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A Scientific Appraisal of "*Aya Chooranam*" - Siddha herbo mineral drug through Physicochemical and Phytochemical Profiling

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ABSTRACT

Siddha Medicine is one of the oldest traditional systems of medicine, originated in Tamil Nadu. According to *siddha*, total number of diseases are said to be 4448 by the sage *(siddhar) Yugimuni*. "*Paandu Noi*" (பாண்டு நோய் - Anaemia) is one among the disease. Various medicines are available for *Paandu noi* in *Siddha*. One kind of medicine is *Aya chooranam*. *Chooranams* are to be standardized by physicochemical and phytochemical analyses as per PLIM guidelines. They play a crucial role in the standardization, validation, and quality control of both herbal and Herbo-mineral formulations. Physicochemical analysis, phytochemical screening, and particle size analysis were carried out in accordance with PLIM (Pharmacopoeia Laboratory of Indian Medicine) guidelines for *Aya chooranam*. The tested sample exhibited quality standards as per the norms of PLIM guidelines. Based on these analytical parameters, the identity and authenticity of *Aya Chooranam* (AC) is established. The findings support the use of AC as a reference standard for the preparation of standardized pharmaceutical formulations. This study further suggests that by incorporating traditional Siddha system with modern analytical techniques can aid in establishing comprehensive quality specifications and will be helpful to establish new drug discoveries in Indian systems of medicines.

Keywords: *Siddha, Paandu noi, Aya chooranam, physicochemical analysis, phytochemical analysis, herbal drug standardization*

INTRODUCTION

Siddha Medicine is one of the oldest traditional systems of medicine known to humanity, originating in South India, particularly Tamil Nadu. It is considered one of the native medical systems of India and is deeply rooted in Tamil culture and philosophy. The word "*Siddha*" is derived from the Tamil word *"Siddhi,"* meaning Spiritual attainment of perfection. In *Siddha* medicine, diseases are classified based on etiology, body humours imbalance, affected organs, and causative factors. The *Siddha* system follows a holistic approach that considers physical, mental, spiritual, seasonal and environmental aspects of disease. According to *siddha*, 4448 disease classifications mentioned.[1] "*Pandu Noi*" (பாண்டு நோய்-Anaemia), is one among the disease characterized by pallor, fatigue, weakness, and poor nourishment of the body tissues. It is considered a derangement of the three vital humours-*Vali (Vata), Azhal (Pittha), and Iyyam (Kapha)*-with a predominant involvement of *Pittha* and also blood, which is one of the 7 vital constituents of the body. In India nearly 59.1% of adolescent girls are affected with anaemia.[2] Modern drugs which contains iron are available, but they are not cost effective. *Aya chooranam* is one *siddha* Herbo- mineral formulation indicated for anaemia. To meet modern scientific and regulatory standards, analytical techniques are integrated into the traditional practices to screen herbal and Herbo mineral drugs.

In indigenous medical systems, physicochemical and phytochemical analyses play a crucial role in the standardization, validation, and quality control of both herbal and Herbo-mineral formulations. *Siddha* pharmacology is based on the use of plants, minerals and animal products, which require careful processing and purification (*Sudhi*) to ensure their safety and effectiveness. Physicochemical analysis helps in determining parameters such as moisture content, ash value, pH, solubility, and extractive values, which are essential for identifying adulterants, ensuring shelf-life, and maintaining batch-to-batch quality of the drug. On the other hand, phytochemical analysis allows the identification and quantification of bioactive plant compounds such as alkaloids, flavonoids, tannins, phenols, and saponins, which are responsible for the therapeutic effects of *Siddha* formulations. Particle size is an important parameter under the physicochemical standardization of powdered herbal drugs and formulations. Passes through an **80-mesh (<180 µm)** or finer.

Such standard analytical procedures are the need of hour in the modern scientific world. Nowadays increasing awareness of drug safety in traditional systems of medicines is very common. Moreover, globalization of traditional medical practices needs the fulfilment of modern analytical standards to get universal acceptance. In this view, it is necessary to screen all medicines in the traditional systems with modern parameters and it is one of such endeavour to reveal the drug safety and therapeutic efficacy of *Aya chooranam* (AC).

