QUANTITATIVE ANALYSIS OF AGNIMUGA CHOORANAM- A SIDDHA HERBOMINERAL FORMULATION

Dr Manjuladevi S[1],Dr Anbu N[2]

[1]Post Graduate, Department of PothuMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.

[2]HOD, Department of PothuMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.

ABSTRACT:

One of the oldest medicinal systems, the Siddha system has many mono-herbal, poly-herbal, herbo-mineral, and animal-based formulations for a variety of diseases.Agnimugachooranam (AMC) is one of the medicines described in Agathiyarvaithiyarathinasurukam 360, for the treatment of Rheumatoid Arthritis (Uthiravaathasuronitham).Thin layer chromatography studies are one of the most important identity tests in most pharmacopoeial monographs. High-performance thin layer chromatography (HPTLC), a TLC extension, is a simple, fast, and effective technique for quantitative compound analysis.Heavy metal toxicity has far-reaching ill effects on living organisms.Aflatoxin is naturally occurring mycotoxins produced by fungi. The existence of aflatoxin in herbal medicines is a well-known issue. The detection of aflatoxin with good sensitivity and also that is reliable in complex.

AIM:

To evaluate the quantitative analysis and safety parameters of the polyherbal siddha formulation Agnimugachooranam.

METHODS:

The drug was screened for TLC,HPTLC fingerprinting ,heavy metal analysis and aflatoxin assay to estimate the quantity and safety efficacy of the drug.

RESULTS:

This study revealed the therapeutic safer level of heavy metal lead and mercury within the maximum limit as per AYUSH Pharmacopoeial Laboratory for Indian medicine guidelines whereas aflatoxin were absent in AMC which showed this formulation was free from toxicity.And also this drug has four prominent peak corresponds to the presence of four versatile phytocomponents present with in it which denotes the abundant existence of drug at minimum peak level.

KEYWORDS:

Siddha formulation,Agnimugachooranam, TLC,HPTLC analysis, Heavy metal analysis, aflatoxin.

INTRODUCTION:

Rheumatoid Arthritis is a chronic disease which willaffect the normal person to live a normal healthy life, dueto its worst complications[(1)]. RA affects nearly 1% ofpopulation worldwide due to its debilitating nature, inadvance stages; the disease burden is considerable ineconomics and health expenditure terms. In developedcountries 0.5-1% of adults are affected. Incidence of newcases ranges from 5-50 per 100,000 adults in developedcountries[(2)].

In Siddha system of medicine, the disease "UTHIRAVADHA SURONITHAM" is brought under the types of vadha diseases. Here the clinical features of Rheumatoid arthritis are correlated with "UTHIRAVADHA SURONITHAM" according to the Siddhar YUGI MUNI classification. In this study AgnimugaChooranam(AMC) was selected and screened for standardization methods as per the procedure.The medicine is composed of 13 herbs Chithiramoolam (verpattai), Chukku, Milagu, Thippili, Omam, Kandubharangi, Yaanaithippili, Kostam, Vaaividangam, Kadugu,Perungayam,Vasambhu(sutta kari), Sarkarai and one mineral drug such as Sodium chloride impura ,otherwise known Rock salt(Inthuppu). These ingredients possess Immunomodulatory and anti inflammatoryactivity.It is effective in the treatment of Rheumatoid arthritis.

The contamination of these herbal products reduces their effectiveness and also poses serious health hazards to consumers[(3)].As a result, it is critical to standardise Siddha medicines using scientific methodologies to demonstrate their safety and quality, which may aid in creating confidence in their potential usage as a therapeutic medicine among people and for global acceptability. The current investigation was conducted as part of the above-mentioned goal to ensure its safety.

MATERIALS AND METHODS:

The trial drug AgnimugaChooranam was taken from AGATHIYAR VAITHIYARATHINA SURUKKAM 360[(4)]for the treatment and management of Uthiravaathasuronitham and its related symptoms[(5)]. This drug comprises of the following ingredients

1. Chithiramoolaverpattai(*Plumbago indigo)* – 1 Palam(35gms)
2. Chukku(*Zingiber officinale)* -1 Palam(35gms)

3. Milagu *(Piper nigrum)*- 1 Palam(35gms)
4. Thippili *(Piper longum)*- 1 Palam(35gms)
5. Omam*(Carum copticum*)- 1 Palam(35gms)
6. Kandubharangi (*Clerodendrumserratum)*- 1 Palam(35gms)
7. Yaanaithippili *(Scindapsus officinalis)*- 1 Palam(35gms)
8. Kostam*(Costusspeciosus*)- 1 Palam(35gms)
9. Vaaividangam(*Embeliaribes)*- 1 Palam(35gms)
10. Kadugu *(Brassica nigra*)-1/2 palam(17.5 gms)
11. Perungayam (*Ferulaasafoetida)*- ¼ palam(9gms)
12. Vasambhu(sutta kari)(*Acorus calamus)*- ¼ palam(9gms)
13. Indhupu (Rock salt/Sodium chloride impure)- 1 palam(35gms)
14. Sarkarai (*Sugar/ Saccharum officinalis)*- equal to all above drugs

COLLECTION AND AUTHENTICATION OF DRUG:

All the raw materials (mentioned above) were collected from a Raw-drug store, Chennai and identified and authenticated by the Botanist, Govt. Siddha medical college, Arumbakkam, Chennai- 106.

