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Standardization of the Siddha Polyherbal Formulation *Kandangkathari Nei*

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Abstract: The aim of this study was to standardize the Siddha polyherbal formulation *kandankathari Nei* by following modern scientific quality control procedures for finished medicated ghee. *Kandankathiri Nei* was subjected to organoleptic evaluations, physiochemical evaluations, Biochemical evaluations. TLC, HPTLC analysis was carried out as per the PLIM guidelines and WHO guidelines to fix the quality standards of this drug. This study results a set diagnostic character essential for its standardization. The saponification value is 164.73 mh KOH/g neutralize the fatty acids resulting from complete hydrolysis of 1 g of sample. The iodine value is 96.52 mgI_2/g indicates that it is not too much hard in texture. The acid value was found to be 0.6171 mg KOH/g. The peroxide value is 3.6 meq/kg comparatively very low and long shelf-life period. The drug was free from specific pathogen, microbial contamination, heavy metals, aflatoxin and pesticide were below deductible limit. The values obtained after physiochemical parameters study showed that these values should be helpful to develop, new pharmacopeial standards. The physiochemical and phytochemical constituents found to be present in raw material used for preparations of *Kandankathari Nei* which facilitate the therapeutic efficacy of poly herbal medicinal formulation.

Keywords: *Kandankathiri Nei*, Standardization, Physiochemical analysis, Siddha medicine, Swasakasam

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Introduction

Siddha system of medicine is one such ancient traditional system of India and practiced mostly in southern India for treating various diseases including even chronic diseases. The raw material used in Siddha medicines are plant, metals, minerals and animal origin. Major formulation of siddha medicines is based on herbs. Siddha literatures are well provided with references on the use of herbs with medicinal properties which are very effective and have therapeutic value but there is lack of proper quality specifications and standardization. Siddhars also explain how a patient should be treated, great emphasis given to herbs by ancient siddhars. The preparations of majority of the medicines involve tedious process which results in physiochemical transformations

of particles size, chemical composition which regulates bio-mechanism of the drug in the body, thus enhances the efficacy and reduce toxicity.

Herbal drugs usually contain plenty of bacteria and fungi, often originating from soil and atmosphere, it generally contaminates the drugs and increases health hazard to human. The determination of *E. coli* and other enterobacteria moulds may indicate the quality of production. The presence of microbial contaminant in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicines. So, it is important to estimate the microbial contamination of the herbal products. Hence there is need for standardization of all herbal drugs to maintain their quality. Therefore, it is highly desirable that these drugs should be characterized with modern instruments, based on which the specifications of such drugs can be well standardized on a scientific basis.

Under the internal medicine, *Nei* is one of the easily palatable drug dosage form with the shelf life period of 6 months. *Kandankathiri Nei* is a form of medicated ghee which is easy to administer, assimiliates quickly and also nutritive.

In this study *Kandankathiri Nei* is used to treat the *kaba thodam*. It cures *swasakasam, Ulaimaanthai, Sunangu maanthai, shayam, Irumal, Salathodam*. Most of the ingredients of *Kandankathiri Nei* possess expectorant, tonic and stimulant and also have anti-inflammatory, anti-oxidant, anti-asthmatic activity.

The present study is aimed to standardize the organoleptic characters, physiochemical, and biochemical analysis of *Kandankathari Nei* based on PLIM guidelines, WHO guidelines. The outcome of this study may help to use a standard reference for further study.

Materials and Methods

The drug *Kandankathari Nei* was prepared in the Gunapadam laboratory of National Institute of Siddha, Chennai, Tamil Nadu, India. The standardization methods are carried out on standard research laboratory in Chennai, and Haryana, India.

Ingredients of Kandankathari Nei

Part I: Kandankathiri whole plant; Vilvam; Amukkara; Peipudal; Adathodai; Sangankuppi; Siruvazhuthalai**.**

Part II: Cow'milk; Cow's ghee; Poosanikai neer

Part III: Thalisapathri; Milagu; Seviyam; Elam; Lavangapattai; Lavangapathri; Thippili; Vilaamichuver; Sirunaagapoo; Sadamaanjil; Karkadagasingi; Pereechampazham; Thippili.

