International Journal of Reverse Pharmacology and Health Research (IJRPHR)

Research article

Standardization of Siddha drug formulation Nayuruvi Nei for the management of Kokki Puzhu (Kudal Kirumi)

***Ajay M[1], Sindhu V[1], Soundararajan DK[2], Shyamala K[3]**

[1*] PG Scholar, Department of Kuzhanthai maruthuvam, [2] Head of the Department, Department of Kuzhanthai Maruthuvam,

[3] Lecturer, Department of Kuzhanthai Maruthuvam, Government Siddha Medical College, Palayamkottai., Tirunelveli.

ABSTRACT

Siddha system of Medicine is one of the most Ancient Medical systems of India. The Siddha system based on a combination of Ancient Medical Practices and Spiritual Disciplines. According to our Siddha Medicine Diseases are classified as 4448 in numbers and in that 100 of them comes under Pediatric Diseases. *Nayuruvi Nei* is a poly herbal formula in siddha is used to treat *Kudal Kirumi (Kokki Puzhu)*-Worm infestation in children, which is mentioned in Book of Siddhar Maruthuvam Part 7. Therefore is a need to analyze the drug as per latest technique. Data were collected and prepared drug sent to Noble Research Solution for evaluating the biochemical, phytochemical HPTLC Analysis and sterility as per PLIM guidelines. This paper describes identifying Biochemical, HPTLC Analysis, Phytochemical analysis and Sterility test properties respectively. Further studies should be done in future.

Keywords:

Biochemical Analysis, Kudal Kirumi ,Nayuruvi Nei, Phytochemical, Sterility, Siddha System

Address for correspondence:

Ajay M

PG Scholar

CODEN : IJRPHR

To access this article online

Website : http://www.ijrphr.com/

DOI : 10.121/ijrphr/05.0101.523

Quick response code

INTRODUCTION

Siddha system of Medicine is one of the most Ancient Medical systems of India. The Siddha system based on a combination of Ancient Medical Practices and Spiritual Disciplines. According to our Siddha Medicine, Diseases are classified as 4448 in numbers and in that 100 of them comes under Pediatric Diseases.

There are 32 types of Internal and 32 types of External Medicines to treat these type of disease. Among this *Nei* is also a well-known type of Medicine. ***Nayuruvi nei*** is a Polyherbal formulation used for the Management of ***Kokki puzhu (kudal kirumi).***

Kokki puzhu (kudal kirumi) is characterised by Loss of appetite, Gastric distension, Perianal itching, Tastelessness, Diarrhoea or Constipation, Wheezing, Coughing, Fatigue, Anemia and Malnutrition. The major causes for ***kokki puzhu (kudal kirumi)*** are as follows conduct with an infected surface such as soil containing eggs or germs at a playground or touching pets infected food with worms, improper hygiene and improper hand washing, thumb sucking, nail biting, pica which causes worm infestation and so on. The two most common types of hook worm that infect humans are *Ancylostoma duodenale* and *Necator americanus*. ***Nayuruvi nei*** is used for treating these symptoms and this *nei* has been prepared under the strict standards and parameters given by PLIM Guidelines.

This article can also be used as an initiative for research areas for identifying Biochemical, HPTLC Analysis, Phytochemical analysis and Sterility test properties respectively.

MATERIALS AND METHODS

Drug Selection:

The siddha formulation drug ***Nayuruvi nei*** selected from the ***Siddha Maruthuvam part-7 (Kuzhanthai maruthuvam)*** and this medication is indicated for treating worm infestation called ***Kokki Puzhu* (*kudal kirumi*).**

Ingredients of *Nayuruvi nei:*

This poly herbal formulation contains plant leaf, raw drugs and the ingredients of the drug and its quantity are listed below Table 1

Table 1 Ingredients of Nayuruvi nei

S.NO	NAME	BOTANICAL NAME	FAMILY	PART USED	QUANTITY
1.	*Nayuruvi ilai*	*Achyranthes aspera*	Amaranthaceae	Leaf	3gm
2.	*Kuppaimeni ilai*	*Acalyphaindica*	Euphorbiaceae	Leaf	3gm
3.	*Thuthuvalai ilai*	*Solanum trilobatum*	Solanaceae	Leaf	3gm
4.	*Vasambu*	*Acorus calamus*	Acoraceae	Rhizome	3gm
5.	*Chukku*	*Zingiber officinale*	Zingiberaceae	Rhizome	3gm
6.	*Manjal*	*Curcuma longa*	Zingiberaceae	Rhizome	3gm
7.	*Pasu Nei*	*Cow ghee*			335 ml

COLLECTION OF RAW DRUG AND PLANT:

The raw drugs were brought from a well reputed raw drug store in Tirunelveli town. The plants were collected in our native place Tirunelveli.

