World Journal of Current Medical and Pharmaceutical Research
Content available at www.wjcmpr.com ISSN: 2582-0222

PHYTOCHEMICAL, PHYSICOCHEMICAL AND HPTLC ANALYSIS OF SIDDHA HERBAL FORMULATION MUPPIRANDAI CHOORANAM

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Article History

Received on: 15-06-2023
Revised on: 04-07-2023
Accepted on: 26-08-2023

Abstract

Aim: The aim of the study was to evaluate the Phytochemical, Physicochemical, HPTLC analysis of Siddha herbal formulation *Muppirandai Chooranam* (MRC), indicated for various ailments including menorrhagia.

Study design

Place of study: The Phytochemical and Physicochemical analysis were conducted at the Tamilnadu Dr. MGR Medical University, located at no.69, Anna salai, Guindy, Chennai-32. The high-performance thin layer chromatography (HPTLC) was carried out at Noble research solutions, Kolathur, Chennai -99.

Methodology: Siddha formulation M*uppirandai Chooranam* was prepared as per good manufacturing practices (GMP) guidelines and the phytochemical analysis, physicochemical analysis were carried out at the Tamilnadu Dr. MGR Medical University, located at no.69, Anna salai, Guindy, Chennai-32. As per PLIM guidelines (Pharmacopeia Laboratory Of Indian Medicine) in accordance with the guidelines established by AYUSH (Ayurveda, Yoga, Unani, Siddha, Homoeopathy), the governing body for traditional health systems in India. The HPTLC analysis was conducted at Noble Research Solutions, Kolathur, Chennai -99.

Results: The Phytochemical screening of MRC shown the presence of Alkaloids, Carbohydrates, Saponins, Flavanoids, Diterpenes, Gum and Mucilage. The physicochemical analysis of MRC revealed that it had a loss on drying of 0.42% at 105°C, a total ash content of 23.3%, an acid-insoluble ash content of 3.02%, a water-soluble ash content of 2.30%, and a water-soluble extract content of 17.67%, and alcohol soluble extract content of 5.23%.HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to the presence of five versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.37.

Conclusion: From the findings, it is concluded that it shown the compendious understanding of presence of Phytochemical components, Physicochemical characteristics, and HPTLC analysis of MRC and it is competent to assess the quality profile of *Muppirandai Chooranam* as a reference standard for the development of the standardised pharmaceutical product.

Keywords: *Muppirandai Chooranam, Menorrhagia, Perumbadu, Siddha, Phytochemical, Physicochemical.*

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DOI: https://doi.org/10.37022/wjcmpr.v5i4.284

1. Introduction

Tamil Nadu, South India, is home to an antiquated Siddha system of medicine. Siddha medicines arrest the body's cells from ageing.The word "*Marunthu*" (drug) itself refers to scented roots or leaves in Tamil literature. The Siddha system of Medicine is primarily focused on developing medications with high potencies and long-lasting lives for usage in the future. Additionally, it tries to sustain lifespan and improve cell regeneration. The Siddha Sutra emphasizes the line of usage of medicines as "*Verpaaru thazhaipaaru minginikaal Mellamella parpachenduram paare*" meaning, It has been suggested to use the pure herbs in liquid, powder, tablet, or paste form first. If such is not able to manage the illness, doctors will employ the combination of Purified animal products, metals, and minerals in addition to the herbs [1].

Since the system uses multiple combinations of medicines, increasing the possibility of adulteration and replacement, a minimal amount of quality control, usage of current technologies for the generation of standards is needed for these medications to be accepted globally. One such ways include standardization of Siddha medicines which ensures the safety and quality through various scientific parameters.

Depending on the type of medicine, the product has to be examined for its suitable parameters [2].

On the other hand, incidence of diseases is increasing every year than its prognosis. One such prevailing gynecological disease includes Menorrhagia (Heavy Menstrual Bleeding-HMB). HMB can be stated as an excessive amount of menstrual blood loss that affects a woman's physical, emotional, social, and material well-being which can happen on its own or in conjunction with other symptoms [3]. This leads to reduction in hemoglobin levels and other consequences, which is also another matter of concern. In recent times, among people, there has been a positive ray of hope in getting treatment from Siddha in gynecological diseases also.

Siddha system of Medicine has unique combination of medicines, which have solution to the present scenario of excessive or prolonged menstrual bleeding. In Siddha, the symptoms of Menorrhagia can be correlated to *Perumbadu* specifically *Pitha Perumbadu*, according *Yugi Vaidhiya Sindhamani* [4]. Also, various Siddha medicines indicated for *Perumbadu* were also evident in Siddha literature. With that importance, one such herbal formulation *Muppirandai Chooranam* which is mentioned in Siddha literature *Yagobu Vaidhiya Sindhamani* for Menorrhagia [5], has been analysed for its Phytochemical, Physicochemical and High-Performance Thin Layer Chromatography in order to promote its credibility through scientific way.

