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Research Article

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Formulation and Standardization of Siddha drug - Kana Mantha Mathirai (KMM)

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Abstract

Background: Siddha system of medicine is founded mainly on the basic principles of nature and its elements after careful and thorough study of the human system. The system has remarkable strength in treating diseases, especially in Paediatric age group also. One of such medicine is Kana mantha mathirai which is mentioned in Aathma ratchamirtham ennum vaiythiya sarasangiragam- used in the treatment of kana mantham (Gastroenteritis in children). It is a traditional Siddha formulation which consist of six major herbs. Often investigation on Siddha preparations attempted on reverse pharmacology basis. Hence nearly 80% of the formulation already have proven track record clinically and now several investigations are being made on its preclinical aspect. Considering the global need the modern standardization method adopted for identity, purity and shelf life of the preparation. samples are collected and subjected to standardization on the basis of Physiochemical analysis and Phytochemicals analysis it result obtained from this study is very much useful data for my further research. This paper attempts to describe the need for standardizing the drugs since the efficacy of medicines.

Methods: The drug was screened for Physiochemical, phytochemical analysis and HPTLC to estimate the quality of study drug.

Results: The results obtained from standardization and physiochemical analysis clearly reveals that the loss on drying value was1.533%, total ash value was 3.767%, and acid insoluble ash is 0.21%.The alcohol soluble extractive value was 7.467%and water soluble extractive was 13.3%.Now-a-days, there is a constant need to explore their medicinal uses and also to conduct phytochemical and bioactivity studies to prove their therapeutic properties. To know any information about any medicinal plant, there is a necessary to go through all the available texts of siddha and also the previous reviews from recent research. Phytochemical investigations and biological reviews on the plants will lead to the valuable information which can help the scientists to know more advanced knowledge about these plant species. The result of the phytochemical analysis indicates that the formulation KMM shows the alkaloid, coumarins, saponins, tannins, glycosides, flavanoids, steroids, Triterpenoids, aanthocyanin,Carbohydrate, proteins. The result of HPTLC analysis shows phytoconstituents present in each sample and has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 7.94 ppm, Arsenic at 0.51ppm and Mercury 0.59 ppm. he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.It was observed form

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the results of In-vitro anti-microbial assay that the formulation KMM possesses significant antimicrobial activity against *E.coli*, Solmonella, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The results also shows no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamtes and pyrethroids.

Conclusion: From the results of the study, it was evident that the Siddha formulation KMM complies with the standard and may be used for clinical management of kana mantham. But further studies need to be carried out to ascertain the exact role of phytotherapeutics present in the formulation might responsible for the expected pharmacological action in animals and humans as well.

Keywords: Siddha Medicine, Polyherbal Formulation, Kana mantha mathirai, Physiochemical analysis, Phytochemical analysis and HPTLC.

Introduction

Siddha medicines are very effective and have therapeutic value in nature but lack of standardization, it is required to develop the standardization technique. In this study, kana mantha mathirai was selected and screened for standardization technique as per procedures. Ingredients of Kmm are Acrous *calamus*, *Piper cubeba*, *Magnolia champaca*, *Allium sativum*, *Syzygium aromaticum*, *Trachyspermum ammi*. *Acorus calamusis* called sweet flag, belonging to Acoraceae. It has anti spasmodic, anti oxidant, anti microbial activity. (Sandeep B Rajput et.al. Phytomedicine, 2014). *Piper cubeba* is called as tailed pepper and the family is Piperaceae. It has a hepatoprotective. (Thukaa Zuhair Abduljalil. et.al, 2000). *Trachyspermum ammi* is called as thymol seed and the family is Apiaceae.It has digestive, stimulant activity,anti bacterial.(A.k.pathaket.al 2010). *Allium sativum* belonging family is alliaceae.It has anti viral, anti protozoal activity. (Gakel.el. Sabar). *Magnolia champaca* has anti microbial activity.(M.R. Khan et.al. Fitoterapia, 2002). *Syzygium aromaticum* has the anti fungal, anti oxidant, anti bacterial activity.

