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STANDARDIZATION OF THE DRUG CHUKKU NEI – A SIDDHA POLYHERBAL FORMULATION

Dr. M. Lavanya*[1], K. Suresh[2], M. Meenakshi Sundaram[3] and V. Banumathi[4]

[1]PG Scholar, Dept. of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai 47.
[2]Lecturer, Dept. of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai 47.
[3]HOD, Dept. of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai 47.
[4]Director, National Institute of Siddha, Chennai 47.

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***Corresponding Author**
Dr. M. Lavanya
PG Scholar, Dept. of Kuzhandhai Maruthuvam, National Institute of Siddha, Chennai 47.

ABSTRACT

The Aim of this study was to standardize the Siddha formulation-Chukku nei by following modern scientific quality control procedures both for the raw material and the finished product. Chukku nei was subjected to macro-microscopic, Physicochemical, preliminary phytochemical, TLC and HPTLC analysis was carried out as per the WHO guidelines to fix the quality standards of this drug. This study results a set of diagnostic characters essential for its standardization. The iodine value is 172.08mgI_2/g indicates it is not too much hard in texture. The saponification value is 262.43 mh KOH/g to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of sample. The pH is found to be 4 that is weakly acidic. The acid value was found to be 3.104 mg KOH/g, the peroxide value is 2.325meq/kg comparatively very low and has long shelf life. The drug was free of from specific pathogen, microbial contamination, heavy metals, aflotoxin and pesticide were below the deductible limit. The values obtained after physicochemical parameters study showed that these values should be helpful to develop new pharmacopoeial standards. The physicochemical and phytochemical constituents found to be present in raw material is used for the preparation of Chukku nei possibly facilitate the desirable therapeutic efficacy of the medicinal formulation.

KEYWORDS: Chukku nei, Physicochemical analysis, Standardization, Siddha medicine.

INTRODUCTION

Siddha is the oldest healing system of medicine and it has fundamental aspects for drug formulation. Major formulations used in Siddha are based on herbs. Our herbal medicines are devoid of serious adverse effects commonly associated with synthetic drugs. Many plant products plays an important role in various diseases. Herbal medicines have often retained popularity for historical and cultural reasons. Since the usage of these herbal medicines has increased. siddha literatures are well provided with references on the use of herbs with medicinal properties are very effective and have therapeutic value in nature but there is a lack of proper quality specifications and standardization .Herbal drugs usually contain plenty of bacteria and fungi, often originating from soil and atmosphere, it generally contaminate the drugs and increases health hazard to human. The determination of E. coli and other entero bacteria 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.

The present study deals with the standardization of polyherbal siddha formulation Chuku nei is mentioned in the classical siddha text book felected drug Chukku nei is a classical siddha formulation is used in the treatment of kattu mantham.

There are no systemic protocols for standardization of Chukku nei hence, it was decided to evaluate the qualitative and quantitative analysis scientifically. For the standardization of this drug Organoleptic, Physico-chemical, phytochemical, HPTLC and TLC were also prepared to evaluate its quality.

2. MATERIALS AND METHODS

The drug Chukku Nei was prepared in the Gunapadam laboratory of National Institute of Siddha, Chennai, Tamilnadu, India. The standardization methods are carried out on standard research laboratory in Chennai, India.

Ingredients of chukku nei

- Chukku (Zingiber officinale)
- Citrarathai (Alpinia officinarum)
- Vetrilai (piper betel)
- Omum (Trachyspermum ammi)
- Murunga pattai (Moringa oleifera)
- Vembu nei (Azadirachta indica)

2.1. Identification of Raw drugs

The required drugs were purchased from Ramaswamy Chettiyar country drug shop, kandha swamy kovil street Paris, Chennai. Raw drugs were authenticated by the Medicinal Botanist in National Institute of Siddha, Chennai. The test drug Chukku nei was Purified and prepared at Gunapadam lab, National Institute of Siddha. Chennai-47.

Purification Method

- Arathai - Remove the outer layer,cut into small pieces and dry it under shadow
- Omum - Soaked in limestone water and dry it
- Murungapattai - Clean and remove the outer layer
- Chukku - Double amount of limestone will be added with an amount of chukku fried for a period of 3 hours after that wash it, remove the outer layer and dry it
- Vembu nei - It will be boiled with equal quantity of neem bark decoction.

2.2. Preparation of the drug

All the ingredients except vembu nei were taken and grind with sufficient quantity of water and make it as surasam (325 ml) then vembu nei (325ml) will be mixed together and boiled and filtered it.

