IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Qualitative Phytochemistry Of Siddha Formulation Mahaanaluruva Chooranam

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Abstract: **Introduction:** The venerable Siddha system, rooted in the Indian subcontinent, is among the most ancient documented medical traditions. Its progenitors, the Siddhars, have chronicled countless remedies since antiquity. Mahaanaluruva Chooranam (MAC), a polyherbal formulation, is reputedly indicated for all types of vatha diseases, as per classical Siddha texts. Rheumatoid arthritis (RA), one of these 80 vatha diseases, is a condition that may benefit from MAC. Drug standardization and dissemination are pivotal for ensuring authenticity in the contemporary globalized milieu. MAC was standardized using PLIM criteria, a critical step toward Westernization**. Material and methods:** MAC was manufactured in strict adherence to Good Manufacturing Practices (GMP). Drug standardization encompassed phytochemical analysis, pesticide residue determination, sterility testing via the pour plate method, aflatoxin assay, and heavy metal analysis. The investigation was conducted at Noble Research Solution's facility, adhering to the meticulous guidelines of the PLIM criteria. Phytochemical analysis was performed at the esteemed Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai. **Results:** Research findings indicate the presence of alkaloids, carbohydrates, saponins, phenols, phytosterols, diterpenes, flavonoids, tannins, quinones, glycosides, gum & mucilage are in phytochemical analysis, mild traces of malathion which is not alarming, no colonies or growth observed. **Conclusion:** Future clinical research and standardization would benefit from the published data. **Key word:** Mahaanaluruva Chooranam (MAC), Rheumatoid arthritis (RA), Musculoskeletal, phytochemistry, Dr.M.G.R Medical university, Chennai, heavy metal analysis, pesticide residue, sterility test.

Index Terms **– Mahaanaluruva Chooranam (MAC), Rheumatoid arthritis (RA), Heavy metal analysis, Aflotoxins, PLIM.**

QUALITATIVE PHYTOCHEMISTRY OF SIDDHA FORMULATION MAHAANALURUVA CHOORANAM

1.INTRODUCTION

Maha Analuruva Chooranam (MAC), a traditional Siddha medicine used for vatha disorders, was analyzed for safety and quality. The study assessed its phytochemical composition, potential contaminants, and microbial burden. Heavy metals, pesticides, and microbial contamination were evaluated using AAS and sterility testing. The findings were compared to AYUSH/WHO guidelines to ensure the safety and suitability of MAC for broader use, contributing to its standardization and wider acceptance in modern healthcare.

2.MATERIAL AND METHODS

The classic work "Agathiyar Vaithiya Vallathi 600"[2] details the polyherbal formulation known as Maha Analuruva Chooranam. A list of the ingredients used in this preparation can be found in Table 1[3-12].

TABLE - 1 INGREDIENTS OF MAC

S.NO	INGREDIENTS	BOTAMNICAL NAME/CHEMICAL NAME	QUANTITY
1	KODIVELI VER	*Plumbago zeylanica*	1 Palam(35gms)
2	PUNGAN VER	*Pongamia glabra*	1 Palam(35gms)
3	AAYILIAM PATTAI	*Holoptelia intergrifolia*	1 Palam(35gms)
4	AAYILIAM VER	*Holoptelia intergrifolia*	1 Palam(35gms)
5	VAIVILANGAM	*Emblica ribes*	1/2 Palam(17.5gms)
6	THIPPILI	*Piper longum*	1/2 Palam(17.5gms)
7	KADUKKAI	*Terminalia chebula*	1/2 Palam(17.5gms)
8	KADUGU	*Brassica nigra*	1/2 Palam(17.5gms)
9	CHUKKU	*Zingiber officinale*	1/2 Palam(17.5gms)
10	KARUNJEERAGAM	*Nigella sativa*	1/2 Palam(17.5gms)

3.COLLECTION, IDENTIFICATION AND AUTHENTICATION OF THE DRUG

All requisite plant materials were procured from a purveyor of raw botanicals situated at Parry's Corner in Chennai, Tamil Nadu. These materials were subsequently verified and confirmed by botanical (GSMC/MB 646-655) [13] and pharmacological experts at the Government Siddha Medical College Hospital in Arumbakkam, Chennai – 106.

