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Research Article

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Analytical standardisation and Evaluation of pirali ennai for the treatment of Aama Kalichal (Bacillary Dysentry)

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Abstract

A Poly herbal siddha formulation, piraliennai was prepared as per the siddha literature for the treatment of aamakalichal. The physicochemical standards available for the standardization of siddha medicated oils are insufficient. For preparation of any herbal formulation identification, evaluation and standardization is necessary. This article reports on analytical study report on piraliennai and standardization on the basis of organoleptic characters, physicochemical properties and analysis of High performance thin layer chromatography. These parameters are crucial for preparation of accurate and potent information.

Keywords: Herbal formulation, standardization, analytical study.

Introduction

Siddha system of medicine is a gift to mankind. Hence it is unique when compared to any other Indian Medical system. It includes the use of herbs, metals and minerals. Siddha system emphasizes not only a healthy body but a peaceful mind and pure immortality.

Optimal nutrition and healthy atmosphere for the mother is essential for the survival and development of a healthy child. In siddha literature the diseases of the children are classified into

1) Agakaarananoigal - due to intra uterine factors (develops congenitally)
2) Purakaarananoigal - due to external factors (acquired)

Aamakazhichal is one of the kazhichalnoigal occurring in infants and children. It is a disease of the alimentary canal caused by microorganisms. The etiological factors, Pathogenesis, clinical features of this disease explained in siddha literature are merely related to bacillary dysentery described in modern system of medicine. Bacterial infection are by far the most common causes of dysentry. These infections include Shigella, *E coli*, Camphylobacter, and salmonella

species of bacteria. Bacillary dysentry is a bacterial dysentry caused by Shigella infection.

Siddha system of medicine which has an indigenous effect in clearing the root cause of the diseases, will be unique in clearing the peculiar symptoms of dysentery. Even though there are many more medicines described in siddha literature for Aamakalichal, Piraliennai was selected the ingredients of Piraliennai have the property of controlling Aamakalichal without any adverse effects. Also it is purely a herbal medicine, easily available, low cost of price and harmless to infants and children.

Materials and Methods

1. Drug selection:

The drug selection of "***PIRALI ENNAI***" in treating " *Aamakalichal*" is to be safe, economic and efficient in reducing the symptoms of Aamakalichal and bring quick recovery. The drug is selected from the classical siddha literature Agathiyarvaithiyakaaviyam 1500, pg: 631.

General protocol for preparation of the herbal drug formulation in siddha involves the following steps:

1. Authentication of raw material
2. Purification
3. Preparation
4. Authentication of final drug

2. Authentication of raw material

This will be performed by geologists in case of Metal/ Mineral ingredients and by a taxonomists in case of herbal ingredients.

3. Purification

Purification process will be performed as per the classical siddha texts.

4. Preparation

1.3 Liters of Aamanakkuennai is taken in an earthern pot and the following 13 raw drugs are added to this earthern pot and these are kept under the flame till it attains the oil consistency.

Preparation:

S.No	Name	Botanical name	Quantity
1	Aamanakkuennai	*Ricinus communis*	1.3Liters
2	Avuriverthol	*Indigofera tinctoria*	10.2gm
3	Manjal	*Curcuma longa*	10.2gm
4	Kadugu	*Brassica nigra*	10.2gm
5	Venthayam	*Trigonella foenum*	10.2gm
6	Vellulli	*Allium sativum*	10.2gm
7	Seeragam	*Cuminum cyminum*	10.2gm
8	Karunjeeragam	*Nigella sativa*	10.2 gm
9	Omam	*Tachyspermum ammi*	10.2gm
10	Milagu	*Piper nigrum*	10.2gm
11	Kadukkai	*Terminalia chebula*	10.2gm
12	Katukarohini	*Picorrhiza kurrooa*	10.2gm
13	Vasambu	*Acorus calamus*	10.2gm
14	Perungayam	*Ferula asafoetida*	10.2gm

5. Administration of the drug

Form of the medicine : Ennai
Route of administration : Internal
Dose : 4 ml thrice a day
Adjuvant : Seeragam, Athimathuram, Chithirapaaladai all these three powder mixed in equal ratio
Indication : Aamakazhichal (Bacillary dysentery)

Authentication of the final drug

The final product of preparation will be authenticated by the trained experts from Gunapadam department for its completion.

Quality assurance of the prepared drug

Quality assurance will be performed as per the PLIM (Pharmacopoeial laboratory of Indian medicine) guidelines and the analytical parameters done as follows.

Physico-chemical evaluation

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.

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. 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

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.

Determination of Refractive Index

Determination of RL was carried out using Refractometer.

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

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) 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.

$$\text{Peroxide value} = 10\ (a - b)/w$$

Sterility 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

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 and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

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 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

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 phytotherapeutics.

Chromotogram 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.

