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Formulation and standardization of Siddha drug - Megari Chooranam

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Abstract

Background:

Siddha system is used to maintain people's health as well as to prevent, diagnose, improve or treat physical and mental illness. When conventional medicine fails to treat chronic diseases such as Diabetes efficaciously and without adverse events, many people seeks herbal medicines. In Siddha literature, Yugi Vaithya Chinthamani Yugi has mentioned about 4448 diseases. Among them 20 types of Meganeer are described under the classification of Neerinaai Perrukkal Noi. Madhumegam is one of the types of Meganeer which is very similar and closely resembles with the chronic metabolic disorder Diabetes Mellitus Type II. Madhumegam is a type of Meganeer which is characterized by passing of sweet urine as per saint Yugi. Several siddha formulation of medicine as documented in Vedic literature is still not explored much. One such novel formulation is Megari Chooranam in AATHMA RATCHAMIRTHAM ENNUM VAITHIYA SASRASANGIRAGAM for the management of (Diabetes Mellitus Type II) Madhumegam. This poly-herbal formulation has gained great importance and rising global attention recently. Now a days the traditional formulation of medicine need to standardization on the basis of Physiochemical analysis and Phytochemicals analysis. This paper is attempt to describe the standardization and efficacy of the drug of the medicine.

Methods:

The drug was screened for physiochemical, phytochemical analysis and HPTLC to estimate the quality of the drug.

Results:

The results obtained from standardization and physiochemical analysis clearly reveals that the volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml. Further we need to prove the medicinal uses and phytochemical and bioactivity studies to prove their therapeutic properties it's also useful in further reference of medicinal plants by go through the Siddha texts and related articles. The results of phytochemical analysis indicates the formulation MEGARI CHOORANAM contains alkaloids, carbohydrates, steroids, phenols, tannins, triterpenoids,

coumarin. The results of HPTLC analysis shows presence of eleven versatile Phyto components present in the sample and haveno traces of heavy metals such as arsenic and cadmium, whereas the sample shows the presence of Mercury at 0.22ppm and Lead at 7.22ppm. The results shown that shows that there were no spots being identified in the test sample MGC loaded on TLC plates when compare to the standard which indicates that the sample MGC were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. The result shows that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

Conclusion:

From the results of the study it was evident that the Siddha formulation Megari Chooranam complies with the standard and may be used for clinical management of Madhumegam. But further studies need to carried out to exact role of phytotherapeutics present in the formulation might responsible for the expected pharmacological action in animals and human as well.

Keywords: Siddha medicine for Madhumegam, Megari Chooranam (MC), phytochemical activity, HPTLC for Siddha drugs.

Introduction

Siddha system is the holistic and unique medical system based on principles for providing preventive, promotive, curative, rehabilitative and rejuvenative health needs. To achieve the goal of human life every human needs healthy body. So they discover medicines to cure human sufferings. This medicine was collectively called as Siddha system medicine. This system of medicine imitate with our mother nature. Siddhars are believed to form the three fundamental components that make up the human constitution. These three components- *Vata*, *Pitta*, and *Kapha* (representing air, fire, and water, respectively)—are known as humors, and their inharmonious interaction produces various pathological states. Though it is curable this system of medicine still trying to proven their drug effectiveness through the standardization methods. In this study Megari Chooranam was selected and screened for standardization methods as per procedure the medicine composed of drugs such as *Cassia auriculata*, *Curcuma longa*, *Coscinium fenestratum*, *Acacia indica* and *Strychnos potatorum*. These drugs possess the Anti-Diabetic property. It is effective against the treatment of Madhumegam (Diabetes Mellitus Type II). It is a group of metabolic disorder characterized by hyperglycemia resulting from defects in Insulin secretion, Insulin action, or both. The chronic hyperglycemia of Diabetes is associated with

