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Research Article

INTEGRATED BIOSCREENING OF THE SIDDHA FORMULATION *POORA MATHIRAI*: PHYSICOCHEMICAL, PHYTOCHEMICAL AND BIOCHEMICAL ANALYSES COUPLED WITH ICP-OES HEAVY-METAL ASSESSMENT

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ABSTRACT

This study presents an integrated Bio screening of *Poora Maathirai*, a Siddha herbomineral formulation traditionally used for *Uthiravatha Suronitham* (rheumatoid arthritis), to establish its quality and safety. Physicochemical analysis revealed a moisture content of 23.79%, total ash of 7.75%, and a slightly acidic pH of 4.39. Phytochemical profiling confirmed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, and tannins in both aqueous and ethanolic extracts, supporting its traditional anti-inflammatory and analgesic claims. Biochemical tests further characterized its profile, identifying calcium, sulphate, chloride, and iron. Crucially, ICP-OES elemental analysis demonstrated that toxic heavy metals like arsenic, cadmium, and lead were below detection limits, validating the efficacy of the *Rasakarpooram* purification process. Mercury was detected at 0.3987mg/L, requiring careful dosage consideration. This comprehensive investigation provides scientific validation for *Poora Maathirai's* quality, safety, therapeutic potential, advocating for its evidence-based pre-clinical and clinical trials.

INTRODUCTION

Siddha, a traditional Indian medical practice, boasts a rich history spanning thousands of years and includes detailed classifications of diseases, such as 80 different *Vadha noigal*, which cover a range of neuro-musculoskeletal disorders. In contemporary discourse, India's Ministry of AYUSH emphasizes the systematic standardization and quality assurance of these age-old formulations. This governmental focus highlights the significant therapeutic potential of traditional remedies while prioritizing patient health. Siddha therapeutics frequently incorporate complex herbo-mineral compounds, merging plant-derived constituents with meticulously purified inorganic substances. Although the clinical efficacy of these preparations-especially in chronic musculoskeletal conditions like *Uthiravatha Suronitham* (a condition bearing symptomatological resemblance to modern Rheumatoid Arthritis)-is widely acknowledged, their inherent complexity mandates rigorous quality control. Sustained administration, often requisite for managing persistent ailments, renders assurances of safety and consistent quality absolutely critical. Current directives for pharmaceutical standardization, notably those promulgated by the Pharmacopoeia Laboratory for Indian Medicine (PLIM), advocate for exhaustive analytical methodologies tailored to such multifaceted formulations. The Siddha preparation, *Poora Maathirai,* prescribed for *Uthiravatha Suronitham*, epitomizes this intricate design. It comprises a blend of botanical elements (including *Syzgium aromaticum, Trachyspermum ammi,* and *Citrullus colocynthis*) alongside the mineral *Rasakarpooram* (Hydrargyrum Subchloride). The inclusion of such mineral components inherently intensifies the necessity for thorough safety assessments, with particular vigilance toward potential heavy metal contamination. Therefore, a comprehensive bio-screening approach-encompassing detailed physicochemical characterization, extensive phytochemical profiling, precise biochemical testing,

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and rigorous heavy-metal analysis using advanced techniques like ICP-OES-is not just beneficial but essential. This thorough investigative framework aligns with current regulatory standards, providing strong scientific validation for *Poora Maathirai's* quality, safety, and claimed therapeutic benefits, thus supporting its move towards evidence-based, cost-effective, and clinically effective use in the long-term management of chronic diseases.

Methodology

Preparation of the Trial Drug (*Poora Maathirai*)

The raw materials for *Poora Maathirai* included: Rasa *Karpooram* (Purified Hydrargyrum subchloride) (35 g), *Ilavangam* (*Syzgium aromaticum*) (35 g), *Omam* (*Trachyspermum ammi*) (35 g), and *Aatru Thumattikai Saaru* (*Citrullus colocynthis* fruit juice).

Purification of *Pooram*: Raw *Pooram* was purified using a specific procedure.

1. Piper betel leaves and Piper nigrum seeds were ground together to form a *Karkam* (poultice).
2. Water was placed in a medium-sized mud pot, and the poultice was mixed into the water.
3. The raw *Pooram* was covered with a piece of clean dry cloth.
4. This cloth containing the *Pooram* was tied with good twine to a bamboo stick placed horizontally over the opening of the mud pot, ensuring the raw drug was dipped in the water/poultice mixture.
5. The vessel was constantly heated until the volume of the mixture was reduced by three-fourths.
6. Finally, the *Pooram* was taken out of the cloth, washed with clean water, and dried in sunlight before being stored.

