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RESEARCH ARTICLE

PHYSICOCHEMICAL EVALUATION OF A SIDDHA POLY HERBAL FORMULATION NELLIKKAI LEGIYAM

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Abstract

Siddha system of medicine is the ancient, holistic system among all the systems. In that one of the excellent polyherbal formulations said in the text `The Pharmacopoeia of siddha research medicine'[(1)] was nellikai legiyam. Standardization of herbal formulations is essential for assessing the quality and efficacy of the particular drugs. This article refers to the physicochemical evaluation of nellikkai legiyam, a polyherbal siddha medicine used for loss of appetite, Anemias, and immunomodulatory diseases. The objective of drug standardization is to ensure the quality and efficacy of medicinal products in terms of their chemical and biological properties. There should be some basic standards as well as methods of preparation for assessing the quality of the finished product. This medicine has been evaluated for organoleptic characters, phytochemical evaluation and physicochemical properties.

Introduction:-

The Siddha system is based on ancient healing traditions and spiritual philosophies and alchemy. Siddha medicine gives importance to the use of both herbals and minerals. Nellikkai legiyam is one of the poly herbal formulation. The ingredients of the nellikkai legiyam were i.e., Phyllathus emblica.Linn (Nellikkai vatral), Zingiber officinalis.Rose (Chukku), Piper nigrum.Linn (Milagu), Piper longum.Linn (Thippili), rootof Piper longum.Linn (Thippili Moolam), Carum copticum Benth&Hook.f (Omam), Syzygiyum aromaticum.Linn (Lavangam), Elattaria cardamomum.Maton (Elarisi), Illicium verum Hook. f. (Thakkolam), Cuminum cyminum.Linn (seeragam), Coriandrum sativum.Linn, (Kothumalli), Maranta arundinacea.Linn (Koogai neer), Glycyrhiza glabra.Linn (Athimathuram), Embelia ribes.Burn.f (Vayuvidangam), Nattu sarkarai (Saccharum officinarum Linn)Cow's ghee, Honey. This research article focuses on the organoleptic characters, phytochemical evaluation and physicochemical properties of Nellikkai legiyam. In this study the parameters for quality assessment have been followed as per CCRAS guidelines for analytical specification of legiyam.[(2)]

Materials And Methods:-

Ingredients:

According to the text the ingredients of the nellikai legiyam are given below:

S.No	Tamil Name	Botanical Name	Parts Used	Quantity

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1	Nellikai vatral	Phyllanthus emblica Linn	Dried fruit	5.4 kg
2	Nattu sarkarai	Saccharum officinarum Linn	Jaggery powder	7 kg
3	Chukku	Zingiber officinale, Rose	Dried rhizome	144 g
4	Milagu	Pipper nigrum Linn	Dried unripe fruit	144 g
5	Thippili	Piper longum Linn	Dried unripe fruit	144 g
6	Thippili moolam	Root of Piper longum Linn	Root	144 g
7	Omam	Carum copticum Benth&Hook.f	Fruit	144 g
8	Jeeragam	Cuminum cyminum.Linn	Fruit	144 g
9	Lavangam	Syzygiyum aromaticum.Linn	Flower buds	144 g
10	Elarisi	Elattaria cardamomum.Maton	Seed	144 g
11	Thakkolam	Illicium verum Hook. f.	Seed	144 g
12	Kothumalli	Coriandrum sativum.Linn	Fruit	144 g
13	Kugaineer	Maranta arundinacea.Linn	Rhizome	144 g
14	Athimathuram	Glycyrhiza glabra.Linn	Root	144 g
15	Vayuvidangam	Embelia ribes.Burn.f	Fruit	144 g
16	Cow's ghee	-	-	1 kg
17	Honey	-	-	½ litre

Collection and authentication:

The raw drugs were purchased from the Chennai raw drug store and authenticated by Assistant Professor, Medicinal botany, National institute of siddha, Tambaram Sanatorium, Chennai-47. (Voucher No NISMB4002019)

Purification of raw drugs:

1**.** Nellikkai (Phyllathus emblica.Linn**)**
Clean in water and removed the seed.
2. Chukku (Zingiber officinale, Rose)
Double the proportion of lime stone [calcium carbonate] solution is poured and boiled for three hours, then wash it, dry and remove the peel.
3. Milagu (Pipper nigrum.Linn)
Soak it in sour butter milk for three hours.
4. Thippili (Piper longum.Linn)
Soak it in plumbago zeylanica,Linn (Kodiveli) leaf juice for twenty-four minutes (1 Nazhigai) and dry it sun.
5. Thippili moolam (Root ofPiper longum.Linn)
Dried under shade.
6. Omam (Carum copticumBenth&Hook.f)
Dried under shade.
7. Jeeragam (Cuminum cyminum.Linn)
Dried in sunlight and fried it like as golden yellow colour.
8. Lavangam (Syzygiyum aromaticum.Linn)
Dried under shade.
9. Elarisi (Elattaria cardamomum.Maton)
Remove the peel, take the seeds and dried under shade.
10.Thakkolam (Illicium verum Hook. f.)
Dried under shade.
11.Kothumalli (Coriandrum sativum.Linn)
Boil with Hotwater & dry it in sunlight
12.Athimathuram (Glycyrhiza glabra.Linn)
Scraped the outer layer and cut in to small pieces then dried into sun light.
13.Vayuvidangam (Embelia ribes.Burn.f)
Cleaned and dried under shade.