2. Materials and Methods

2.1 Collection of Raw Drugs

The Plant *Muppirandai* was collected and the indigeneous herbal 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-603 – 607).

2.2 Ingredients

1. *Cissus quadrangularis* - three sided (*Muppirandai*) - 1 *thooku* (1.75 kg)
2. *Zingiber officinale* Rosc. (Dried ginger) - 1*palam* (35gm)
3. *Piper nigrum* Linn. (Pepper) - 1*palam* (35gm)
4. *Piperlongum*Linn. (Long pepper) - 1*palam* (35gm)
5. *Trachyspermum ammi Linn*. (Ajwain / thymol seeds) - 1*palam* (35gm)

2.3 Purification

Raw drugs were purified as mentioned in *Sikitcha Rathna Deepam Ennum Vaidhiya Nool*[6], *Marundhu Sei Iyalum Kalaiyum*[7].

Muppirandai

Muppirandai was cleaned by removing its *kanu*, outer skin and soaked in buttermilk, added with salt for 3 days and dried in sunlight.

Chukku

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.

Milagu

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

Thippili

Soaked in lime juice and dried.

Omam

Omam was washed with lime stone water and dried.

2.4 Sample Preparation

1 *thooku* (1.75 kg) of *Muppiranda*i, were taken in a mud vessel, boiled with cow's milk, filtered, squeezed and juice was taken. Dried ginger, Pepper, Long pepper, Ajwain(each 35gms) were roasted, powdered and mixed with the above juice.The juice mixed with the powders,were kept sun dried. After drying, contents were finely powdered.

2.5 Phytochemical Analysis

The preliminary phytochemical screening test was carried out at, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai, for each extracts of *Muppirandai Chooranam* as per the standard procedure mentioned hereunder.

2.5.1 Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

2.5.1.1 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.

2.5.1.2 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.

2.5.1.3 Wagner's test

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

2.5.2 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.

2.5.2.1 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.

2.5.2.2 Benedict's test

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

2.5.3 Detection of saponins

2.5.3.1 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.

2.5.4 Detection of phenols

2.5.4.1 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.

2.5.5 Detection of tannins

2.5.5.1 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.

2.5.6 Detection of flavonoids

2.5.6.1 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.

2.5.6.2 Lead Acetate Test

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

2.5.7 Detection of diterpenes

2.5.7.1 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.

2.5.8 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.

2.5.9 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.

2.6 Physicochemical Analysis

The preliminary physicochemical screening test was carried out at, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai for *Muppirandai Chooranam* as per the standard procedures mentioned hereunder.

2.6.1. Loss on Drying

An accurately weighed 1g of *Muppirandai Chooranam 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.

2.6.2. Determination of total ash

Weighed accurately 2g of *Muppirandai Chooranam* formulation was added in crucible at a temperature 600^{0}C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

2.6.3. 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.

2.6.4. 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^{0}C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

2.6.5. Determination of water soluble extractive

5gm of air dried drug, coarsely powered *Muppirandai Chooranam* was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^{0}C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

2.6.6. Determination of alcohol soluble extractive

1 gm of air dried drug coarsely powdered *Muppirandai Chooranam 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 100^{0}C and weighted.The percentage of alcohol soluble extractive was calculated with reference to air dried drug [8].

2.7 HPTLC Analysis

This was conducted at Noble Research Solutions, Kolathur, Chennai -99. Project Id was NRS/AS/0898/09/2022. 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 [9].

2.7.1. Chromatogram Development

The CAMAG TLC SCANNER III device was for performing HPTLC. TLC Plate - Aluminium Coated Silica Gel – Merck, Mobile phase - 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 [9].

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

The Phytochemical screening of MRC shown the presence of Alkaloids in Wagner test, Carbohydrates in Molisch's test, Benedict test, Saponin in Foam test, Flavanoids in lead acetate test, Diterpenes in Copper acetate test, Gum and Mucilage in Gum and mucilage test of the study sample. Also, shown the absence of Tannins, Phenols and Quinones.

