Standardization of Siddha Herbo Mineral Formulation *ArputhaMathirai* for Poly Cystic Ovarian Syndrome (PCOS)

ABSTRACT

Aim

The aim of this study was to examine the physicochemical and phytochemical properties of the *Siddha*herbomineral formulation, *ArputhaMathirai (AM)* for its potential in treating PCOS (Poly Cystic Ovarian Syndrome). The objective was to analyze the tablet and assess its physical and chemical properties, as well as the presence of bioactive compounds derived from plant sources.

Place of Study

The TamilnaduDr.MGR Medical university, No.69, Anna Salai, Guindy, Chennai – 600 032.

Materials and Methods

ArputhaMathirai, the *Siddha*herbomineral formulation, was prepared in compliance with Good Manufacturing Practices (GMP) guidelines. The formulation underwent a thorough analysis of its physicochemical and phytochemical properties at, The TamilnaduDr.MGR Medical university, Guindy, Chennai. The analysis was conducted following the standards set by the Pharmacopoeial Laboratory for Indian Medicine (PLIM) in accordance with the guidelines established by AYUSH (Ayurveda, Yoga, Unani, Siddha, Homoeopathy), the governing body for traditional health systems in India.

Result

Physico-chemical analysis of AM showed 10.08% of loss on drying at 105^0 C, 15.39% total ash value, 2.45% acid-insoluble ash, 12.18% water-soluble ash, 20.55% water-soluble extraction, and 8.46% alcohol-soluble extraction. According to the phytochemical analysis of AM, alkaloids, carbohydrates, saponins, flavonoids, diterpenes, gum, and mucilage were all found in the sample. Additionally, it demonstrated the absence of quinones, tannins, and phenols.

Conclusion

The findings of this study provide a comprehensive understanding of the physicochemical, & phytochemical of *Arputha Mathirai* (AM). These results contribute to establishing the nature of the formulation's composition and its safety profile. Moreover, the standardization of the tablet formulation based on these parameters supports its suitability for therapeutic use in the treatment of PCOS. These outcomes validate the quality and potential effectiveness of AM in addressing PCOS, enhancing its credibility as a viable treatment option.

***Key Words**: Siddha system, ArputhaMathirai, PCOS, physicochemical, phytochemical*

1.INTRODUCTION

Siddha system of medicine is one of the oldest traditional systems of medicine, which has been originated from India and is practiced mostly in the southern part of this country for treating various diseases including even chronic conditions. Traditional systems of medicine have been in vogue for treating various ailments in many countries such as China, Japan and India since immemorial time.

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Despite the remarkable advancements in modern medicine and the development of synthetic drugs, traditional remedies, now referred to as herbal pharmaceuticals or herbal treatments, are still advocated and endorsed by the World Health Organization (WHO). According to the second WHO global survey, Siddha medicine is a popular form of traditional and complementary medicine, recognized by several Member States. [2]

Polycystic ovary syndrome (PCOS) is an endocrine and reproductive disorder affecting 7 to 15% of women of reproductive age. It was first described by Stein and Leventhal in 1935. PCOS etiology is complex, including genetic, environmental and lifestyle factors and remains controversial. PCOS is defined by the presence of at least two of the Rotterdam criteria: oligo-anovulation, clinical or biological hyperandrogenism, and micropolycystic syndrome (ovarian volume > 10 ml and/or more than 12 follicles by the ovary). [3]

The aetiology of this syndrome remains largely unknown, but mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and lifestyle factors [4]. Despite the availability of numerous treatments for PCOS, the development of new drugs remains ongoing.

In recent times, there has been a growing trend in utilizing these medicinal preparations for addressing Poly Cystic Ovarian Syndrome (PCOS). Herbal remedies for PCOS have received attention as a form of lifestyle management in traditional medicine, in which the menstrual cycle and normal serum hormones levels can be recovered. [5]

Herbal remedies are known to have reduce polycystic ovaries and ovarian volume, improve insulin sensitivity, and normalize reproductive cycles [6] [7].

The initiation of clinical trials for any medication is dependent on a comprehensive analysis of its physicochemical and phytochemical properties.

To instill confidence in the therapeutic usage of Siddha medicines on a global scale, it becomes crucial to standardize them using scientific techniques. Through such standardization, the nature of their composition, safety, and quality can be proven, thus fostering trust among people and facilitating global acceptance of these medicines.

In Siddha system of Medicine, there are unique combination of medicines which have solution to manage PCOS. One such formulation is *Arputha Mathirai (AM)*[8], which is mentioned in Siddha literature,*KoshayeeAnubogaVaithiyaBrammaRagasiyam.* The method of preparation of the medicine is easy and the raw drugs are also easily available.

The formulation has been evaluated for its physico-chemical profile such as ashvalue, extractive value in water and alcohol and qualitative phytochemicalanalysis. Therefore the present study was to ensure the standardization of drug.