MATERIALS AND METHODS

Selection of drug

The formulation "*Aya Chooranam* (AC)" is taken for analytical study, mentioned in the *Siddha* literature "*Anuboga Vaithiya Navaneetham"* part-1. Page no:53.[3]

Collection of the drug

The ingredients used to prepare the drug *Aya chooranam* were purchased from reputed *Siddha* raw drug store, at Chennai, Tamil Nadu.

Recognition and Authentication of the drug:

All drugs were recognized and authenticated by *Gunapadam* experts Government siddha medical college, Arumbakkam, Chennai. Each sample has been labelled as 313-325/ PGG/GSMC-CH/2023-2026. To facilitate future reference, specimen samples were taken and archived in the PG *Gunapadam* laboratory.[4]

Table: 1- Ingredients of *Aya Chooranam*(AC)

S.NO	INGREDIENTS	BOTANICAL NAME	QUANTITY
1	Purified *ayam* powder	*Ferrum*	5 Palam(175g)
2	*Chukku*	*Zingiber officinale*	1 Palam(35g)
3	*Milagu*	*Piper nigrum*	1 Palam (35g)
4	*Arisi Tippili*	*Piper longum*	1 Palam (35g)
5	*Manjal kadukkai thol*	*Terminalia chebula*	1 Palam (35g)
6	*Thaantrikai thol*	*Terminalia bellirica*	1 Palam (35g)
7	*Nelli vatral*	*Phyllanthus emblica*	1 Palam (35g)
8	*Kothamalli vidhai*	*Coriandrum sativum*	1 Palam(35g)
9	*Kodiveli verpattai*	*Plumbago zeylanica*	1 Palam(35g)
10	*Narseeragam*	*Cuminum cyminum*	1 Palam(35g)
11	*Sittrarathai*	*Albinia officinarum*	1 Palam(35g)
12	*Omam*	*Trachyspermum ammi*	10 Palam(350g)
13	*Kaiyanthagarai juice*	*Eclipta prostrata*	1.2 ml

. **Figure: 01 - Ingredients of *Aya chooranam* (AC)**

AYA PODI

Ferrum-Iron

CHUKKU

Zingiber officinale

MILAGU

Piper nigrum

THIPPILI

Piper longum

MANJAL KADUKAI THOL

Terminalia chebula

THANTRIKAI THOL

Terminalia bellirica

NELLI VATRAL

Phyllanthus emblica

KOTHAMALLI VIDHAI

Coriandrum sativum

KODIVELI VERPATTAI

Plumbago zeylanica

Narseeragam

Cuminum cyminum

Sittrarathai

Albinia officinarum

Omam

Trachyspermum ammi

Kaiyanthagarai juice

Eclipta prostrata

Purification of the drug

Purification of herbal drugs and iron dust were done as per classical *siddha* literature (*sarakkugalin suthi sei muraigal*)[5] and *Siddha* Materia Medica (*Gunapadam Thathu Jeeva Vaguppu*).[6]

METHOD OF PREPARATION

- Except iron powder, all the ingredients are purified, and slightly roasted. They are powdered and seived with a cullander to get a mixture of very fine powder.
- Then, iron dust was added to the mixture and stirred well for even distribution. This *chooranam* is mixed with the juice of *Kaiyanthagarai* and kneaded gently.
- The above mixture is laid on the oven and the contents were stirred continuously with a ladle without allowing it to burn, until it becames dry.
- Once dried, it was taken out and again kneaded with *Kaiyanthagarai* juice and roasted repeatedly for 21 times. Finally, it was powdered and stored in air tight container.

DRUG PROFILE

- **Drug** : *Aya chooranam(AC)*
- **Route of administration :** Oral

- **Dose** : 10 *Kuntrimani edai* (1300 mg)
- **Adjuvant** : Honey
- **Indications** : ***Ella vagai paandugal(anemia),*** *Ella vagai kiranigal, Sogai, Palavagai kazhichal, Eraichal.*

STANDARDIZATION OF THE DRUG

Standardization ensures the safety, efficacy, and consistency of herbal drugs. Physicochemical analysis assesses quality through parameters like moisture, ash and extractive values, while phytochemical screening identifies key bioactive compounds. These methods support the scientific validation of traditional polyherbal formulations. *Noble Laboratory* Chennai was employed for conducting the standardization parameters of *Aya chooranam*.

