PHYSIOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF PARANGIPATTAI CHOORANAM – A POLYHERBAL SIDDHA FORMULATION

Lalitha Sivasankaran*1, V. Anavarathan2, V. Mahalakshmi1, N. J. Muthu Kumar1

1Department of Sirappu Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai -600047

2Trincomalee Campus, Eastern University, Sri Lanka

Abstract
Phytochemical and physiochemical analysis of the polyherbal Siddha preparation of “Parangipattai chooranam” being used for skin diseases (kutta noigal) especially in kaalanjagapadai (psoriasis). This study is screening of Physiochemical and Preliminary Phytochemical analysis of the Parangipattai chooranam. The powder of this formulation was subjected to physiochemical and preliminary phytochemical analysis. The physiochemical study shows such as Loss on drying is 2.83%, Total ash value is 3.14%, Acid insoluble ash is less than 1%, and Water soluble ash is 1.12%. Water soluble extraction is 47.75%, Alcohol soluble extraction is 12.74% and preliminary phytochemical screening test had reveals the presence of Carbohydrates, Glycoside, Saponin, Flavonoids, Protein and Amino acids, Diterpenes, and Quinones. The results of the study could be used as a diagnostic tool for this polyherbal formulation.

Key words: Polyherbal Siddha formulation, Parangipattai chooranam, Physiochemical analysis, Preliminary phytochemical analysis.
INTRODUCTION

The Siddha system of medicine is the ancient system of medicines and being practiced by a large population in Southern part of India. Herbal based traditional remedies are highly recommended by World Health Organization (WHO) because of their safety, easy availability, low cost in the treatment of various diseases [1, 3]. An herbal based formulation improves the quality of human life through its potent natural antioxidants. World Health organisation (WHO) and National Centre for Complementary and Alternative Medicine (NCCAM) accentuates the need to ensure quality and safety of herbal medicines by modern techniques and applying suitable standards and has proposed guidelines for development of standard herbal medicine. Physiochemical and Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth [4, 9, 10].

*Parangipattai Chooranam* – a poly herbal Siddha formulation from the Siddha text of *Agasthiyar vaithya kaviyam 1500*, is being treated for *kutta noigal* (skin diseases). This poly herbal formulation comprises 23 herbals such as, *Parangipattai* (stem bark of Smilax china), *Ammukara ver* (toot of Withania somnifera), *Sangam kuppi ver* (root of Azima te tracantha), *Sirupirappan kizhangu* (tuber of Calamus rotang), *Kondrai pattai* (stem bark of Cassia fistula), *Saaranai ver* (root of Trianthema decandra), *Kodiveli ver* (root of Plumbago zeylanica), *Adhividaiyam* (stem of Coriandrum sativum), *Oمام* (seeds of Trachyspermum ammi), *Koththa mali* (seeds of Coriandrum sativum), *Vaal milagu* (seeds of Piper cubeba), *Vendhayam* (seeds of Trigonella foenum-graecum), *Sitraratthai* (tuber of Alpinia officinarum), *Akkara kaaram* (root of Anacyclus pyrethrum), *Thippili* (seeds of Piper longum), *Saadhikkai* (seeds of Myristica fragrans), *Saadhhipathiri* (seeds covering of Myristica fragrans), *Kiraambu* (dry fruit of Syzygium aromaticum), *Karuncheeragam* (seeds of Nigella sativa), *Seeragam* (seeds of Cuminum cyminum), *Chukku* (dry tuber of Zingiber officinalae), *Milagu* (seeds of Piper nigrum), *Paal* (Milk), *Sarkarai* (Sugar). The required raw drugs for the trial medicines were purchased from a well reputed raw drug shop and the raw drugs were authenticated by the Department of Medicinal Botany in National Institute of Siddha. After that the raw drugs were purified as per Siddha literatures are *Sigicha rathina theepam* and *Sarakku sudhdi muraigal*, and then
the trial drugs were prepared in the Gunapadam laboratory of National Institute of Siddha by following a Standard Operating Procedures\textsuperscript{[3]}.

