Research Article

Standardization of Siddha Polyherbal Formulation *Kameshwara Karpa Avizhtham* – Siddha Karpa Marunthu

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ABSTRACT

An ancient Siddha medicine practiced in Tamil population around worldwide which has lot of medical preparation. Kameshwara Karpa Avizhtham is one among them, used to treat *Veeriyaviruthi, Thathukuraivu, Thathunattam* as mentioned in *Theraiyar Yamaha Venba.* In order to globalize siddha drugs, standardization is needed. *Kameshwara karpa Avizhtham* is prepared as per siddha classical text *Theraiyar Yamaha Venba* and subjected to Physiochemical and phytochemical analysis as per the pharmacopeia laboratory standards of Indian medicine. *Kameshwara karpa Avizhtham* is Brown in colour, Hard in Nature, contains no pesticide residues and microbial contamination, heavy metals are under below quantification level. According to the findings, it is safe to eat Kameshwara karpa Avizhtham.

Keywords: Kameshwara karpa Avizhtham, Standardization, Thathukuraivu.

INTRODUCTION

The Siddha System of Medicine is a contribution of the Dravidian culture, which is one of the oldest cultures in world. Siddha system of medicine talks more about moral principle and moral guidance which is extremely relevant to our present-day health care system. The hallmark of Siddha system is ***Kaya Karpam*** i.e., imparting immunity to diseases and counteracting the aging process.

A deeper exploration in the areas of *kaya karpam* of the siddhars can fetch us break-through in combating various incurable diseases. Karpa therapy is inclusive of herbal karpam, Mineral karpam, regulatory procedures for life force Circulation, Yoga and more integrated therapies like muppu, (Vaithyamuppu, Vathamuppu, Yoga muppu).

Siddha medical science most distinctly emphasizes the practice of *Kaya karpam* to rejuvenate the body and mind, markedly slowing down the biological ageing. The therapy of *Kaya karpam* is attained through *Karpa-aviztham* (karpa-medicines) and *Karpa yogam* (regimens of life). *Kameshwara Karpa Avizhtham* is one among them, used to treat *Veeriyaviruthi, Thathukuraivu, Thathunattam* as mentioned in *Theraiyar Yamaha Venba.* In order to globalize siddha drugs, standardization is needed

MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE FOR THE PREPARATION OF DRUG

Procurement of Raw Drugs:

The Raw drugs for the preparation of Medicine Kameshwara karpa Avizhtham was purchased from the reputed indigenous drug store at Parry's Corner, Chennai.

Raw drugs

a. *Kattu Maangai Paruppu* (*Buchanania lanzan*) – 35gm

b. *Murungai Paruppu* (*Moringa oleifera*) – 35gm

c. *Munthirigai Paruppu* (*Anacardium occidentalae*)– 35gm

d. *Kothumai* (*Triticum aestivum*) – 35gm

e. *Vaathumai Paruppu* (*Prunus dulcis*)-35gm

f. *Naruvili* (*Cordia dichotoma*)– 35gm

g. *Atthippaal* (*Latex of Ficus carica*) -1260 gm

h. Ghee – required quantity

i. Sakkarai (Country Sugar)– required quantity.

Identification and Authentication

The raw drugs for the preparation of *Kameshwara karpa avizhtham* was identified and authenticated by the experts of Medicinal Botany and Gunapadam, National Institute of Siddha, Chennai – 47.

Procedure

Purification: The raw drug was weighed and purified by getting rid of sand, dust particles, small wooden sticks and other adulterants.

Preparation[1]: *Kattu Maangai Paruppu, Murungai Paruppu, Munthirigai Paruppu Kothumai, Vathumai Paruppu, Naruvili* were taken 35gm (each drug) and placed in mud pot. 1.260 Kilogram of *Atthippaal* was collected and pour into the mud pot. Allowed it to dry at sunlight for 6 days. After that external layer of *Vaathumai paruppu, Kattu maangai paruppu* was removed. Again, the raw drugs were allowed to dry until it completely dried. After that powder it and weighed. Equal quantity i.e., 200gm of Sugar (Brown

Sugar) and 200gm of ghee 24 is added to that mixture. After that the drug is stored in airtight container.