Identification of Raw drugs

The required drugs were purchased from Ramaswamy Chettiyar country drug shop, kandha Swamy Kovil Street Parrys, Chennai, India. Raw drugs were authenticated by the Medicinal Botanist in National Institute of Siddha, Chennai.

The test drug *Kandankathari Nei* was Purified and prepared at Gunapadam lab, National Institute of Siddha. Chennai, India.

Purification

1. *Kandankathiri, Vilvam* - Clean with pure white cloth and reomove debris .
2. *Adathoda, Sangankuppi, Siruvazhuthalai* – Clean with pure white cloth and remove the debris .
3. *Thalisapathri, Lavangapathri, Lavangapattai, Elam, Sadamanjil, Sirunaagapoo* - Clean and dried through the sunlight.
4. *Milagu* - Soaked in sour butter milk and then dreied.
5. *Thippili* - Soaked in *Kodiveli* leaf juice for one *naazhigai (24 minutes)* and dried through the sunlight .
6. *Vilaamichu ver , Peipudal* - Wash well with water then dried .
7. *Karkadakasingi* - Roasted in almond oil .
8. *Pereecham pazham* - Remove the inner seed.
9. *Amukkara* - It is dried and grinded into fine

powder. It is placed in a cloth which is tied above the pot containing milk and closed . After boiling for one *saamam* (3 hours) the powder is dreid, grinded and taken.

Preparation of medicated Ghee

The decoction was made from the part I ingredients. The ingredients of part II were added into the decoction. Part III ingredients were made as a paste by using cow's milk. The grinded paste was added to the above mixed combination. Then allowed to boil until it reached the correct texture and then Thippili, Pereechampazham added into the preparation. It is preserved in an air tight container.

Indication

Swasakasam, Ulaimanthai, Sunangumanthai, Shayam, Irumal, Salaodhodam

Dosage: 3 kazhanchu (15.3 g)

Standardization parameters (*Physiochemical Evaluation*)

Organoleptic characters

Colour, odour, taste and consistency of the drug were noted.

Physio-chemical parameters

All the physio-chemical parameters were carried out as per the methods mentioned in standard books. The parameters are as follows:

Determination of specific gravity: Fill the dry sp. gravity bottle with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50°C and hold for 30 min. Carefully wipe off any substance that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the sample and reference standard.

Determination of Weight per ml: Weight per ml was determined using the comparative weight calibration method, in which the weight of 1 ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation have been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

Determination of Refractive Index: Determination of RL was carried out using Refractometer.

Determination of Viscosity value: Viscosity determination has 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 pH: One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for 2 min, and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred into a 50 ml beaker and tested for pH using digital pH meter.

Determination of Iodine value: About 20 g of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 min. 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 min and refrigerated for an hour. 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 indicated end point. Repeated the above procedure without taking sample and noted the corresponding reading for blank titration.

Determination of saponification value: About 2 g of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added in the round bottomed flask. Repeated the same procedure without taking the sample for blank titration. Refluxed both sample and blank round bottomed flasks for 1 h. After reflux, allowed the round bottomed flasks to cool. Titrated the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicated the end point.

Determination of Acid Value: Accurately 5 g of 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. 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 colour 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)

Determination of Peroxide value: 5 g of the substance accurately weighed into a 250 ml glass-stoppered conical flask, added 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and added 0.5 ml of saturated potassium iodide solution. Allowed to stand for exactly 1 min, with occasional shaking, added 30 ml of water and titrated gradually with continuous and vigorous shaking, with 0.01 M sodium thiosulphate until the yellow colour almost disappeared. Added 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappeard ('a' ml). Repeated 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

High Performance Thin Layer Chromatography Analysis (*HPTLC*)

HPTLC method is a sophisticated and automated selection technique derived from TLC. Pre coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. HPTLC is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. It offers high degree of selectivity, sensitivity and rapidity combined with single step sample preparation. In addition, it is a reliable method for the quantitation of nano grams level of samples. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprints of phytochemicals which is suitable for confirming the identity and purity of medicinal plant raw materials.