2. MATERIALS AND METHODS

2.1 Selection of Drug

Arputha Mathirai is a Siddha preparation that is mentioned in the text *KoshayeeAnubogaVaithiyaBrammaRagasiyam*(Pg. No: 87 & 88)

2.2 Ingredients

The drug composition of *Arputha Mathirai* (AM) consists of nine ingredients, including seven herbal compounds and two mineral compounds. [9] [10]

1) *Cuminumcyminum* L. (Cumin) - 70 g

2) *Piper nigrum*L. (Black Pepper) -70 g
3) *Zingiberofficinale*Roscoe. (Dried Ginger) -70 g
4) *Piper longum* L. (Long Pepper) -70 g
5) *Allium sativum*L. (Garlic) - 70 g
6) *Ferula asafoetida* L. (Asafetida) -70 g
7) Purified rock salt -70 g
8) Purified sulphur - 70 g
9) *Citrus limon*Linn. (Lemon juice) - sufficient quantity.

2.3 Collection of Raw Material

The indigeneous raw materials were procured from areputed drug store, identified and authenticated by the Botanist of GovernmentSiddha Medical College, Chennai, (Voucher number GSMC/MB- 566 – 571 & 614) and HOD of the Department of Gunapadam, Government Siddha Medical College, Chennai,Tamilnadu– 106

2.4 Sample Preparation

2.4.1 Purification of Raw Drugs

Herbal and mineral drugs were purified as mentioned in "*SikitchaRatnaDeepamEnnumVaidhiyaNool"* and "*GunapadamThathuJeevaVaguppu"* respectively. [11][12]

a) Cumin

Unwanted soil particles and dust were removed, winnowed and sun dried.

b) Black Pepper

Soaked in sour buttermilk for 3 hours (1 saamam) and sun dried.

c) Dried Ginger

One part of sukku was bleached with 2 parts of lime stone (kalsunnambu) for 3 hours (1 saamam), washed, dried and the outer skin was peeled.

d) Long Pepper

Soaked in the leaf juice of *Plumbagoindica (Kodiveli)* for 24 minutes (1 *naazhigai*) and then sun dried.

e) Garlic

Outer dry papery skin and the tip were removed and washed.

f) Asafetida

Roasted in coal fire and then powdered.

g) Rock Salt

Soaked in vinegar (kaadi) for 3 days and then sun dried.

h) Sulphur

Crude Sulphur (Gandhagam) was melted in an iron ladle containing little butter and the melted content was poured into a container containing juice of banana stem. This was repeated for 10 times.

i) Lemon Juice

Lemons were washed, pat dry, juice was extracted, and the seeds were removed.

2.4.2 Preparation

The Herbomineral siddha formulation *Arputha Mathirai* (AM) wasprepared as per Siddha text.

- All the purified ingredients except garlic and lemon juice were taken in the said quantities, grounded individually into a very fine powder, and sievedusing a sieving cloth individually.
- Then all the powdered single drugs were mixed together.
- Garlic was grounded and made into a very fine paste, and mixed andgrounded with the above powder.
- The above mixture was grounded together for about 12 hours (4 *saamam*) withadequate lemon juice and then rolled into pills of 500 mg (*sundaialavu*)[13] size.

The phyto chemical screening and physico chemical analysis of AM wascarried out at, The TamilnaduDr.MGR Medical university, Guindy, Chennai.

2.5 Physico-Chemical Parameters

The formulation underwentphysico chemical screeningsuch as percentage of loss on drying, total ash, acid-insoluble ash,water solubleextractive and alcohol soluble extractive, based on the AYUSH PLIM (The results are depicted in Table 1).

2.5.1. Loss on Drying

An accurately weighed 1g of *ArputhaMathirai*formulation was taken in a tarred glass bottle.The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. The Percentage moisturecontent of the sample was calculated with reference to the shade dried material.

2.5.2 Determination of Total Ash

Weighed accurately 2g of *ArputhaMathirai*formulation was added in crucible at a temperature6000^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air drieddrug.

2.5.3 Determination of Acid Insoluble Ash

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burntto a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference tothe air dried drug.

2.5.4 Determination of Water Soluble Ash

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filterpaper was washed with hot water and ignited for 15 min at a temperature not exceeding 4500^0C in a mufflefurnace. The amount of soluble ash is determined by drying the filtrate.

2.5.5 Determination of Water Soluble Extractive

5gm of air dried drug, coarsely powered *Arputha Mathirai*was macerated with 100ml of distilledwater in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml offiltrated was evaporated in a tarred flat bottom shallow dish, further dried at 1000^0C and weighted. Thepercentage of water soluble extractive was calculated with reference to the air dried drugs.

