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Research Article

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Formulation and Standardization of siddha polyherbal formulation–Bala karappan kudineer (BKK)

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Abstract

Bala karappan kudineer is a poly herbal formulation originated from Siddha system of Indian medicine. It is mainly used for Bala karappan (Atopic dermatitis in children). Standardization is mandatory to prove the quality and reliability of traditional medicines. All the ingredients were procured and botanically authenticated. The prepared Bala karappan Kudineer was subjected to analyses. The derived Physico-chemical parameters, TLC profiling, HPTLC profiles serve as diagnostic parameters to identify this polyherbal formulation.
The achieved results of Physico-chemical, TLC profiling, HPTLC profiles will be useful as tools for authentication and standardization profile of Bala karappan Kudineer.

Keywords: siddha medicine, Balakarappan, Atopic dermatitis, Standardization, Bala karappan kudineer

Introduction

Siddha system of medicine is a peculiar science and it is an uncomparable system of medicine. According to the siddha system of medicine total number of diseases are 4448. Of these, 108 diseases are occur during childhood. The sages said 18 types of karappan occurs in childhood. One among them is Balakarappan. Bala karappan kudineer which is mentioned in yugi vaithiya sindhamani (peru nool 800) for the treatment of Balakarappan (Atopic dermatitis in children). Standardization is a process resulting from a consensus based on scientific finding obtained by parties most affected by it. It may refer to a manufacturing process, an analytical test, operational procedure, calibration of an instrument or any set of conditions required for a purpose. It deals with formulating and applying rules for an orderly approach to a scientific activity for the benefit of, and with the co-operation of all concerned. In this study Bala

karappan kudineer was screened for standardization technique as per PLIM guidelines. Ingredients of BKK are *Morinda tinctoria, Vitex negundo, Cleome viscosa, Carum copticum, Phyla nodiflora, Allium cepa, Acorus calamus. Morinda tinctoria*, commonly known as Indian mulberry, is a species of flowering plant in the family of Rubiaceae, native to Southern Asia. *Morinda tinctoria* has a Anti inflammatory activity. *Vitex negundo* commonly known as five leaves chaste tree, It belongs to Verbenaceae family. It has anti inflammatory, Anti oxidant and Alagesic activity. *Cleome viscosa* belongs to Capparaceae family. it has Anthelmintic, Anti microbial activity, Anti inflammatory activity. *Carum copticum* or Ajwain belongs to Apiacaeae family. Ajwain shows Anti inflammatory, Anti microbial and immunomodulatory effects. *Phyla nodiflora* herbal belongs to Verbenaceae family. It has Anti inflammatory activity, Hepatoproductive effects and Anti microbial effects. *Allium cepa* is grown as a edible bulb belongs to Lilliaceae family. Onion has Anti inflammatory, Anti bacterial, Antiseptic, anti oxidant activity. *Acorus calamus* commonly known as Sweet flag, belongs to Acoraceae family. *Acorus calamus* has Anti inflammatory, Antidiarrheal, Antimicrobial activity.

Materials and Methods

Selection of drug

The drug Bala karappan Kudineer was selected from the classical Siddha literature.

Collection and authentication of the drug

The raw materials of this formulation like oomam (*Carum copticum),* vasambu (*Acorus calamus*) were procured from country drug shop at Chennai, Tamilnadu. Fresh Poduthalai (*Phyla nodiflora),* Onion (*Allium cepa*) was collected from Koyambedu market at Chennai, Tamilnadu. Healthy and disease free plant roots of Nuna (*Morinda tinctoria*, Notchi (*Vitex negundo*) was collected from dindigul district Tamilnadu. Root of velai (*Cleome viscosa*) was collected at Chennai, Tamilnadu. They were identified and authenticated by the Botanist, Govt. Siddha Medical College, Arumbakkam, Chennai-106.

Purification of the drug

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

Preparation of the drug

The above mentioned drugs are taken in an equal quantity, then grinded into coarse powder. The decoction powder were stored in clean air tight container and named as BKK with manufacturing date.

