Research Article

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Standardization and Physico-chemical analysis of classical poly-herbal Siddha formulation -"*maaradaippuku chooranam*"

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Abstract

Most of the formulations in the Siddha system is very effective but they have not been standardized. Any drug should be standardized before clinical use. There are various formulations in Siddha to treat cardiovascular diseases. One among them is the poly-herbal formulation *Maaradiappuku Chooranam*, but it has not been validated so far. The principal intention of this current study is to regularize the drug '*Maaradaippuku chooranam'* by physicochemical, phytochemical and biochemical evaluation through modern Analytical techniques. The organoleptic characteristics of the *Chooranam* are in solid form which is pale brownish in colour and soft to touch with a characteristic odour which reveals the quality of the formulation. The aflatoxin assay showed the chooranam is free of aflatoxins. The outcomes of the study for pesticide residue show no traces of pesticide residue in the chooranam. This polyherbal formulation does not contain microbial contamination and displays the existence of steroids, alkaloids, triterpenoids, phenols, tannin, saponin and sugar. The ash value is 9.5 ± 0.4%. Heavy metals like Arsenic, Lead, Mercury and Cadmium are below the detectable limit.

Keywords: *Siddha Medicine, Maaradaippuku chooranam, physicochemical analysis, Standardization.*

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Introduction

Lifestyle disorders are a group of diseases associated with the way a person lives. These diseases are mostly Non-communicable. Non-communicable illnesses (NCDs) kill about 40 million individuals annually, about 70% of all casualties globally [1]. They include cardiovascular diseases, Stroke, Diabetes, COPD, Cancer, neuropsychiatric disorders and others. Among these, cardio vascular diseases have been listed on the top of the WHO list of common lifestyle disorders [2]. Cardiovascular diseases (CVDs) are the major reason for death in the world [3]. *Siddha* medicine's traditional approach to recovery developed in South India and is regarded as one of India's most aging medicine approaches. In recent days, people seeking the traditional medicines to lead their healthy lifestyle.

There are different medications available in *the Siddha* system of medic fore for treating non-communicable diseases**.** Chooranam is a powdered form of medicine. It is one of the 32 internal medicines of the Siddha system. *Maaradaippukku chooranam* is one of the most useful *Siddha* polyherbal formulations mentioned regarding Cardiovascularular diseases. *Maaradaippukku chooranam* which is mentioned in *Theraiyar vaidhiyam 1000*, page no 231, for the treatment of *Maaradaippu* and *Maarbu noi* [4]. In this study, MAC was filtered for standardization procedure as per PLIM procedures. The intention of this research is to give information about the standardization of MAC through physicochemical, HPTLC, biochemical and phytochemical analysis.

Materials and Methods

Table 01 has the detailed information about the Ingredients of *Maaradaippuku chooranam*

Table: 01

S.No	Drug Names	Botanical Name
1.	Parangipattai	*Smilax china*
2.	Kurundhan ver (Kaatu elumichai ver) [5]	*Atalantia monophyllum*
3.	Kazharchikkai	*Caesalpinia bonduc*
4.	Nannari	*Hemidesmus indicus*
5.	Nuna ver	*Morinda tinctoria*
6.	Milagu	*Piper nigrum*

Preparation of *Chooranam*

Collection of the Drugs

The drug ingredients, *Smilax china (Parangipattai), Caesalpinia bonduc (Kazharchikkai), Hemidesmus indicus (Nannari), Piper nigrum (Milagu)* was bought from Authenticated country drug store, Morinda tinctoria (*Nuna ver*) was collected from Thiruvallur district, Tamilnadu and Atalantia monophyllum (*Kurundhan ver)* was gathered from Kolli hills, Tamilnadu.

Drugs Identification and Authentication

All the natural materials were determined and certified by Botanist Government Siddha Medical College, Arumbakkam, Chennai. A specimen sample of all the natural material has been labelled as 1001-1006/PGG/321912101/GSMC/CH/2019-2022 respectively and were kept in the post graduate Department of Gunapadam for future consideration.

Process for Purification

The purification procedure was accomplished as per classical *Siddha* literature.