Method of Preparation of *Poora Maathirai*

1. After purification, 35g of each Purified *Rasakarpooram, Ilavanga thool*, and *Oma thool* were mixed.
2. This mixture was rubbed with *Aatruthumatti* Kai juice little by little for 24 hours (8 *Saamam*) as required.
3. The resulting mixture was made into ½ *Kundrimani alavu maathirai* (tablets).
4. These tablets were dried under shadow.

Preparation of *Poora Maathirai* Tablets

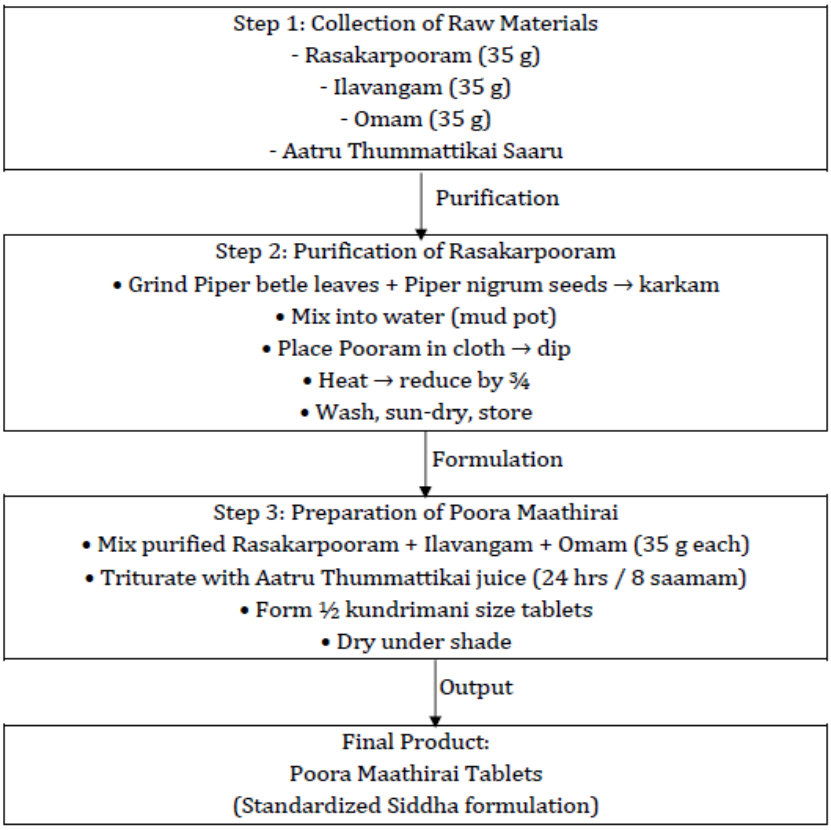

Figure 1: Stepwise Preparation of *Poora Maathirai*

Analytical Methods Employed - Methodology

Preparation of Extract: A 100mg sample of *Poora Maathirai* was treated sequentially with concentrated hydrochloric and nitric acids, evaporated to dryness, and dissolved in 20ml distilled water. The solution was transferred to a 100ml volumetric flask, diluted to volume, mixed, and filtered prior to analysis.

Physicochemical Analysis: Standard parameters assessed included loss on drying at 110°C (moisture

content), total ash (inorganic matter), acid-insoluble ash (siliceous content), water- and alcohol-soluble extractives, and pH of a 5% solution to determine acidity/alkalinity.

Phytochemical Screening: Aqueous and ethanolic extracts were evaluated for alkaloids, carbohydrates, reducing sugars, glycosides, proteins, amino acids, flavonoids, phenolics, tannins, phytosterols, terpenoids, lignin, quinones, emodins, gums, mucilage, and resins.

Biochemical Analysis: Qualitative tests were performed to detect calcium, sulphate, chloride, ferric and ferrous iron, unsaturation, carbonates, starch, phosphate, albumin, tannic acid, reducing sugars, amino acids, and zinc.

ICP-OES Heavy-Metal Assessment: A 0.472 g sample was analyzed using a Perkin Elmer Optima 5300 DV ICP-OES at the Sophisticated Analytical Instrument Facility, IITM, Chennai. Elements quantified included As, C, Ca, Cd, Cu, Fe, Hg, K, Mg, Na, Pb, and P, with "BDL" indicating below detection limit.