State	Solid	Liquid
Nature	Coarse Leafy Materials	Non- Viscous
Odor	Characteristic	Characteristic
Touch	Hard Texture	Non- Greasy
Flow Property	Non free flowing	Free flowing
Appearance	Greenish Black	Dark brownish

Profile of BalakarappanKudineer – BKK

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	Hexane	Insoluble
6	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.

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 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 (%)***	5.267 ± 1.002
2.	***Total Ash (%)***	3.433 ± 2.001
3.	***Acid insoluble Ash (%)***	0.39 ± 0.041
4.	***Water soluble Extractive (%)***	24.63 ± 1.159
5.	***Alcohol Soluble Extractive (%)***	20.2 ± 1.345

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 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.

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

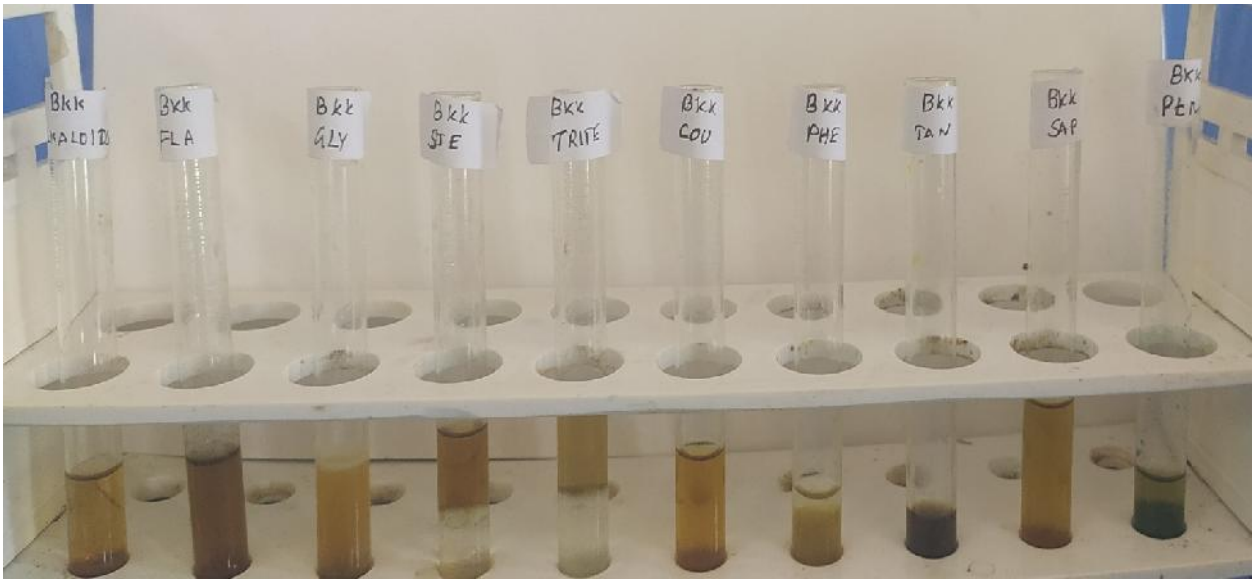
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

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



Extraction 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.

Test Result Analysis of the Sample BKK Pesticide Residue	Sample BKK	AYUSH Limit (mg/kg)
I.Organo Chlorine Pesticides		
Alpha BHC	ND	0.1mg/kg
Beta BHC	ND	0.1mg/kg
Gamma BHC	ND	0.1mg/kg
Delta BHC	ND	0.1mg/kg
DDT	ND	1mg/kg
Endosulphan	ND	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	ND	1mg/kg

Chlorpyriphos	ND	0.2 mg/kg
Dichlorovos	ND	1mg/kg
III. Organo carbamates		
Carbofuran	ND	0.1mg/kg
III.Pyrethroid		
Cypermethrin	ND	1mg/kg

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

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

Test Report Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	6.65 PPM	10 ppm
Arsenic	193.7 nm	1.54 PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.98 PPM	1 ppm

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 6.65 ppm, arsenic at 1.54 ppm and mercury at 0.98 ppm.