Drugs Purification [6]

The *Smilax china* root was parched and powdered and then steamed in cow's milk. The root of *Atlantia monophyllum* was washed and dried. The outer shell of *Caesalpinia bonduc* was removed and the seed was cleansed with hot water and dried. The root of *Hemidesmus indicus* and *Morinda tinctoria* was washed and the impurities was removed and dried. *Piper nigrum* seed was bathed in buttermilk for 1hour 30 minutes and dried.

Preparation of the Trial Drug *Maradaippuku Chooranam*

Method of Preparation

Equal quantity of purified ingredients was powdered separately by pounding in an Iron mortar and sieved through a mesh 86. Then all the powders were assorted and preserved in an air-tight container, and the drug was labeled as MAC.

Dose: 1 to 2 g

Adjuvant: sugar

Indication: Chest pain and Ischemic heart disease.

Organoleptic Character

State, Color, Odor, Taste, Texture and other morphological characters were noted.

All the following studies were accomplished in Noble Research Solution, Perambur, and Chennai.

- Physicochemical Analysis was done [7,8]
- Finding total ash was done
- Determination of acid insoluble was done
- Acid soluble extractive was done
- Water soluble determination extractive was done
- pH determination also done
- solubility test was done
- Particle size determination was done [9]

Phytochemical Analysis was done for following tests [10]

- Alkaloids analysis – was performed with Mayer's Test
- Coumarins
- .Saponin
- Tannins
- Glycosides - Borntrager's Test
- Flavonoids
- phenols - Lead acetate test
- steroids
- Triterpenoids
- Cyanins

  A. Anthocyanin

B. Betacyanin

- Carbohydrates - Benedict's test
- Proteins (Biuret Test)
- TLC [11]
- HPTLC was done [12]
- Chromatogram Development was done
- Scanning was done
- Biochemical analysis was done [13]
- Heavy Metal Analysis By AAS Standard was done [14]

Standard preparation

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

Test for Sterility was done by Pour Plate Method [15]

Specific Pathogen Test [16]

Table: no: 2 – complete info about Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus aureus	ST	Mannitol salt agar
Pseudomonas /aeruginosa	PS	Cetrimide Agar

- **Pesticide Residue was done [17, 18]**
- **Test for Aflatoxins was done [19]**
- **Standard**

Table: no: 3 – Aflatoxins

1	Aflatoxin B_1
2	Aflatoxin B_2
3	Aflatoxin G_1
4	Aflatoxin G_2

- **Solvent was done for analyzing the combination of chloroform and acetonitrile.**

Result and Discussions

Organoleptic characters

The organoleptic characters of MAC were determined and the results are tabulated in Table: 2

Fig No. 1. Organoleptic Characters of *MAC*

Table:4

Parameter	Results
State	Solid
Nature	Fine
Odor	Characteristic
Touch	Soft
Flow Property	Non Free flowing
Appearance	Pale Brownish
Taste	Bitter

Results for physicochemical

The physicochemical parameters of MAC were determined and the results given in Table: 5 and Table:6

Table:5

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	5.9 ± 0.781
2.	Total Ash (%)	9.5 ± 0.4
3.	Acid insoluble Ash (%)	0.04 ± 0.004
4.	Water-soluble Extractive (%)	10.73 ± 0.7024
5.	Alcohol Soluble Extractive (%)	6.933 ± 1.79
6.	pH	5.5

Observation of Particle Size in the Microscope for the sample MAC

Microscopic observation of the particle dimension examination indicates that the moderate particle size of the sample was found to be 15.49 ± 2.97 µm. additionally, the sample has particles with a size range of the most subordinate 10 µm to the most elevated 18 µm.

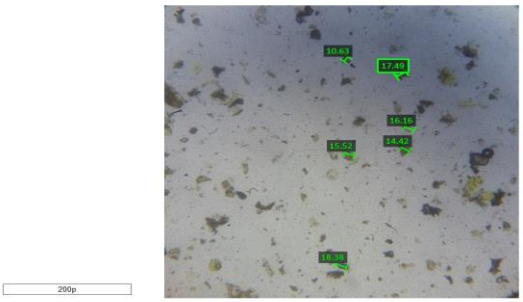

Table: 06 Profile for Solubility

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Results for Analysis of phytochemical

The phytochemical parameters of MAC were determined and the results are tabulated in Table: 07

Table: 07

S.No	Test	Observation
1	ALKALOIDS	+
2	STEROIDS	+
3	TRITERPENOIDS	+
4	PHENOL	+
5	TANNIN	+
6	SAPONINS	+
7	SUGAR	+
8	BETACYANIN	+

