the oldest forms of medicine in South India. The Siddha System's distinctiveness is highlighted by its unwavering service to humanity for over 5,000 years in fighting diseases and promoting physical, mental, and moral well-being, even as many of its contemporaries have long since disappeared[3]. It describes about 4448 diseases and countless number of medicine preparations. There are 32 types of internal medicines described in Siddha system. *Ilagam* is one among them. *Aswaganthi ilagam* is an herbal drug mentioned in *Chikicha ratna deepam* book by *Kannusaamy pillai*. It is indicated for the treatment of dropsy, anaemia and jaundice. It is also used to improve general health of the body. It is indicated in the text for usage during pregnancy and in children[4]. The WHO has recognized the crucial role of medicinal plants in public health care for developing countries and has developed guidelines to assist member states in creating national policies on traditional medicines. These guidelines also support the study of their potential benefits, including evaluation of their safety and efficacy[5]. In today's world standardization of traditional medicines is important to ensure their global acceptance. The aim of the study to analyse AGL by PLIM guidelines through physicochemical and high-performance thin layer chromatograph (HPTLC)[6]. This study will make a fingerprint for the AGL for further research in future.

MATERIALS AND METHODS

Preparation of *Aswaganthi ilagam*

The raw drugs were purified as described in Siddha literature and medicine prepared in the *Gunapadam* laboratory of National Institute of Siddha, Chennai. Sugar was added to the milk and boiled till the string consistency (*thandhupadham)*[4] was obtained. The remaining drugs were powdered and added to the milk slowly with continuous stirring. It was cooled and ghee was added followed by addition of required quantity of honey.

