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Exploration of Physicochemical, Phytochemical, and HPTLC Analysis of Arputha Mathirai: A Siddha Herbo Mineral Formulation for Poly Cystic Ovarian Syndrome (PCOS)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to explore the physicochemical, phytochemical and HPTLC analysis of the *Siddha* herbomineral formulation, *Arputha Mathirai (AM)*, in tablet form, for its potential in treating PCOS (Poly Cystic Ovarian Syndrome). The objective was to analyze the tablet and assess its physicochemical properties, as well as the presence of bioactive compounds derived from plant sources.
Place of Study: The physicochemical and phytochemical analysis was conducted at The Tamilnadu Dr.MGR Medical University, located at No.69, Anna Salai, Guindy, Chennai – 600 032.

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The High-Performance Thin Layer Chromatography (HPTLC) was carried out at Noble Research Solutions in Kolathur, Chennai – 600 099.

Materials and Methods: *Arputha Mathirai,* 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 Tamilnadu Dr.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. HPTLC was peformed at Noble Research Solutions, Kolathur, Chennai – 600 099.

Results: 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. HPTLC finger printing analysis of the sample revealed the presence of five prominent peaks corresponding to the presence of five versatile phytocomponents present with in it. Rf value of the peaks ranged from 0.02 to 0.56.

Conclusion: The findings of this study provided a comprehensive understanding of the physicochemical properties, bio active phytochemicals and phytocomponents 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.

Keywords: Siddha system; Arputha Mathirai; 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. Siddha system of medicine is practiced mostly in India's southern part for treating various diseases including even chronic conditions" [1].

"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 properties and phytochemical components.

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, *Koshayee Anuboga Vaithiya Bramma Ragasiyam.* 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 ash value, extractive value in water and alcohol and qualitative phytochemical analysis and HPTLC analysis. Therefore the present study was to ensure the standardization of drug.

2. MATERIALS AND METHODS

2.1 Selection of Drug

In the Siddha text "Koshayee Anuboga Vaithiya Bramma Ragasiyam" (Pg. No: 87 & 88) [8], numerous formulations are mentioned with various indications. Arputha Mathirai is one among them. This herbomineral preparation is known to be effective for treating conditions such as gastric ulcer, PCOS, and abdominal diseases. In this preliminary study, the author's specific focus was to examine the effects of Arputha Mathirai in the context of PCOS. The aim was to gather data that could be utilized in subsequent preclinical and clinical evaluations.

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. *Cuminum cyminum* L. (Cumin) - 70 g
2. *Piper nigrum* L. (Black Pepper) -70 g
3. *Zingiber officinale* 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 herbal and mineral raw drugs were procured from a reputed raw drug store, identified and authenticated by the Botanist of Government Siddha Medical College, Chennai, (Voucher number GSMC/MB- 566 – 571 & 614) and HOD of the Department of Gunapadam, Government Siddha Medical College, Chennai, Tamilnadu – 106, respectively.

2.4 Sample Preparation

2.4.1 Purification of raw drugs

Herbal and mineral drugs were purified as mentioned in "*Sikitcha Ratna Deepam Ennum Vaidhiya Nool"* and "*Gunapadam Thathu Jeeva Vaguppu"* respectively [11,12].

2.4.1.1 Cumin

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

2.4.1.2 Black pepper

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

2.4.1.3 Dried ginger

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

2.4.1.4 Long pepper

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

2.4.1.5 Garlic

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

2.4.1.6 Asafetida

Roasted in coal fire and then powdered.

2.4.1.7 Rock salt

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

2.4.1.8 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.

2.4.1.9 Lemon juice

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

2.4.2 Sample preparation

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

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

The phyto chemical screening and physico chemical analysis of AM was carried out at, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai.

2.5 Physico-Chemical Parameters

The formulation underwent physico chemical screening such as percentage of loss on drying, total ash, acid-insoluble ash, water soluble extractive 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 *Arputha Mathirai* formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0 C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material" [14].

2.5.2 Determination of total ash

Weighed accurately 2 g of *Arputha Mathirai* formulation was added in crucible at a temperature 600° C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

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%

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 ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug" [14].

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 filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. The amount of soluble ash was 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 distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 1000^0 C and weighed. The percentage 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 in closed 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 1000^0 C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug" [14].

