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Research Article

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Formulation and Standardization of Siddha drug- Vali Kana Kudineer (VKK)

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Abstract

The aim of the present study was to evaluate physiochemical,phytochemical analysis Heavy metal analysis, Sterility, TLC analysis, HPTLC, Pesticide, Aflatoxin and Specific pathogen of ‘‘VALI KANA KUDINEER’’. The physicochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble extraction value were carried out.The Heavy metal such as Lead, Arsenic, Cadmium and Mercury were carried out.The phytochemical properties such as tannins, Alkaoid, saponins, flavonoids, phenols, coumarins, Triterpenes, cyanins, carbohydrate, protein were also carried out.The present study provides the details of physiochemical , Phytochemical and heavy metal properties of ‘‘VALI KANA KUDINEER’’ which is useful in ‘‘PHARYNGITIS’’.

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

Result: The results obtained from the physicochemical analysis clearly reveals that the Loss on Drying value was3.367 ± 0.2082,total ash value was 8.5 ± 0.2646, Acid insoluble Ash value was 0.44 ± 0.1153, Water soluble Extractive value was 15.4 ± 2.2.The result of HPTLC analysis shows phytoconstituents present in each sample. The result of Heavy metal shows that the sample has no traces of heavy metals such as Arsenic and Cadmium, whereas the sample shows the presence oflead at 7.18 ppm and mercury at 0.23 ppm.It was observed from the result of In-Vitro anti-microbial assay that the formulation VKK possesses significant antimicrobial activity against *E-coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa.*

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

Keywords: Physicochemical, phytochemical, Heavy metal, vali kana kudineer.

Introduction

Siddha medicine, traditional system of healing that originated in South India and is considered to be one of India's oldest systems of medicine. This system considers the human body as the replica of the universe and so are the food and drugs irrespective of their origin. Siddhars classified diseases in different categories which accounts for 4448 disease in human body. According to the ancient siddha texts, the human body is made up of several elements. There are three humors or the DOSHAS called,

- Vatham
- Pitham
- Kapham

The three dos has are considered the three pillars of health and support the structures and functions of the body. As per siddha aspect paediatric diseases are carried from gene. It defines that the pediatric diseases occur at the time of fertilization to gestational period those paediatric disease where classified in to Agakkarana and Purakarananoigal.

Pharyngitis is being 1/3 of the primary system of the upper respiratory tract infection in children. Acute pharyngitis inflammation of mucous membranes and underlying structures of the throat, characterized by fever, sore throat and pharyngeal exudates. Most common infectious agents are Group A Streptococcus and various viral agents. Every year, 10% of children are affected by Acute pharyngitis. Peak prevalence of Acute Pharyngitis in children aged between 5-10 years.

Clinical features of acute pharyngitis correlates with the symptoms of valikanam fever, cold, cough, loss of appetite, sore throat, urinary infection described in the siddha text. In siddha literature valikanam is one of the 24 types of ''kanam'' that occurs in children. The medicine was chooses for treatment and management of the valikanam was valikana kudineer 15-30ml internally, twice a day after food described in pillaipini maruthuvam. In this study valikana kudineer was selected and standardization technique as per procedures. Ingredients of vali kana kudineer are *Trigonella foenum graecum, Allium cepa, Aegle marmelos.* Vali kanakudineer considerably high antimicrobial activity against the tested pathogenic micro organisms as well as Anti inflammatory activity. This biological activity due to the presence of phytochemical, HPTC and physiochemical analysis of ''Vali kana kudineer''.

