https://doi.org/10.48047/AFJBS.6.13.2024.7365-7379

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Qualitative and Quantitative analysis of Siddha Polyherbal formulation "*Kadukkai nei*"

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Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.7365-7379

Abstract:

The Siddha system of medicine gaining its attention globally due to its vast healing property, therapeutic effect and minimal side effect etc., and different forms of internal medicine and external medicine are available. Ghee is one form of internal medicine as mentioned in ancient Siddha classical text. Safety is main criteria for drug development; physiochemical analysis is the preliminary step to ensure drug safety. *Kadukkai nei* is indicated for *Athithoolam*. *Athithoolam/* Obesity is nothing but abnormal or excessive accumulation of fat in body which results in over weight. In India obesity is major life style disorder and accounts for 20 % of total population and it is the major risk factor for the development of diabetes mellitus, hypertension and other life style disorder. *Kadukkai nei* is prepared as per ancient Siddha classical text *Agasthiyar vaithiya vallathy 600* and its physiochemical characters like Colour, Odour, viscosity, Rancidity, Iodine value, Saponifiction value, pesticide residues, microbial contamination, heavy metals analyses, Chromatography were tested. *Kadukkai nei* is Yellow in colour, greasy in consistency, contains no pesticide residues and microbial contamination, heavy metals are under below quantification level. From the results, Kadukkai nei is safe to consume.

Keywords: *Kadukkai nei*, Athithoolam, Physiochemical properties, Siddha medicine**.**

INTRODUCTION

Siddha medicine, originating from the ancient healing traditions of South India, represents a profound system of healthcare that integrates natural medicine with spiritual and lifestyle practices. Siddha medical system doesn't consider treatment and prevention separately. The Siddha medical dictionary –Mr.T.V.Sambasivam pillai had described *Athithoolam* as, excessive accumulation of fat in

the system. Many diseases are emerging as a result of lifestyle modifications due to stress, sedentary lifestyle and food habits. One among them is *Athithoolam* which may be correlated to Obesity[1]. The prevalence of Obesity among Indians increased in 2019 to 2021 compared to 2015 -2016 as per National Family Health Survey (NFHS – 5). The percentage of obese men between the age of 15 and 49 increased to 22.9 percentage from 18.9 percentage, among women, the number increased from 20.6 percent to 24 percent[2]. In our system of medicine classified in 32 internal medicine and 32 external medicines, under the internal medicine *nei* is one of excellent drug category to treat many diseases which lifespan is 6 months[3]. My study medicine *Kadukkai nei* is indicated *Thoolanoi, Veekkam, Pithasokai, Sobai, Akkinimantham, Malasalakattu, Pithaveppu, Elaippu*[4]. The main aim of the study is to ensure *Kadukkai Nei* quality and safety through Physiochemical, Microbiological assays.

MATERIALS AND METHODS

1. **Standard Operative Procedure for the preparation of *Kadukkai nei***

A. Procurement of Raw Drugs:

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

Raw drugs

A. 1. *Kadukkai* (*Terminalia chebula*) - 20 nos.
2. *Keezhanelli* (*Phyllanthus niruri*) - 128g

B. 1. *Shenbaga poo* (*Magnolia champaca*) - 4g
2. *Elakkai* (*Elettaria cardamomum*) - 4g
3. *Sivathai ver* (*Operculina turpethum*) - 4g
4. *Chukku* (*Zingiber officinale*) - 4g
5. *Manjal* (*Curcuma longa*) - 4g
6. *Seeragam* (*Cuminum cyminum*) - 4g
7. *Kaduku Rohini* (*Picrorhiza kurroa*) - 4g
8. *Seenthil* (*Tinospora cordifolia*) - 4g
9. *Thippili* (*Piper longum*) - 4g

C. 1. Cow's ghee - 70 ml

D. 1. Cow's milk - 140 ml

B. Identification and Authentication

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

C. Procedure

Preparation

The raw drugs were purified as per ancient siddha literature. *Kadukkai, Keezhanelli samoolam* are smashed well and put into a vessel. Added 2 liters of water, and boiled until it reduced to 400 ml. Then 200gms of pure cow's ghee is added. Grinded the drugs *Sivathai ver, Manjal, Chukku, Kadukorohini, Seeragam, Seenthil, Thippili, Elam, Shenpaga poo* and added to the vessel. Added 400 ml of cow's milk to the vessel and boiled until it reached ghee's consistency. It is then filtered and stored in an airtight container.