long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. India currently faces an uncertain future in relation to the potential burden that Diabetes may impose upon the country. Many influences affect the prevalence of disease throughout a country, and identification of those factors is necessary to facilitate change when facing health challenges. This is the most common form of Diabetes Mellitus and is highly associated with a family history of Diabetes, older age, obesity and lack of exercise. It is more common in women, especially women with a history of Gestational Diabetes. Most individuals with Type II Diabetes exhibit intra-abdominal (visceral) obesity, which is closely related to the presence of Insulin resistance. In addition, hypertension and dyslipidemia (high triglyceride and low HDL-cholesterol levels, postprandial hyperlipidemia) often are present in these individuals. Clinical symptoms include Polyuria, Polyphagia, Polydipsia, Emaciation, Pruritis vulvae, Balanitis, Genital Candidiasis, Skin Sepsis, Unhealed wounds and frequent changes in Refractive error.

Materials and Methods

2.1 Selection of drug:

The drug Megari Chooranam was collected from the classical Siddha literature.

2.2 Collection and authentication of the drug:

The raw materials included in the formulation are *Cassia auriculata*, *Curcuma longa*, *Coscinium fenestratum*, *Acacia indica* and *Strychnos potatorum* were procured from the country drug shop at Chennai, Tamilnadu. They were identified and authenticated by the Botanist, Govt, Siddha medical college, Arumbakkam, Chennai-106.

2.3 Purification of drug:

The purification of drugs was done by procedures mentioned in Siddha literature. This Megari Chooranam is polyherbal medicine. The aim of

this study was carried out to standardize the drug Megari Chooranam by evaluating physiochemical properties.

2.3 Preparation of the drug:

Aavarai, Manjal, Maramanjai, Thetran vithai and Karuvelam pisinthese five ingredients were taken purified and dried. Then the purified ingredients are powdered separately by mortar. Then the powders are mixed together. The mixed powders are sieved by using a cloth. Then the powder is stored in an air tight container.

Physio- chemical analysis

Fig 1: Physio- chemical analysis



Table 1: Physio- chemical analysis

State	Solid
Nature	Fine
Odour	Characteristic
Touch	Soft
Flow Property	Non Free flowing
Appearance	Pale Brownish

Table 2: Solubility Profile

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

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Final Test report

S.No	Parameter	Mean (n=3) SD
1.	<i>Loss on Drying at 105 °C (%)</i>	1.783 ± 0.1365
2.	<i>Total Ash (%)</i>	8.733 ± 1.102
3.	<i>Acid insoluble Ash (%)</i>	0.02 ± 0.02646
4.	<i>Water soluble Extractive (%)</i>	13.9 ± 1.637
5.	<i>Alcohol Soluble Extractive (%)</i>	10.97 ± 2.228

Phytochemical analysis

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarin is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky

white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

A. Anthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

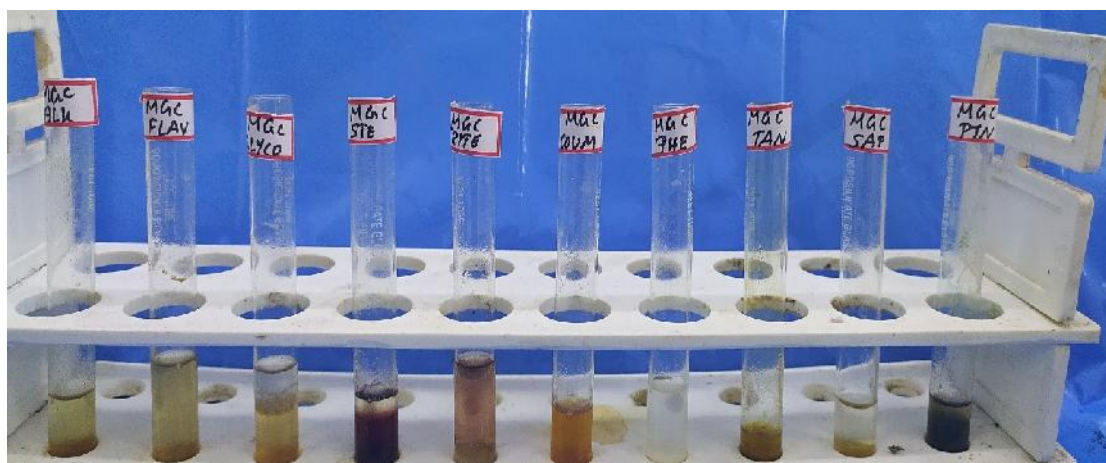
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Results

