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Phytochemical and physico-chemical analysis of Thulasi ennai - Siddha preparation

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Abstract

Thulasi Ennai is a special Siddha formulation for the treatment of childhood asthma (Sooli kanam). The drug was prepared as per the mentioned in the classical Siddha literature Balavagadam. The physicochemical analysis such as loss of dying, total ash and the phytochemical analysis such as alkaloids, steroids, triterphenoids, phenols, tannins, saponins. The physicochemical analysis reveald that the loss of dying was 2.7+- 0.75, total ash was 0.4+- 0.08 and the phytochemical analysis such as alkaloids, steroids, triterphenoids, phenols, tannins, saponins.

Keywords: Siddha, Thulasi Ennai, childhood asthma, phytochemical and physic-chemical analysis.

Introduction

Siddha medicine is mainly practiced in Southern part of India. It is the oldest system of medicine. Most of the drugs not standardized. The standardization of medicine is very essential and mandatory requirement, so that the quality of medicine can be achieved as per the standard of modern scientific society. Standardisation is a vast area to cover, that can be achieved by evaluating the medicine with the required modern standardization rules and regulations.

Thulasi Oil is beneficial for childhood asthma but there is a requirement of standardization to find out its efficacy. Thulasi oil is one of the herbal formulation mentioned in classical literature balavagadam. This drug was analysed physically- chemically and phytochemically.

Aim and objective:

The aim of the study is to do physico-chemical analysis and preliminary phytochemistry for the drug Thulasi Ennai

Materials and Methods

Collection and identification of drug:

The required drug of Thulasi Oil would be purchased from a well reputed country shop, Chennai, and raw drugs were authenticated by the medicinal botanist of National Institute of Siddha. The medicine will be prepared in Gunapadam lab of National Institute of

Siddha after proper purification. The prepared medicine would also be authenticated by the concerned head of the department for its completeness.

Ingredients of Thulasi Oil:

1. Chitramanakku nei (*Ricimus communis*)
2. Thulasi (*Ocimum sanctum*)
3. Nilathuasi (*Ocimum prostratum*)
4. Kanchakorai (*Ocimum canum*)
5. Thalisapathiri (*Taxus buccata*)
6. Vilvam (*Aegle marmelos*)
7. Eerulli (*Allium cepa*)
8. Chukka (*Zingiber officinale*)
9. Thippili (*Piper longum*)
10. Milagu (*Piper nigrum*)
11. Then

Preparation of drug

Take 352ml of Castor oil, and 160 ml of Thulasi, Nilathulasi juice, Kanchakorai juice, Thalisapathiri juice, Vilvam fruit leaves juice, Onion juice. Mix the juices with oil in a mud vessel. Take 8gms of dried ginger, Long pepper, Black pepper fry it and make it fine powder mix 160ml of honey and heat it till muster form appears and filter it.

Administration

Dosage: 5 ml /twice a day/after meal.

Phytochemical analysis of Thulasi ennai

Percentage Loss on Drying

10gm of test drug (weight equivalent to oil) was accurately weighed in evaporating dish .The sample was dried at 105 for 5 hours and then weighed.

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

Determination of Total Ash

3 g of test drug (weight equivalent to oil) was accurately weighed in silica dish and incinerated at the furnace a temperature 400ºC 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.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of pH

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

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 a hour. 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 (weight equivalent to oil) 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.

Preliminary phytochemical screening

Sample Preparation

Thulasi oil (TO) was extracted with ethanol and the extract was subjected to the following analysis

Test for alkaloids:

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

Test for coumarins:

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

Test for saponins:

To 1 ml of the extract, 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 extract, 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 0.1ml of 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: The extract was taken; 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 solution 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.

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.

Test for Cyanins

A. Anthocyanin:

To 2 ml of the leaf extract, 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.

B. Betacyanin:

To 2 ml of the leaf extract, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of yellow colour indicates the presence of betacyanin.

Test for Carbohydrates - Benedict's test

To 0.5 ml of test drug 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.