PURIFICATION OF DRUG:

The purification of drugs was done by procedures mentioned in Siddha literature **Sikitcha Rathna DeepamEnnumVaithiya Nool**[(6)].

1.Chittiramoola verpattai(Plumbago indigo)

Remove the inner vein and upper bark of the root before powdering it. Pour milk into a large vessel, cover with a cloth, then place the above powder in that cloth and cover with a lid. Burn for 3 hours to dry the powder and grind it into a fine powder.

2.Chukku (Zingiber officinale)

Twice the dose of limestone was taken for single dose of chukku were seasoned and after 3 hours wash and dry then comb the epidermis and make it into powder.

3. Milagu (*Piper nigrum*)

Pepper was soaked into sour butter milk for 3 hours and dried to get purified form of pepper.

4.Thippili(Piper longum)

Thippili soaked in kodiveli(Plumbago indigo) extract for 1 naazhi and dry it in sunlight.

5. Omam (*Carum copticum*)

Take omam and soaked into lime water then dried to get purified form.

6. Kandubharangi (Clerodendrumserratum)

Clean without any debris and keep it dry in sunlight & get purified form.

7. Yaanaithippili (Scindapsus officinalis)

Soak this in vinegar for 3 hrs and keep it in sunlight.

8. Kostam(Costusspeciosus)

Remove all dirts and keep it in sunlight.

9. Vaaividangam (Embeliaribes)

Remove all dirts and keep it in sunlight.

10.Kadugu(Brassica nigra)

Clean it well and keep it in sunlight for 2 days.

11.Perungayam (Ferulaasafoetida)

Soak it in charcoal water or lotus leave extract for 1 naazhi and dry it.

12. Vasambhu (Acorus calamus)

Make it charcoal and powder it.

13. Indhuppu(Sodium chloride impura)

Soak it in vinegar for 3 days and keep it in daylight.

14. Sarkkarai

Grind it into fine powder.

METHOD OF PREPARATION:

All the purified ingredients was taken in above mentioned quantity, mildly roasted, pounded and finely powdered. Obtained powder is sieved well using sieving cloth.For the above powder equal amount of powdered sugar was added and mixed well. The powder were stored in the clean air tight container.

DOSAGE : Thirikadipramanam(800 – 1000mg)

ADJUVANT : Hot water

DURATION :48 Days

AGNIMUGA CHOORANAM

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 were 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[(7)].

High Performance Thin Layer Chromatography Analysis

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[(8)].

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.