Fig 2: Qualitative Phytochemical Investigation



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.

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.

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.

Fig 3: TLC Visualization of SGYC at 366 nm

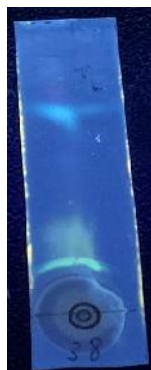


Fig 4:3D – Chromatogram

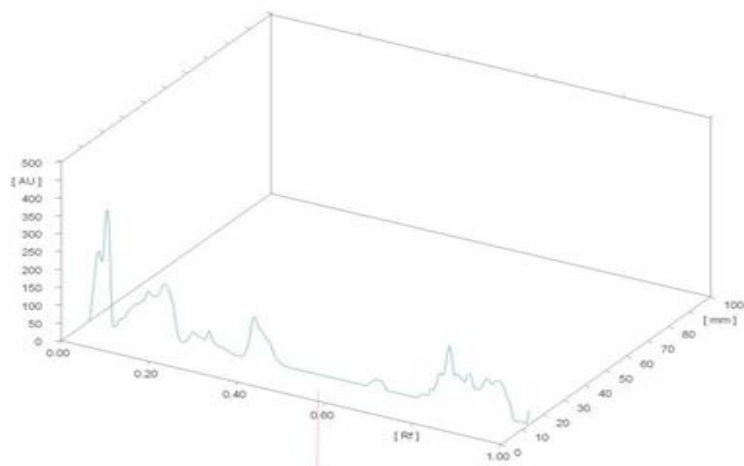
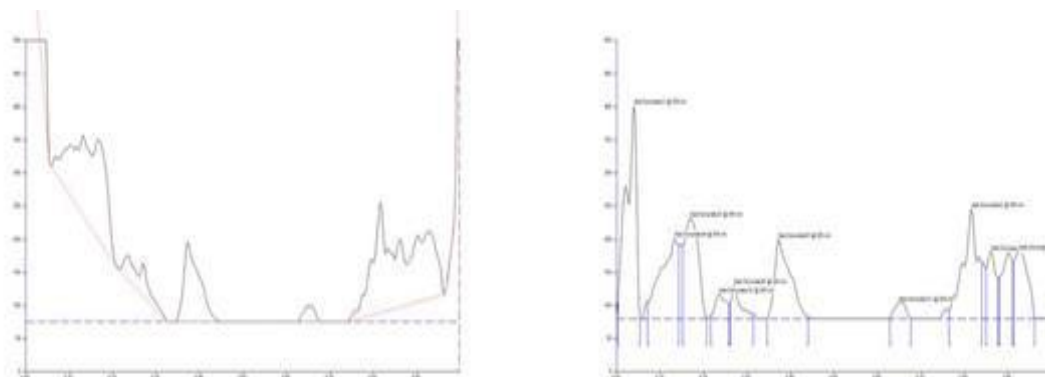


Fig 5: HPTLC finger printing of Sample SGYC



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.