Results

Results for TLC and HPTLC

TLC Visualization of MAC at 366 nm

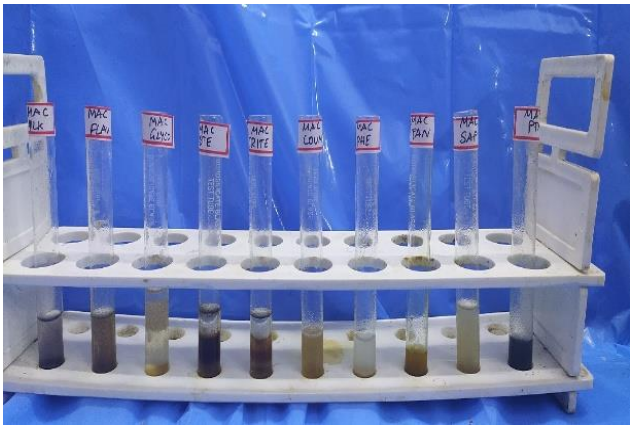

Figure: 2 Qualitative Phytochemical Investigation

Figure: 3

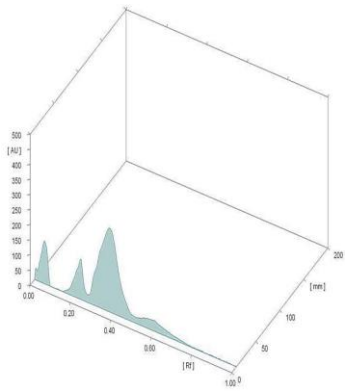

Figure: 4

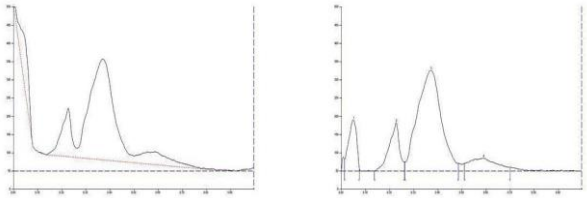

Figure: 5 HPTLC fingerprinting of sample MAC

REPORT

HPTLC fingerprinting analysis of the model demonstrates that four major peaks correspond to four versatile image features. Rf value of the peaks ranges from 0.01 to 0.51, and it was tabulated in Table: 08

Table: 08

Peak table

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.01	33.1	0.05	140.4	24.01	0.08	6.6	2232.4	11.50
2	0.14	0.4	0.23	132.9	22.74	0.26	25.5	2624.5	13.53
3	0.26	25.8	0.37	276.1	47.22	0.49	21.0	12663.4	65.26
4	0.51	21.2	0.59	35.3	6.03	0.70	10.4	1884.0	9.71

Results for Biochemical Analysis

Test for Acid Radicals

Specific Radical

Test report

Test for carbonates Positive – indicates the presence

Test for sulfates Positive – indicates the presence

Results for Heavy Metal Analysis

Heavy metal analysis of MAC was determined and results are given in Table: 09

Table: 09 Report for Test

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL-Below Detection Limit

Report and Inference

Results of the current study include show that the model has no hints of heavy metals such as Lead, Arsenic, Mercury, and Cadmium.

Results for Sterility

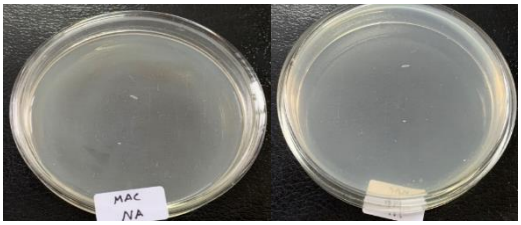

Figure: 06 **Figure: 07**

Observation

No change was observed after the incubation time. Indicates the lack of precise pathogen.

Result

No growth/colonies were marked in any of the plates inoculated with the test sample and are given in Table: 10

Table: 10

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	

Results For Specific Pathogens:

Detail of Specific Medium and their abbreviation are tabulated in Table: 11

Table: 11

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus aureus	ST	Mannitol salt agar
Pseudomonas aeruginosa	PS	Cetrimide Agar

Observation

No change was observed after the incubation period. Indicates the lack of a specific pathogen.