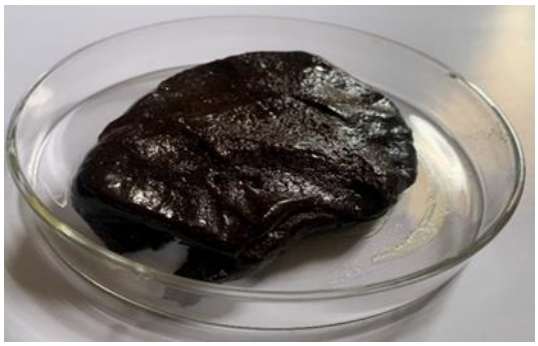


Figure 1: *Aswaganthi ilagam*

Table 1: Ingredients of the study drug AGL

S. NO	Tamil vernacular name	Botanical name	Quantity
1	*Aswaganthi*	*Withania somnifera Dunal (Solanaceae)*, Root	2 *palam* (70g)
2	*Sarkkarai*	*Saccharum officinarum Linn. (Poaceae)*, Jaggery	1 ½ *veesai* (2.1 kg)
3	*Seeragam*	*Cuminum cyminum Linn. (Apiaceae),* Fruit	¼ *palam* (8.75 gm*)*
4	*Dhiratchai*	*Vitis vinifera Linn. (Vitaceae),* Dried fruit	¼ *palam* (8.75 gm*)*
5	*Perinthu*	*Phoenix dactilifera Linn. (Arecaceae*), Dried fruit	¼ *palam* (8.75 gm*)*
6	*Chandhanam*	*Santalum album Linn. (Santalaceae)*, Heart wood	¼ *palam* (8.75 gm*)*
7	*Jathikai*	*Myristica fragrans Houtt. (Myristicaceae),* Nut	¼ *palam* (8.75 gm*)*
8	*Chithiramoolam*	*Plumbago zeylanica Linn. (Plumbaginaceae),* Root	¼ *palam* (8.75 gm*)*
9	*Naagesuvaram*	*Mesua ferrea Linn.(Clusiaceae)* Flower	¼ *palam* (8.75 gm*)*
10	*Korai kizhangu*	*Cyperus rotundus Linn. (Cyperaceae)*, Tuber	¼ *palam* (8.75 gm*)*
11	*Kadukkai poo*	*Terminalia chebula Retz. (Combretaceae*), Gall	¼ *palam* (8.75 gm*)*
12	*Koogaineeru*	*Maranta aurundinacea Linn. (Marantaceae),* Rhizome powder	¼ *palam* (8.75 gm*)*
13	*Chukku*	*Zingiber officinale Rosc. (Zingiberaceae),* Dried rhizome	¼ *palam* (8.75 gm*)*
14	*Milagu*	*Piper nigrum Linn. (Piperaceae),* Fruit	¼ *palam* (8.75 gm*)*
15	*Ilavangapattai*	*Cinnamomum verum Presl. (Lauraceae),* Bark	¼ *palam* (8.75 gm*)*
16	*Pachilai*	*Garcinia xanthochymus Hook.F. (Clusiaceae),* Leaves	¼ *palam* (8.75 gm*)*
17	*Thippili*	*Piper longum Linn. (Piperaceae)*, Fruit	¼ *palam* (8.75 gm*)*
18	*Vilamichu ver*	*Plectranthus vettiveroides (Jacob) Singh & Sharma (Poaceae*), Root	¼ *palam* (8.75 gm*)*
19	*Kirambu*	*Syzygium aromaticum (Linn.) Merr. & L.M. Perry (Myrtaceae),* Flower bud	¼ *palam* (8.75 gm*)*
20	*Kandathippili*	*Piper longum Linn. (Piperaceae),* Root	¼ *palam* (8.75 gm*)*
21	*Chevviyam*	*Piper nigrum Linn. (Piperaceae)*, Root	¼ *palam* (8.75 gm*)*
22	*Elam*	*Elettaria cardamomum Maton. (Zingiberaceae),* Seed	¼ *palam* (8.75 gm*)*[7]
23	*Pasum paal*	Cow's milk	2 *padi* (2.68 litres)
24	*Pasu nei*	Cow's ghee	1 *padi* (1.34 litres)[8]
25	*Thaen*	Honey	Required quantity

PHYSICOCHEMICAL EVALUATION

Organoleptic characters

The organoleptic characters such as state, nature, odour, touch, flow property and appearance of the drug was noted.

Solubility profile

The drug was mixed with various solvents such as chloroform, ethanol, water, ethyl acetate and DMSO and the solubility was noted.

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400°C until it turns white in color which indicates absence of carbon. Percentage of total ash was calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6 mins. Then the insoluble matter is collected in crucible and was washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105°C, to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of Water-Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105°C, to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

pH determination

Required quantity of test sample was admixed with distilled water and subjected to screening using pH meter.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm[9].

High Performance Thin Layer Chromatography Analysis

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated[10].

OBSERVATION AND RESULTS

AGL appeared to be dark brown in colour with a characteristic sweet taste. The results are mentioned below in the following table

Table 2: Organoleptic characters of AGL

State	Semisolid
Nature	Soft
Odour	Characteristic
Touch	Greasy
Flow Property	Non free flowing
Appearance	Dark Brownish

Table 3: Solubility profile

S. No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

The results obtained from the physicochemical evaluation reveals the following:

Loss on drying:

The loss of drying value at *105 °C* was found to be 11.41 %.

Total ash:

The total ash value of AGL was found to be 3.967 %.

Acid insoluble ash:

The acid insoluble ash obtained from AGL was found to be 0.026 %.

Water soluble and alcohol soluble extractive:

The water-soluble extractive and alcohol soluble extractive were found out to be 12.64% and 7.96% respectively.

pH:

The pH of AGL was found to be 5.88.

Table 4: Physicochemical analysis of AGL

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	11.41 ± 0.99
2.	Total Ash (%)	3.967 ± 0.50
3.	Acid insoluble Ash (%)	0.026 ± 0.02
4.	Water soluble Extractive (%)	12.64 ± 0.76
5.	Alcohol Soluble Extractive (%)	7.96 ± 0.47
6.	pH	5.88