METHOD OF PREPARATION

As per the Siddha literature (Agastiyar vaithya kaaviam 1500), 175 grams (5 palam) Parangipattai was taken, the above mentioned six roots were taken in each 35 grams (1 palam), and remaining raw drugs were taken in each 15.3 grams (3 kazhanju). Paal (Milk) 5.3lit and an half the amount of Sarkarai (sugar) was needed for the chooranam.

The above mentioned first seven ingredients are dried, and make it into the fine powder. Take required quantity of milk in pot, the top of the pot covered with cloth. The fine powdered placed over the cloth and it closed by another pot. Allowed to boil the milk. Then the fine powder mixed with the remaining part of powdered ingredients. Finally add sugar which is half the amount of powder. Then the medicine stored in an air tight container and to be administered 2 grams (Verukadi) with warm water at morning and night in 48 days \textsuperscript{[2]}.

2. MATERIALS AND METHODS

All the physiochemical analysis was carried out as per standard guidelines \textsuperscript{[4, 5, 7]}.

2.1) Loss on Drying:

An accurately weighed 2g of Parangipattai Chooranam formulation was taken in a tarred glass bottle. The crude drug was heated at 105\textdegree{}C for 6 hours in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.

2.2) Determination of total ash:

Weighed accurately 2g of Parangipattai Chooranam formulation was added in crucible at a temperature 600\textdegree{}C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

2.3) Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.
2.4) **Determination of water soluble ash:**

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

2.5) **Determination of water soluble Extractive:**

5gm of air dried drug, coarsely powered Parangipattai Chooranam was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100°C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

2.6) **Determination of alcohol soluble extractive:**

2.5gm. of air dried drugs, coarsely powdered Parangipattai Chooranam was macerated with 50 ml. alcohol in closed flask for 24 hrs. With frequent shaking it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

3. **PRELIMINARY PHYTOCHEMICAL SCREENING**

Primary metabolites like carbohydrates, Proteins, fixed oil, fats, gums and mucilage were analysed for their presence of standard procedures \[3, 6, 8\].

3. 1. **Detection of alkaloids:**

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) **Mayer’s Test:**

Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) **Wagner’s Test:**

Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
c) **Dragendroff’s Test:**

Filtrates were treated with Dragendroff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

**d) Hager’s Test:**

Filtrates were treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

### 3.2 Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**a) Molisch’s Test:**

To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid are added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

**b) Benedict’s Test:**

Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

### 3.3 Detection of glycosides:

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

**a) Modified Borntrager’s Test:**

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

**b) Cardiac glycoside (Keller-Killiani test):**

Extract was shaken with distilled water (5 ml). To this, glacial acetic acid (2 ml) containing a few drops of ferric chloride was added, followed by H2SO4 (1 ml) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.
3.4 Detection of saponins

a) Froth Test:
Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test:
0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

3.5) Detection of phytosterols

a) Salkowski’s Test:
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

3.6. Detection of phenols Ferric Chloride Test:
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

3.7. Detection of tannins Gelatin Test:
The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatine containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

3.8. Detection of Flavonoids

a) Alkaline Reagent Test:
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test:
Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
3.9. Detection of proteins and amino acids

a) Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

3.10. Detection of diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

3.11. Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

3.12. Test for Fixed oils and Fats

a). Spot test:

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

3.13. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

The Preliminary phytochemical studies of aqueous extract of Parangipattai Chooranam were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of Parangipattai Chooranam.

RESULTS

Physiochemical analysis results had shown, Loss on drying is 2.83%, Total ash value is 3.14%, Acid insoluble ash is less than 1%, and Water soluble ash is 1.12%. Water
soluble extraction is 47.75%, Alcohol soluble extraction is 12.74% and Preliminary phytochemical screening test was carried out for each extracts of Parangipattai chooranam in Molisch’s Test, Benedict’s test had shown in the presence of Carbohydrates, Modified Borntrager’s test that shows the presence of Glycosides, Froth Test reveals the presence of Saponin, Alkaline Reagent Test, Lead acetate Test shows the result of Flavonoids. Xanthoproteic Test reveals the presence of Protein and amino acids, Copper Acetate Test and NAOH+ Extract shows the results of Diterpenes and Quinones.