Physicochemical Evaluation[2,3]

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.

pH determination

Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

TLC Analysis[4]

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 was 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[5]

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 phyto therapeutics.

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.

Test 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 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

Sterility Test by Pour Plate Method

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°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° 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.

Test for pesticide residue[6,7]

Extraction Test sample were extracted with 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 milli Liters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Heavy metal analysis by AAS Standard:

Hg, As, Pb and Cd – Sigma 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 HNO3. Standard preparation As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO3.

Test for Aflatoxins[8]

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.

RESULTS

KKA Sample Description

State	Solid
Nature	Rough
Odour	Strong Characteristic
Touch	Slightly Hard
Flow Property	Non-Free flowing
Appearance	Brownish

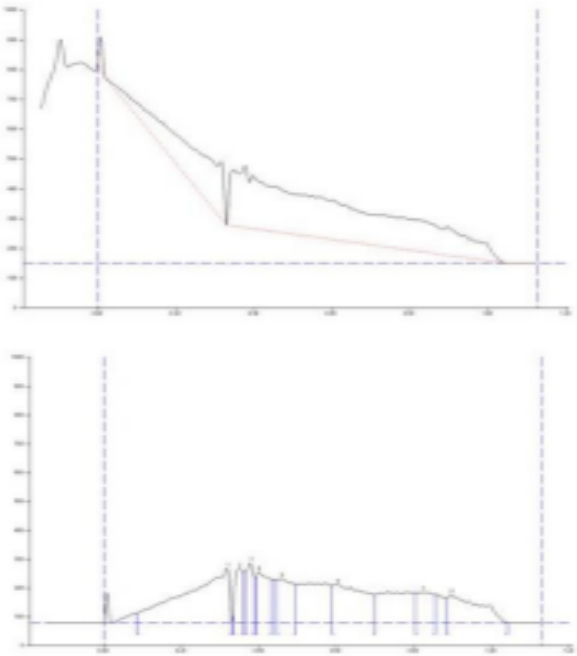
Final Test report

Parameter	Mean (n=3) SD
Loss on Drying at 105 °C (%)	6.333 ± 0.8622
Total Ash (%)	10.77 ± 0.7638
Acid insoluble Ash (%)	0.0313 ± 0.007
Water soluble Extractive (%)	14 ± 1.345
Alcohol Soluble Extractive (%)	10 ± 1.136
pH	6.5

TLC Visualization of KKA at 366 nm

3D – Chromatogram

HPTLC finger printing of KKA



HPTLC finger printing analysis of the sample reveals the presence of eight prominent peaks corresponds to the presence of eight versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.09 to 0.89.

Test for Specific Pathogen

Observation

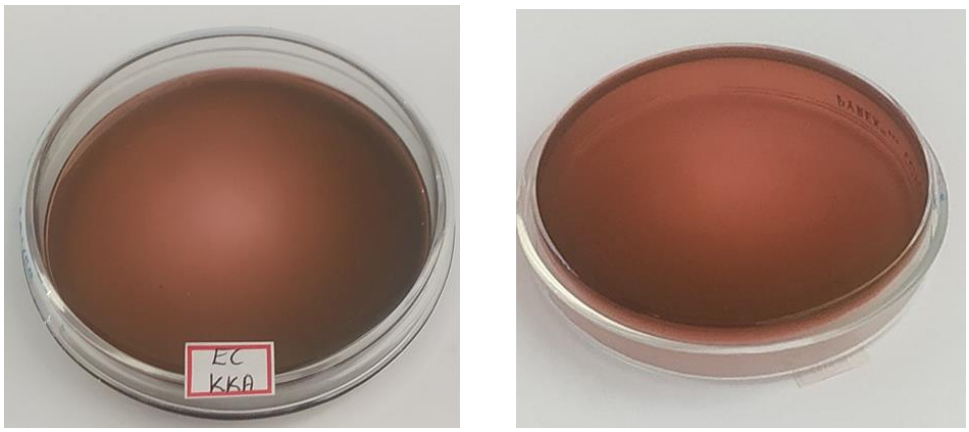
No growth was observed after incubation period. Reveals the absence of specific pathogen.

