PHYSICO CHEMICAL, PHYTO CHEMICAL AND CHEMICAL ANALYSIS OF VILAAM PAZHA THIRAVAGAM - A SIDDHA PREPARATION

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ABSTRACT

Vilaam Pazha Thiravagam is a Siddha herbal formulation plays an important role in treating Diabetes. Thiravagam Means an acid - They are all process of destructive distillation of salts and alkalis with or without any addition of fluids in a peculiar distillation set up called “Vaalai yanthiram”. The drugs required for making Vilampazha Thiravagam are collected and authenticated by experts. Then it is prepared as per siddha literature. Vilam Pazha Thiravagam is then subjected to Physio chemical, Chemical and phytochemical analysis for standardization. The results obtained are described briefly in this paper.

KEYWORDS: Vilaam pazha Thiravagam, Siddha Medicine, Diabetes.

INTRODUCTION

Siddha medicine is claimed to revitalize & rejuvenate dysfunctional organs, that cause the disease. (Vatham, pitham, kabam) The siddha medicine in practice includes leaves, flowers, fruit & various root in a formulary medicine. The author of this paper have brought out the efficacy of herbal drug vilaam pazha thiravagam for the treatment of Mathumegam. The dosage for thiravagam is 2 ½ to 3 ½ palam (87.5g to 122.5 g) along with water as adjuvant. The prepared formulation was subjected to physico chemical, phytochemical analysis for standardization.
MATERIALS AND METHODS

PREPARATION OF THE TRIAL DRUG

DRUG SELECTION

*Vilampala Thiravagam* is taken as a trial drug for *Hypoglycemic Acitivity* from the siddha literature *Anupoga Vaithiya Navaneetham* (8th part).

INGREDIENTS

1. Naturally ripped fruit pulp of limonia acidissima Linn - 40 palam (1400g) (*vilampazham*).
2. Crushed stem bark of Acacia nilotica Linn – 40 palam (1400g) (*karuvelampattai*).
3. Palm jaggery – powdered (40 palam).

COLLECTION OF THE PLANT MATERIALS

All plant materials were freshly collected from in and around Tirunelveli, Tamilnadu.

IDENTIFICATION AND AUTHENTICATION OF THE DRUG

The identification of herbal drug is authenticated by the faculties of PG Gunapadam Department and Herbal Botany Department, Government Siddha Medical College, palayamkottai.

PURIFICATION OF THE DRUGS

All the drugs mentioned here were purified as per the siddha literature.

- Fruits of Limonia acidissima were purified by removing the outer thick and the flesh is obtained.
- Bark of Acacia nilotica is first dusted with a clean cloth and then purified by gently removing the outer skin with a knife.

PROCEDURE

The above said three drugs are placed in a large mud pot and is mixed with 10.4 litres of water. Mouth of the mud pot is covered by a thin white cloth and kept it in sunlight for 5-10 days depending upon the day light stir it daily 2-3 times. After that the mixture is subjected to distillation process. The obtained product is “Thiravagam” and stored it in a glass bottle.

STORAGE OF THE DRUG

The prepared test drug was stored in a clean air tight glass container for further studies.
PHYSICO CHEMICAL PARAMETERS

_Vilam Pazha Thiravagam_ was evaluated for various physico-chemical parameters such as physical appearance (Color, odor) pH and specific gravity.

COLOUR EXAMINATION

5 ml of _Thiravagam_ was taken in a watch glass and placed against white background in white tube light. It was observed for its color by naked eye.

ODOUR EXAMINATION

2ml of _Thiravagam_ was smelled individually the time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

DETMINATION OF PH

Operate the pH meter and electrode system according to the manufacturer’s instructions. Standardize the meter and electrodes with 0.05m sodium borate when measuring the alkaline solution. At the end place an accurately measured amount 10ml of _Thiravagam_ in a 100ml volumetric flask and add 100ml of distilled water the solution was sonicated for about 10 min. PH was measured with the help of digital PH meter.