IDENTIFICATION AND AUTHENDICATION OF THE DRUG:

The raw drugs and plants herbarium were identified and authenticated by the Head of the Department of Post graduate department of Gunapadam, Government Siddha Medical College, Palayamkottai. The sample of each raw drug is stored in the PG Department of Gunapadam for the future reference.

PURIFICATION OF THE RAW DRUG AND PLANTS:

Purification of raw drugs and plants were done as per classical Siddha literature.

PREPARATION OF THE TRIAL COMPOUND DRUG NAYURUVI NEI:

1-6 are grinding with water. It is then mixed with cow ghee in a vessel and is heated. Once the ghee attains "*MEZHUGU*" consistency, the heating should be stopped and *nei* is stored in glass container.

ADMINISTRATION OF THE DRUG:

Form of the Medicine: *Nei*

Route of Administration: Oral

Dose: 1.5-3ml (twice a day)

Adjuvant: Hot water

Indication: ***Kokki puzhu (Kudal kirumi)***

HPTLC ANALYSIS OF *NAYURUVI NEI*:

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 a ssessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

BIOCHEMICAL ANALYSIS OF *NAYURUVI NEI*

The Phytochemical screening analysis was carried out for the extract of *Nayuruvi nei* as per the standard procedure by the experts of Biochemistry Department of Government Siddha Medical College, Palayamkottai.

Preparation of the Extract

5 grams of the drug was weighed accurately and placed in a 250 ml clean beaker. Then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and then it is made to 100 ml with distilled water. This fluid is taken for analysis.

Test for Calcium

2ml of the above prepared extract is taken in a clean test tube. To this add 2 ml of 4% Ammonium oxalate solution. No white precipitate is formed absence of calcium.

Test for Sulphate

2 ml of the extract is added to 5% Barium chloride solution. Formation of White colored precipitate indicates the presence of Sulphate.

Test for chloride

The extract is treated with Silver nitrate solution. Formation of White colored precipitate indicates the presence of chloride.

Test for Carbonate

The substance is treated with concentrated HCL. No brisk effervescence is formed Absence of Carbonate.

Test for Starch

The extract is added with weak iodine solution. formation of Blue color indicates the presence of Starch.

Test for Ferric Iron

The extract is acidified with Glacial acidic acid and potassium ferrocyanide. No Blue color is formed Absence of ferric iron.

Test for Ferrous Iron

The extract is treated with Concentrated Nitric acid and Ammonium thiocyanate solution. No Blood red color is formed Absence of ferrous iron.

Test for Phosphate

The extract is treated with Ammonium molybdate and concentrated nitric acid. No Yellow color precipitate is formed Absence of Phosphate.

Test for Albumin

The extract is treated with Eshbach's reagent. No Yellow color precipitate is formed Absence of Albumin.

Test for Tannic Acid

The extract is treated with Ferric Chloride. Formation of Blue- black colored indicates the presence of Tannic acid.

Test for Unsaturation

Baeyer's test Potassium permanganate solution is added to the extract. If it gets decolorized, it indicates the presence of unsaturated compounds.

Test for Reducing Sugar

5 ml of Benedicts qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8-10 drops of the extract again boil it for 2 minutes. No colour change occurs absence of Reducing sugar.

Test for Amino acid

One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% Ninhydrin is sprayed over the filter paper and again dried. Formation of violet colour indicates the presence of amino acid.

Test for Zinc

The extract is treated with Potassium Ferrocyanide. No white colored precipitate Absence of Zinc.

PHYTOCHEMICAL ANALYSIS

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and

the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

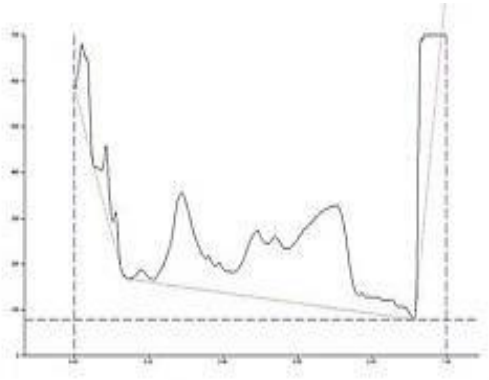
Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.