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.

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.

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.

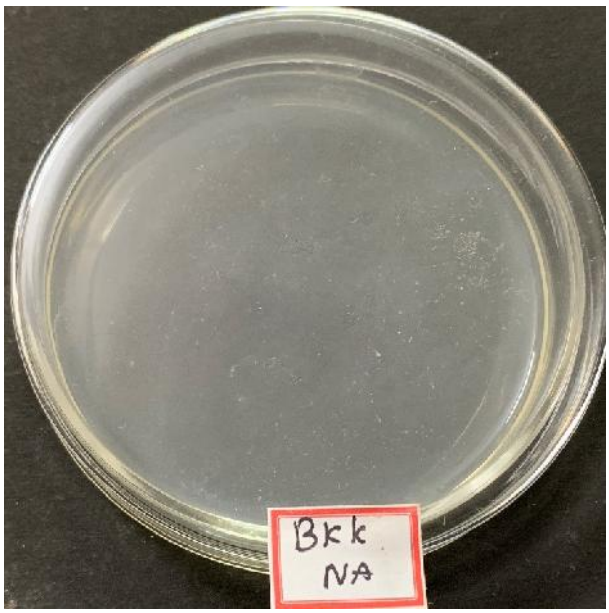
Sterility test by pour plate method

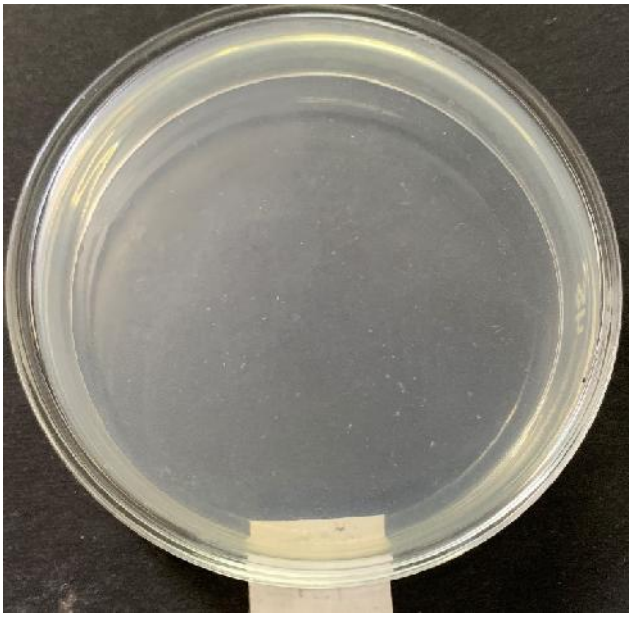
Objective

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).

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.





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	

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 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.