Materials and Methods

2.1 Selection of drug

The drug Kana Mantha Mathirai was collected from the classical Siddha literature.

2.2 Collection and authentication of the drug

The raw materials included in the formulation are *Piper Cubeba, Syzygium aromaticum,Michelia champaca, Trachyspermum ammi, Allium sativum, Acorus calamus* were procured from the country drug shop at Chennai , Tamilnadu. Fresh raw Poduthalai (Phyla Nodiflora) was procured from Koyambedu market at Chennai, Tamilnadu. They were identified and authenticated by the Botanist, Govt. Siddha Medical College, Arumbakkam,chennai-106.

2.3 Purification of the drug

The purification process was done according to the procedures mentioned in the classical Siddha literature.

This Abstract Kana Mantha mathirai traditional Siddha polyherbal medicine. The aim of this study was carried out to standardize the drug Kana mantha mathirai by evaluating physico chemical properties.

2.4 Preparation of the drug

Valmilagu, vellulli, omam, lavangam, sembaga poo are taken in the equal quantity and made into fine powder. Then this mixture is grinded for12 hours by adding poduthalai juice and then made into pill form are rolled in 700mg size and dried in shade. The pills were stored in clean airtight container and named as KMM.

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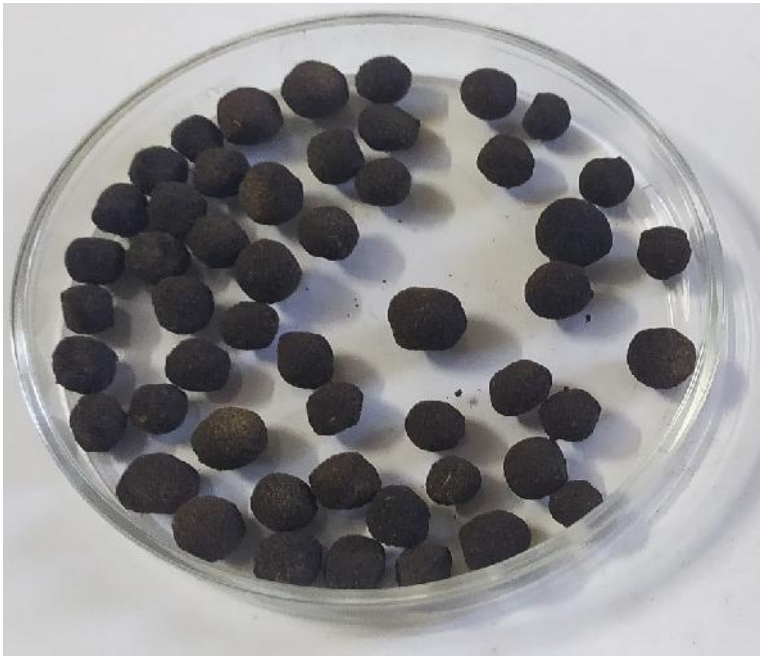
Physio-Chemical analysis



State	Solid
Nature	Hard Solid with smooth surface
Odour	Mild Characteristic
Touch	Rigid and Hard
Flow Property	Free flowing
Appearance	Blackish

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^{o}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 ^{o}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.

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.

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Determination of Acid Insoluble 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 105ºC, 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 105ºC, 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.	Loss on Drying at 105 °C (%)	1.533 ± 0.2517
2.	Total Ash (%)	3.767 ± 0.5033
3.	Acid insoluble Ash (%)	0.21 ± 0.035
4.	Water soluble Extractive (%)	13.3 ± 1.3
5.	Alcohol Soluble Extractive (%)	7.467 ± 2.003

Qualitative Phytochemical Investigation

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 coumarins 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 hydrolysed 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 choloroform is added and shaken, choloroform layer is separated and 10% ammomia 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.