High performance thin layer chromatography analysis

TLC Visualization of AGl at 366 nm

3D – Chromatogram

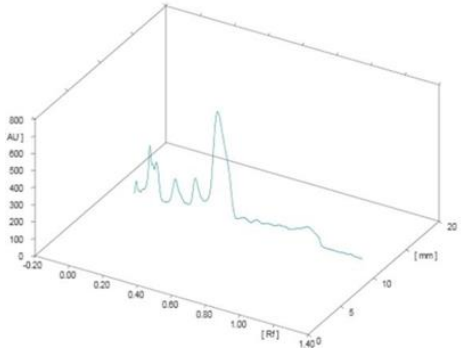
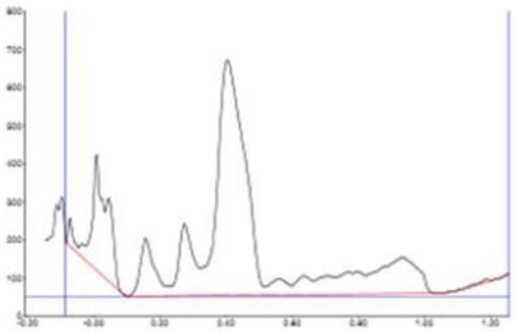


Table 6: HPTLC finger printing of AGl



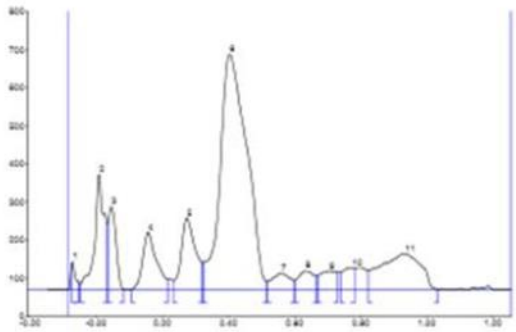


Table 6: Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	69.3	-0.07	73.3	3.97	-0.05	21.0	383.8	0.81
2	-0.05	15.6	0.01	302.5	16.40	0.03	172.3	4275.1	9.02
3	0.04	175.8	0.05	218.1	11.82	0.08	0.2	2346.9	4.95
4	0.11	0.4	0.16	151.6	8.22	0.22	25.8	3021.8	6.37
5	0.23	24.6	0.28	188.8	10.23	0.32	71.2	3872.3	8.17
6	0.32	71.2	0.40	618.1	33.50	0.52	21.4	23694.2	49.97
7	0.52	21.5	0.56	42.5	2.30	0.60	24.6	1109.3	2.34
8	0.60	24.8	0.63	49.2	2.67	0.67	36.3	1123.0	2.37
9	0.67	36.5	0.71	48.5	2.63	0.73	45.9	1169.4	2.47
10	0.74	46.9	0.78	57.7	3.13	0.78	55.5	967.8	2.04
11	0.82	49.3	0.94	94.6	5.13	1.04	0.3	5448.7	11.49

Interpretation:

HPTLC finger printing analysis of the sample reveals the presence of nine prominent peaks corresponds to the presence of nine components present with in it. Rf value of the peaks ranges from 0.04 to 0.82

DISCUSSION

The physicochemical analysis of *Aswaganthi Ilagam* (AGL), a traditional Siddha herbal drug, reveals important insights into its quality and standardization. This analysis is crucial for ensuring the efficacy and safety of AGL, especially given its use for vulnerable populations such as pregnant women and children. The organoleptic properties of AGL, which include its dark brown colour, characteristic sweet taste, and semi-solid, greasy texture, align with traditional descriptions. These characteristics are essential for identifying the drug and ensuring its authenticity[11]. The solubility profile indicates that AGL is soluble in ethanol, water, and DMSO, but insoluble in chloroform and ethyl acetate. This solubility pattern is consistent with the nature of many traditional herbal formulations, suggesting the presence of diverse phytoconstituents with varying solubility profiles[12].