Result

No growth/colonies were marked in any plates inoculated with the test sample. The results of typical pathogens are given in Table: 12.

Table: 12

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

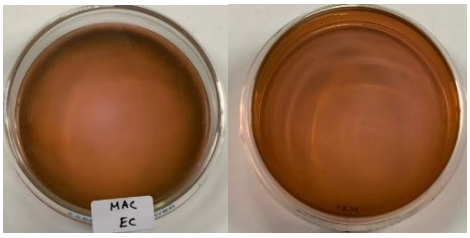

Figure: 08 Culture plate with E-coli (EC) specific medium

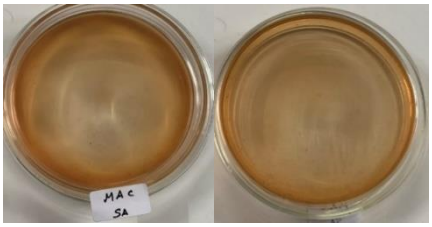

Figure: 09 Culture plate with Salmonella (SA) specific medium

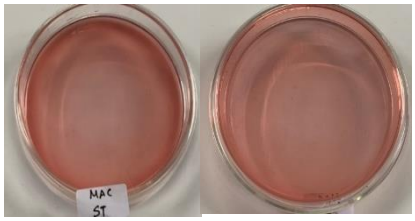

Figure: 10 Culture plate with Staphylococcus aureus (ST) specific medium

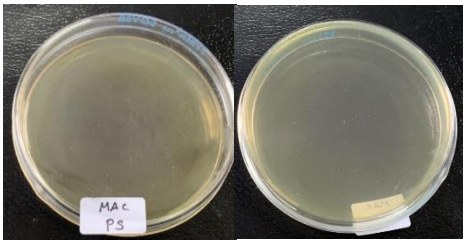

Figure: 11 Culture plate with Pseudomonas aeruginosa (PS) specific medium

Results for Pesticides residues

The results demonstrated no traces of pesticide residues such as Organochlorine, Organophosphorus, Organo carbamates, and pyrethroids in the sample supplied for analysis. The outcomes are tabulated in Table: 13

Table: 13 Test Result Analysis of the Sample Mac

Pesticide Residue	Sample MAC	AYUSH Limit (mg/kg)
I.**Organo chlorine pesticides**		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1mg/kg
Gama BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
III. Organo phosphorous pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbomates		
Carbofuran	BQL	0.1mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Results for Aflatoxin Assay

Result: The outcomes show that no spots were recognized in the test sample packed on TLC plates compared to the standard, suggesting that the sample was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. These are tabulated in Table: 14

Table: 14

Aflatoxin	Sample MAC	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

Discussion

Standardization of herbal formulations is essential to assess the drug's grade, effectiveness, and potency. The standardization of *Maaradippuku chooranam* was acquired via numerous methods by dissecting the organoleptic characters, physicochemical qualities, and elements current in the drug. The organoleptic parameters like State, Nature, Odor, Touch, Flow property, and appearance show that it is solid, soft to touch, pale brownish with a characteristic odor. This drug MAC is excellent and safe to consume. The results received from the physicochemical study of *Maaradaippuku chooranam* (MAC) apparently show that soluble in primary solvents proves the efficacy of solubility in the stomach indirectly and improves the bioavailability. The pH of the drug is 5.5, which is acidic. The acidic drug is essential for bioavailability and its effectiveness. So, the drug MAC will be absorbed better in the stomach [20]. The loss on drying value was 5.9% indicates the stability and shelf life of the drug MAC are good. The total Ash value was 9.5%, which indicates that the drug MAC has no impurities. It is safe to treat thrombosis; the acid insoluble ash value of MAC was 0.004% which was less than 1% suggesting the less content of siliceous matter in the *Chooranam*. The water-soluble extractive is 10.73%, conveying easy facilitation of diffusion and osmosis mechanism, and the alcohol-soluble extractive is 6.933%, which reveals that the drug has good quality and purity. It indicates no impurity in the raw drug MAC.