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.

2.6.1.1 Mayer's test

Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids. Such precipitate was not formed.

2.6.1.2 Dragendroff's test

Filtrate was treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids. Red precipitate was not formed.

2.6.1.3 Wagner's test

Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated 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.

2.6.2.1 Molisch's test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube. A violet ring indicated the presence of carbohydrates.

2.6.2.2 Benedict's test

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.

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

2.6.3 Detection of saponins

2.6.3.1 Foam test

0.5 gm of extract was shaken with 2 ml of water. The foam produced persisted for ten minutes indicated the presence of saponins.

2.6.4 Detection of phenols

2.6.4.1 Ferric chloride test

Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols. No such color was formed, indicating the absence of phenols.

2.6.5 Detection of tannins

2.6.5.1 Gelatin test

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

2.6.6 Detection of flavonoids

2.6.6.1 Alkaline reagent test

Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.

2.6.6.2 Lead Acetate Test

Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

2.6.7 Detection of diterpenes

2.6.7.1 Copper acetate test

Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

2.6.8 Test for quinones

Extract was treated with sodium hydroxide. Blue or red precipitate indicates the presence of quinones. No such precipitate was formed indicating the absence of quinones.

2.6.9 Gum and mucilage

To 1ml of extract 2.5ml of absolute alcohol was added and stirred constantly. Then the precipitate was dried in air and examined for its swelling properties. Swelling was observed, which indicated the presence of gum and mucilage.

2.7 HPTLC Analysis

This was carried out at Nobel research Solutions, Chennai. Project ID was NRS/AS/0901/09/2022**.** **"**HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics" [15].

2.7.1 Chromatogram development

CAMAG TLC SCANNER III instrument was to carry out HPTLC. It was carried out in CAMAG Twin Trough chambers. Aluminium coated silica gel – Merck, TLC plate was used. The mobile phase was chloroform:n-Butanol:Methanol: Water:Acetic Acid (4:1:1:0.5:0.5). 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 [15].

2.7.2 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.

3. RESULTS AND DISCUSSION

Physico chemical analysis of the current study concluded 10.08% of loss on drying (LOD) 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.

The moisture content analysis (LOD) revealed a less amount of moisture, indicated by a value of 10.08%. The total ash value of 15.39% indicated the presence of a substantial quantity of inorganic residue in the formulation. The acid insoluble ash value of 2.45% was within the safety margin limit, suggesting the absence of contamination. The water soluble ash value of 12.18% indicated the presence of water-soluble components in the formulation. Finally, the alcohol soluble extract value of 8.46% indicated the exhausted drug in the formulation.

Phytochemical screening showed the presence of bioactive components such as alkaloids, carbohydrates, saponins, flavonoids, diterpenes, gum, and mucilage in the sample. Additionally, it demonstrated the absence of quinones, tannins, and phenols.

HPTLC finger printing analysis of the sample revealed the presence of five prominent peaks corresponding to the presence of five versatile phytocomponents present with in it. Rf value of the peaks ranged from 0.02 to 0.56.

Prior research studies have extensively examined the specific ingredients present in *Arputha Mathirai* and their individual therapeutic effects. Some of these studies have investigated the ovulation-inducing properties of asafetida [16], the hypolipidemic effects of cumin and black pepper [17,18,19], the anti-diabetic, anti-atherosclerotic, anti-thrombotic, and anti-hyperlipidemic activities of garlic as well as its role as an oxidative stress marker [20,21], and the emmenagogue properties of asafetida [22,23]. These studies have collectively established the therapeutic significance of these specific ingredients within the formulation.

The current physicochemical, phytochemical, and HPTLC analysis of AM yielded valuable evidence regarding the presence of certain compounds. These compounds are believed to be active components that have the potential to intervene in the pathological mechanisms associated with PCOS, thereby offering a potential means of managing the condition.

The purpose of conducting this study was to determine the presence 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.

This standardization process ensured the consistency, safety, and efficacy of the formulation, thus enhancing its value as a therapeutic intervention.