The *Kadukkai nei* was labelled, stored in an airtight container and sent for laboratory analysis.

2. Qualitative and quantitative analysis of *Kadukkai nei*

Determination of Iodine value[5,6,7]

About 20 gm weight equivalent of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for an hour. T About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow colour. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

Determination of saponification value[5,6,7]

About 2 gm weight equivalent of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

Determination of Viscosity value[5,6,7]

Viscosity determination was been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a

capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured.

Determination of Refractive Index[5,6,7]

Determination of RI was carried out using Refractometer. 29 5. Determination of Weight per ml Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation were been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

Acid Value[5,6,7]

Accurately 5 g weight equivalent of the test sample was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed KOH solution was determined and the titration of test sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression. Acid value = Titter Value X 0.00561X 1000 / Wt. of test sample (g)

Peroxide value[5,6,7]

5 g weight equivalent of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide soluton. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Peroxide value = 10 (a-b)/ w

Microbial contamination test (Sterility test)

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 30 fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

TLC Analysis[8]

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 litre 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[9]

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 analysed. After evalution, 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 tabulated10.

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 37 ^{0}C 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. 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.

Sterility test by pour plate method

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45 ^{0}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 ^{0} 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[11,12]

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 millilitres of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Test for aflatoxins[10]

Solvent Standard samples were 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.

RESULTS

Sample Description

S. No	**State**	Liquid
1	**Nature**	Viscous
2	**Odour**	Strong characteristic
3	**Touch/ Consistency**	Greasy
4	**Flow Property**	Free flowing
5	**Appearance**	Yellowish

Solubility Profile

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

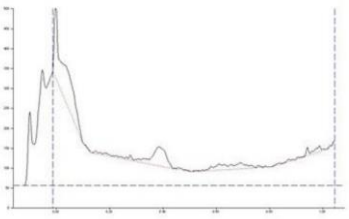
Analytical Report

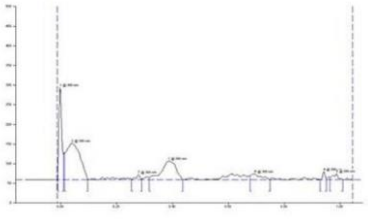
S.No	Parameter	*KAN*
1	Viscosityat50°C(Pa s)	82.40
2	Refractive index	1.78
3	Weight per ml(gm/ml)	0.833
4	Iodoine value(mgI2/g)	91.44
5	Saponification Value (mg of KOH to saponify 1gm of fat)	173.40
6	Acid Value mgKOH/g	1.2903
7	Peroxidase Value mEq/kg	3.38

TLC Visualization of Kadukkai Nei at 366nm

3D Chromatogram

HPTLC FINGER PRINTING OF KADUKKAI NEI





PEAK TABLE

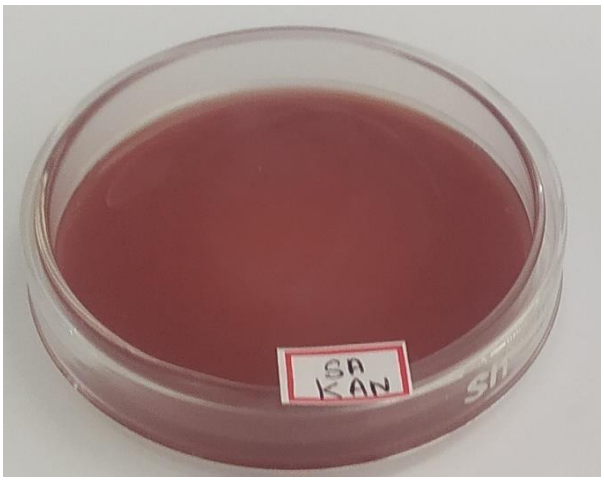
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	23.7	-0.00	234.2	53.49	0.01	64.8	1384.4	22.90
2	0.01	70.1	0.04	92.8	21.20	0.10	1.1	2596.6	42.96
3	0.26	1.8	0.28	12.9	2.94	0.29	2.3	99.9	1.65
4	0.32	5.8	0.39	47.4	10.84	0.44	0.5	1384.5	22.90
5	0.68	10.2	0.70	14.8	3.38	0.75	0.7	299.6	4.96
6	0.93	0.6	0.94	20.5	4.68	0.95	3.4	108.1	1.79
7	0.97	6.1	0.99	15.2	3.47	1.01	0.6	171.7	2.84

HPTLC finger printing analysis of the sample reveals the presence of seven prominent peaks corresponds to the presence of seven versatile phytocomponents present within it. Rf value of the peaks ranges from 0.01 to 0.97.