Detail of 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***

Observation

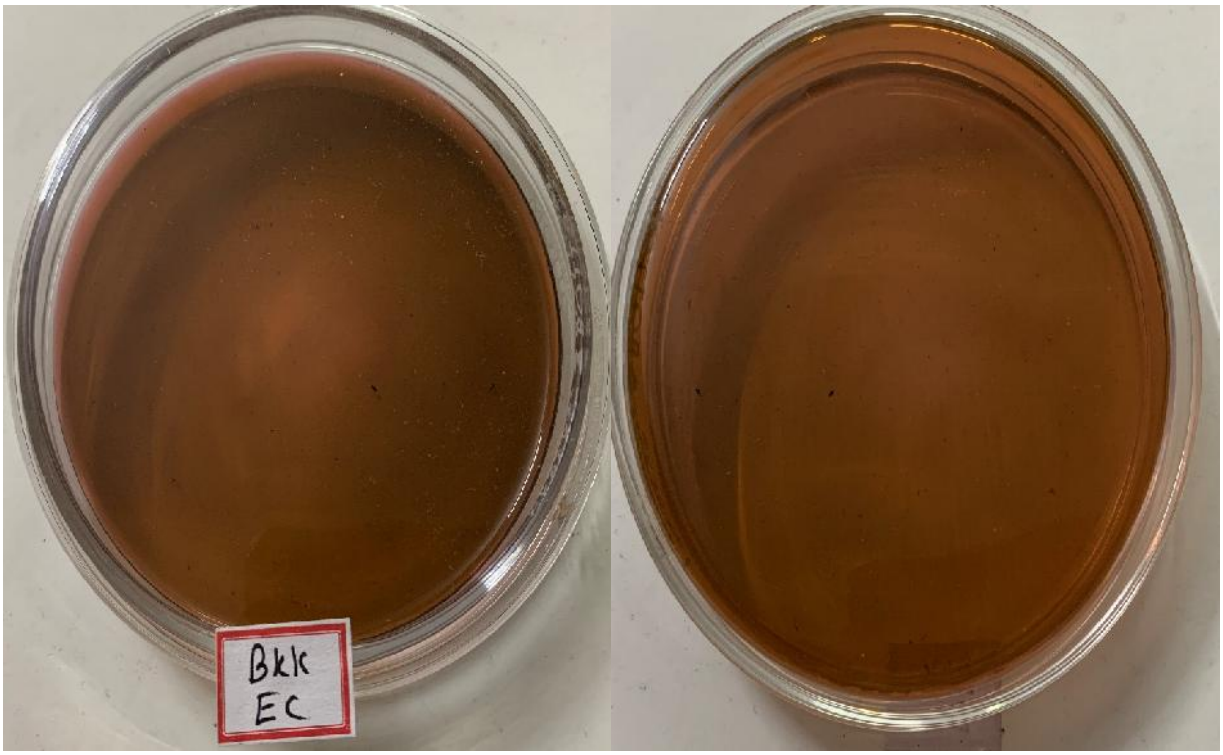
No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

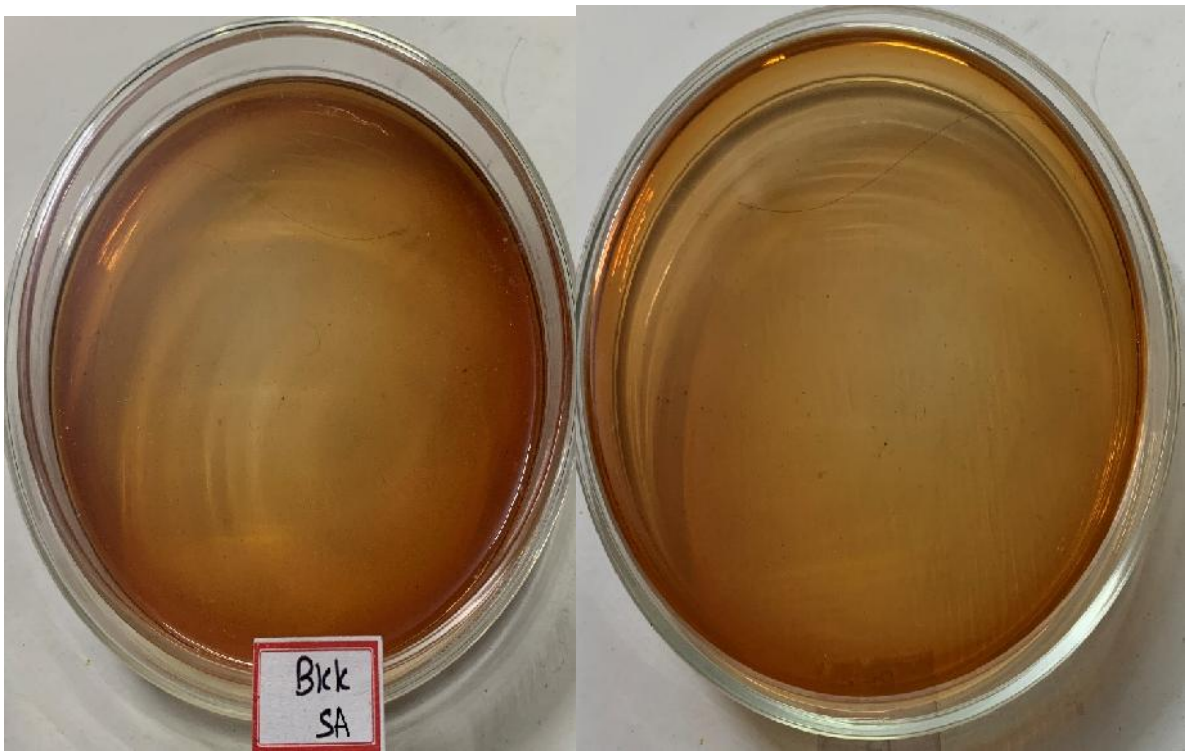
No growth / colonies were observed in any of the plates inoculated with the test sample.