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 elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint ewes developed for the detection of Phyto constituents present in each extract and Rf value were tabulated.

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 was used to spot the sample for TLC applied sample volume 10 µl by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent

system [Toluene: Ethyl Acetate: Acetic Acid (1.5:1:0.5)]. After the run plates are dried it was observed using visible light Short-wave UV light 254 nm and light long-wave UV light 365 nm.

Test for Heavy metal 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. Determination of heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test sample was performed.

Standard preparation: Standard Hg, As, Pb and Cd was obtained from Sigma. Standard were prepared -- As and Hg- 100 ppm sample in 1 mol/l HCl; Cd and Pb- 100 ppm sample in 1mol/l HNO_3.

Sterility test by pour plate method

The pour plate techniques were adopted to determine the sterility of the product. Contaminated/unsterile 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: About 1ml of the 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 min). Plates were then inverted and incubated at 37°C for 24-48 h. Grown colonies of organism was then counted and calculated for CFU.

Test for specific pathogen

Methodology: 0.5 ml of the test sample was directly inoculated into the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 – 72 h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media (Table 1).

Test for Organochlorine pesticide, organophosphorus pesticide and pyrethroids

About 10 g of test substance 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 added a few millilitres of toluene R and heated again until the acetone is completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter.

Aflatoxin Assay by TLC

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 allowed 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 was moved not less than 15 cm from the origin. Removed the plate from the developing chamber, marked the solvent from and allowed the plate to air-dry. Located the spots on the plate by examination under UV light at 365 nm.

Results and Discussion

Organoleptic Evaluation

Table 2 illustrates the organoleptic characteristics of *Kandankathari Nei.*

Physiochemical parameters

The viscosity (the time required for a given

Table 1: Detail of specific organism 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*

Table 2: Organoleptic characters

State	Semisolid
Nature	Slightly Viscous
Odour	Mild Characteristic
Touch	Greasy
Flow Property	Non free flowing
Appearance	Yellowish

volume of liquid) at 50 c/pas was 84.87 (Table 3). The refractive index was found to be 1.62. The index of the weight per ml was 0.8333 The saponification value was 164.73 mh KOH/g neutralize the fatty acids resulting from complete hydrolysis of 1 g of sample. The iodine value was 96.52 mgI_2/g indicates that it is not too much hard in texture. The acid value was 0.6171mg KOH/g. The peroxide value was 3.6 meq/kg comparatively very low and long shelf-life period.

HPTLC / TLC Analysis

HPTLC finger printing revealed the presence of eight prominent peaks corresponds to presence of eight versatile phytocomponents present with in it. Rf value of peaks was from 0.05 to 0.99 (Table 4). Further the peak 1 occupies the major percentage of area 40.10% and peak 5 occupies the second major percentage of area 20.63% which denotes abundant existence of such compound (Figs. 1, 2).

Heavy metal/pescticide analysis

Table 5 depicts the analysis of heavy metals. Pesticide residue analysis is shown in Table 6.

Test for microbial contamination

No growth after incubation period reveals the absence of specific pathogen (Table 7). No growth/colonies was observed in any of the plates inoculates with the test sample.

Test for specific pathogen

No growth was observed after incubation period. Reveals the absence of specific pathogen (Table 8). No growth/colonies were observed in any of the plates inoculated with the test *sample* (Table 9).