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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. Aanthocyanin:

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

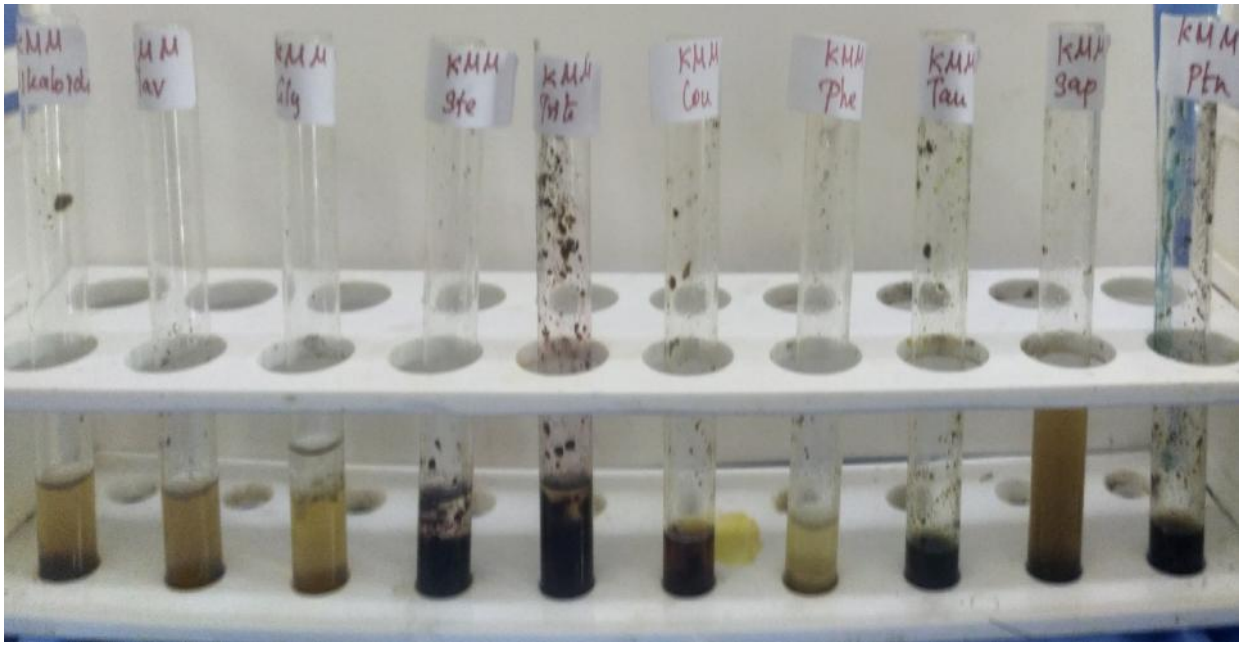
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

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

Qualitative Phytochemical Investigation



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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.

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.

Methodology for 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 HNO_3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl
Cd &Pb- 100 ppm sample in 1mol/L HNO_3

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Name of the heavy metal	Absorption max	Result analysis	Maximum limit
Lead	217.0 nm	7.94 PPM	10 ppm
Arsenic	193.7 nm	0.51PPM	3ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.59 PPM	1ppm

BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 7.94 ppm, Arsenic at 0.51ppm and Mercury 0.59 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 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.

Result:

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

Methodology for aflatoxin:

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.

Aflatoxin Sample KMM 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

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 for Sterility

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}C 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 37^{o} 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.