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

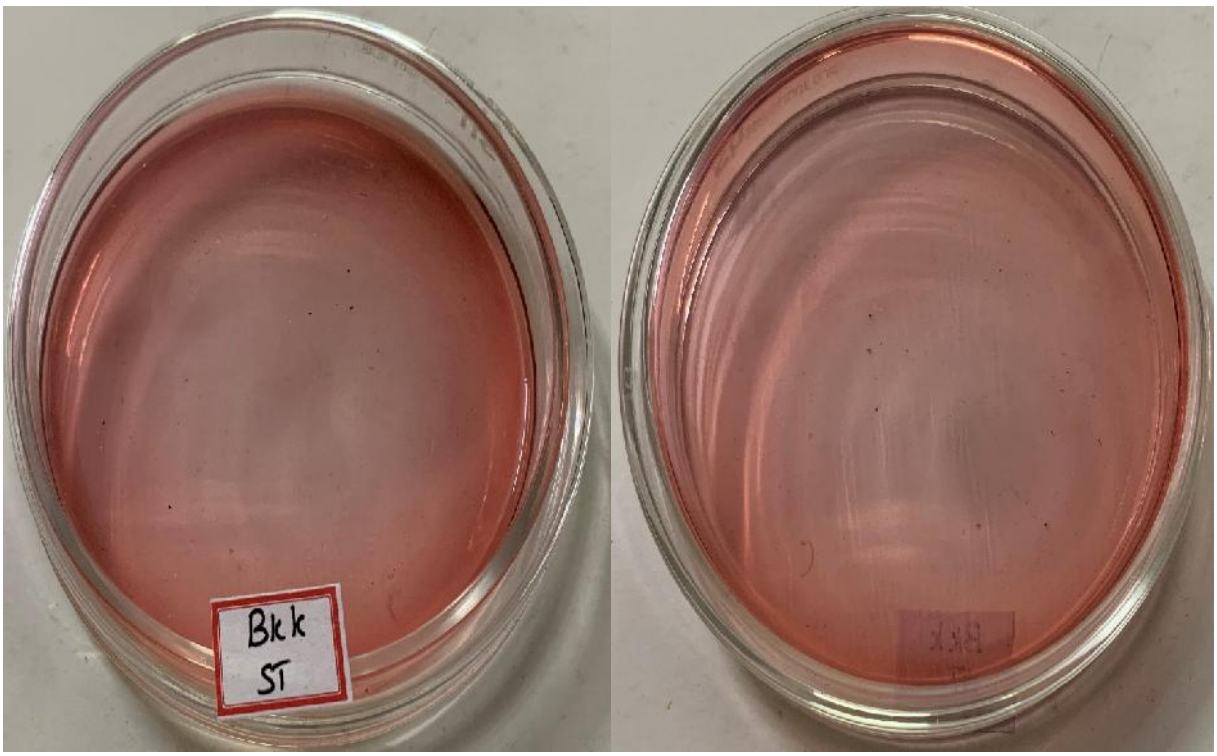
Culture plate with E-coli (EC) specific medium