The physicochemical analysis provides critical data on AGL's composition and quality. The total ash value of 3.967% suggests a moderate level of inorganic material, which is typical for herbal preparations. The loss on drying value of 11.41% indicates a relatively high moisture content, which is important for understanding the stability and shelf-life of the drug. The acid-insoluble ash percentage of 0.026% is minimal, reflecting the low content of siliceous matter, which is beneficial for ensuring the purity of the formulation. The water-soluble extractive of 12.64% and alcohol-soluble extractive of 7.96% highlight the presence of significant quantities of soluble phytoconstituents. This information is crucial for evaluating the drug's therapeutic potential, as these extracts often contain the active principles responsible for the drug's medicinal properties. The pH value of 5.88 suggests that AGL is slightly acidic, which is consistent with many herbal preparations and may influence its stability and compatibility with biological systems[13,14]. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) analyses reveal the presence of nine prominent peaks, indicating the presence of various phytoconstituents with Rf values ranging from 0.04 to 0.82. This chromatographic fingerprint is essential for the standardization and quality control of AGL, as it provides a unique profile for the drug and helps in identifying its key chemical components[15].

CONCLUSION

The physicochemical analysis of Aswaganthi Ilagam provides a comprehensive profile of its quality, consistency, and authenticity. The organoleptic and solubility characteristics confirm the traditional descriptions and expected behaviour of the herbal drug. The physicochemical parameters, including total ash, loss on drying, and extractive values, offer insights into the drug's composition and purity, which are crucial for ensuring its efficacy and safety. The TLC and HPTLC analyses further support the standardization of AGL by identifying its unique

chemical fingerprint. These findings are instrumental in validating the quality of AGL and can aid in the development of standardized methods for its preparation and quality control. Future research could focus on further elucidating the specific phytoconstituents present in AGL and their pharmacological activities. Additionally, exploring the stability of AGL under various storage conditions and its interactions with other substances could provide valuable information for its safe and effective use in traditional medicine. Standardization and validation through rigorous scientific methods will enhance the global acceptance and utilization of Siddha herbal drugs, ensuring their benefits are accessible and reliable.

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Conflict of Interest

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

REFERENCES

1. D. S. Fabricant, N. R. Farnsworth, The value of plants used in traditional medicine for drug discovery, Environmental Health Perspectives, 2001, 109, 69-75.
2. Bhardwaj AK, Kashyap K, Hait M, Bera SK, Dewangan H. Physicochemical Characterization of Rhizome of *Curcuma caesia* Roxb. ES Food & Agroforestry. 2023 Feb 11;11(7):813.
3. Karunamoorthi K, Jegajeevanram K, Xavier J, Vijayalakshmi J, Melita L. Tamil traditional medicinal system-siddha: an indigenous health practice in the international perspectives. CellMed. 2012;2(2):12-.
4. Kannusamy pillai, Chikicha ratna deepam -Rathna naicker and sons.2nd ed. Chennai: 2018. p.180,181.
5. Organization Mondiale De La Sante, Quality control methods for medicinal plants materials, 559, rev. 1, Original English, World Health Organization. 1992; 159.
6. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization an evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH Ministry of Health & Family Welfare, Govt of India.
7. Thiagarajan R. Gunapadam Thathu – Jeeva Vaguppu. Vol. 1, 1st ed. Chennai: Indian Medicine and Homeopathy Department; 2009. p.53.
8. Ragavi R. Phytochemical and biochemical analysis of Siddha preparation Aya Chendhooram. Journal of Traditional and Integrative Medicine. 2020 Mar 27;3(1):312-6.
9. Lukasz Komsta, Monika Waksmundzka-Hajnos, Joseph Sherma. Thin Layer Chromatography in Drug Analysis . CRC Press, Taylor and Francis.
10. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305,227.
11. Liu WJ, editor. Traditional herbal medicine research methods: identification, analysis, bioassay, and pharmaceutical and clinical studies. John Wiley & Sons; 2011 Mar 29.
12. Mohapatra D, Agrawal AK, Sahu AN. Exploring the potential of solid dispersion for improving solubility, dissolution & bioavailability of herbal extracts, enriched fractions, and bioactives. Journal of Microencapsulation. 2021 Nov 17;38(7-8):594-612.
13. Kumari R, Kotecha M. A review on the standardization of herbal medicines. International journal of pharma sciences and research. 2016 Feb;7(2):97-106.
14. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol. 2008;2(3):195-203.
15. Gunjal Sanket B, Dighe PR. Analysis of herbal drugs by HPTLC: A review. Asian Journal of Pharmaceutical Research. 2022;10(2):125-8.