Result

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

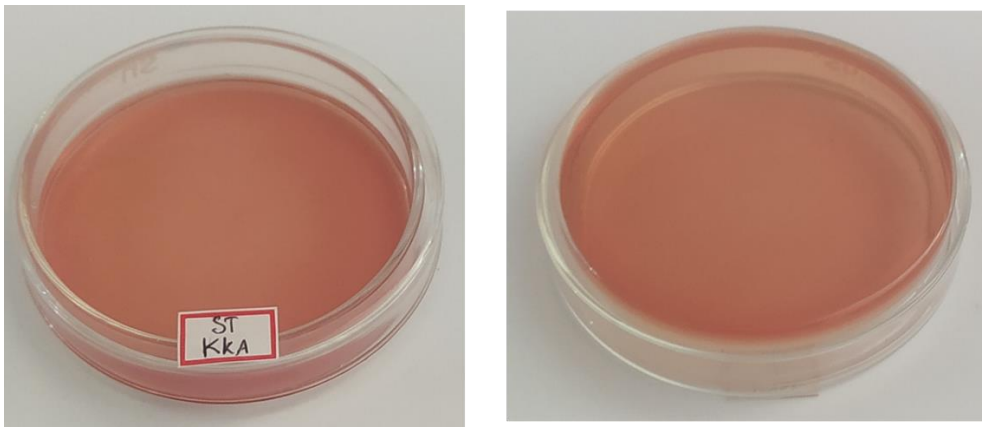
Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Culture plate with E-coli (EC) specific medium

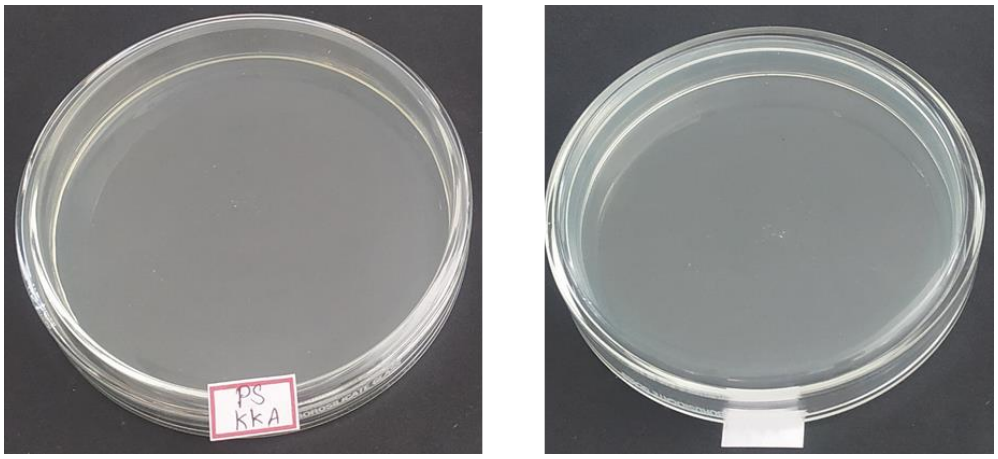


Culture plate with Salmonella (SA) specific medium

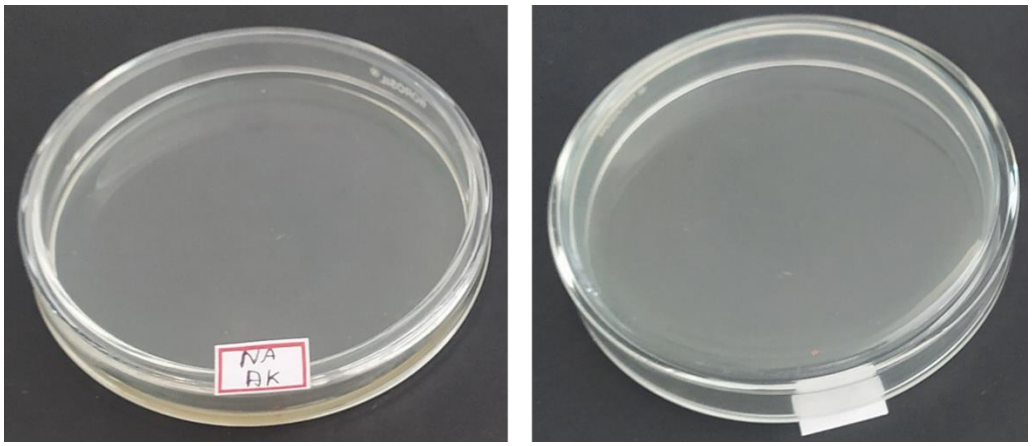
Culture plate with Staphylococcus Aureus (ST) specific medium