Materials and Methods

Selection of drug

The drug vali kana kudineer was collected from the Pillaipinimaruthuvam part 2

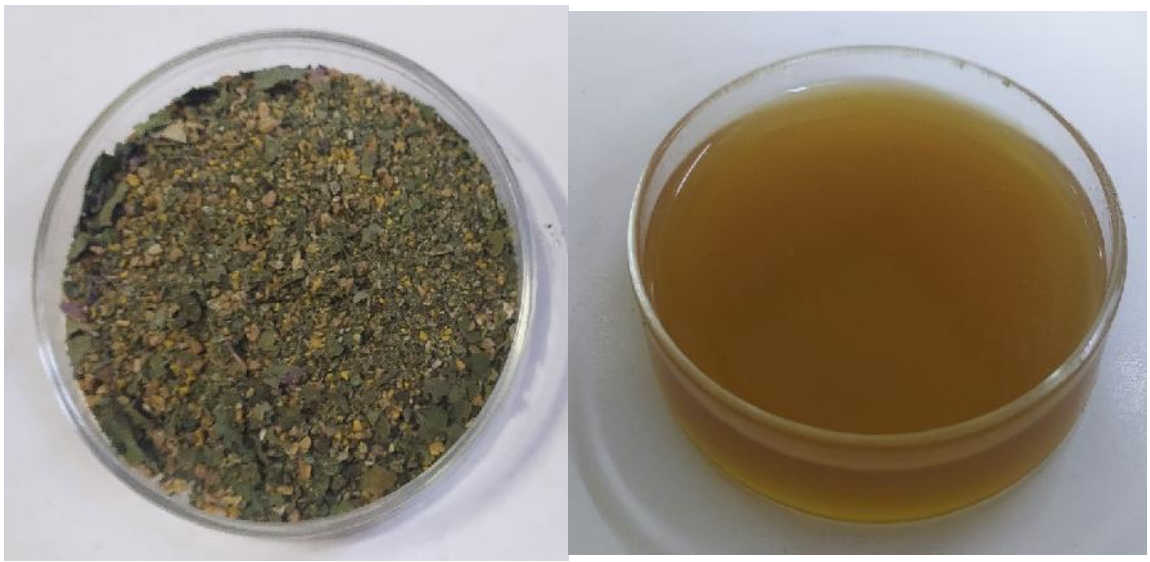
Collection and authentication of the drug

The raw materials included in the formulation are *Trigonella foenum graecum* were procured from the local drug shop at Chennai. Fresh *Allium cepa, Aegle marmelos* was procured from Koyambedu market at Chennai, Tamilnadu. They were identified and authenticated by the Botanist, Government Siddha Medical College, Arumbakkam, Chennai-106.

Purification and Preparation of the drug

The mentioned drugs are purified as in Sigicha Rathna Deepam Suthimuraigal text book procedure. Then the dried vilvam leaves and vendhayam are made intocoarse powder (decoction powder) . Then fresh pieces of *Alium cepa* 2gm is taken and added to 5gm of decoction powder in a vessel filled with 240ml of water. It is then boiled in medium flame and reduced into 30ml (1/8 part).Then, the decoction is filtered.

Physio-chemical analysis



State	Solid	Liquid
Nature	Coarse Leafy Materials	Non- Viscous
Odor	Characteristic	Strong characteristic
Touch	Hard Texture	Non- Greasy
Flow Property	Non free flowing	Free flowing
Appearance	Greenish	Brownish orange

Solubility Profile of Valikana Kudineer – VKK

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 (%)	3.367 ± 0.2082
2.	Total Ash (%)	8.5 ± 0.2646
3.	Acid insoluble Ash (%)	0.44 ± 0.1153
4.	Water soluble Extractive (%)	15.4 ± 2.2
5.	Alcohol Soluble Extractive (%)	12.43 ± 2.515

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.

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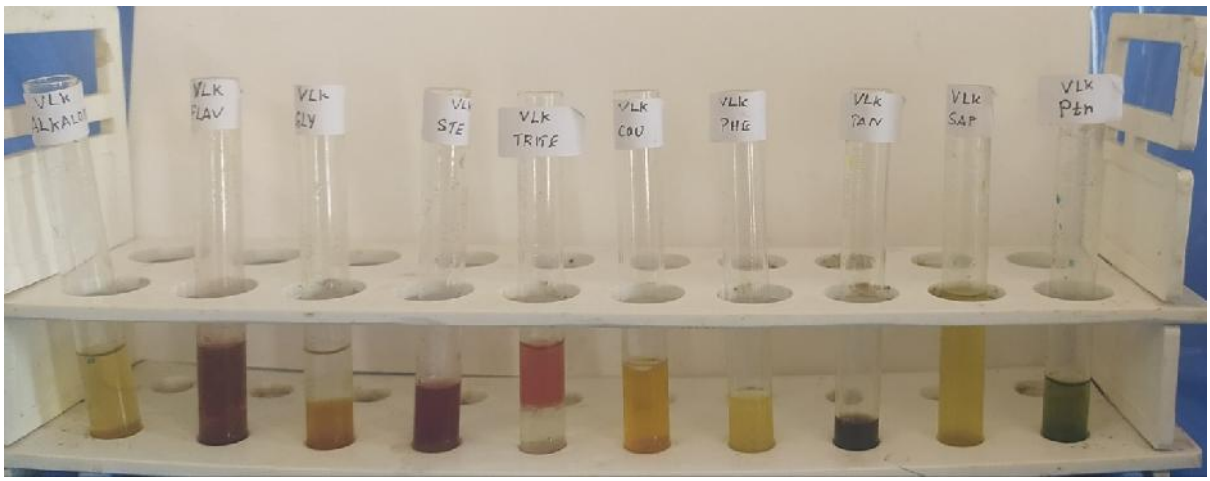
Results

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.