2.5.6 Determination of Alcohol Soluble Extractive

1 gm of air dried drug coarsely powdered *Arputha Mathirai*was macerated with 20 ml alcohol inclosed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 1000^0C and weighted. Thepercentage of alcohol soluble extractive was calculated with reference to air dried drug.

2.6 Phytochemical Screening

The preliminary phytochemical screening test was carried out for each extracts of*Arputha Mathirai*as per the standard procedure mentioned hereunder.

(The results are depicted in Table 2)

2.6.1 Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow colour precipitate indicates the presence of alkaloids.

b) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (PotassiumBismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

c) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in PotassiumIodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2.6.2 Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used

to test for the presence of carbohydrates.

a) Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and few drops of concentrated sulphuricacid is added slowly along the sides of test tube. A violet ring indicates the presence ofcarbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange

red precipitate indicates the presence of reducing sugars.

2.6.3 Detection of Saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten

minutes it indicates the presence of saponins.

2.6.4 Detection of Phenols

Ferric Chloride Test **:**Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colorindicates the presence of phenols.

2.6.5 Detection of Tannins

Gelatin Test **:**The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

2.6.6 Detection of Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxidesolution. Formation of intense yellow color, which becomes colorless on addition of dilute acid,indicates the presence of flavonoids.

***b) Lead Acetate Test*:** Extracts were treated with few drops of lead acetate solution.Formation of yellow color precipitate indicates the presence of flavonoids.

2.6.7 Detection of Diterpenes

***Copper Acetate Test* :**Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution.Formation of emerald green color indicates the presence of diterpenes.

2.6.8Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence ofQuinones.

2.6.9 Gum and Mucilage

To 1ml of extract 2.5ml of absolute alcohol was added and stirred constantly. Then the precipitatewas dried in air and examine for its swelling properties. Swelling was observed thatindicated the presence of gum and mucilage.

3. RESULTS

Table 1 : Results of Physico chemical analysis of AM

S.No	*Parameters*	*Percentage*
1	Loss on drying	10.08%
2	Total ash value	15.39%
3	Acid insoluble ash	2.45%
4	Water soluble ash	12.18%
5	Water soluble extraction	20.55%
6	Alcohol soluble extraction	8.46%

Table 2 Results of Phytochemical analysis of AM

S.No	Phytochemicals	Test Name	H_2O Extract
1	Alkaloids	Mayer's Test	**-ve**
		Dragendroff's Test	**-ve**
		Wagner Test	**+ve**
2	Carbohydrates	Molisch's Test	**+ve**
		Benedict test	**+ve**
3	Saponin	Foam Test	**+ve**
4	Phenols	Ferric Chloride Test	**-ve**
5	Tannins	Gelatin Test	**-ve**
6	Flavonoids	Alkaline Reagent Test	**+ve**
		Lead Acetate	**+ve**
7	Diterpenes	Copper Acetate Test	**+ve**
8	Quinones	Test for Quinones	**-ve**
9	Gum & Mucilage	Test for Gum & Mucilage	**+ve**

+ve/-ve present or absent if component tested

4. DISCUSSION

The purpose of conducting phytochemical analysis was to determine the presence and concentration of bioactive components within a formulation, thus establishing its efficacy and therapeutic significance. By identifying and quantifying these phytoconstituents, researchers can gain insights into the potential mechanisms of action and therapeutic properties of the formulation.

On the other hand, physicochemical analysis aimed to assess the macroscopic and microscopic properties of the formulation, providing valuable information about its physical characteristics, such as color, texture, solubility, and stability. This analysis helped in ensuring the consistency and quality of the formulation.

Previous studies have already investigated the ovulation-inducing, hypolipidemic, hypoglycemic and emmenagogue (menstrual flow promoting) activities of specific ingredients present in *Arputha Mathirai*. These studies have demonstrated the therapeutic importance of these individual components within the formulation.

Considering these factors, the Siddha herbomineral formulation, *ArputhaMathirai (AM),* underwent standardization to evaluate its quality and to determine the beneficial phytoconstituents present in it. This standardization process ensured the consistency, safety, and efficacy of the formulation, thus enhancing its value as a therapeutic intervention.

5. CONCLUSION

Physico chemical analysis of the current study concluded 10.08% of loss on drying at 105°C, 15.39% of total ash value, 2.45% of acid insoluble ash, 12.18% of water soluble ash, 20.55% of water soluble extraction and 8.46% of alcohol soluble extract. Phytochemical screening showed the presence of bioactive componentssuch as alkaloids, carbohydrates, saponins, flavonoids, diterpenes, gum, and mucilage in the sample. Additionally, it demonstrated the absence of quinones, tannins, and phenols. Presence of these bio active compounds suggests that it can be a better drug ofchoice for PCOS. But invivo studies and clinical studies in largesamples have to be done in future to prove its efficacy.

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