Table 4: HPTLC Peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	33.7	0.04	322.1	24.86	0.05	3.0	5353.3	20.59
2	0.07	25.8	0.13	121.4	9.37	0.14	111.1	3344.5	12.86
3	0.15	111.5	0.17	151.4	11.68	0.21	0.2	3363.5	12.93
4	0.22	4.7	0.24	37.2	2.87	0.26	22.9	663.1	2.55
5	0.26	23.8	0.27	50.6	3.91	0.31	7.2	713.3	2.74
6	0.35	0.1	0.37	119.6	9.23	0.44	3.1	3007.4	11.56
7	0.63	0.9	0.65	24.3	1.88	0.68	0.2	441.2	1.70
8	0.77	12.2	0.82	165.8	12.79	0.84	84.8	3761.1	14.46
9	0.85	74.3	0.86	101.4	7.83	0.88	62.2	1479.7	5.69
10	0.88	62.3	0.90	100.3	7.74	0.91	88.3	1657.3	6.37
11	0.92	88.3	0.93	101.6	7.84	0.96	2.1	2220.3	8.54

Report

HPTLC finger printing analysis of the sample reveals the presence of eleven prominent peaks corresponds to presence of eleven versatile phytocomponents present within it. Rf value of the peaks ranges from 0.07 to 0.92.

Methodology of heavy metal analysis

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

Table 5: Heavy metals analysis

Name of the Heavy Metal	Absorption Max max	Result Analysis	Maximum Limit
Lead	217.0 nm	7.22 PPM	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.22 PPM	1 ppm

BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as arsenic and cadmium, whereas the sample shows the presence of Lead at 7.22 ppm and Mercury at 0.22 ppm

Methodology for pesticide:

Test sample were extracted with 100 ml of 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 40°C 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.

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.

Methodology of aflatoxin:

Standard Aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5µL, 5µL, 7.5µL and 10µL. 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 front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Aflatoxin sample MGC AYUSH specifications limit

- B1 Not Detected-Absent 0.5ppm
- B2 Not Detected-Absent 0.1ppm
- G1 Not Detected-Absent 0.5ppm
- G2 Not Detected-Absent 0.1ppm

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, Aflatoxin G2.

Test Report

Test for Specific Pathogen

Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media.

Table 6: Detail of Specific Medium and their abbreviation

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

Observation

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

Result

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

Table 7: Absence of Specific Pathogen

Organism	Specification	Result	Method
<i>E.coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus aureus</i>	Absent	Absent	
<i>Pseudomonas aeruginosa</i>	Absent	Absent	