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

Test Report

Name of the heavy metal	Absorption Max	Result Analysis	Maximum Limit
Lead	217.0nm	7.18PPM	10 ppm
Arsenic	193.7nm	BDL	3 ppm
Cadmium	228.8nm	BDL	0.3 ppm
Mercury	253.7nm	0.23PPM	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 7.18 ppm and mercury at 0.23 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 valikanakudineer	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

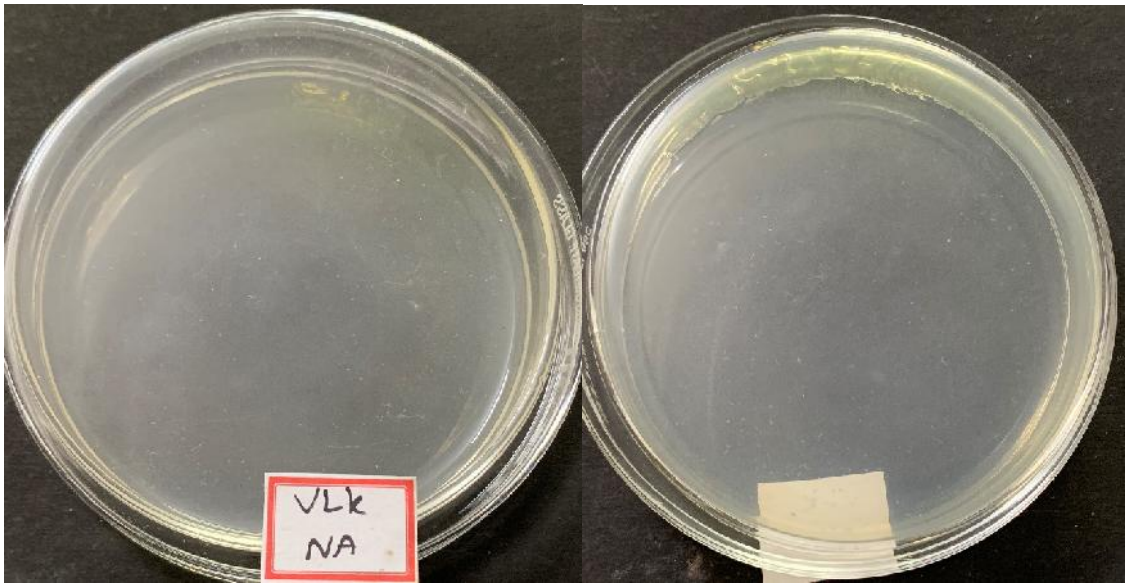
Result

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 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.



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 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Methodology of 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.

Observation

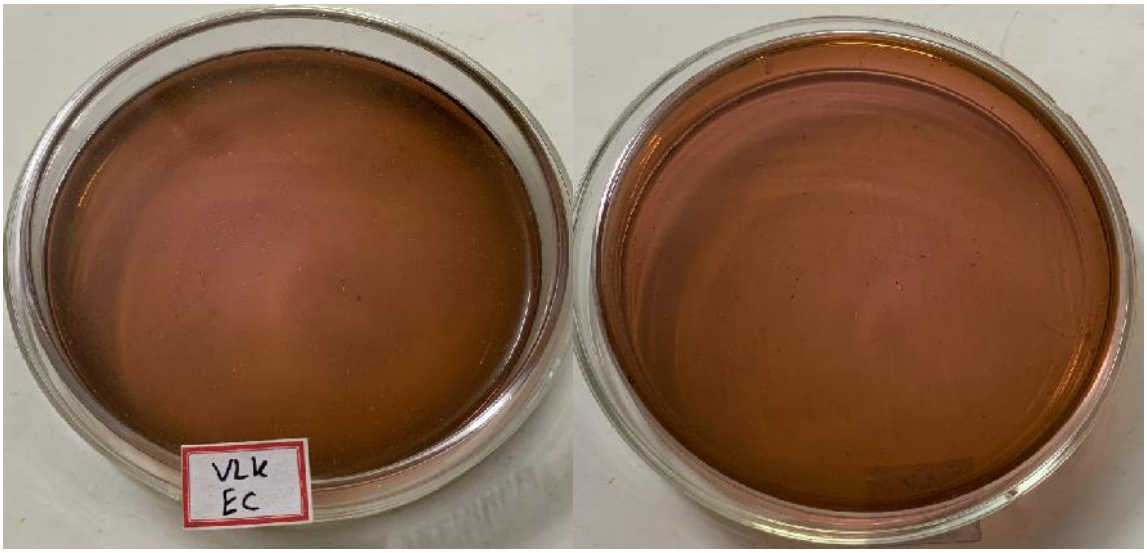
No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