2.3. Organoleptic characters

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

2.4. Physico-chemical parameters

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

2.4.1. 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 $50^{O}C$ 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.

2.4.2. 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.

2.4.3. Determination of Refractive Index

Determination of RL was carried out using Refractometer.

2.4.4. Determination of Viscosity value

Viscosity determination were 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.

2.4.5. 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 two minutes and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transfered in to a 50 ml beaker and tested for pH using digital pH meter .

2.4.6. Determination of Iodine value

About 20 gm 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.

2.4.7. Determination of saponification value

About 2 gm 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. Repeatthe same procedure with out 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.

2.4.8. 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 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)

2.4.9. Determination of Peroxide value

5 g 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.

Calculation: Peroxide value = 10(a-b)/w

2.5. Preliminary phytochemical tests

Chukku nei was extracted with ethanol and the extracted was subjected to following analysis as per the following guidelines.

2.5.1. 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.

2.5.2. 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.

2.5.3. 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.

2.5.4. 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.

2.5.5. 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.

2.5.6. 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.

2.5.7. 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.

2.5.8. 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.

2.5.9. 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.

2.5.10. Test for Cyanins

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.

2.5.11. 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.

2.5.12. 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.

2.6. 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 finger prints of phytochemicals which is suitable for confirming the identity 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, platees 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 finger print ewas developed for the detection of phyto constituents present in each extract and Rf value were tabulated.

2.7. 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 different solvent system Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5). 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.

2.8. Flourescent analysis

Fluorescence analysis in dried powder

Sample was subjected to fluorescence analysis under visible light and UV – Light at 365 nm under closed circuit cabinet. Each fluorescence characteristic of the treated sample was observed underordinary light and then under UV light of wave lengths 365 nm. The drug was treated with acids viz., Conc. HCl, Conc. H2SO4, Conc. HNO3 and glacial acetic acid. The drug was treated with alkaline solutions viz., aqueous NaOH and ferric chloride. They were subjected to fluorescence analysis in visible light and in short UV- light (254 nm) and long UV- light (365 nm.

2.9. Test for Heavy metal Analysis

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 KN 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 sample.

Sample Digestion

Test sample 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 reparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO_3

2.10. Sterlity 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

About 1ml of the 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. Grown colonies of organism was then counted and calculated for CFU.

2.11. Test for specific pathogen

Methodology

0.5 ml of the 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.

2.12. Test for Organochlorine pesticide, organophosphorous pesticide and pyrethroids

About 10 g of test substance were extracted with 100 ml of acetone and followed byhomogenization 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 R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

2.13. Aflotoxin Assay by TLC

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.

Test solution: Concentration 1 µg per ml

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.

3. RESULTS

3.1. Organoleptic characters

The drug chukku nei was a liquid reddish brown in colour with strong odour, bitter and slightly sweet in taste (figure 1). The inference are tabulated in table 1.

Fig. 1: ***Prepared drug.***

Table 1: ***Organoleptic characters.***

State	Liquid
Appearance	Reddish Brown
Nature	Viscous Liquid
Odor	Strong Characteristic

3.2. Physicochemical parameters

The specific gravity of the drug is 0.9471**.** Viscosity (the time required for a given volume of liquid) at 50 c/pas is 33.019. The refractive index is found to be 1.46. The index of weight per ml is 0.068 gm/ml. The iodine value is 172.08mgI_2/g indicates it is not too much hard in texture. The saponification value is 262.43 mh KOH/g to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of sample. The pH is found to be 4 that is weakly acidic and safe in pH. The Acid value is used to quantify the substance, which is an index of free fatty acid content due to enzymatic activity. The acid value of chukku nei was found to be 3.104 mg KOH/g. the peroxide value is 2.325meq/kg and it is comparatively very low. Therefore, it is cleared that the oil has long shelf life. The observed results were tabulated in table 2.

Table 2: ***Physicochemical characters of Chukku nei.***

S. No.	Parameters	Results
1	Specific gravity	0.9471
2	Viscosity at 50c (pa s)	33.019
3	Refractive index	1.46
4	Weight per ml (gm/ml)	0.068 gm/ml
5	Iodine value (mg I2/g)	172.08
6	Saponification value (mh of KOH to saponify 1gm of fat)	262.43
7	PH	4
8	Acid value mg KOH/g	3.104
9	Peroxidase value mEq/kg	2.325

3.3. Preliminary phytochemical analysis

The drug has high polar secondary metabolites like Alkaloids, Flavonoids, Steroids, Triterpenoids, Saponins, Beta cyanin are shown in table 3.