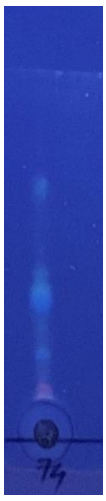

Fig. 1. TLC Visualization of AM at 366 nm

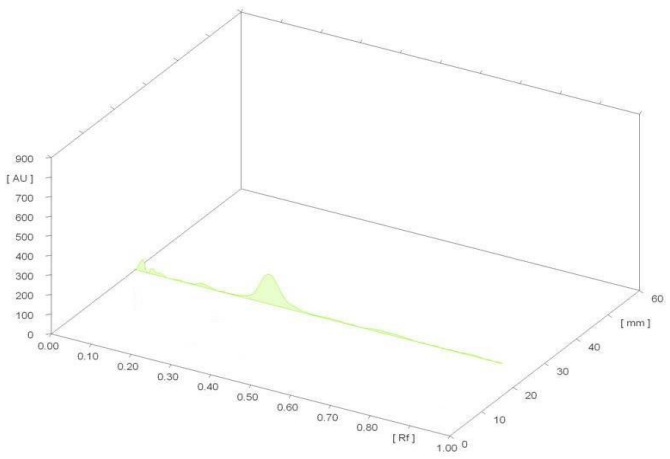

Fig. 2. 3D Chromatogram

Table 3. HPTLC Peak Table of AM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	53.2	0.02	59.2	22.22	0.04	15.9	276.5	5.66
2	0.04	24.9	0.04	28.3	10.61	0.08	1.2	218.3	4.47
3	0.13	0.2	0.17	16..6	6.21	0.20	1.7	222.0	4.54
4	0.23	0.7	0.34	151.6	56.89	0.45	1.5	3881.9	79.41
5	0.56	0.2	0.61	10.8	4.06	0.66	7.8	289.7	5.93

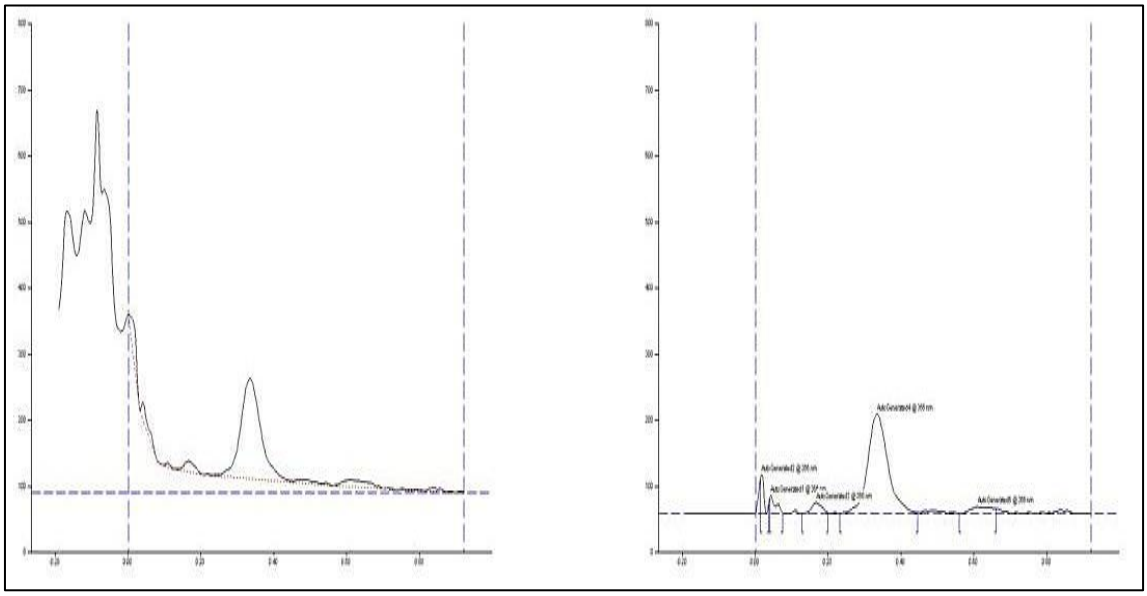

Fig. 3. HPTLC Fingerprinting of Sample AM

4. CONCLUSION

The standardization process implemented in this study was successful in confirming the presence of bioactive phytocomponents in the formulation. The presence of these bioactive compounds enhances the therapeutic potential of *Arputha Mathirai* (AM) as an intervention for PCOS. However, it is important to note that further in vivo studies and clinical trials involving larger sample sizes are necessary in order to substantiate its efficacy as a preferred treatment for PCOS.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ram A, Joseph DA, Balachandar S, Singh VP. Medicinal plants from Siddha system of medicine useful for treating respiratory diseases. International Journal of Pharmaceuticals Analysis. 2009;1(2):20.
2. Available:https://www.who.int/publications/i/item/9789240064973
3. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments. Gynecological Endocrinology. 2021;37(10):869-74.
4. Escobar-Morreale HF. Polycystic ovary syndrome: Definition, aetiology, diagnosis