Test for terpenoids:

Salkowski test: 5ml of extract was mixed in 2ml of chloroform, and concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Quantitative estimation of phytoconstituents of TO

Determination of total Phenol content

The total phenol content was determined using Folin–Ciocalteu reagents with analytical grade Gallic acid as the standard. 1 ml of sample was added to deionized water (10 ml) and Folin–Ciocalteu phenol reagents (1ml). After 5 minutes, 20% sodium carbonate (2 ml) was added to the mixture. After being kept in total darkness for 1 hr, the absorbance was measured at 750 nm using a spectrophotometer.

Amounts of total Phenol was calculated using Gallic acid calibration curve. The results were expressed as Gallic acid equivalents (GAE) mg/g of dry plant matter.

Estimation of Alkaloid

To weight equivalent to 5 gm was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Tannin

The tannin content was determined using Folin Ciocalteu assay. Sample TO of 100 µL was added to 750 µL of distilled water, 500 µL Folin-Ciocateu reagent and 1000 µL of 35 % sodium carbonate (Na2CO3). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm. Distilled water was used as blank. Tannic acid standard solutions were prepared and standard calibration curve was plotted with varying concentration. The total tannins content were expressed as Tannic acid mg/gm, as calculated from the prepared standard curve.

Results and Discussion

Physico-Chemical analysis:

The result of physico chemical parameters given in the Table 1.

Table 1: The result of Physicochemical parameters

Parameter	Observation
Color	Greenish
Smell	Characteristic Odour
Touch	Oily
Appearance	Clear

S.No	Parameter	Mean (n=3) SD
1	Loss on Drying at 105 °C (%)	2.7 ± 0.75
2	Total Ash (%)	0.45 ± 0.08

S.No	Specific Test	MO
1	pH	6
2	Refractive index	1.41
3	Iodoine value (mg I2/g)	119
4	Saponification Value (mg of KOH to saponify 1gm of fat)	224
5	Specific Gravity	0.97468
6	Viscosity	50.06 Centipoise (CP)

Physio-chemical analysis was done as preliminary evaluation on Thulasi Ennai .The method of measuring the moisture content in liquid materials is loss on drying (LOD). Low moisture content is always desirable for higher stability of drugs. In, the loss on drying at 105 was found to be 2.7±0.7%.So the determination of moisture content shows Thulasi Ennai the good stability of the drug.

The ash value represent the purity of the drugs. The total ash includes both "physiological ash", which is derived from the organic matter, and "non-physiological" ash, which is the residue of the extraneous matters like sand/soil, inorganic material.

Phytochemical analysis:

The result of phytochemical parameters given in the Table 2.

Phytochemical analysis of the trial drug Thulasi Ennai reveals presence of certain phyto-compounds. They are named as alkaloids, steroids, triterpnoids, phenols, tannins, saponins. These constituents enhance the activity of Thulasi Ennai.

Table 2: Phytochemical analysis

Phyto- constituents	MO
Total phenols (GAE mg/gm)	0.63 ± 0.04
Total alkaloids(mg/gm)	0.49 ± 0.06
Total tannins(mg/gm) (Tannic acid mg/gm)	0.266 ± 0.03

Mean with 3 replicates ± SD.

Phytocomponents	TO
Alkaloids	+
Flavonoids	-
Glycosides	-
Steroids	+
Carbohydrates	-
Triterepnoids	+
Coumarins	-
Phenols	+
Tannins	+
Saponins	+
Proteins	-
Anthocyanin	-
Betacyanin	-

\+ Indicates positive / Presence
\- Indicates Negative / Absence

Conclusion

The current study investigation

The current study investigation is done to meet the standardization of WHO and other scientific communities. The present analysis revealed that physicochemical analysis such as loss of dying, total ash and the phytochemical analysis reveals presence of certain phyto component they are named as alkaloids, steroids, triterphenoids, phenols, tannins, saponins. It can be assumed the drug Thulasi Ennai has validated the traditional claim. This research work is done for future studies to be carried out in future.

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Conflict of interest

The author declared no potential conflicts of interest with respect to the research and publication of this article.

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