3.1 PURIFICATION OF THE DRUGS (14-17)

All drugs mentioned in this study were purified according to traditional Siddha practices. The following purification methods were employed:

- **Root Purification:** The inner vein and outermost bark of kodiveli (Plumbago zeylanica) root, pungan (Pongamia glabra) root, and aayiliam (Holoptelia integrifolia) root and stem were removed. The remaining portions were ground into a fine powder and purified using the pittavial steam boiling process.
- **Fruit Purification:** Vaivilangam (Emblica ribes) were carefully cleaned to remove dust particles and dried in sunlight. Thippili (Piper longum) seeds were soaked in lemon juice and dried in sunlight. Kadukkai (Terminalia chebula) were soaked in water, the yellow water was discarded, and the remaining fruit was dried.
- **Seed Purification:** Kadugu (Brassica nigra) and Karunjeeragam seeds (Nigella sativa) were cleaned and dried in sunlight.
- **Rhizome Purification:** Chukku (Zingiber officinale) was treated with lime stone for three hours, washed, dried, and the outer skin was removed [14-17].

3.2 PREPARATION OF THE DRUG

The refined raw materials listed in Table 1 were meticulously pulverized into a fine powder using a mortar and pestle. This powder, designated as Maha Analuruva Chooranam (MAC) [2], was subsequently stored in an airtight container for preservation. Lastly, adhering to the guidelines outlined in a time-honored Siddha text, the Chooranam underwent a steam purification process known as pittavial[18].

4. STANDARDIZATION OF THE DRUG

I. PRELIMINARY PHYTOCHEMICAL SCREENING OF MAHAANALURUVA CHOORANAM

The preliminary phytochemical screening test was carried out for each aqueous extract of **MAHA ANALURUVA CHOORANAM** as per the standard procedure mentioned hereunder.

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. **Wagner's Test**: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
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 sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.
   b. **Benedict's Test**: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
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.
4. **Detection of phytosterols:**
   Salkowski's test
   Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow color indicates the presence of Triterpenes
5. **Detection of phenols Ferric Chloride Test:**
   Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.
6. **Detection of tannins Gelatin Test:**
   The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.
7. **Detection of Flavonoids**
   Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.
8. **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.
9. **Gum and Mucilage:**
   To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.
10. **Test for glycosides; Liebermann's test**
    2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, violet color change into blue and green indicates the presence of glycosides
11. **Test for Quinones:**
    Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

The Preliminary phytochemical studies of aqueous extract of **MAHA ANALURUVA CHOORANAM** were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of **MAHA ANALURUVA CHOORANAM.**

Table 2: Phytochemical analysis of MAC

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

+ve/-ve present or absent if component tested

Certified that the above stated are the phytochemical properties for the given sample.

Fig:1 Indicates phytochemical screening

II. HEAVY METAL ANALYSIS BY AAS

Standard: Hg, As, Pb and Cd – Sigma

a. 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.

b. 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.

c. Standard reparation

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

d. BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Cadmium were as the sample evident the presence of Arsenic, Lead and Mercury at 0.156, 5.017 and 0.28 ppm as listed in the table.