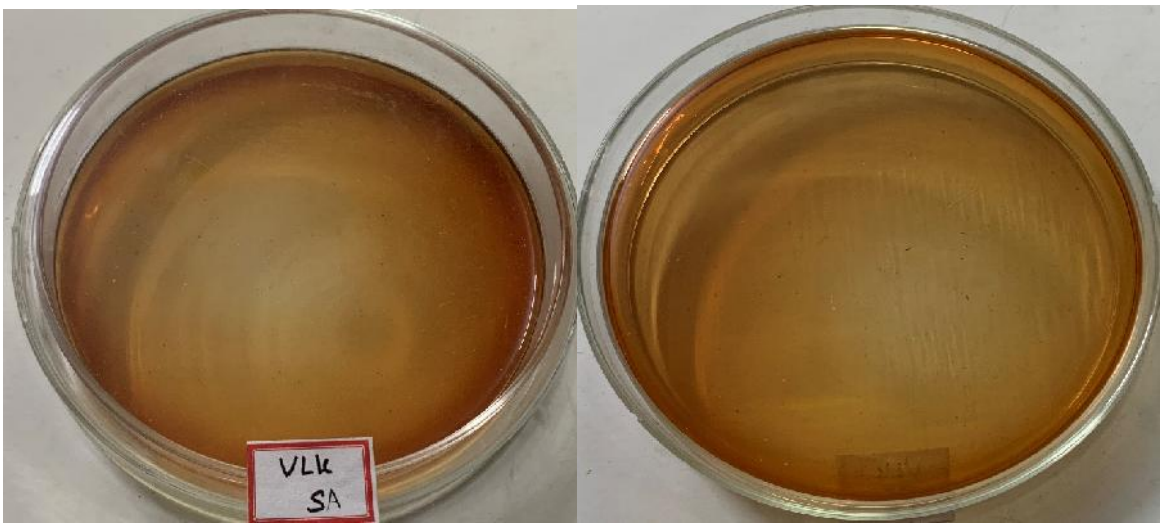
No growth / colonies were observed in any of the plates inoculated with the test sample.

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

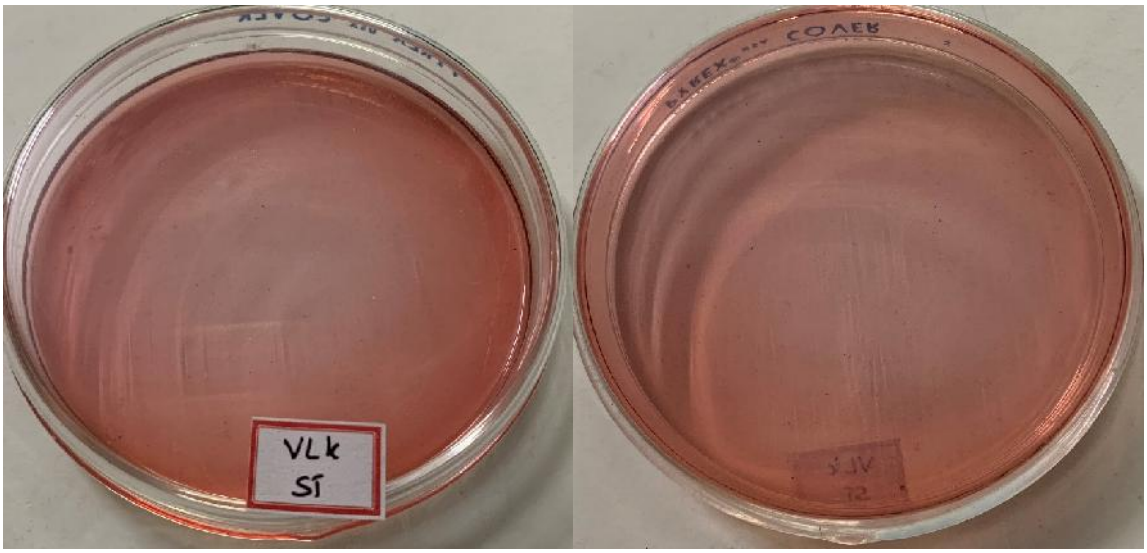
Culture plate with E-coli (EC) specific medium