Physicochemical analysis:[7,8]

The following physicochemical analyses were carried out and the results were documented:

Loss on Drying (Percentage):

An accurately weighed quantity of the test drug was placed in an evaporating dish and dried in a hot air oven at 105°C for 5 hours. The sample was then reweighed to determine the percentage loss due to moisture content.

Determination of Total Ash

A precisely weighed quantity of the test sample was placed in a silica crucible and gradually incinerated in a muffle furnace at 400°C until a consistent white residue was obtained, signifying complete oxidation and elimination of organic matter. The residue, representing the total ash, was weighed, and the total ash content was calculated as a percentage of the original air-dried sample, reflecting the total inorganic constituents present.

Determination of Acid-Insoluble Ash

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 6 minutes. The insoluble residue was collected in a crucible, washed with hot water, and ignited to a constant weight. The acid-insoluble ash was measured and expressed as a percentage relative to the weight of the air-dried sample.

Determination of Alcohol-Soluble Extractive

The test sample was macerated with 100 ml of alcohol in a sealed flask for 24 hours, involving frequent shaking during the initial 6 hours, followed by undisturbed standing for the remaining 18 hours. After filtration, 25 ml of the filtrate was transferred to a pre-weighed shallow dish, evaporated to dryness, and dried at 105°C until a constant weight was obtained. The percentage of alcohol-soluble extractive was calculated relative to the air-dried sample.

Determination of Water-Soluble Extractive

The test sample was macerated with 100 ml of chloroform water in a sealed flask for 24 hours, with intermittent shaking during the initial 6 hours, followed by static immersion for the remaining 18 hours. After filtration, 25 ml of the filtrate was evaporated to dryness in a tared shallow dish and dried at 105°C to a constant weight. The percentage of water-soluble extractive was calculated relative to the air-dried sample.

pH Determination

A specified amount of the test sample was mixed with distilled water and the pH was measured using a calibrated pH meter.

Solubility Test

A small quantity of the sample was placed in a dry test tube, followed by the addition of 2 ml of each solvent. The mixture was shaken vigorously for about one minute, and solubility was observed. The test was conducted separately using chloroform, ethanol, water, ethyl acetate, hexane, and dimethyl sulfoxide (DMSO).

PHYTOCHEMICAL ANALYSIS[9]

Test for Alkaloids – Mayer's Test

To the test sample, 2 ml of Mayer's reagent was added. The formation of a dull white precipitate indicated the presence of alkaloids.

Test for Coumarins

To the test sample, 1 ml of 10% sodium hydroxide solution was added. The development of a yellow color confirmed the presence of coumarins.

Test for Saponins

The test sample was mixed with 22.5 ml of water and shaken vigorously. The formation of lasting and dense foam was indicative of the presence of saponins.

Test for Tannins

The addition of ferric chloride solution to the test sample led to the appearance of a dark blue or greenish-black color, indicating the presence of tannins.

Test for Glycosides – Borntrager's Test

The test sample was hydrolyzed with concentrated hydrochloric acid for 2 hours in a water bath and then filtered. To 2 ml of the resulting filtrate, 3 ml of chloroform was added and the mixture was shaken thoroughly. The chloroform layer was carefully separated, and 10% ammonia solution was added. The appearance of a pink color in the ammoniacal layer confirmed the presence of glycosides.

Test for Flavonoids – Alkaline Reagent Test

Two to three drops of sodium hydroxide were introduced into 2 ml of the extract, resulting in the emergence of an intense yellow hue. The subsequent addition of a few drops of dilute hydrochloric acid caused the yellow color to fade, confirming the presence of flavonoids.

Test for Phenols – Lead Acetate Test

Three ml of 10% lead acetate solution were added to the test sample. The development of a thick white precipitate indicated the presence of phenolic compounds.

Test for Steroids

A qualitative test for steroids was performed by adding 2 ml of chloroform and 3 ml of concentrated sulfuric acid to the test sample, followed by thorough shaking. The presence of steroids was indicated by the emergence of a red tint in the upper chloroform layer, accompanied by a yellow hue with green fluorescence in the underlying sulfuric acid layer.