Results

The results of the physicochemical, phytochemical, biochemical, and ICP-OES analyses of *Poora Maathirai* are enlisted below:

Results

Table 1: ICP-OES Heavy-Metal Assessment

Elements Symbol	Wavelength (nm)	Concentration (mg/L)
As	188.979	BDL
C	193.030	201.021
Ca	315.807	BDL
Cd	228.802	BDL
Cu	327.393	BDL
Fe	238.204	01.160
Hg	253.652	**03.987**
K	766.491	01.987
Mg	285.213	BDL
Na	589.592	01.821
Pb	220.353	BDL
P	213.617	71.309

Results of ICP-OES Heavy metal analysis of *Poora Maathirai* performed in Institute: Sophisticated Analytical Instrument Facility, IITM, Chennai-36, BDL: Below Detection Limit.

Table 2: Physico-Chemical Analysis

S.No	Parameters	Results of analysis
1.	Loss on drying at 110°C	23.79%
2.	Total ash	07.75%
3.	Acid insoluble ash	01.91%
4.	Water soluble extractive	26.41%
5.	Alcohol soluble extractive	16.85%
6.	pH (5% solution)	4.39

Results of Physicochemical analysis of *Poora Maathirai*, **Institute:** Research and Development Wing for ISM (Directorate of Indian medicine & Homeopathy, Govt. of Tamil Nadu), AAGHIM west campus, Arumbakkam, Chennai-106.

Table 3: Phyto Chemical Analysis

S.No	Name of the Phyto constituents	Aqueous extract of *Poora Mathirai*	Ethanolic extract of *Poora Mathirai*
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Reducing sugar	+	+
4.	Glycosides	+	+
5.	Proteins and amino acids	-	+
6.	Flavonoids	+	+
7.	Phenolic compounds	+	+
8.	Tannins	+	+
9.	Phytosterols	-	+
10.	Terpinoids	-	-
11.	Lignin	-	-
12.	Quinone	-	-
13.	Emodins	-	-
14.	Gums and mucilage	-	+
15.	Resins	-	-

Phytochemical analysis of *Poora Maathirai*, **Institute:** Research and Development wing for ISM (Directorate of Indian medicine & Homeopathy, Govt. of Tamil Nadu), AAGHIM west campus, Arumbakkam, Chennai-106

Table 4: Biochemical Analysis (Qualitative)

S.No	Experiment	Observation	Inference
1.	Test for calcium	A white precipitate is formed	Indicates the presence of calcium
2.	Test for sulphate	A white precipitate is formed	Indicates the presence of sulphate
3.	Test for chloride	A white precipitate is formed	Indicates the presence of chloride
4.	Test for carbonate	No brisk effervescence is formed	Absence of carbonates
5.	Test for starch	No blue color is formed	Absence of Starch
6.	Test for ferric Iron	Blue color is formed	Indicates the presence of ferric Iron
7.	Test for ferrous Iron	Blood red color is formed	Indicates the presence of ferrous Iron
8.	Test for phosphate	No yellow precipitate is formed	Absence of phosphate
9.	Test for albumin	No yellow precipitate is formed	Absence of albumin
10.	Test for tannic acid	No blue-black precipitate is formed	Absence of tannic acid
11.	Test for unsaturation	It gets decolorized	Indicates the presence of unsaturated compound
12.	Test for reducing sugar	No color change occurs	Absence of reducing sugar
13.	Test for amino acid	No violet color is formed	Absence of amino acid
14.	Test for zinc	No white precipitate is formed	Absence of zinc

Biochemical screening of *Poora Maathirai*, **Institute:** Dept of Biochemistry, Government Siddha medical college, Palyamkottai.

DISCUSSION

The physicochemical analysis revealed key parameters defining the general quality and stability of *Poora Maathirai.* A loss on drying of 23.79% indicates the moisture content, which is important for drug stability and prevention of microbial growth. The total ash content of 7.75% and acid-insoluble ash of 1.91% fall within expected ranges for herbomineral preparations, reflecting the inorganic material present, including mineral constituents and potential earthy matter. A low acid-insoluble ash suggests minimal

siliceous impurities, indicating good manufacturing practice. The water-soluble extractive (26.41%) and alcohol-soluble extractive (16.85%) provide an indication of the amount of soluble active principles present in the formulation, which can be crucial for therapeutic efficacy and consistent dosage. The slightly acidic pH of 4.39 suggests a stable environment for its components, and this acidity might also play a role in the solubility and bioavailability of certain active compounds. Phytochemical profiling demonstrated a rich array of secondary metabolites in *Poora Maathirai*.