and treatment. Nature Reviews Endocrinology. 2018;14(5):270-84.
5. Lee JH, Jo J. Successful treatment with Korean herbal medicine and lifestyle management in an obese woman with polycystic ovarian syndrome. Integrative Medicine Research. 2017;6(3):325-8.
6. Phipps WR, Martini MC, Lampe JW, Slavin JL, Kurzer MS. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab.1993;77:1215–1219.
7. Kudolo GB, Wang W, Javors M, Blodgett J. The effect of the ingestion of Ginkgo biloba extract (EGb 761) on the pharmacokinetics of metformin in non-diabetic and type 2 diabetic subjects—A double blind placebo-controlled, crossover study. Clinical Nutrition. 2006;25(4):606-16.
8. .Mohan RC, Koshayee Anuboga Vaithiya Bramma Ragasiyam, First Part, 2014;87-88.
9. Nadkarani KM, Indian Material Medica., 3rd revised edition. 2005;2.
10. The Wealth of India. I: Third Reprint; 2009.
11. Kannusamypillai C, Sikitcharatnadeepam Ennum Vaithiya Nool; 1931.
12. Thiyagarajan R, Gunapadam Thathu Seeva Vaguppu Paagam 2 & 3. 1st revised edition; 2013.
13. Sayujya PS, Waheeda Banu. Phytochemical Analysis Of Palasatwagadi Kashaya Churna. World Journal of Advance Healthcare Research. 2022: 6(12).
14. Sornamariammal I, Siddha Marunthakiyal Vithigalum Sei Muraigalum Second Print; 2018.
15. Wagner H. Plant drug analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium. 2002:305,227.
16. Shieh A, Bagheri SM, Yadegari M, Javidmehr D, Farhadi Z. Therapeutic effect of Ferula assa-foetida oleo-gum resin in rats with letrozole-induced polycystic ovary syndrome. Clinical and Experimental Reproductive Medicine.2022;49(4):239.
17. Surya Dhandapani, Vijayakumar Ramasamy Subramanian, Senthilkumar Rajagopal, Nalini Namasivayam, Hypolipidemic Effect of *Cuminum Cyminum* L. on Alloxan-induced diabetic Rats. Pharmacological Research. 2002; 46(3):251-255, ISSN 1043-6618.
18. Mnif S, Aifa S. Cumin (*Cuminum cyminum* L.) from traditional uses to potential biomedical applications. Chem Biodivers. 2015;12(5):733-42. DOI:10.1002/cbdv.201400305.PMID: 26010662
19. Parim B, Harishankar N, Balaji M, Pothana S, Sajjalaguddam RR. Effects of Piper nigrum extracts: Restorative perspectives of high-fat diet-induced changes on lipid profile, body composition, and hormones in Sprague-Dawley rats. Pharm Biol. 2015; 53(9):1318-28. DOI:10.3109/13880209.2014.980585.Epub 2015 PMID:25856709.
20. Majewski M. Allium sativum. facts and myths regarding human health. Rocz Panstw ZaklHig. 2014;65(1):1-8. PMID:24964572.
21. Zadhoush R, Alavi-Naeini A, Feizi A, Naghshineh E, Ghazvini MR. The therapeutic impact of garlic (*Allium Sativum*) on oxidative stress markers among polycystic ovary syndrome patients: A randomized, double-blinded clinical trial. International Journal of Preventive Medicine. 2023;14.
22. Mahendra P, Bisht S. Ferula asafetida. Traditional uses and pharmacological activity. Pharmacogn Rev. 2012;6(12):141-6. DOI:10.4103/0973-7847.99948.PMID:23055640;PMCID:PMC3459456.
23. Poonam Mahendra P, Ferula asafetida. Traditional uses and pharmacological activity. 2012;6(12):141-146.

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