Table 3:

S. No.	Test	Observation
1	Alkaloids	+
2	Flavanoids	+
3	Glycosides	-
4	Steroids	+
5	Triterpenoids	+
6	Coumarin	-
7	Phenol	-
8	Tanin	-
9	Protein	-
10	Saponins	+
11	Sugar	-
12	Anthocyanin	-
13	Betacyanin	+

3.4. High Performance Thin Layer Chromatography

HPTLC finger printing analysis of the sample Chukku nei reveals the presence of ten prominent peaks corresponds to presence of ten versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.09 to 0.91. Further the peak 4 occupies the major percentage of area of 30.12% which denotes the abundant existence of such compound. Followed by this peak 2 and 7 occupies the percentage area of 19.64 and 14.61%. fig 2,3.

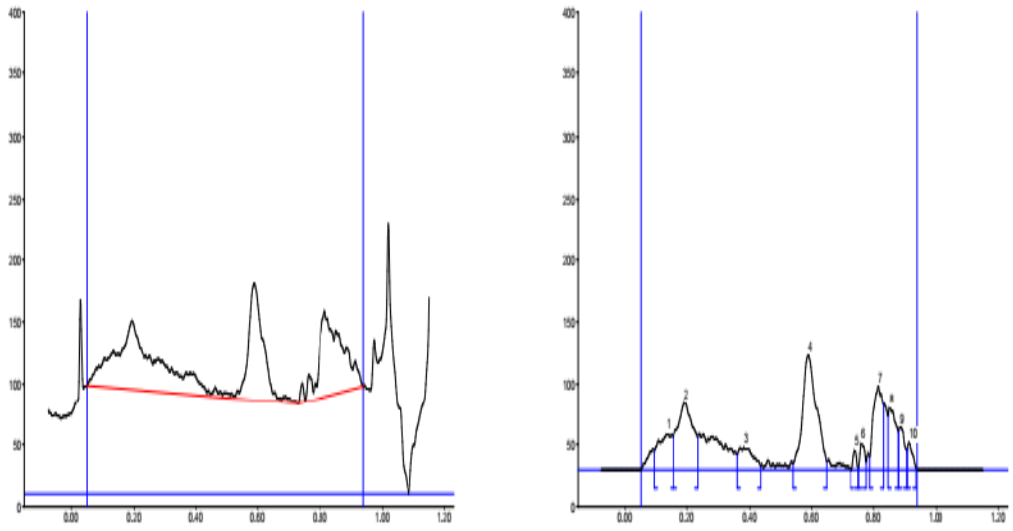

Fig. 2: ***HPTLC finger print.***

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	15.6	0.13	29.8	7.21	0.16	26.4	1030.9	9.86
2	0.16	27.4	0.19	55.2	13.36	0.24	26.6	2053.7	19.64
3	0.36	12.8	0.38	18.1	4.38	0.44	2.9	649.1	6.21
4	0.54	5.3	0.59	94.2	22.80	0.65	6.8	3150.1	30.12
5	0.73	0.3	0.74	16.4	3.97	0.75	1.4	126.6	1.21
6	0.75	2.0	0.76	21.8	5.27	0.78	6.3	239.9	2.29
7	0.79	10.5	0.82	69.5	16.83	0.83	53.2	1527.7	14.61
8	0.85	43.4	0.85	50.8	12.29	0.88	31.1	935.9	8.95
9	0.88	31.5	0.89	35.2	8.52	0.91	16.2	487.2	4.66
10	0.91	17.2	0.92	22.1	5.36	0.94	1.9	257.6	2.46

HPTLC high peak table

Fig. 3: TLC Analysis at 254 nm. TLC Analysis at 366 nm.

3.5. Fluorescence Analysis

Table 4: fluorescence report.

S. No.	Experiment	Visible light	Short UV – Light 254 nm	Long UV – Light 365 nm
1	Sample + Conc. Hcl	Yellow	Yellow	Fluorescent Yellow
2	Sample + Conc. Sulphuric Acid	Greenish brown	Dark reddish brown	Brown
3	Sample + Conc. Nitric acid	Creamy white	Creamy yellow	Fluorescent yellow
4	Sample + Sodium hydroxide in water	Whitish yellow	Fluorescent yellow	Fluorescent yellow
5	Sample + Ferric chloride	Brownish orange	Fluorescent green	Orange
6	Sample + glacial acetic acid	Creamy white	Lime Yellow	Yellow

3.6. Heavy metal analysis

Results of the present investigation has clearly shows that Chukku nei has no traces of Mercury and Arsenic. The level of lead and cadmium was found to be 0.010 ppm and 0.005 ppm. Reported heavy metals (lead and cadmium) seems very low when compare to the allowed recommended limit. The result were shown in table 5.

