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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF SIDDHA HERBAL MEDICINE KARAPPAN ENNAI

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ABSTRACT

Standardization of Siddha drug is necessary one in this scientific word 'Karappan ennai' is a polyherbal formulation indicated for skin diseases particularly for Karappan (Atopic dermatitis)'. Pharmacognostical standardization of herbal formulation is essential in order to assess the quality of drug, based on the concentration of their active principles. The present study was attempt to evaluate the Physicochemical and phytochemical analysis of the siddha medicine Karappan ennai. But there is no standardization work report on Karappan ennai. The standardization of the drugs is a key factor in assessing the quality control of the drugs to establish the medicine in a valuable mode.

KEYWORDS: Karappan ennai, Physicochemical, Phytochemical analysis, Siddha polyherbal formulation.

INTRODCTION

A Siddha system of medicine is oldest holistic management system and being practiced by a large population in South India. The World Health Organization[1] (WHO) defines herbal medicine as those containing plants parts or plant materials in raw state or processed form

containing active principles. Standardization of drug is essential to exhibit conformation of its identity and determination of its purity, quality and quantity.

It is based on the concentration of their active principles, physicochemical, phytochemical and In-vitro, In-vivo parameters. Thus, the present study deal with standardization of Siddha polyherbal formulation Karappan ennai[2] is mentioned in the classical Siddha literature for the treatment of Skin diseases particularly Karappan[3] (Atopic dermatitis[4]).

There are no systemic protocols for standardization of Karappan ennai. Hence, it was decided to evaluate the physicochemical and phytochemical analysis for Karappan ennai scientifically to prevent its adulteration.

MATERIALS AND METHODS

Trial drug preparation

The fresh herbals of Karuppura valli, Siru cheruppadai, Erulli, Narimiratti, Cheppu nerunjil and vilakkennai was collected from Thiruvannamalai district 600 905, Tamilnadu, India.

Identification and authentication of the Drug

All the herbals were authenticated by the pharmacognosist, Siddha Central Research Institute (SCRI), Arumbakkam, Chennai 106, Tamilnadu, India.

Purification of the drugs

All the herbals were purified as per the methods mentioned in Siddha literature.[5]

Ingredients[6]

Karuppura valli Charu - 1.3 liters
Siru cheruppadai Charu - 1.3 liters
Eerulli Charu - 1.3 liters
Narimiratti Charu -1.3 liters
Cheppu nerunjil Charu -1.3 liters
Vilak- Ennai -1.3 liters

Method of preparation

The above mentioned ingredients were whole plant extracts are taken in a mud pot and boiled in a small flame. When the waxy consistency (Mezhugu patham) is obtained, the oil is taken out of the flame and cooled. Then preserved in a clean dry and air tight container.

Therapeutic usage

Karappan ennai are given topical application. Oil is applied over the affected skin lesions.

PHYSICOCHEMICAL ANALYSIS

Preparation of standard solution

0.2g of ferric ammonium Sulphate was dissolved in distilled water containing 10ml of concentrated hydrochloric acid and the volume was made up to 250ml with distilled water 3. From this stock solution 1, 2, 3, 4 & 5ml was pipette out into 5 different 50ml volumetric flask and 5ml of 10% aq. Hydroxyl ammonium chloride solution was added and the pH was adjusted between 3 to 5 using 2M sodium acetate buffer solution and 4ml of 1, 10-phenanthrolin was added and finally the volume was made up to 50ml with distilled water. After 15-20 min. the absorbance was noted at 515nm. The standard curve of concentration Vs absorbance was plotted.

Preparation of Test Solution

0.21g of test sample was taken with 50ml of 6N hydrochloric acid and boiled for 2-3 min. Then it was filtered and the volume was made up to 250ml with distilled water. From this 5ml of solution was pipette out into 50ml volumetric flask and the same procedure was followed as in the preparation of standard solution. After 15-20 min. the absorbance was noted at 515nm. From the absorbance the corresponding concentration was determined by extrapolation of calibration curve.

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 oil 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 Iodine value

About 20 gm of oil 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 1hour. Then about 10 ml of KI solution was added to this and

titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow color.1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue color 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.

Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish .The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

Determination of pH

Sample being oily in nature the direct litmus evaluation method was adopted to check the pH of the sample.

Table 1: Result of organoleptic character.

S.No	Parameter	Observation
1.	Color	Greenish color
2.	Smell	Characteristic Odour
3.	Touch	Oily
4.	Appearance	Translucent

Table 2: Result of physicochemical analysis.