SPECIFIC GRAVITY

A thoroughly clean and dry pycnometer was selected and calibrated by filling it with recently boiled and cooled water at 25°C and weighing the contents. Assuming that, the weight of 1 ml of water at 25°C when weighed in air density 0.0012g/ml was 0.99602g. The capacity of the pycnometer was calculated. Adjusting the temperature of _Thiravagam_ to about 20°C and the pycnometer was filled with it. Then the temperature of the filled pycnometer was adjusted to 25°C any _Thiravagam_ was removed and weight was taken. The tare weight of the pycnometer was subtracted from the filled weight. The weight per milliliter was determined by dividing the weight in air expressed in gm of the quantity of the _Thiravagam_ which fills the pycnometer at the specified temperature. Specific gravity of the _thiravagam_ was obtained by dividing the weight of the _Thiravagam_ contained in the pycnometer by the weight of water contained both determined at 25°C.
MICROBIAL LIMIT TEST
DETERMINATION OF TOTAL AEROBIC MICROBIAL COUNT
For Bacteria and Fungi

Test for Specified Micro-Organisms
Escherichia coli.
Salmonella.
Pseudomonas aeruginosa.
Staphylococcus aureus.

CHEMICAL ANALYSIS
PROCEDURE
The drug (Thiravagam) is directly used as the extract

QUALITATIVE ANALYSIS FOR BASIC RADICALS
TEST FOR CALCIUM
2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

TEST FOR IRON (FERRIC)
The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

TEST FOR IRON (FERROUS)
The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

TEST FOR ZINC
The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS
TEST FOR SULPHATE
2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.
TEST FOR CHLORIDE
The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

TEST FOR PHOSPHATE
The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

TEST FOR CARBONATE
On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

TEST FOR STARCH
The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

TEST FOR ALBUMIN
The extract is treated with Esbach’s reagent. Formation of yellow precipitate indicates the presence of albumin.

TEST FOR TANNIC ACID:
The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

TEST FOR UNSATURATION:
The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

TEST FOR THE REDUCING SUGAR
5ml of Benedict’s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

TEST FOR AMINO ACID
One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.
PHYTOCHEMICAL SCREENING

Phyto-chemicals, chemical compounds that occur naturally in plants (phyto means "plant" in Greek), are responsible for colour and biological properties. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients.

The following tests are used for the analysis of phytochemicals as described by Harborne, and Onwukaeme and coworkers, 1999 were carried on alcoholic extract of plant.

1. **ALKALOIDS-DRAGANDROFFS TEST**

8g of Bi (NC>3) 3. 5H2O was dissolved in 20 ml HNO3 and 2.72g of potassium iodide in 50 ml H2O. These were mixed and allowed to stand. When KNO3 crystals out, the supernatant was discarded off and made up to 100 ml with distilled, water. The alkaloids were regenerated from the precipitate by treating with Na2CC>3 followed by extraction of the liberated base with ether. To 0.5 ml of alcoholic solution of extracts were added to 2.0 ml of HC1. To this acidic medium 1.0 ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

2. **FLAVANOIDS**

In a test tube containing 0.5 ml of alcoholic extract 5-10 drops of dilute HC1 and a small piece of ZnCl2 or Mg were added and the solution was boiled for few minutes. In the presence of flavanoids reddish pink or dirty brown color was produced.

3. **TANNIN-FERRIC-CHLORIDE TEST**

To 1 -2 ml of aqueous extract, few drops of 5% ferric chloride solution was added. A bluish black colour which disappears in addition of a few ml of sulfuric acid, there is no formation of yellowish brown precipitate.

4. **PHENOLS-LEAD ACETATE TEST**

1 ml of alcoholic extract was diluted to 5ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed, which indicate the presence of phenols.

5. **GLYCOSIDES**

A small amount of alcoholic extract was dissolved in 1 ml of H2O and the aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.
6. TERPENOIDS

To 2ml of chloroform extract 1 ml of concentrated E^SC^ was added carefully along the sides of the test tube in the presence of terpenoids a red color was produced in the chloroform layer.

RESULTS AND DISCUSSION

PHYSICOCHEMICAL ANALYSIS

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oraganoleptic characters</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Colour</td>
<td>Colourless</td>
</tr>
<tr>
<td>b.</td>
<td>Odour</td>
<td>Odourless</td>
</tr>
<tr>
<td>c.</td>
<td>Sense of touch</td>
<td>Water</td>
</tr>
<tr>
<td>d.</td>
<td>Appearance</td>
<td>Liquid</td>
</tr>
<tr>
<td>e.</td>
<td>Taste</td>
<td>L.Sweet</td>
</tr>
<tr>
<td>2.</td>
<td>Physio chemical standard</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Loss on drying</td>
<td>98.3%</td>
</tr>
<tr>
<td>b.</td>
<td>specific gravity</td>
<td>0.99/1ml</td>
</tr>
<tr>
<td>c.</td>
<td>PH</td>
<td>6.78</td>
</tr>
<tr>
<td>3.</td>
<td>Microbial Limit test</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Total viable aerobic count</td>
<td>2.1 x 10^3 col./g</td>
</tr>
<tr>
<td>b.</td>
<td>Total Enterobacteriaceae</td>
<td>5 x 10^3 col./g</td>
</tr>
<tr>
<td>c.</td>
<td>Total fungal count</td>
<td>3.4 x 10^1 col./g</td>
</tr>
<tr>
<td>d.</td>
<td>Test for specific pathogen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella sp</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureas</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>Nil</td>
</tr>
</tbody>
</table>