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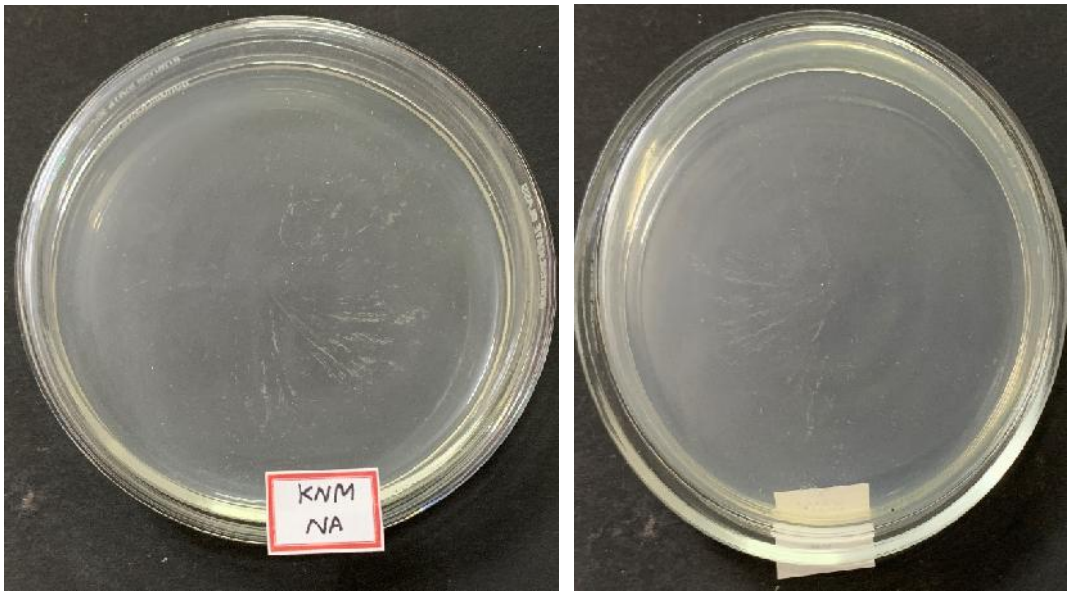

Result:

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

Test	Result
Total Bacterial Count	Absent
Total Fungal Count	Absent

Methodology for specific pathogen

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37^{o}C 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.