III. STERILITY TEST BY POUR PLATE METHOD

a. Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

b. Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

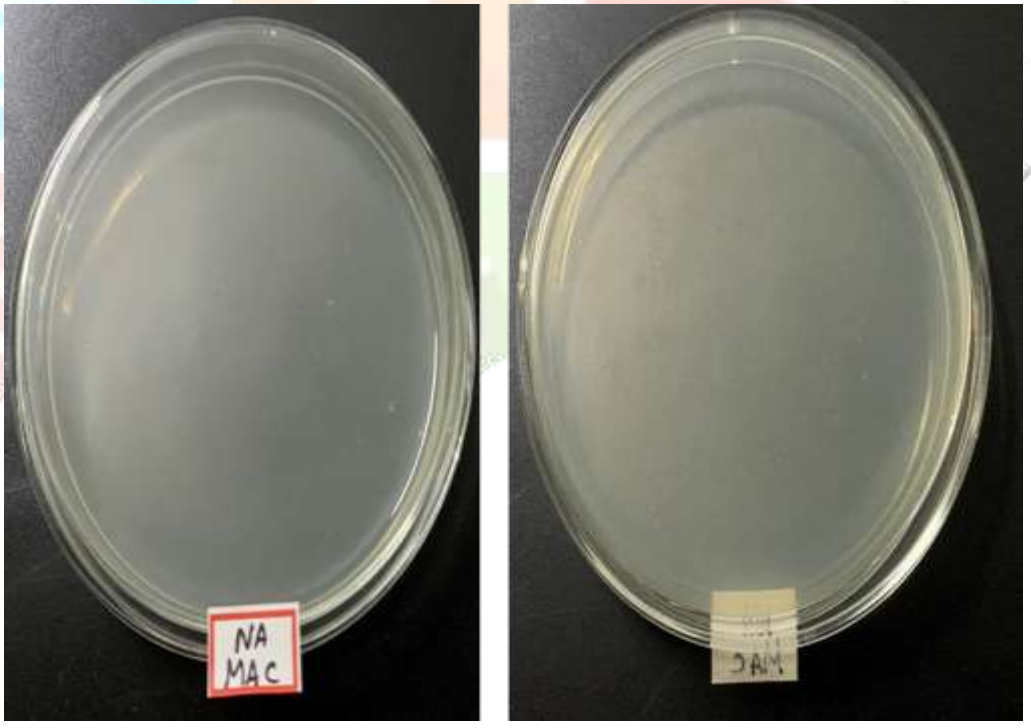

Fig 2: Sterility test of MAC

Observation

No growth was observed after incubation period reveals the absence of specific pathogen

d. Result

No growth / colonies were observed in any of the plates inoculates with the test sample.

Table 3: Sterility test

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

e. Standard

AflatoxinB1
AflatoxinB2
AflatoxinG1
AflatoxinG2

f. 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.

g. 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.

Table 4: Table for Aflotoxin

Aflatoxin	Sample MAC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

h. Result:

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 and Aflatoxin G2.

IV. PESTISIDE RESIDUE

a. Extraction

Test sample were extracted with acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Table 5: Test result analysis of the sample MAC

Pesticide Residue **I. Organo Chlorine Pesticides**	**Sample MAC**	**AYUSH Limit (mg/kg)**
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	50 µg/kg	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

b. **Result:** The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. Whereas the sample reveals the presence of mild traces of Malathion at 50 µg/kg which belongs to the category of Organo Phosphorus Pesticide.

5. DISCUSSION

The present study investigated the physicochemical properties, heavy metal content, sterility, aflatoxin content, and pesticide residue of Mahaanaluruva Chooranam (MAC), a polyherbal formulation.

Phytochemical Screening: The aqueous extract of MAC revealed the presence of various bioactive compounds, including alkaloids (except for Mayer's test), carbohydrates, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, and quinones. The absence of gum and mucilage, and glycosides (as indicated by Liebermann's test) suggests specific characteristics of the formulation.

Heavy Metals: The analysis showed the presence of Arsenic (0.156 ppm), Lead (5.017 ppm), and Mercury (0.28 ppm) in the sample. While these levels were reported, their safety within the context of Ayurvedic or WHO standards is not mentioned. Further investigation is required to determine if these levels pose any health concerns.

Sterility and Aflatoxin Content: The sterility test indicated no bacterial or fungal growth, signifying that the product meets sterility standards (as per AYUSH/WHO) for safe consumption. Additionally, no aflatoxins (B1, B2, G1, or G2) were detected, ensuring the absence of these harmful toxins.

Pesticide Residue: The analysis identified trace amounts of Malathion (50 µg/kg) – an organophosphate pesticide. While the level is below the AYUSH limit (1 mg/kg), further studies to minimize or eliminate even these trace amounts might be beneficial. No other pesticide residues (organochlorine, other organophosphorus, carbamates, or pyrethroids) were found.

This exploratory study delves into the fundamental phytochemical characteristics of Mahaanaluruva Chooranam (MAC). The findings presented herein serve as a preliminary framework for future investigations into this polyherbal formulation. By building upon the insights gleaned from this study, subsequent research can delve deeper into the intricate properties and potential therapeutic applications of MAC.

6. CONCLUSION

This study provides preliminary insights into the physicochemical properties and quality of Maha Analuruva Chooranam. The presence of various bioactive compounds suggests its potential for therapeutic applications. However, the presence of heavy metals (Arsenic, Lead, and Mercury) necessitates further investigation to determine safety. The absence of bacterial/fungal growth, aflatoxins, and most pesticide residues indicates good quality standards. Trace amounts of Malathion were detected, and efforts to minimize or eliminate these traces might be considered. Overall, this study establishes a foundation for further research to explore the efficacy and safety of Maha Analuruva Chooranam.

7. ACKNOWLEDGEMENT

I would like to extend my heartfelt thanks to the esteemed faculty members of the Pg Pothu Maruthuvam department at Government Siddha Medical College, Chennai. Their invaluable mentorship and insightful feedback have been instrumental to the success of my research project. I am also deeply grateful to Dr. K. Kanakavalli, Principal of Government Siddha Medical College, Chennai, and The Tamil Nādu Dr. M.G.R. Medical University for their unwavering support. Additionally, I would like to express my sincere appreciation to my colleagues and friends for their constant guidance and encouragement throughout this endeavour.

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