Fig 6: Culture plate with *E. coli* (EC) specific medium

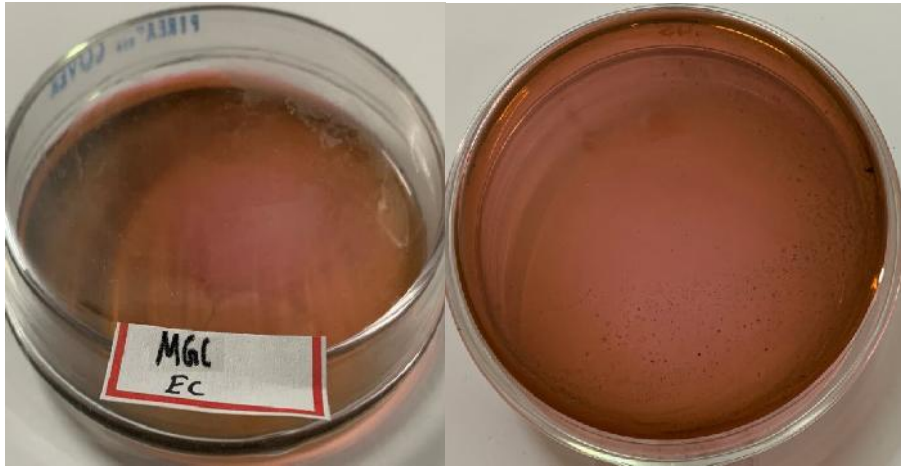


Fig 7: Culture plate with *Salmonella* (SA) specific medium

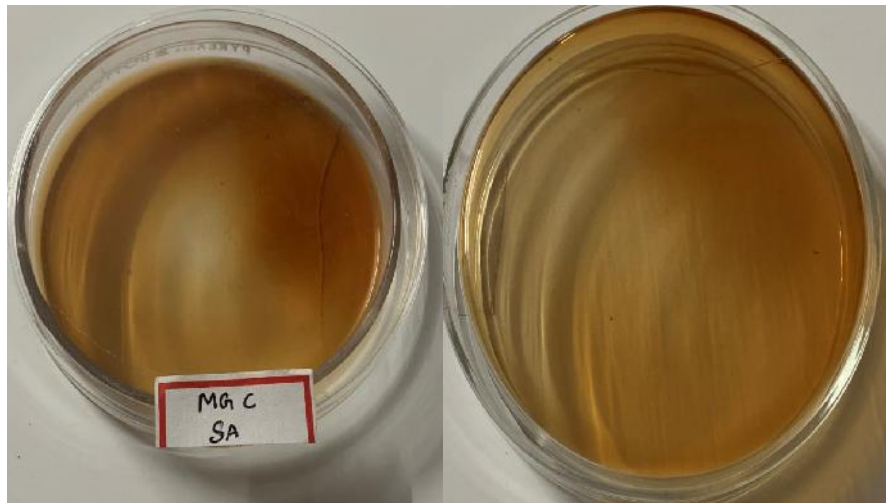


Fig 8: Culture plate with *Staphylococcus aureus* (ST) specific medium

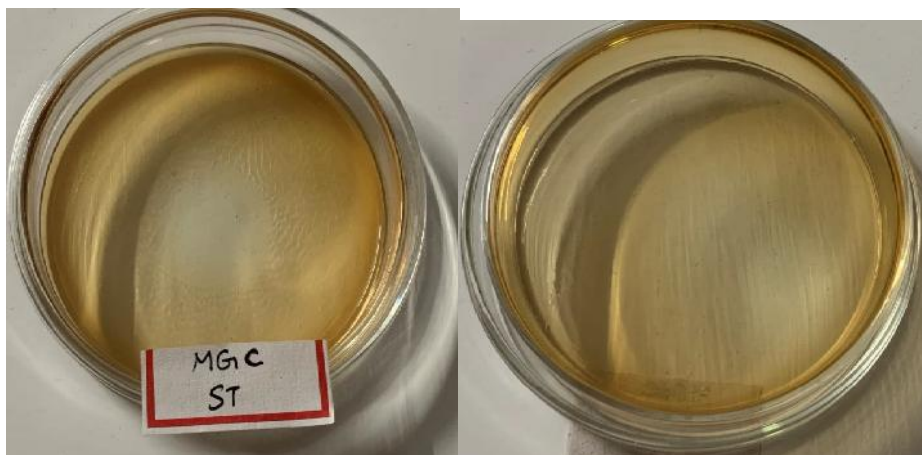
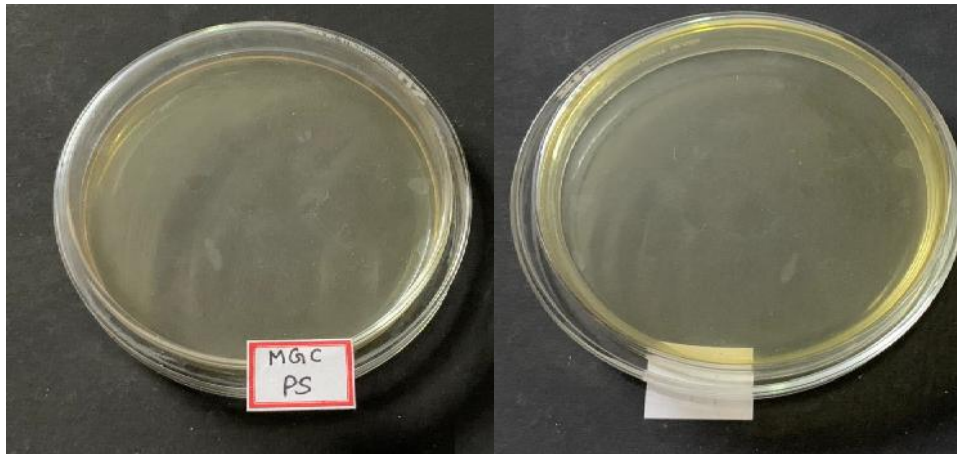