Result

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

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

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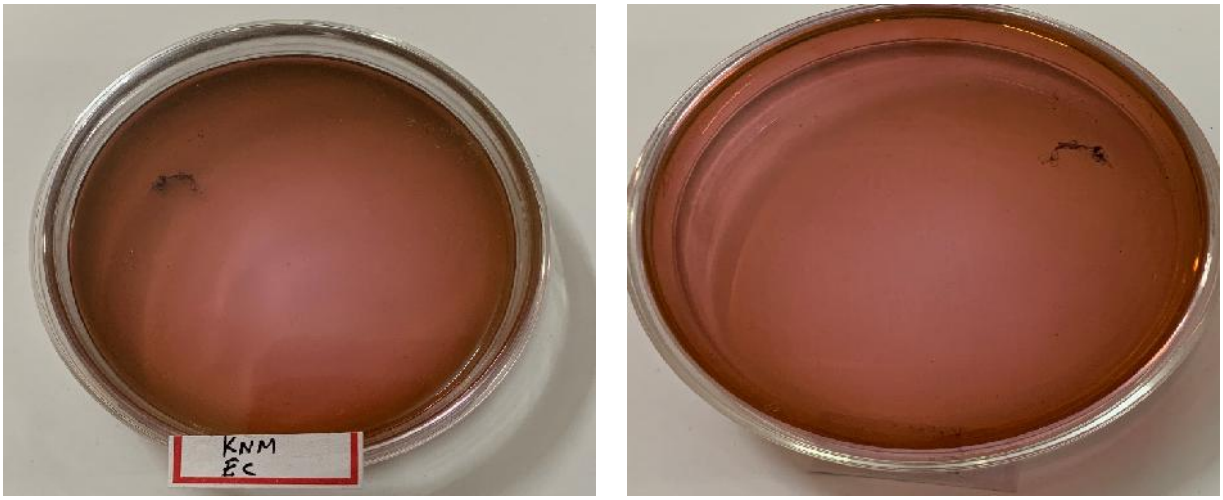
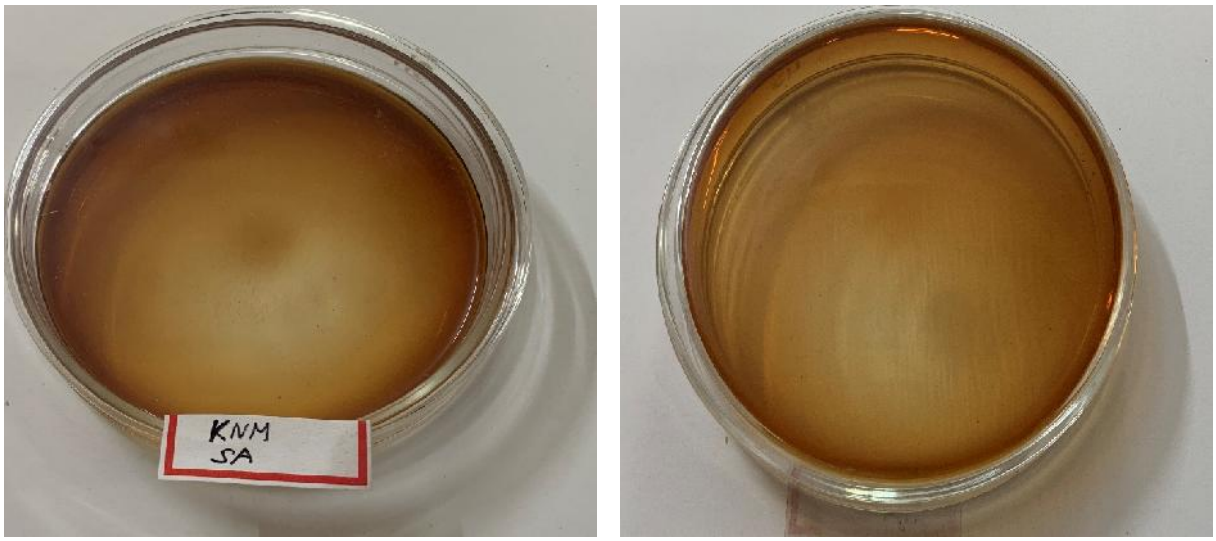
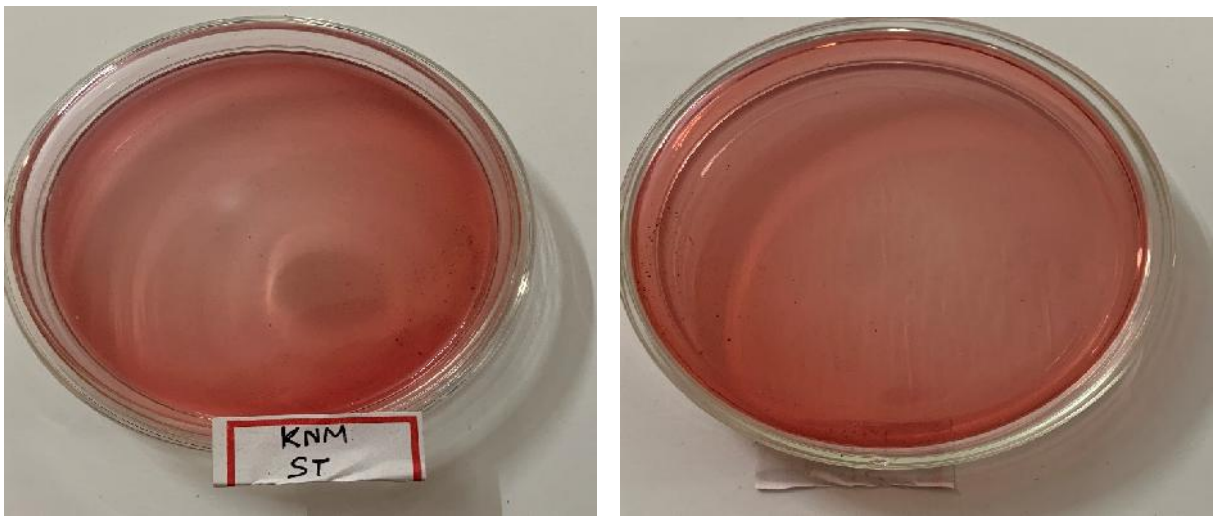
Culture plate with E-coli (EC) specific medium



Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus aureus (ST) specific medium