Preparation of nellikai legiyam:[(1)]
Step 1:
Nellikai vatral is added with 8 parts of water and reduced into 1/8 parts of decoction.

Step 2:
The decoction is added with Nattu sarkarai and boiled until it reaches kambi patham.

Step 3:
The paagu is added with other powdered drugs and stirred till it attains legiyam patham.

Step 4:
Finally add cow's ghee and honey.

Dose:
½ thola(6g) bd with Hot water

Analytical Study:
Organoleptic characters, preliminary phytochemical screening, physicochemical evaluation, were done by following the standard procedure in Noble Research solutions, Perambur, Chennai and the biochemical analysis were done in National institute of Siddha, Chennai.

Biochemical Analysis Of Trial Drug:
Test for silicate:
A little sample was shaken well with distilled water and then with con.HCL/con.H2SO4, insoluble indicated the absence of silicates.

Test for Carbonate:
A small amount of the sample was taken in a dry test tube and heated gently at first and then strongly, formation of white fumes indicated the presence of carbonate.

Test for Sulphate:
2 ml of the extract was taken in a test tube add 2ml of 4% Ammonium Oxalate solution, formation of cloudy appearance indicated the presence of sulphate.

Test for Chloride:
2ml of the extract was treated with 2ml of dilute HNO_3, until the effervescence ceases off. Then 2ml of silver nitrate was added, no formation of cloudy appearance indicated the absence of chloride.

Test for carbonate:
2ml extract was treated with 2 ml of Magnesium Sulphate solution. Formation of cloudy appearance indicated the presence of carbonate.

Test for Nitrate:
3 drops of the extract was placed on a filter paper, on that 2 drops of acetic acid and 2 drops of Benzidine solution was placed. No characteristic change was observed, indicating the absence of nitrate.

Test for Sulphide:
1 gm of the extract was treated with 2ml of conc.HCL, presence of rotten egg smelling gas indicated the presence of sulphide.

Test for Lead:
2ml of the extract was added with 2ml of Potassium Iodide solution. No yellow precipitate was formed, indicating the absence of Lead.

Test for Copper:
2 ml of extract was added with excess of ammonia solution. No blue precipitate was formed, indicating the absence of Copper.

Test for Aluminium:
To the 2ml of the extract, Sodium Hydroxide was added in drops to excess. No characteristic change was observed, indicating the absence of Aluminium.

Test for Iron:
2 ml extract was treated with 2ml of Ammonium Thiocynate solution and 2ml of Conc. HNO3 was added. The formation of blood red colour indicated the presence of Iron.

Test for Zinc:
To the 2ml of the extract Sodium Hydroxide solution was added in drops to excess. White precipitate was formed, indicating the presence of Zinc.

Test for Magnesium:
To the 2ml of the extract Sodium Hydroxide solution was added in drops to excess. No white precipitate was formed, indicating the absence of Magnesium.

Test for Calcium:
2ml of extract was taken in a clean test tube and 2 ml of 4% Ammonium Oxalate solution was added. White precipitate was formed, indicating the presence of Calcium.

Test for Ammonium:
To 2ml of extract 2ml of Nessler's reagent and excess of NaOH solution were added. The appearance of Brown colour indicated the presence of Ammonium.

Test For Potassium :
A pinch of substance was treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid. No yellowish precipitate was formed, indicating the absence of Potassium.

Test for Sodium:
2 pinches of the substance was made into paste by using HCL and introduced into the blue flame of Bunsen Burner. No yellow colour flame was formed, indicating the absence of Sodium.

Test for Mercury:
2ml extract was treated with 2ml of NaOH solution. No yellowish precipitate was formed, indicating the absence of Mercury.

Test for Arsenic:
2ml of the extract was treated with 2ml of Sodium Hydroxide solution. No brownish red precipitate was formed, indicating the absence of Arsenic.

Test for Starch:
2ml extract is treated with weak Iodine solution. The formation of blue colour indicated the presence of starch.

Test for Reducing sugar:
5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and 8-10 drops of the extract was added and again boil it for 2 minutes. The colour change is noted. No brick red colour was developed, indicating the absence of reducing sugar.

Test for Alkaloids:
2ml extract was treated with 2ml of Potassium Iodide solution and 2ml Picric acid was added. Yellow colour was developed indicating the presence of Alkaloids.