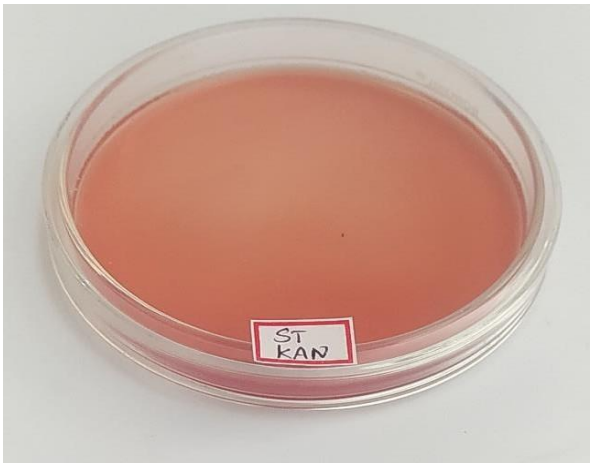
Test for Specific Pathogen

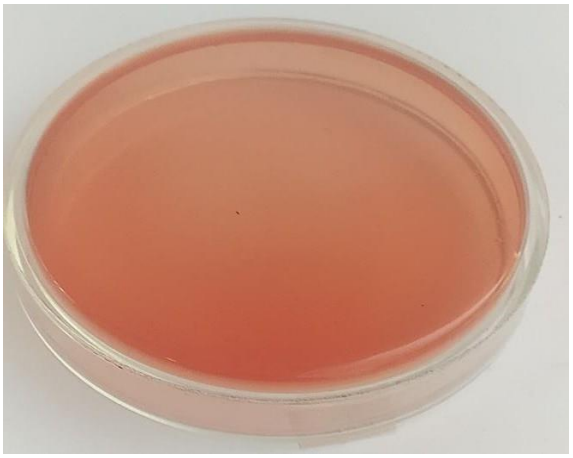
Culture plate with *E-coli(EC)* specific medium

Culture plate with ***Salmonella (SA)*** specific medium

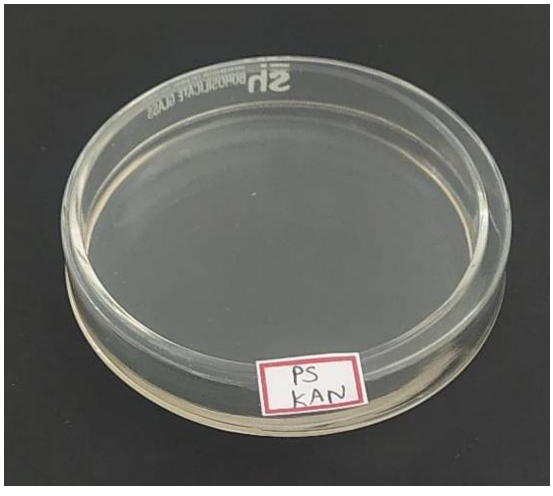


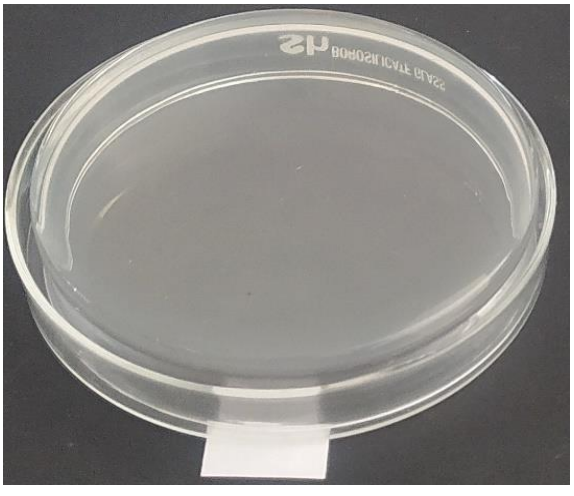
Culture plate with ***Staphylococcus Aureus (ST)*** specific medium