Test for Cyanins

A. Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

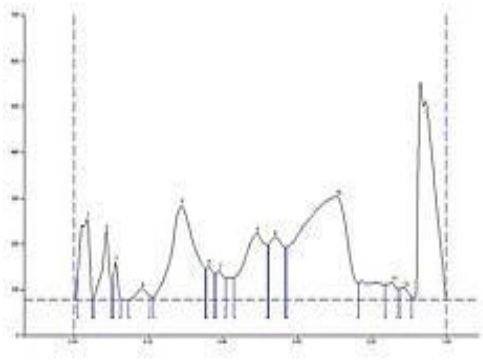
Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

STERILITY TEST BY POUR PLATE METHOD

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).

Researcher's study method is 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.



RESULTS AND DISCUSSION

HPTLC finger printing of Sample NN

Peak table 2

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	53.6	0.03	177.1	13.12	0.05	3.6	2896.7	6.49
2	0.05	1.9	0.09	149.6	11.08	0.10	34.7	1843.7	4.13
3	0.10	35.0	0.11	81.6	6.05	0.13	1.0	617.0	1.38
4	0.15	0.2	0.18	24.7	1.83	0.20	8.0	429.8	0.96
5	0.21	6.8	0.29	205.6	15.23	0.35	67.7	8647.7	19.39
6	0.36	69.0	0.36	74.9	5.55	0.38	55.8	860.8	1.93
7	0.38	57.3	0.39	64.2	4.76	0.41	46.7	949.0	2.13
8	0.43	48.9	0.49	145.4	10.77	0.52	120.4	5556.9	12.46
9	0.52	120.8	0.54	138.0	10.22	0.57	115.0	3551.6	7.96
10	0.57	115.5	0.71	225.7	16.72	0.76	37.0	18190.2	40.78
11	0.84	33.5	0.86	37.2	2.75	0.87	23.9	681.7	1.53
12	0.88	24.8	0.89	25.9	1.92	0.91	7.5	376.9	0.84

REPORT

HPTLC finger printing analysis of the sample reveals the presence of twelve prominent peaks corresponds to presence of twelve versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.01 to 0.88.

BIOCHEMICAL ANALYSIS:

The Biochemical analysis of ***Nayuruvi nei*** reveals the presence of **Sulphate, Chloride, Starch, Tannic acid, Unsaturated Compound and Amino acid.**