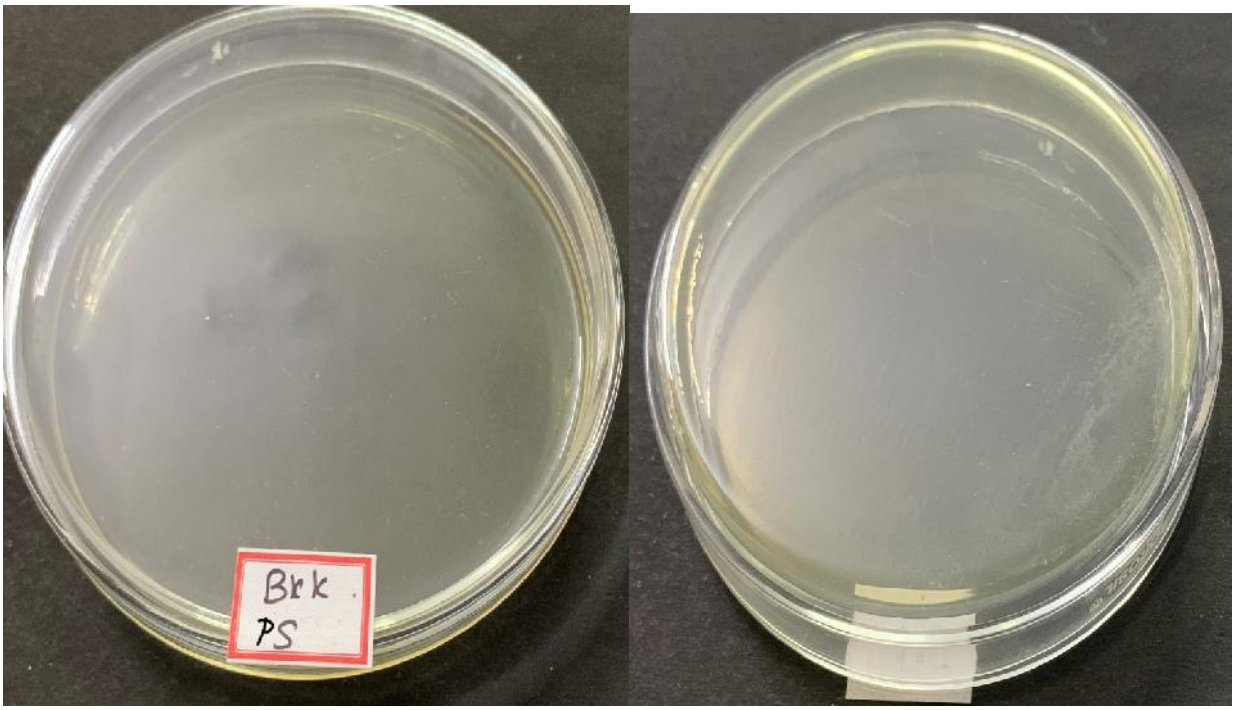
Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus aureus (ST) specific medium



Culture plate with Pseudomonas aeruginosa (PS) specific medium



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.

Discussion

Standardization is an essential factor for Siddha preparations in order to assess their quality based on the concentration of chemicals present in that. So, The results obtained from physiochemical analysis of siddha formulation BKK, clearly reveals that the loss on drying value was 5.267%, total ash value was 3.433%, and acid insoluble ash is 0.39%.The alcohol soluble extractive value was 20.2%. The result of the phytochemical analysis indicates that the formulation BKK shows the alkaloid, Triterpenoids coumarins, phenol, tannin, saponins, sugar, betacyanin. The result of HPTLC analysis shows phytoconstituents present in each sample and has no traces amount of heavy metal cadmium, whereas the sample shows the presence of Lead at 6.65ppm, Arsenic at 1.54 ppm and Mercury 0.98 ppm. this sample was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.The results also shows no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamtes and pyrethroids.It can be concluded that the formulation of BKK was in accordance with the standards limits. The findings of this study also highlighted the safety of the Bala karappan kudineer. Such kind of standardization studies are needed in promoting the safe use of genuine drugs, thus contributing to human health. thus, it can be concluded that the parameters studied in the present work may be used for quality evaluation and standardization of BKK to achieve genuine and standard drug for further preclinical and clinical research.

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