Test for Triterpenoids – Liebermann–Burchard Reaction

To detect the presence of triterpenoids, the test sample dissolved in chloroform was first treated with a few drops of acetic anhydride and thoroughly mixed. Following this, 1 ml of concentrated sulfuric acid was gently layered along the inner wall of the test tube. The appearance of a distinct red ring at the boundary between the two liquid layers suggested the presence of triterpenoid components.

Test for Cyanins (Anthocyanins)

To the test sample, 1 ml of 2N sodium hydroxide was added and the mixture was heated at 100°C for 5 minutes. The appearance of a bluish-green coloration signalled the presence of anthocyanin compounds.

Test for Carbohydrates – Benedict's Test

Approximately 0.5 ml of Benedict's reagent was introduced to the test sample, and the mixture was subsequently heated in a boiling water bath for 2 minutes to facilitate the reaction. The generation of a noticeably colored sediment indicated the presence of carbohydrate constituents.

Test for Proteins – Biuret Test

To the extract, 1% copper sulfate solution was added followed by 5% sodium hydroxide solution The development of a violet or purple color confirmed the presence of proteins.

Particle Size Determination by Microscopic Method [10]

Particle size analysis was carried out using the optical microscopy method. The sample was diluted with sterile distilled water at a ratio of approximately 1:100 and mounted on a glass slide. Microscopic observations were performed using an ocular micrometer, and measurements were taken at appropriate focal levels. A minimum of 30 particles were measured to determine the mean particle size.

RESULT

Physicochemical analysis:

Table 2: The results of the physicochemical analysis of ***Aya Chooranam(AC)*** are summarized in the table below.

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	4.36 ± 0.73
2.	Total Ash (%)	0.60 ± 0.24
3.	Acid insoluble Ash (%)	0 ± 0
4.	Water soluble Extractive (%)	16.5 ± 0.3
5.	Alcohol Soluble Extractive (%)	12.5 ± 0.36
6.	pH	6.15

Table 3: Solubility Profile of AC

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

Phytochemical analysis :