Both aqueous and ethanolic extracts showed the presence of alkaloids, carbohydrates, reducing sugars, glycosides, flavonoids, phenolic compounds, and tannins. Proteins and amino acids, as well as gums and mucilage, were detected in the ethanolic extract but not the aqueous, suggesting differential solubility of these constituents based on solvent polarity. The presence of flavonoids, phenolic compounds, and tannins is particularly significant given the traditional claims of anti-inflammatory, analgesic, and anti-nociceptive actions for *Poora Maathirai.* These classes of compounds are well-known in modern pharmacology for such activities. For instance, phenolic compounds and flavonoids from *Syzgium aromaticum* and *Trachyspermum ammi* are documented to possess strong antioxidant and anti-inflammatory properties, which would be beneficial in mitigating the inflammatory cascade associated with rheumatoid arthritis. The absence of phytosterols, terpinoids, lignin, quinone, emodins, and resins in both extracts (or specifically noted as absent) provides a comprehensive phytochemical fingerprint, aiding in standardization and distinguishing this formulation from others. The biochemical qualitative tests confirmed the presence of calcium, sulphate, and chloride, which are often found as part of mineral preparations or as natural constituents. The detection of both ferric and ferrous iron indicates the presence of iron in different oxidation states, potentially contributing to its overall elemental composition. Crucially, the absence of carbonates, starch, albumin, tannic acid (in this specific test), reducing sugars, amino acids, and zinc provides further specific characterization, helping to establish a unique biochemical profile for *Poora Maathirai* and ensuring consistency in future batches. The positive result for unsaturation (decolorization of KMnO4) hints at the presence of compounds with double or triple bonds, common in many active plant secondary metabolites. A magnonimous concern with herbomineral formulations is the presence of heavy metals, especially in preparations containing mercury-based ingredients like *Rasakarpooram*. The ICP-OES analysis revealed critical information regarding the elemental composition. Encouragingly, heavy metals such as Arsenic (As), Cadmium (Cd), and Lead (Pb) were all found to be Below Detection Limit (BDL). This finding is highly significant, demonstrating the effectiveness of the *Pooram* purification process outlined in the methodology. The purification method involving Piper betel leaves and Piper nigrum seeds, followed by heating, appears to be efficacious in mitigating harmful elements, aligning with traditional Siddha pharmaceutical detoxification practices *(Suthimurai).*

Mercury (Hg) was detected at 0.3987mg/L. Given that *Rasakarpooram* (Hydrargyrum Subchloride) is a primary ingredient, the presence of mercury is expected. In the context of the total dosage and administration regimen, this level must be carefully assessed to ensure patient safety during long-term use. Other detected elements included Carbon (201.021 mg/L), Iron (1.160mg/L), Potassium (1.987mg/L), Sodium (1.821mg/L), and Phosphorus (71.309mg/L). The relatively high concentration of phosphorus could be indicative of its role as a mineral constituent, potentially contributing to the therapeutic action. The detection of iron, sodium, and potassium are also common given their ubiquitous presence in biological systems and importance as micronutrients. The absence of calcium, magnesium, and copper at detectable levels by ICP-OES, despite calcium being qualitatively identified in biochemical tests, might be due to the specific extraction method for ICP-OES analysis or concentrations being below the ICP-OES detection limit for that particular sample preparation, highlighting the complementary nature of different analytical techniques.

CONCLUSION

The integrated bio screening confirms that *Poora Maathirai* is a complex, multi-component formulation with a diverse phytochemical profile and a controlled elemental composition. The rigorous purification of *Rasakarpooram* is crucial in minimizing toxic heavy metal contamination, ensuring the safety of the final product. The observed presence of flavonoids, phenolics, and tannins supports its traditional uses for anti-inflammatory and analgesic activities against conditions like *Uthiravatha Suronitham*. This study provides a foundational scientific basis for the quality, safety, and potential efficacy of *Poora Maathirai*, moving towards an evidence-based framework for its clinical application in chronic musculoskeletal disorders. Further research involving quantitative analysis of active phytochemicals and in-vivo pharmacological studies would further elucidate its therapeutic mechanisms and optimize its clinical utility.

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