CHEMICAL ANALYSIS

PRELIMINARY BASIC AND ACIDIC RADICAL STUDIES

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>PARAMETERS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Calcium</td>
<td>Absent</td>
</tr>
<tr>
<td>2.</td>
<td>Iron (ferric)</td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td>Iron (ferrous)</td>
<td>Absent</td>
</tr>
<tr>
<td>4.</td>
<td>Zinc</td>
<td>Absent</td>
</tr>
<tr>
<td>5.</td>
<td>Sulphate</td>
<td>Present</td>
</tr>
<tr>
<td>6.</td>
<td>Chloride</td>
<td>Absent</td>
</tr>
<tr>
<td>7.</td>
<td>Phosphate</td>
<td>Absent</td>
</tr>
<tr>
<td>8.</td>
<td>Carbonate</td>
<td>Absent</td>
</tr>
<tr>
<td>9.</td>
<td>Starch</td>
<td>Absent</td>
</tr>
<tr>
<td>10.</td>
<td>Albumin</td>
<td>Absent</td>
</tr>
<tr>
<td>11.</td>
<td>Tannic acid</td>
<td>Absent</td>
</tr>
<tr>
<td>12.</td>
<td>Unsaturated compounds</td>
<td>Absent</td>
</tr>
<tr>
<td>13.</td>
<td>Reducing sugar</td>
<td>Absent</td>
</tr>
<tr>
<td>14.</td>
<td>Amino acid</td>
<td>Absent</td>
</tr>
</tbody>
</table>
PHYTOCHEMICAL ANALYSIS

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids (Dragendroff’s test)</td>
<td>Orange red precipitate was found</td>
<td>Presence of alkaloid (+)</td>
</tr>
<tr>
<td>2.</td>
<td>Flavanoids</td>
<td>No characteristic change was observed</td>
<td>Absence of Flavanoid (-)</td>
</tr>
<tr>
<td>3.</td>
<td>Phenols</td>
<td>Yellow precipitate was observed</td>
<td>Presence of Phenols (+)</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>No characteristic change was observed</td>
<td>Absence of glycosides (-)</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoids</td>
<td>No characteristic change was observed</td>
<td>Absence of terpenoids (-)</td>
</tr>
<tr>
<td>6.</td>
<td>Tannins</td>
<td>No characteristic change was observed</td>
<td>Absence of tannins</td>
</tr>
</tbody>
</table>

DISCUSSION

The physical parameters like odour, colour, pH, specific gravity revealed that the vilam pazha Thiravagam is a transparent, odorless, weak acid having pH 6.78 which is near to the pH of biological secretion saliva, urine and also milk. It also plays a role in enzyme activity by maintaining the chemical environment thus regulating the homeostasis; it is also an important factor for drug absorption. Being weak acidic the drug is more readily absorbed in an acid medium like stomach which enhance the bio availability of the drug.

MICROBIAL LIMIT TEST

- Total viable aerobic counts within the normal level (Normal 1 x 10^5 col./g)
- Total Enterobacteriaceae counts within the normal level (Normal 1 x 10^3 col./g)
- Total fungal count within the normal level (Normal 1 x 10^4 col./g)
- Specific pathogens salmonella sp, Staphylococcus aureus, E.coli, Pseudomonas aeruginosa are Nil.

The Chemical analysis of Vilampaza thiravagam contains the chemical constituent sulphate

Biological importance of sulphate.

- Nutritionally essential element
- Functional in the form of sulphur containing amino acids like cysteine and methionine
  Phytochemicals are natural bioactive compound found in plants and fibers which act as a defense system against diseases and more accurate to protect against diseases and more accurate to protect against diseases. (James Anderson MD (1983). The phytochemical analysis reveals the presence of alkaloids and phenols.

CONCLUSION

The achieved results of Physio-Chemical, Chemical and Phyto-Chemical will be useful as a tool for authentication, standardization profile and quality control assessment of herbal formulation.
ACKNOWLEDGEMENT
The authors are thankful to Gunapadam department, Government Siddha Medical College, Palayamkottai.

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