Table.1 Phytochemical analysis result of MRC

S.No.	Phytochemicals	Test Name	H_20 Extract
1	Alkaloids	Mayer's Test Dragendroffs Test Wagner Test	-ve -ve +ve
2	Carbohydrates	Molisch's Test Benedict Test	+ve +ve
3	Saponin	FoamTest	+ve
4	Phenols	Ferric Chloride Test	-ve
5	Tannins	Gelatin Test	-ve
6	Flavonoids	Alkaline Reagent Test Leadacetate	-ve +ve
7	Diterpenes	Copper Acetate Test	+ve
8	Quinones	Testfor Quinones	-ve
9	Gum & Mucilage	Testfor Gum & Mucilage	+ve

*+ve/ -ve indicates the present or absent if component tested

The physicochemical characteristics of MRC are found to be a loss on drying of 0.42% at 105°C, which is used to determine the moisture content of the sample. Ash values represent the presence of inorganic residues, in which the total ash value was found to be 23.30%, acid insoluble ash 3.02%, and water soluble ash 2.30%. Water soluble extraction was found to be 17.67%, whereas alcohol soluble extraction was 5.23%.

The observed values of the physico-chemical properties are given below:-

Table.2 Physicochemical analysis result of MRC

S.No	Parameters	Percentage
1.	Loss on drying	0.42%
2	Total ash value	23.30%
3	Acid in soluble ash	3.02%
4	Water soluble ash	2.30%
5	Water soluble extraction	17.67%
6	Alcohol soluble extraction	5.23%

HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to the presence of five versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.37.

Prior studies related to *Muppirandai Chooranam* are Functional groups identification through FTIR characterization, which identified some organic functional groups such as alcohols / phenols, alkyl groups, carboxyl groups, amides, aromatic, alkyne, alkane, alkene [10]. The results of the present study shown the physicochemical, phytochemical, and HPTLC analysis of MRC provided important proof that certain bioactive compounds were present which enrich the quality profile of the drug.

Fig.1 TLC Visualization of MRC at 366 nm

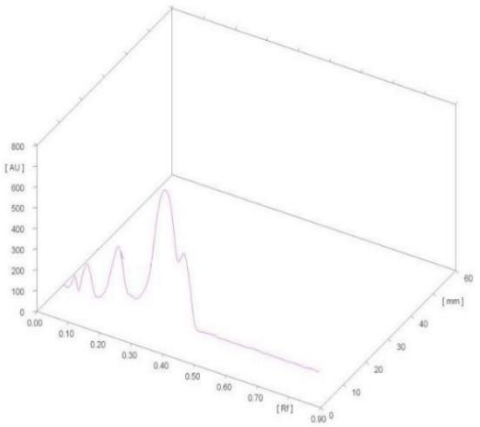

Fig.2 3D - Chromatogram

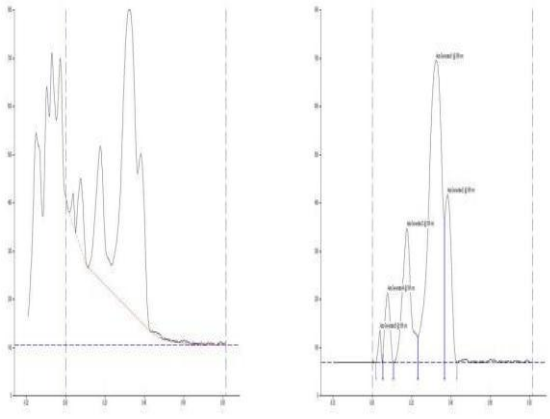

Fig.3 HPTLC finger printing of Sample MRC

Table.3 Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	1.3	0.04	67.2	4.59	0.05	6.1	450.8	1.28
2	0.06	21.2	0.08	144.4	9.86	0.11	1.8	1882.1	5.35
3	0.11	0.4	0.18	278.6	19.02	0.23	54.1	6183.2	17.59
4	0.23	54.6	0.33	626.3	42.75	0.37	300.5	21065.1	59.92
5	0.37	303.6	0.38	348.4	23.78	0.43	0.4	5575.8	15.86

4. Conclusion

From the findings, it is concluded that it shown the compendious understanding of presence of Phytochemical components, Physicochemical characteristics, HPTLC analysis of MRC and it is competent to assess the quality profile of *Muppirandai Chooranam*as a reference standard for the development of the standardised pharmaceutical product. However in addition to the results of present study, other preclinical and clinical studies are necessary to affirm the efficacy of *Muppirandai Chooranam* for Menorrhagia. This can be a valuable basic source of data for future research.

Acknowledgement

This publication is a part of the MD program of Government Siddha Medical College, Arumbakkam, Chennai-106 affiliated with The Tamil Nadu Dr. MGR Medical University, Guindy, Chennai, Tamil Nadu, India.

Consent

It is not applicable.

Funding

Self Funding.

Ethical Approval

It is not applicable.

Competing Interests

Authors have declared that no competing interests exist.

Author Contribution

Dr. Shanthini R, performed the study and prepared the manuscript. Dr. Anbu N, guided the study and approved the manuscript.

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