Table 5: Heavy metal analysis result.

Name of the heavy metal	Absorption Max	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1 PPM
Lead	217.0 nm	0.010PPM	10 PPM
Arsenic	193.7 nm	BDL	3 PPM
Cadmium	228.8 nm	0.005 PPM	0.3 PPM

3.7. Sterlity Test by pour plate method

No growth / colonies were observed in any of the plates inoculates with the test sample shown in table 6.

Table 6: Sterlity 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	

3.8. Test for specific pathogen

No growth was observed after incubation period. Reveals the absence of specific pathogen are shown in table 7.

Table 7: *Specific pathogen report.*

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

3.9. Analysis of organochlorine pesticide, organophosphorus pesticide and pyrethroid

The results showed that there were no traces of pesticides residues such as Organo chlorine and Organo phosphorus Pesticides in the sample CN. Further sample shows the presence of Cypermethrin belongs to pyrethroid type of pesticide at the concentration of 0.1 mg/kg which was low when compare the AYUSH prescribed limit of 1mg/kg. Table 8.

Table 8: *Pesticide residue report.*

Pesticide residue I. Organo Chlorine Pesticide	Sample PPC	AYUSH Limit (mg/kg)
Alpha BHC	BQL	0.1
Beta BHC	BQL	0.1
Gamma BHC	BQL	0.1
Delta BHC	BQL	1
DDT	BQL	3
Endosulphan	BQL	

II. Organo Phosphorous Pesticide		
Malathion	BQL	1
Chlorpyriphos	BQL	0.2
Dichlorovos	BQL	1
III. Pyrethroid		
Cypermethrin	0.1 mg/kg	1

BQL – Below Quantification Limit

3.10. Aflotoxin assay by TLC (B1,B2,G1,G2)

The results shown that there was no spots were been identified in the test sample loaded TLC plated when compare to the standard indicates that he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 were shown in table 9.

Table 9: ***Aflotoxin report.***

Aflotoxin	Sample	Ayush Specification
B1	Not Detected- Absent	0.5 ppm
B2	Not Detected- Absent	0.1 ppm
G1	Not Detected- Absent	0.5ppm
G2	Not Detected- Absent	0.1ppm

DISCUSSION

From this study of preclinical standardization of Chukku nei mentioned in siddha text book shows that the drug was liquid, reddish brown in clour with strong odour, slightly sweet in taste. The specific gravity of the drug is 0.9471**.** Viscosity (the time required for a given volume of liquid) at 50 c/pas is 33.019. The refractive index is found to be 1.46. The index of weight per ml is 0.068 gm/ml. The iodine value is 172.08mgI_2/g indicates it is not too much hard in texture. The saponification value is 262.43 mh KOH/g to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of sample. The pH is found to be 4 that is weakly acidic and safe in pH. The Acid value is used to quantify the substance, which is an index of free fatty acid content due to enzymatic activity. The acid value of chukku nei was found to be 3.104 mg KOH/g. the peroxide value is 2.325meq/kg and it is comparatively very low. Therefore, it is cleared that the oil has long shelf life. HPTLC finger printing reveals the presence of ten prominent peaks corresponds to presence of ten versatile phytocomponents present with in it. This study also reveals that the drug was sterile and free of bacteria, fungi and specific pathogen like Salmonella, Staphylococcus aureus, E.Coli, Pseudomonos aeruginosa and pesticide residues. In heavy metal analysis there is no traces of Mercury and Arsenic. lead and cadmium seems very low when compare to the allowed recommended limit. There was no spots of Aflotoxin. like B1,B2,G1,G2.

CONCLUSION

The obtained results of pre clinical standardization of chukku nei such as physico-chemical parameters, preliminary phytochemicals analysis, TLC photo documentation, HPTLC finger print profiling at UV 254 nm provide valuable information. The parameters are sufficient to standardize Chukku nei. As a result Chukku nei was proved its safety over the defined standardization method. The results obtained could be utilized as reference for developing standard formulation of great efficacy.

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