Fig 9: Culture plate with *Pseudomonas aeruginosa* (PS) specific medium



Sterility test by pour plate method

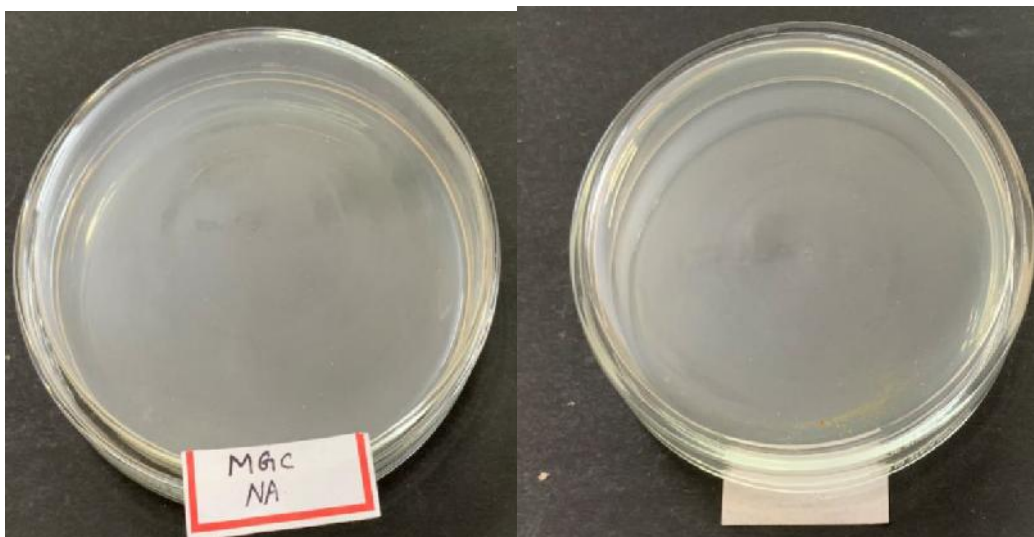
Objective

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).

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 9: Sterility test by pour plate method



Observation

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

Result

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

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

Discussion

The results obtained from standardization and physiochemical analysis clearly reveals that the loss on drying value was 1.78%, total ash value was 8.73%, and acid insoluble ash is 0.02%. The alcohol soluble extractive value was 10.97% and water soluble extractive was 13.9%. We need to study the medicinal uses and phytochemical and bioactivity analysis to prove their therapeutic properties. The phytochemical analysis indicates that the formulation of Megari Chooranam contains alkaloids, carbohydrates, steroids, phenols, tannins, triterpenoids, and coumarin. HPTLC finger printing analysis of the sample presence of eleven versatile Phyto components present in the sample and have no traces of heavy metals such as arsenic and cadmium, whereas the sample shows the presence of Mercury at 0.22ppm and Lead at 7.22ppm. The results shown that shows that there were no spots being identified in the test sample MGC loaded on TLC plates when compare to the standard which indicates that the sample MGC were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. The result shows that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. It was observed from the results of In-vitro anti-microbial assay that the formulation MGC possesses significant antimicrobial activity against *E.coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Hence this paper is an attempt that was made to evaluate & standardize Megari Chooranam by identifying the ingredients with using chemical parameters such as Physico-chemical parameters, preliminary

phytochemical analysis, TLC photo documentation & HPTLC finger printing profile. It is also useful in further reference of medicinal plants by going through the Siddha texts and related articles.

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