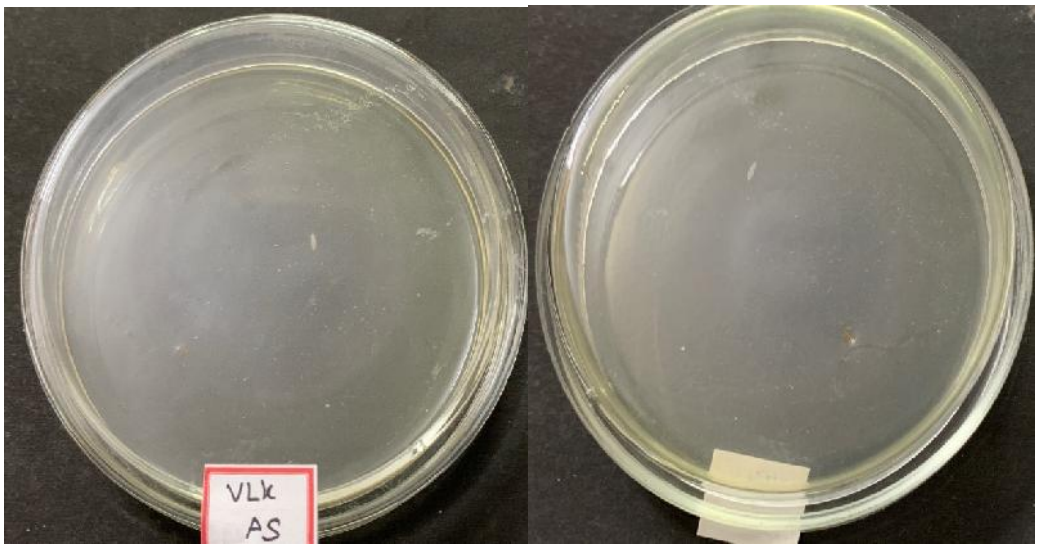
Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus aureus (ST) specific medium



Culture plate with Pseudomonas aeruginosa (PS) specific medium



Discussion

The siddha medicines acts mostly as rejuvenation and prevention. The ACUTE PHARNYGITIS once cured will have less chance of reoccurrence. The commonly elemental analysis included is evaluation of vatham, pitham and kabam proportions. The results obtained from the physicochemical analysis clearly reveals that the Loss on Drying value was3.367 ± 0.2082,total ash value was 8.5 ± 0.2646, Acid insoluble Ash value was 0.44 ± 0.1153, Water soluble Extractive value was 15.4 ± 2.2.

Now a days purpose of phytochemical analysis chemically constituents may be therapeutically active or inactive. The phytochemical research approach is considered effective in discovering bioactive profile of plants with therapeutic importance. The result of the phytochemical analysis indicates that the formulation VKK shows the alkaloid, coumaries, saponins, tannins, phenol, glycosides, flavonoids, steroids, triterpenoids, aanthocyanin, carbohydrate, protein.

The result of HPTLC analysis shows phytoconstituents present in each sample. The result of Heavy metal shows that the sample has no traces of heavy metals such as Arsenic and Cadmium, whereas the sample shows the presence oflead at 7.18 ppm and mercury at 0.23 ppm.

It was observed from the result of *In-Vitro* anti-microbial assay that the formulation VKK possesses significant antimicrobial activity against *E-coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa.*

The *Aegle marmelos* leaves extract, shows higher action against the microorganisms, *E.coli* followed by *Salmonella typhi, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa* in all the concentration.

The present study carried out to determine the antibacterial activity of Fenugreek's leaves, seeds and stem in aqueous, methanol and acetone extract against *E. coli* and *Staphylococcus*.

The *Allium cepa* essential oil shows a moderate Antimicrobial activity present.

In the present study specific pathogenic bacteria and Aflotoxin, heavy metals, pesticides, sterility are absent in VKK formulation. It is as per WHO norms. So it proves that VKK is free form microbial contamination. The findings of this study also highlighted the safety of the vali kana kudineer. The information obtained from preliminary screening will be useful in finding out the reality of the drugs.

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