The result of the phytochemical analysis indicates the presence of Alkaloids, Steroids, Triterpenoids, Phenol, Tannin, Saponins, Sugar, and Betacyanin. Alkaloids have potent antiplatelet, antithrombotic, and anticoagulation effects [21]. The presence of alkaloids in *MAC* confirms the thrombolytic potency of the drug. The natural phenolic potential is used as a medication for thrombosis and cardiovascular diseases. Thus

inhibition of cyclooxygenase enzymes (COX-1 and COX-2) action by inhibitors decreases the presentation of TXA2 and after that inhibits platelet accumulation [22]. Since the trial drug contains phenols, it confirms the thrombolytic potency of the drug. Phytosterols effectively reduce plasma cholesterol levels and reduce cardiovascular risk [23]. Since this drug contains steroids, it helps in the thrombolytic activity of the trial drug. Tannins inhibit thrombin-induced platelet aggregation [24]; thus, the tannin in the trial drug can enhance the thrombolytic potency of the trial drug. The nonsugar portion of the saponins has natural antioxidant activity, resulting in additional usefulness that suggests lowering the chance of heart diseases and cancer [25]. The presence of saponin confirms the thrombolytic activity of MAC, A plant-based diet with more whole carbs and unsaturated fats is proven to reduce the risk of heart disease by ten percent [26, 27]. Since the trial drug shows the presence of sugar, it can strengthen the thrombolytic activity of the drug. Betacyanin is a promising alternative to supplement therapies in oxidative stress and dyslipidemia-related diseases like artery stenosis, atherosclerosis, and hypertension [28]. Since this drug has the presence of Betacyanin, the thrombolytic activity is strengthened.

HPTLC fingerprinting study of the example demonstrates four major peaks compared to four versatile Phyto-components present within it. Rf significance of the peaks varies from 0.01 to 0.51. So, the presence of medicinally important phytochemicals in the sample drug MAC was strengthened by TLC and comparing the *Rf* of the corresponding spot with that of standards. This result supports the ethnomedical uses of the drug MAC in treating thrombosis. Biochemical analysis shows the presence of carbonates and sulfates. Carbonates can reduce serum cholesterol levels which are responsible for cardiovascular diseases [29]. Since this drug *MAC* has carbonates, it enhances the thrombolytic potential of the drug. Sulfates are present in heparin which has anticoagulant properties. Heparin instantly quells thrombin action, starts antithrombin, and fires the thrombin from circulation. So it inactivates the active state of clotting factors. Since the trial medication has Sulphate, it may work on this mechanism. Heavy metal analysis results include establishing that the sample MAC contains no traces of heavy metals like Lead, Arsenic, Mercury, and Cadmium. These outcomes indicate that the trial medication is positively secure as it has heavy metals below detection limitations. This reveals the drug is safe to consume. The sterility test outcome shows no evolution/colonies in any plates inoculated with the trial sample MAC. This demonstrated that the medication MAC is free from microorganisms and the absence of total bacterial and fungal count, which indicates that the drug MAC is of good quality and safety. A specific pathogen test showed that the drug MAC prevented the growth of microorganisms such as E.*coli*, *Salmonella species, Staphylococcus aureus, and Pseudomonas aeruginosa*, indicating that the drug MAC can be used to reduce the morbidity and mortality from chronic diseases. The aflatoxin assay outcomes revealed no spots seen in the test sample MAC crowded TLC plates compared to the standard, which indicates that the sample MAC was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2. So, this drug MAC is non-toxic, there is no contamination, and it does not act as a carcinogen. This sample drug, MAC, is safe for treating cardiovascular diseases. The pesticide precipitate outcomes demonstrated that the drug MAC has no hints of pesticide remains, such as Organochlorine, Organophosphorus, and Pyrethroids. So, this drug MAC has no toxicity and bioaccumulation. Hence, the drug MAC is a safer drug for human health in treating thrombosis.

Conclusion

It can be concluded that the Analysis of *Maaradaippuku chooranam* has been taken out to propose benchmarks for assessing its grade and righteousness. The analytical parameters, TLC image documentation, and HPTLC fingerprinting profile choice be essential in improving its Pharmacopoeial norms. As a result, *Maaradaippuku chooranam*, a Siddha poly-herbal formulation, is subjected to many studies to validate its effectiveness and safety via a defined standardization process. It is advised to bring the formulation to the subsequent investigation status via pharmacological studies and clinical trials.

Conflict of Interest

The authors declared No conflict of interest.

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Author Contribution

All authors contributed equally

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