Results and Discussion

Physicochemical Evaluation

State	Liquid
Nature	Viscous
Odor	Characteristic
Touch / Consistency	Greasy
Flow Property	Free flowing
Appearance	Yellowish

Solubility Profile

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

Analytical Report

S.No	Parameter		PIE
1	Viscosity at 50^{o}C (Pa s)		70.85
2	Refractive index		1.22
3	Weight per ml (gm/ml)		0.131
4	Iodoine value (mg I2/g)		82.27
5	Saponification (mg of KOH to saponify 1gm of fat)	**Value**	170.57
6	Acid Value mg KOH/g		0.4488
7	Peroxidase Value mEq/kg		4.752

Observation

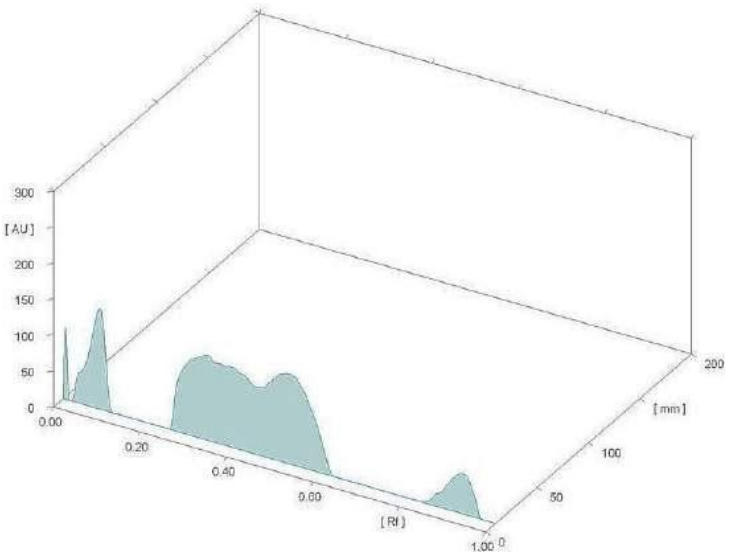
No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

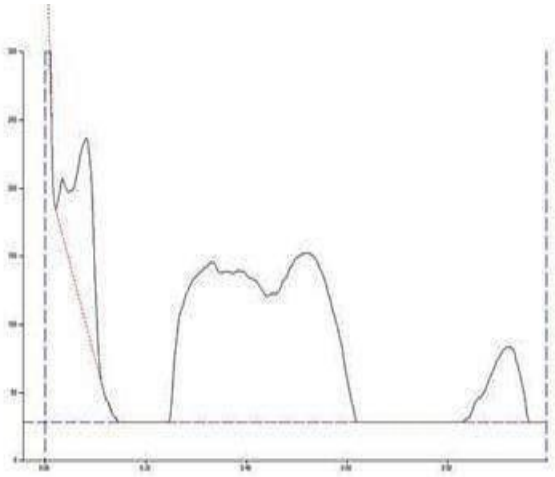
No growth / colonies was observed in any of the plates inoculates with the test sample.

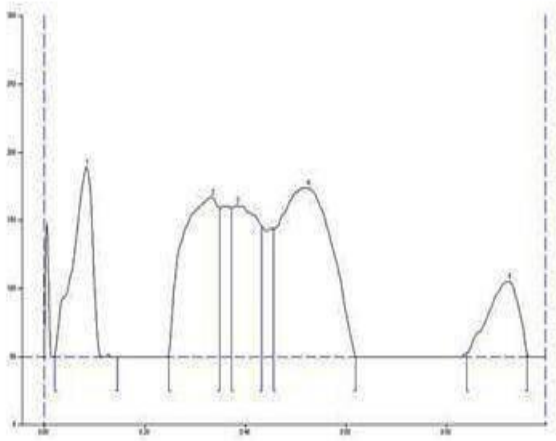
Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUS specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

TLC Visualization of PIE - TLC plate visualization at 366 nm



HPTLC finger printing of Sample PIE





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	0.8	0.08	139.5	25.43	0.15	0.0	2442.1	15.26
2	0.25	2.3	0.33	117.6	21.45	0.35	109.6	3709.1	23.18
3	0.37	109.4	0.38	111.5	20.34	0.43	96.8	2558.7	15.99
4	0.46	94.4	0.52	124.4	22.69	0.62	0.4	5780.6	36.12
5	0.84	3.3	0.93	55.3	10.09	0.96	2.2	1512.6	9.45

Report

HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to presence of five versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.84.

Conclusion

Thus the above studies concluded that the drug **PIRALI ENNAI** was subjected to many studies to validate its efficacy and safety through the defined standardization procedure. Preparation should be made according to the formulary published by Govt. of India. The parameters for each category of medicine should be followed uniformly along with the high performance thin layer chromatography.

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