Table 1: Physiochemical properties of Parangipattai chooranam

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>2.83%</td>
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<tr>
<td>2</td>
<td>Total ash value</td>
<td>3.14%</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>Less than 1%</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>1.12%</td>
</tr>
<tr>
<td>5</td>
<td>Water soluble extraction</td>
<td>47.75%</td>
</tr>
<tr>
<td>6</td>
<td>Alcohol soluble extraction</td>
<td>12.74%</td>
</tr>
</tbody>
</table>

Table 2: Preliminary phytochemical screening of parangipattai chooranam

<table>
<thead>
<tr>
<th>S.no</th>
<th>Phytochemicals</th>
<th>Test name</th>
<th>H2O extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
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<tr>
<td></td>
<td></td>
<td>Wagner’s Test</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Dragendorff’s Test</td>
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<tr>
<td></td>
<td></td>
<td>Hager’s Test</td>
<td>-ve</td>
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<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Molisch’s Test</td>
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<td></td>
<td></td>
<td>Benedict’s Test</td>
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<tr>
<td>3</td>
<td>Glycoside</td>
<td>Modified Borntrager’s Test</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Keller Killiani</td>
<td>-ve</td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td>Froth Test</td>
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<td></td>
<td></td>
<td>Foam Test</td>
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<tr>
<td>5</td>
<td>Phytosterol</td>
<td>Salkowski’s Test</td>
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</tr>
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<td>6</td>
<td>Phenols</td>
<td>Ferric chloride Test</td>
<td>-ve</td>
</tr>
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<td>7</td>
<td>Tannins</td>
<td>Gelatin Test</td>
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<td>8</td>
<td>Flavonoids</td>
<td>Alkaline Reagent Test</td>
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<td></td>
<td></td>
<td>Lead acetate Test</td>
<td>+ve</td>
</tr>
<tr>
<td>9</td>
<td>Proteins and Amino acids</td>
<td>Xanthoproteic Test</td>
<td>+ve</td>
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<td>10</td>
<td>Diterpenes</td>
<td>Copper Acetate Test</td>
<td>+ve</td>
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<tr>
<td>11</td>
<td>Gum &amp; Muclilage</td>
<td>Extract + Alcohol</td>
<td>-ve</td>
</tr>
<tr>
<td>12</td>
<td>Fat &amp; Fixed Oil</td>
<td>Spot Test</td>
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<tr>
<td>13</td>
<td>Quinones</td>
<td>NAOH + Extract</td>
<td>+ve</td>
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</tbody>
</table>
DISCUSSION AND CONCLUSION

Siddha system of medicines and being practiced by a large population in south India. The development of this traditional system of medicines with perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in health care. Plants serve as vast source for varied phytoconstituents exhibiting pharmacological property. Identifying such potential plants is of significance in medicine. In physiochemical analysis Ash value are used to determine the quality and purity of drugs, the successive extractive value showed that having more extractive which indicate the more solubility of phytoconstituents. The moisture content is found to be less than ten which indicates the dryness of the drug and pH value access acidity or alkalinity and Preliminary phytochemical screening test was carried out for each extracts of Parangipattai chooranam in standard procedure had reveals the presence of Carbohydrates, Glycoside, Saponin, Flavonoids, Protein and Amino acids, Diterpenes, and Quinones and Physiochemical analysis results had shown, Loss on drying is 2.83%, Total ash value is 3.14%, Acid insoluble ash is less than 1%, Water soluble ash is 1.12%. Water soluble extraction is 47.75%, Alcohol soluble extraction is 12.74%.

REFERENCES


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