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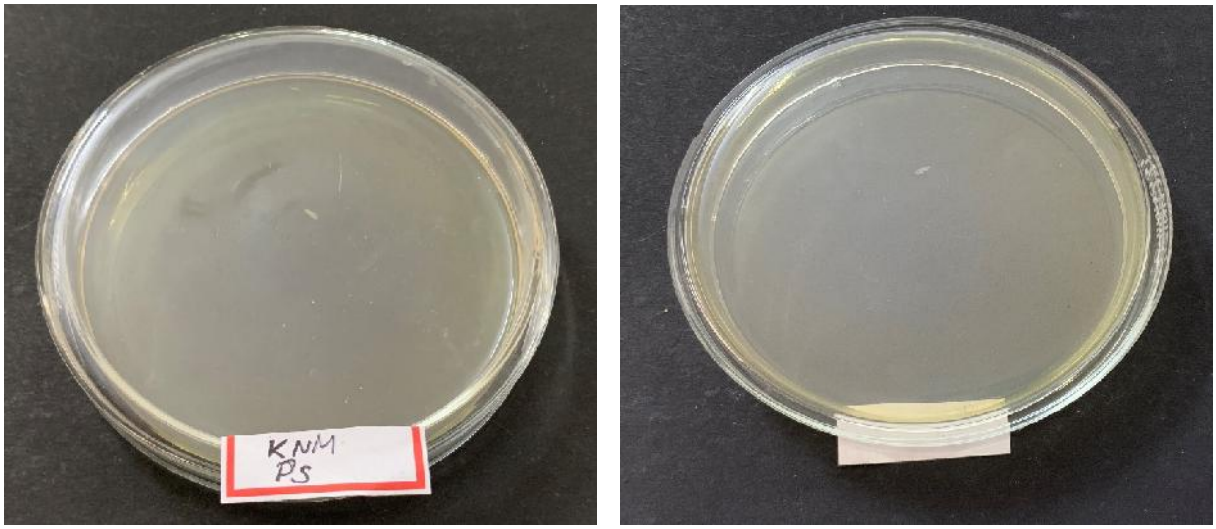
Culture plate with Pseudomonas aeruginosa (PS) specific medium



Discussion

Polyherbal preparation or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutic role of number of herbal drugs in disease management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants Siddha system of traditional medicine has numerous formulations which are utilized for the treatment ofdisease in mankind since several years. Till now there are several formulations which need to be standardized and evaluated for its potency.

The results obtained from standardization and physiochemical analysis clearly reveals that the loss on drying value was1.533%, total ash value was 3.767%, and acid insoluble ash is 0.21%.The alcohol soluble extractive value was 7.467%and water soluble extractive was 13.3%.Now-a-days, there is a constant need to explore their medicinal uses and also to conduct phytochemical and bioactivity studies to prove their therapeutic properties. To know any information about any medicinal plant, there is a necessary to go through all the available texts of siddha and also the previous reviews from recent research. Phytochemical investigations and biological reviews on the plants will lead to the valuable information which can help the scientists to know more advanced knowledge about these plant species. The result of the phytochemical analysis indicates that the formulation KMM shows the alkaloid, coumarins, saponins, tannins, glycosides, flavanoids, steroids, Triterpenoids, aanthocyanin, Carbohydrate, proteins. The result of HPTLC analysis shows phytoconstituents present in each sample and has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 7.94 ppm, Arsenic at 0.51ppm and Mercury 0.59 ppm. The sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.It was observed form the results of *In-vitro* anti-microbial assay that the formulation KMM possesses significant antimicrobial activity against *E.coli*, Solmonella. *Staphylococcus aureus, Pseudomonas aeruginosa*.

Siddha also has standardized protocols for purification and detoxification of certain phytocomponents used in specific formulations. As this will greatly reduce the toxicity and also enhance the therapeutic efficacy of the formulation. The chances of occurrence of an adverse event are very minimal in Siddha when compare to any other therapies in the world this is mainly because the 90 % of ingredients used in the preparing formulations are compatible with the biological system of the humans and animals.

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In the present study specific pathogenic bacteria and Aflotaxin, heavy metals, pesticide, Sterility are absent in KMM formulation. It is as per WHO norms. So it proves that kmm is free from microbial contamination. The findings of this study also highlighted the safety of the kana mantha Mathirai. The information obtained from preliminary phytochemical screening will be use full in finding out the reality of the drugs.

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