HEAVY METAL ANALYSIS BY AAS

Standard: Hg, As, Pb and Cd – Sigma

Methodology

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

AFLATOXIN ASSAY BY TLC

Standard

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

Solvent

Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Results

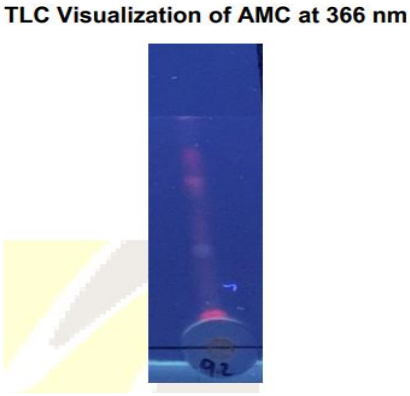

Fig 1: TLC Histogram of AMC

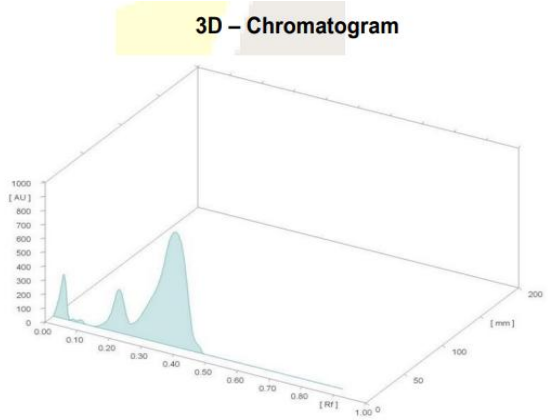

Fig 2: 3D Chromatogram of AMC

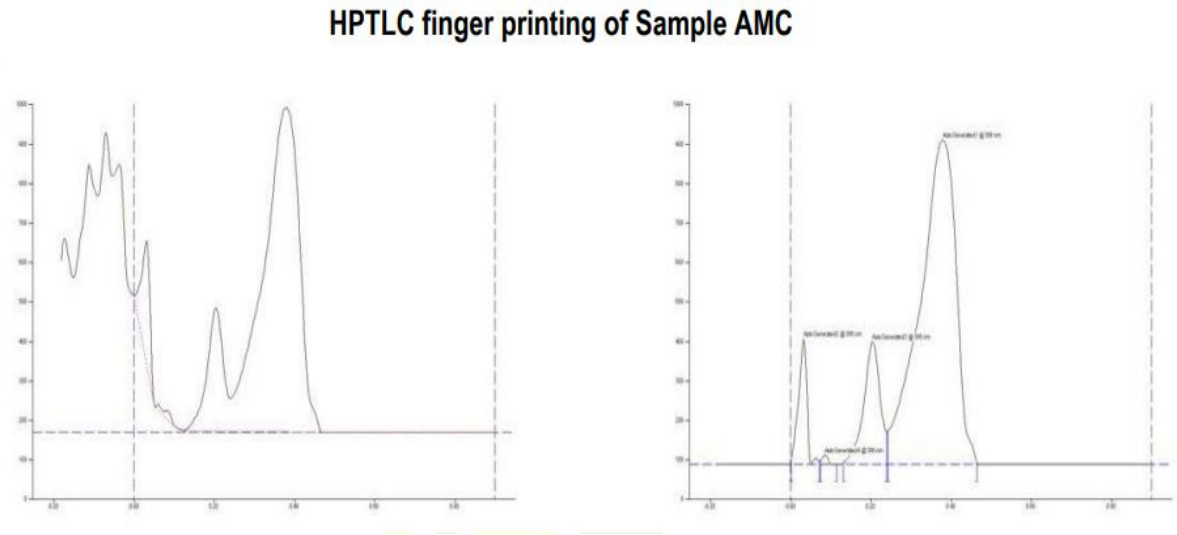

Fig 3 : HPTLC Chromatogram of AMC

Tab 1: R_f value of AGNIMUGA CHOORANAM

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End height	Area	Area %
1	0.00	13.2	0.03	318.6	21.61	0.07	9.5	3207.1	7.08
2	0.07	10.0	0.09	24.3	1.65	0.12	0.0	177.5	0.39
3	0.13	4.1	0.20	310.2	21.03	0.24	83.7	6194.1	13.68
4	0.24	84.0	0.38	821.6	55.71	0.47	2.0	35692.9	78.84

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to the presence of four versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.07 to 0.24.

Tab 2: Heavy Metal Analysis report

Name of the Heavy metal	Absorption Max ^max	Result Analysis	Maximum limit
Lead	217.0 nm	6.41 PPM	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.12 PPM	1 ppm

BDL- Below Detection Limit

Report and Inference:

Results of the present investigation have clearly shows that the sample shows the presence of Lead and mercury at 6.41 and 0.12 PPM level as listed in the table.

Tab 3: Aflatoxin analysis report

Aflatoxin	Sample AMC	AYUSH Specification limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 ppm
G2	Not Detected – Absent	0.1 ppm

Report and inference:

The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

DISCUSSION:

Thin layer chromatography (TLC) is the preliminary step to identify the phytochemical constituents in a sample. High performance thin layer chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample[(9)]. Both the methods are efficient, faster, reliable and reproducible. HPTLC is an analytical technique based on TLC, but with modifications to increase the resolution of the chemicals to be separated and to allow quantitative analysis of the compounds[(10)].In this study,TLC visualisation of AMC at 366nm shows blue color. HPTLC finger printing of AMC at UV 366nm shows Rf value of peak range from 0.07 to 0.24. AAS analysis for heavy metals revealed that the sample shows the presence of Lead and Mercury(6.41PPM, 0.12 PPM) below the maximum limit. Hence this result ensure the safety usage of the drug, free from

toxicity there by proving the safety of its utilization in Siddha system. Similarly, aflatoxins have emerged as a major hazard to human health due to a variety of harmful side effects such as hepatotoxicity, carcinogenicity, and immunological suppression. As a result, the World Health Organisation has established a permitted limit for their content in plant [(11)]. This study shows that Aflotoxins(B1;B2;G1&G2) were found to be absent in AMC.

CONCLUSION:

While there are several medicines stated in Siddha literature, there is no single effective treatment mentioned to treat RA. Based on the conclusions of the prior discussion, the Siddha formulation AMC possesses powerful physiologically biologically active elements that could aid in the treatment of RA. Many of the Siddha drugs mentioned in the literature were effective in treating RA, but they need to assess the safety parameters and quantitative analysis of the drug..In this study . Heavy metal content was found below the permissible limit and Aflatoxins were not detected. Hence, the present study assures the formulation was safe for therapeutic use and free from toxicity.

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