Figure 2: Phytochemical analysis of AC

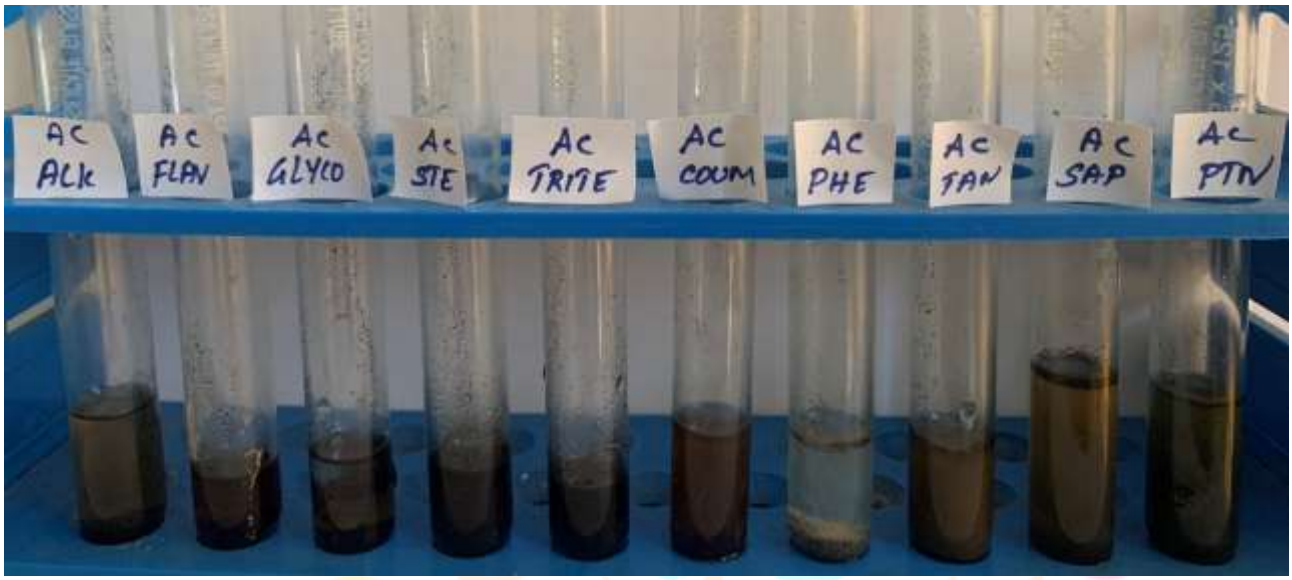


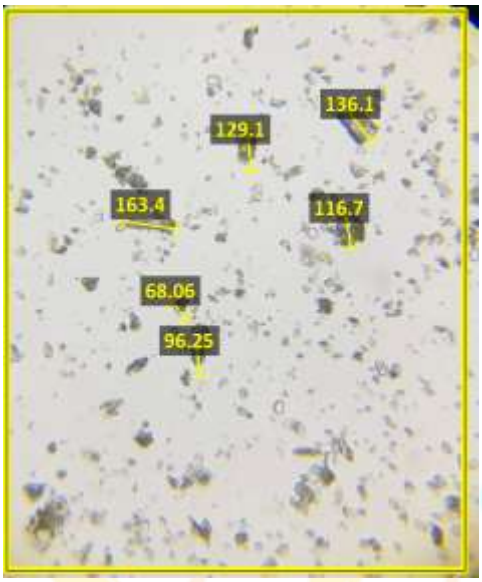
Table 4: Result of Phytochemical analysis of AC

S.NO	TEST	OBSERVATION
1.	ALKALOIDS	+
2.	FLAVANOIDS	-
3.	GLYCOSIDES	-
4.	STEROIDS	+
5.	TRITERPENOIDS	+
6.	COUMARIN	-
7.	PHENOL	+
8.	TANIN	+
9.	PROTEIN	-
10.	SAPONINS	-
11.	SUGAR	+
12.	ANTHOCYANIN	-
13.	BETACYANIN	-

(+) -> Indicates Positive and (-) -> Indicates Negative

Particle size determination:

Figure 3: Microscopic view for Particle size determination of AC



Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 132.8 ± 28.9 µm.

DISSCUSSION

- The present study focused on the phytochemical and physicochemical properties of AC. One of the primary steps in standardizing the drug AC is ensuring its quality through physicochemical analysis. The loss on drying was found to be 4.36 ± 0.73, indicating good stability and quality. The total ash content was 0.60 ± 0.24 suggesting a minimal presence of siliceous matter. The water-soluble extractive value was 16.5 ± 0.3, while the alcohol-soluble extractive value was 12.5 ± 0.36, indicating the presence of various phytoconstituents in AC.
- Solubility is a fundamental requirement for a medication to be effectively absorbed in the gastrointestinal tract. Solubility studies indicate that *Aya Chooranam* is soluble in ethanol, DMSO and sparingly soluble in water.
- The results of the phytochemical analysis confirm the presence of various bioactive compounds in the drug, including alkaloids,steroids,triterpenoids,phenol,tannin and sugar, which may contribute to its therapeutic properties.
- Nanoparticles enhance drug solubility and bioavailability, reduce macrophage clearance, and minimize side effects, leading to lower drug dosage and improved therapeutic efficiency.

CONCLUSION

- This study concludes that the sample *Aya chooranam (AC)* was prepared and purified under standard guidelines, confirming its quality. Standardizing a drug is a crucial step in the drug development process. For AC, this was carried out following the analytical specifications for *chooranam* (fine powder) as per PLIM guidelines. Based on the findings, the formulation of *Aya chooranam* is identified to meet out the quality standards specified in PLIM guidelines.
- This analytical study establishes the standardization of *Aya chooranam* and highlights its medicinal potential due to the presence of secondary metabolites. Further preclinical and clinical evaluations are necessary to support its therapeutic use.

Declaration by Authors:

Ethical approval

Approved

Acknowledgement:

I am deeply grateful to all those who have supported and guided me throughout the successful completion of this research work on "A Scientific Appraisal of *Aya Chooranam*-Siddha herbo mineral drug through Physicochemical and Phytochemical Profiling".

Conflict of interest

The author has no conflict of interest

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