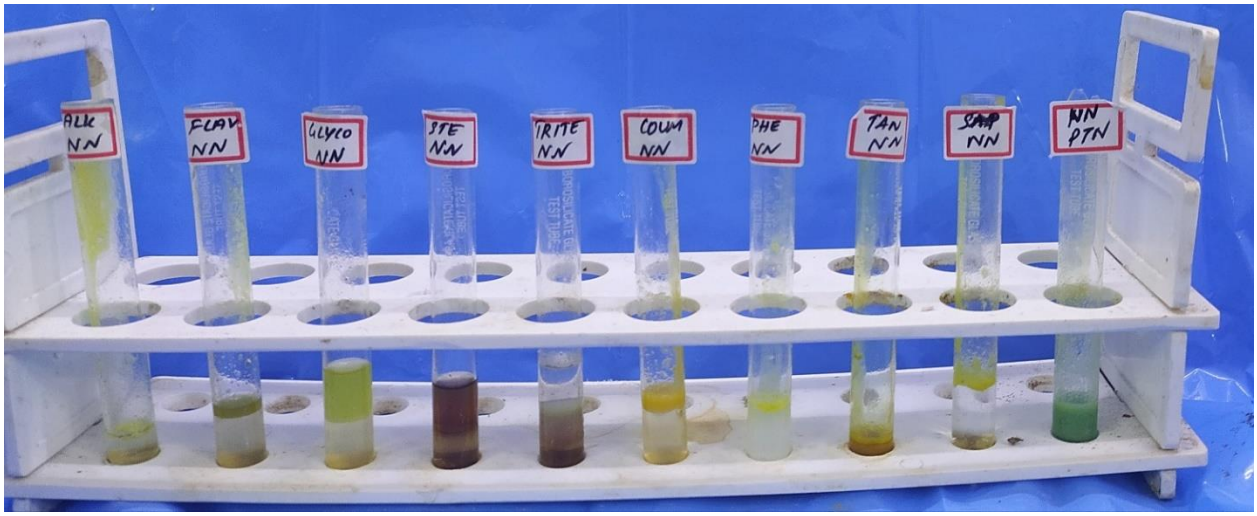
Table 3 Biochemical analysis

S.NO	BIOCHEMICALS	RESULT
1.	Calcium	Absent
2.	**Sulphate**	**Present**
3.	**Chloride**	**Present**
4.	Carbonate	Absent
5.	**Starch**	**Present**
6.	Ferric iron	Absent
7.	Ferrous Iron	Absent
8.	Phosphate	Absent
9.	Albumin	Absent
10.	**Tannic Acid**	**Present**
11.	**Unsaturated compounds**	**Present**
12.	Reducing sugar	Absent
13.	**Amino acid**	**Present**
14.	Zinc	Absent

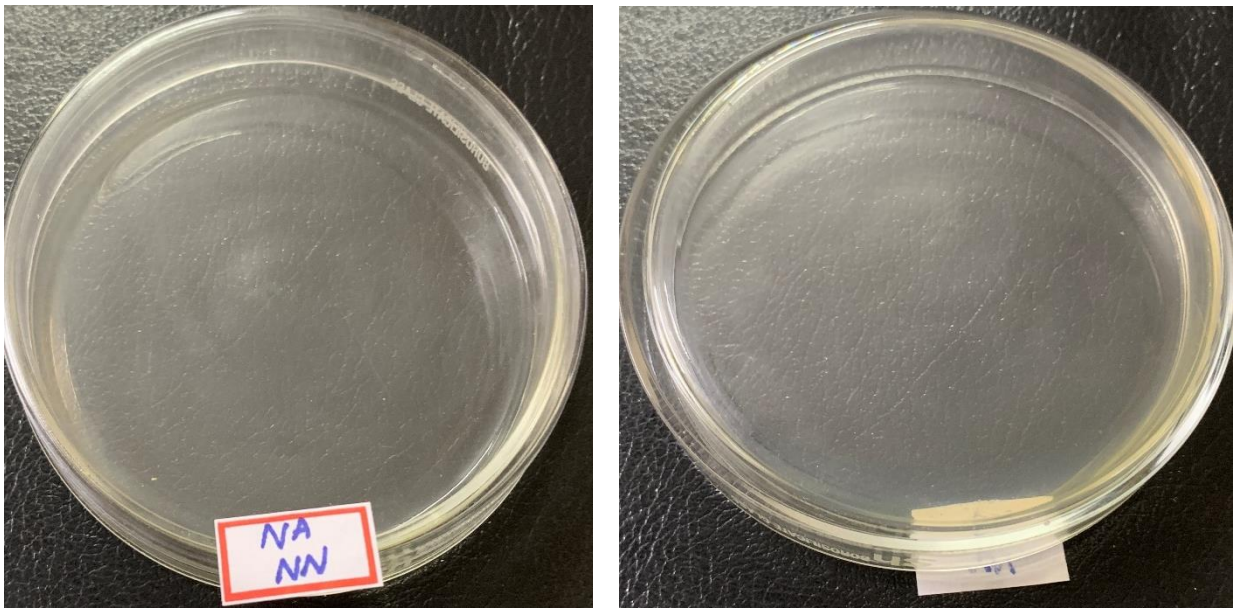
Table 4 Qualitative Phytochemical Investigation

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	-

+ -> Indicates Positive and - -> Indicates Negative



STERILITY TEST BY POUR PLATE METHOD



No growth / colonies was observed in any of the plates inoculates with the test sample.

Table 5. Total bacterial count test

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	

CONCLUSION

Physiochemical and Phytochemical property of *Nayuruvi nei* reveals that the drug is safety and effective. Further preclinical and clinical trials should be done in future to know the value of the drug.

REFERENCES

1. Dr.KalayanasundaramT.K.- Siddha Maruthuvam Part - 7 Kuzhanthai Maruthuvam First Edition -2011 pg.no:270
2. Dr.sundarrajan- Pillai Pini Maruthuvam part-1 First Edition,Department of Indian Medicine and Homeopathy
3. Pongurusironmani – Balavagadam- Department of Indian Medicine and Homeopathy
4. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
5. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH Ministry of Health & Family Welfare, Govt. of India
6. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305, 227.
7. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975:36-45