S.NO	PARAMETERS	KARAPPAN ENNAI
1.	Specific gravity	0.84g/cm^3
2.	Viscosity at 50° C	0.6533 mPa.s (millipascal-second
3.	Refractive index	1.468
4.	Weight per ml (gm/ml)	2.2±0.33
5.	Iodine value	74.4
6.	Saponification value (mg of KOH to saponify 1gm of fat)	186.4
7.	Loss on drying at 105° c	0.23 % by mass
8.	Viscosity	35.88mm^2/sec
9.	PH	6.6

PHYTOCHEMICAL ANALYSIS

Sample Preparation

The Hydro-alcoholic extract of *Karappan Ennai* drug was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

1. Test For Alkaloids

The extract was treated with dilute hydrochloric acid and filtered.

Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

2. Test For Carbohydrates

Benedict's test: (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

3. Test For Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

5. Test For Sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

6. Test For Phenols

The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

7. Test For Flavonoid's

5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes In each test tube, development of yellow color demonstrated the presence of flavonoids.

8. Test For Diterpenes

Salkowski test: 5ml of extract was mixed in 2ml of chloroform and concentrated sulphuric acid was carefuly added to form a layer. A reddishbrown colouration of the interference indicates the presence of diterpenes.

9. Test For Quinones

The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

10. TEST FOR TRITERPENES

5ml of extract was mixed in 2ml of chloroform and concentrated sulphuric acid was carefuly added to form a layer. A reddishbrown colouration of the interference indicates the presence of triterpenes.

Table 3: Results of phyto chemical analysis of 'Karappan ennai'.

S.no	Phyto - components	Inference
1	Alkaloid	Present
2	Carbohydrate	Absent
3	Glycoside	Absent
4	Saponins	Present
5	Phytosterols	Present
6	Phenols	Present
7	Flavonoids	Present
8	Diterpenes	Present
9	Quinones	Present
10	Triterpenes	Present

The above table shows the presence of secondary metabolites such as alkaloid, saponin, phytosterols, phenols, flavonoids, quinones, di and tri terpenes. Presence of this phytochemicals indicates their clinical validation

Plants description and active principles of Karappan ennai[7]

1. Tamil name : Karuppura valli

Botanical name : *Plectrathus ambonicus*

English name : Indian borage

Family : Lamiaceae

Part used : Whole plant

Chemical constituents: Carvacrol, Chlorogenic acid, Rosmarinic acid, Caffeic acid.

Actions: Stimulant, Diaphoretic, Expectorant.

Activity[8] **:** Anti inflammatory

2. Tamil name **:** Siru cheruppadai
Botanical name **:** *Coldenia procumbens*
English name **:** Prostrate shrup
Family **:** Boraginaceae
Part used **:** Whole plan
Chemical constituents: Tannins, Flavanoids, Mucilage, Essential oil, Alpha humulene.
Actions: Stimulant.
Activity[9] **:** Anti inflammatory

3. Tamil name **:** Eerulli
Botanical name **:** *Allium cepa*
English name **:** Onion
Family **:** Lilliaceae
Part used **:** Bulb
Chemical constituents: Sapogenin cepagenin, Tropeosides A1 /A2, Kaempferol, Luteolin, Myricetin, Quercetin, Apigenin.
Actions: Stimulant, Demulcent.
Activity[10] **:** Anti inflammatory

4. Tamil name **:** Narimiratti
Botanical name **:** *Crotolaria verrucosa*
English name **:** Rattle wort
Family **:** Fabaceae
Part used **:** Whole plant.
Chemical constituents: Secopyrrolizidine alkaloids, Crotaverrine, o- Acetyilcrotaverine.
Actions: Germicides.
Activity[11] **:** Anti inflammatory

5. Tamil name **:** Cheppu nerunjil
Botanical name **:** *Indigofera enneaphylla*
English name **:** Birdsville Indigo
Family **:** Fabaceae
Part used **:** Whole plant.

Chemical constituents: Alkaloids, Flavanoides, Tannins, Steroides, Quinine, Terpenoides and Fixed oil.

Actions: Febrifuge.

Activity[12] **:** Anti inflammatory

6. Tamil name : Vilak-ennai

Botanical name : *Ricinus communis*

English name : Castor oil

Family : Euphorbiaceae

Chemical constituents: Glycerides, Ricinoleic, Isoricinoleic and Stearic acids, Dihydro cystearic acids, Lipase and Alkaloid ricinine.

Actions: Laxative, Emolient.

Activity[13]**:** Anti inflammatory

DISCUSSION

In the present study it is concluded that the organoleptic characters and physicochemical parameters[14] such as the specific gravity (0.84g/cm^3), Iodine value (74.4), viscosity (35.88mm^2/sec), PH (6.6).The phytochemical analysis of the drug Karappan ennai has the presence of secondary metabolites such as alkaloid, saponin, phytosterols, phenols, flavonoids, quinones, di and tri terpenes.

CONCLUSION

The present study on physicochemical parameters, preliminary phytochemical analysis and active principles provides important information which can be used as a fingerprint of polyherbal formulation of 'Karappan ennai'. The physicochemical and phytochemical analysis of the Karappan ennai shows the safe and effectiveness of the drug.

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