The present preclinoical study of *Kandankathiri Nei* was standardized as per Siddha formulations. It is a greenish-brown, semi-solid preparation with a mild characteristic odor. Its viscosity at 50 c/pas is 84.87, and the refractive index of 1.62 confirms purity without adulteration. The specific gravity is 0.068 g/ml. The iodine value of 96.52 mg I_2/g indicates moderate texture and a high content of polyunsaturated fatty acids (PUFA), which

Table 3: Physio-Chemical Evaluation

S. No.	Parameter	*KKN*
1	Viscosity at 50°C (Pa s)	84.87
2	Refractive index	1.62
3	Weight per ml (g/ml)	0.833
4	Iodine value (mg I2/g)	96.52
5	Saponification Value (mg of KOH to saponify 1g of fat)	164.93
6	Acid Value (mg KOH/g)	0.6171
7	Peroxidase Value (Eq/kg)	3.6
8	Free Fatty acid value	0.22 %
9	Mineral Oil Test	Absent
10	Total fatty matter	98.79%

Table 4: HPTLC Peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	11.5	0.03	221.2	61.89	0.04	4.7	2090.1	48.10
2	0.05	15.7	0.05	24.8	6.94	0.08	0.1	216.3	4.98
3	0.11	0.6	0.13	20.1	5.62	0.15	0.9	207.0	4.76
4	0.20	2.4	0.23	12.1	3.39	0.26	3.8	198.5	4.57
5	0.36	5.9	0.40	34.6	9.68	0.45	13.3	896.3	20.63
6	0.85	7.9	0.87	14.8	4.15	0.90	3.4	221.0	5.09
7	0.90	4.2	0.94	16.7	4.69	0.96	10.6	347.8	8.00
8	0.99	8.2	0.99	13.0	3.65	1.03	0.2	168.5	3.88

Fig. 1: HPTLC Analysis.

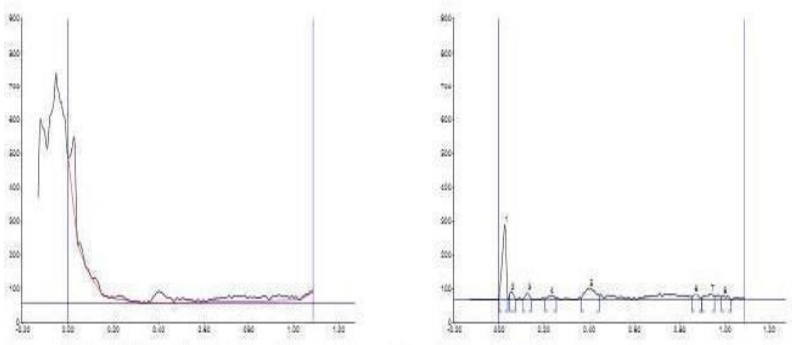

Fig. 2: HPTLC finger printing.

Table 5: Heavy metals/toxic metals

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	8.703	10 ppm
Arsenic	193.7 nm	0.902	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.513	1 ppm

Table 6: Pesticide residue results

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

Table 7: microbial contamination report

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	

Table 8: specific pathogen results

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

Table 9: Aflatoxins report

Aflatoxin	Sample KKN	AYUSH Specification Limit
B1	0.01 mg/kg	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)

may help reduce LDL cholesterol levels. The saponification value (164.93 mg KOH/g) suggests a high proportion of short-chain fatty acids (SCFA), beneficial for colonic health and easy digestibility. The acid value (0.6171 mg KOH/g) and low peroxide value (2.325 meq/kg) imply minimal free fatty acid content and prolonged shelf life. The preparation contains no mineral oil, with total fatty matter at 98.79%.

HPTLC fingerprinting identified eight major phytocomponents with Rf values ranging from 0.05 to 0.99; peak 1 (40.10%) and peak 5 (20.63%) represent the dominant compounds.

Microbial analysis confirmed sterility and the absence of pathogens, including *Salmonella*, *Staphylococcus aureus*, *E. coli,* and *Pseudomonas aeruginosa*, as well as pesticide residues.

Heavy metal testing showed no detectable mercury or arsenic, and lead and cadmium were below quantification limits. Aflatoxins (B1, B2, G1, G2) were absent, confirming the safety and purity of *Kandankathiri Nei*.

Conclusion

The results obtained from standardization parameters of the siddha poly herbal drug formulation *Kandankathiri Nei* ensures the identity, purity and quality of the prepared medicine. The study drug has anti-inflammatory

activity and anti-asthmatic activity. The results can be used as reference for further researches and clinical trials.

Ethical Statement

No animal has been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Each author has contributed equally in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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