Culture plate with Pseudomonas Aeruginosa (PS) specific medium



Sterility Test by Pour Plate Method



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 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	

Test Result Analysis of the Sample KKA Pesticide Residue

Pesticide Residue	**Sample KAN**	**AYUSH Limit (mg/kg)**		**Pesticide Residue**	**Sample KAN**	**AYUSH Limit (mg/kg)**
I. Organo Chlorine Pesticides				II. Organo Phosphorus Pesticides		
Alpha BHC	BQL	0.1mg/kg		Malathion	50 µg/kg	1mg/kg
Beta BHC	BQL	0.1mg/kg		Chlorpyriphos	BQL	0.2mg/kg
Gamma BHC	BQL	0.1mg/kg		Dichlorovos	BQL	1mg/kg
Delta BHC	BQL	0.1mg/kg		III. Organocarbamates		
DDT	BQL	1mg/kg		Carbofuran	BQL	0.1mg/kg
Endosulphan	BQL	3mg/kg		IV. Pyrethroid		
				Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

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.

Heavy Metal Analysis Test Report:

Name of the Heavy Metal	Absorption Max - Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	7.232	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.215	1 ppm

Report and Inference

Results of the present investigation have clearly shown that the sample has no traces of heavy metals such as Arsenic and Cadmium, whereas the sample shows the presence of Lead and Mercury at 7.232 and 0.215 PPM level as listed in the above table.

Test for Aflatoxin:

Aflatoxin	Sample KKA	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

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 and Aflatoxin G2.

DISCUSSION

Kameshwara Karpa Avizhtham (KKA) is hard in nature, brown in colour, Solid based medicine. Analytical test values *Loss on Drying at 105 °C (%) is* 6.333 ± 0.8622, *Total Ash (%)* - 10.77 ± 0.7638, *Acid insoluble Ash (%)* - 0.0313 ± 0.007, *Water soluble Extractive (%)* -14 ± 1.345, *Alcohol Soluble Extractive (%)* - 10 ± 1.136, *pH – 6.5* – all the parameters are analysed and submitted as per the guidelines. Kameshwara karpa aviztham is subjected to TLC and HPTLC fingerprint test and the values and bio markers are documented. The peak and RF values (0.09 to 0.89) with 7 prominent peaks indicated the unique bio markers or phytochemical present in the drug. The Rf value was unique and didn't resemble with other plant finger prints. The 7 prominent peaks of Rf value were compared to that of the raw drugs Kameshwara karpa aviztham. The similarities in the peak values prove that which raw drug was responsible for the efficacy of the Kameshwara karpa aviztham. KKA is also analysed by microbiological test, where no specific pathogens were grown in the medium. This clearly implies that no microbial contamination was present and it indicates the purity. Pesticide residue in KKA had pesticide content below the quantification level. The malathion organo - phosphorous pesticides present in traces. Trace amount of lead and mercury was present but these also under limited quantity. Aflatoxins were absent in the KKA. All the parameters of the standardization technique in KKA were evaluated and documented. From the above results it is proved that this medicine is much safer because it does not contain any harmful substance and microorganism and their pesticide contents were below limited value.

CONCLUSION

Theraiyar yemaha venba, a work of Siddha classical literature, states that the *Kameshwara karpa Avizhtham* was developed in a traditional manner. In addition, all standardization procedures were applied, and values were turned in. Only the baseline is included in this study. It upholds the purity and quality criteria. Further preclinical and clinical studies will be needed in the future to evaluate the effectiveness of the developed medicine.

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