Culture plate with ***Pseudomonas Aeruginosa (PS)*** specific medium





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**	CetrimideAgar

Observation

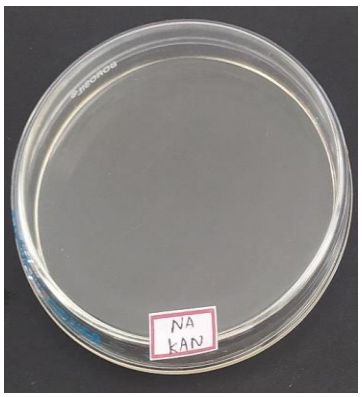
No growth was observed after incubation period. Reveals the absence of specific pathogen.

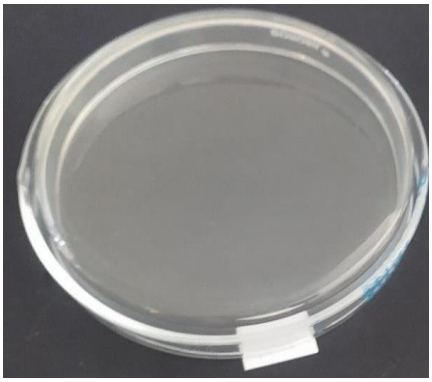
Result

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

Organism	Specification	Result	**Method**
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
StaphylococcusAureus	Absent	Absent	
PseudomonasAeruginosa	Absent	Absent	

STERILITY TEST BY POURPLATE 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 in occulates with the test sample.

Test	**Result**	**Specification**	**As per AYUSH/WHO**
TotalBacterial Count	**Absent**	NMT 10^5CFU/g	As per AYUSH specification
TotalFungal Count	**Absent**	NMT 10^3CFU/g	

Pesticide Residue Test Results

PesticideResidue I.Organo Chlorine Pesticides	Sample KAN	AYUSH Limit (mg/kg)
AlphaBHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
GammaBHC	BQL	0.1mg/kg
DeltaBHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	50 µg/kg	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III.Organocarbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

Below the Quentification Level.

RESULTS

The results showed that there were no traces of pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrthroids in the sample provided for analysis. Wheresthe samplerevelsthepresence ofmildtracesofmalathionat 50ug/kgwhich blongs to the category of Organo Phosphorus pesticide.

Aflotoxins Test Results

Aflatoxin	SampleKAN	AYUSHSpecificationLimit
B1	Not Detected-Absent	0.5ppm(0.5mg/kg)
B2	Not Detected-Absent	0.1ppm(0.1mg/kg)
G1	Not Detected-Absent	0.5ppm(0.5mg/kg)
G2	Not Detected-Absent	0.1ppm(0.1mg/kg)

RESULTS

The results shown that there were no spots were being identified in the test sample loaded on TLC plate when compare to the standard which indicates that the sample were free from aflotoxin B1, B2, G1, G2.

DISCUSSION.

Kadukkai Nei is viscous in nature, greasy yellowish colour, liquid form ghee based medicine. This character matched with ghee medicines criteria. Kadukkai Nei (KN) is soluble in chloroform and ethyl acetate and insoluble in water and DMSO. This nature reveals that it is a lipid based medicine. Analytical test values are viscosity at 50oC (Pa s) 82.40, refractive index 1.78, saponification value 173.40, acid value1.2903, peroxidase value 3.38 – all the parameters are analysed and submitted as per the guidelines. Kadukkai nei is subjected to TLC and HPTLC fingerprint test and the values and bio markers are documented. The peak and RF values (0.01 - 0.97) 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 was compared to that of the raw drugs of Kadukkai nei. The similarities in the peak values prove that which raw drug was responsible for the efficacy of the Kadukkai nei. KN 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 KN had pesticide content below the quantification level. The malathion organo - phosphorous pesticides present in traces. Aflatoxins were absent in the KN. All the parameters of the standardization technique in KN 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

According to the Siddha Classical literature, *Agathiyar vaithiya vallathi* - 600, the *Kadukkai Nei* was constructed using a traditional manner. It was also subjected to all standardizing processes and values were submitted. This study just covers the baseline. It supports the standards for purity and quality. In the future, more preclinical and clinical research is required to assess the prepared medicine's efficacy.

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