Test for Tannic acid:
2ml of the extract was treated with 2ml of Ferric Chloride solution. Black precipitate was formed indicating the presence of Tannic acid.

Test for Amino acid:
2drops of the extract was placed on a filter paper and dried well.No violet colour was developed, indicating the absence of Amino acid.

Preliminary phytochemical tests:[4]
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 was 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. Copiouslather formation indicates the presence of Saponins.

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

Test for glycosides (Borntrager's Test)
Test drug was hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:
To the test sample about 5 ml of dilute ammonia solution where 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
Anthocyanin:
To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}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 Benedict's reagent was 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.

Physicochemical evaluation:[5,6]
Determination of Loss on Drying:
Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash:
Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash:
The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter was collected in crucible and washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive:
Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water-Soluble Extractive:
Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Results and Discussion:-

Organoleptic characters:
The organoleptic characters of Nellikkai legiyam are tabulated as Table 1.

Biochemical analysis:
The biochemical analysis results of Nellikkai legiyam are tabulated as Table 2.

Preliminary phytochemical analysis:
Preliminary phytochemical analysisresults of Nellikkai legiyam are tabulated as Table 3.

Physicochemical evaluation:
Physicochemical evaluation results of Nellikkai legiyam are tabulated as Table 4.

Table 1:- Organoleptic characters of Nellikkai legiyam.

S.No	Organoleptic Characters	Observation
1	State	Semisolid
2	Odor	Characteristic
3	Touch	Greasy
4	Flow Property	Non free flowing
5	Appearance	Dark brownish

Table 2:- Biochemical evaluation of Nellikkai legiyam.

S.No	TEST	INFERENCE
1	Silicate	Absence of silicate
2	Sulphate	**Presence of Sulphate**

3	Chloride	Absence of Chloride
4	Phosphate	**Presence of Phosphate**
5	Carbonate	**Presence of Carbonate**
6	Nitrate	Absence of Nitrate
7	Sulphide	**Presence of Sulphide**
8	Nitrite	Absence of Nitrite
9	Borate	Absence of Borate
10	Lead	Absence of Lead
11	Copper	Absence of Copper
12	Aluminum	Absence of Aluminium
13	Iron	**Presence of Iron**
14	Zinc	**Presence of Zinc**
15	Calcium	**Presence of Calcium**
16	Magnesium	Absence of Magnesium
17	Ammonium	**Presence of Ammonium**
18	Potassium	Absence of Potassium
19	Mercury	Absence of Mercury
20	Arsenic	Absence of Arsenic
21	Starch	**Presence of Starch**
22	Reducing sugar	Absence of Reducing sugar
23	Alkaloids	**Presence of Alkaloids**
24	Tannic acid	**Presence of Tannic acid**
25	Amino acid	Absence of Amino acid

Table 3:- Phytochemical evaluation of Nellikkai legiyam.

S.No	TEST	OBSERVATION
1.	Alkaloids	**Positive**
2.	Flavonoids	**Positive**
3.	Glycosides	**Positive**
4.	Steroids	**Positive**
5.	Triterpenoids	**Positive**
6.	Coumarin	**Positive**
7.	Phenols	**Positive**
8.	Tannins	**Positive**
9.	Protein	Negative
10.	Saponins	**Positive**
11.	Sugar	**Positive**
12.	Anthocyanin	Negative
13.	Betacyanin	**Positive**

Table 4:- Physicochemical evaluation of Nellikkai legiyam.

S.No	Parameter	Mean (n=3) SD
1.	**Loss on Drying at 105 °C (%)**	20.4 ± 6.183
2.	**Total Ash (%)**	0.3433 ± 0.080
3.	**Acid insoluble Ash (%)**	0.093 ± 0.011
4.	**Water soluble Extractive (%)**	14.4 ± 1.153
5.	**Alcohol Soluble Extractive (%)**	8.183 ± 0.56

Conclusion:-

In biochemical analysis of Nellikkai legiyam, the presence of carbonate, sulphate, Iron, Zinc, Calcium, Ammonium, Sulphide, Starch, Alkaloids & Tannic acids were detected. In phyto chemical analysis of Nellikkai legiyam, Alkaloids, Flavonoids, Glycosides, Steroids, Triterpenoids, Coumarin, Phenols, Tannins, Saponins, Sugar, Betacyaninwere detected. The physicochemical evaluation like loss on drying at 105 ^{0}C, Ash, Acid insoluble Ash, Water soluble extractive, Alcohol soluble extractive and pH of Nellikai legiyam were 20.4 ± 6.183, 0.3433 ±

0.080, 0.093 ± 0.011, 14.4 ± 1.153, 8.183 ± 0.56 respectively. All the secondary metabolites present in the nellikkai legiyam are responsible for its immunomodulatory, antioxidant, anti-inflammatory and hematinic actions.

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