ORGANOLEPTIC CHARACTERS, PHYSIOCHEMICAL AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THRIKADUGU, PARANGIPATTAI AND MASIKKAI CHOORANAM

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ABSTRACT

AIM: The aim of the study is to evaluate Organoleptic characters, Physiochemical parameters and to screen the phytochemicals present in three Siddha formulations – Thrikaduku chooraram (TKC), Parangippattai choorangam (PPC), Masikai choorangam (MSC). Thrikadugu chooraram is widely used for fever due to multiple etiology, Parangippattai chooraram is indicated for all types of skin diseases and MSC is prescribed for gastric ulcer and any bleeding disorder. Method: Initially, organoleptic characters like appearance, colour, taste and odour of three siddha formulations were noted. Three samples were screened for total ash value, acid insoluble ash, water soluble ash to estimate the quality of study drug. Each extraction of the sample was taken by dissolving the 4 gram of respective sample with 40 ml of water and heated it in water bath. Then filtered and used the filtrates for testing. Preliminary phytochemical test was done following the standard procedure. Each sample was tested for 12 phytochemicals. Results: The results of physiochemical analysis of TKC, PPC, MSC were found within normal limits. The results showed presence of alkaloids, carbohydrates, phytosterols, phenols, flavonoids, proteins and amino acids, diterpenes, gum and mucilage and quinones for TKC, Presence of alkaloids, carbohydrates, saponins, phytosterols, phenols, tannins, flavonoids, gum and mucilage and quinones for PPC, Presence of saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids, diterpenes, and quinones for MSC.

KEYWORDS: Organoleptic characters, physiochemical Parameters, Preliminary Phytochemical, Thrikadugu chooraram, Parangippattai chooraram, Masikai chooraram.

INTRODUCTION

Ayurveda and Siddha medicines are very effective and have therapeutic value in nature but lack of standardization, it is required to develop the standardization technique. In this study, an attempt has been made to test the organoleptic characters, physiochemical Parameters and phytochemical screening of three siddha formulations ie Thrikadugu chooraram, Parangippattai chooraram and Masikai chooraram. Thrikadugu chooraram is prescribed for all types of fever and pain[5] Parangippattai chooraram is indicated for all types of skin disorders.[2] Venerreal diseases, leprosy, leukodermia, gives complexion to the skin.[3] Masikai chooraram is mainly used for the treatment of dysentery, gastric ulcer, and to arrest any bleeding.[4]

Zingiber officinale possess the analgesic and anti-inflammatory efficacy.[1] The hexane extract from piper longum presented the highest activity against S. aureus, E.coli and Klebsiella Sp.[5] The Piper nigrum extracts have antibacterial activity against gram positive organism than gram negative organism.[6]

Aqueous extract of Quercus infectoria possess optimum antibacterial activity.[7] HPTLC fingerprinting detected the presence of diosgenin, a biomarker compound in the alcohol and aqueous extract of Smilax zeylanica.[8]

In this study organoleptic characters, physiochemical Parameters and Phytochemical test for alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids, diterpenes, gum and mucilage and quinones of three Siddha formulations were done.

MATERIALS AND METHODS

Thrikadugu chooraram, Parangippattai chooraram and Masikai chooraram were collected from Outpatient Department of The TN Dr MGR Medical University, Chennai and used for testing.
Ingredients of thrikatu chooranam are Zingiber officinale, Piper nigrum and Piper longum. Parangipattai chooranam and Masikai choornam is also a single drug preparation.

Initially, organoleptic characters like appearance, colour, taste and odour of three siddha formulations were noted. Three samples were screened for total ash value, acid insoluble ash, water soluble ash to estimate the quality of study drug. Preliminary Phytochemical test was done following the standard procedure. Each sample was tested for 12 phytochemicals

**Evaluation of Organoleptic Characters**
Organoleptic characters refer to the evaluation of formulations by appearance, colour, odour, taste, etc. Organoleptic evaluation of three Siddha formulations were carried out using traditional and standard techniques.

**Physicochemical Analysis**
Physicochemical evaluation of the study drug was done following the standard procedure. (B. Lavanya 2016., WHO 1998) Three samples screened for, total ash value, acid insoluble ash, water soluble ash to estimate the quality of study drug.

**Determination of Total Ash values**
The ash remaining following ignition of sample is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash.

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non-physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Procedure: 4 gm of sample weighed, placed evenly in a previously ignited and tarred silica dish. Ignited in a muffle furnace at 600° C until it turned white in color. It indicated the absence of carbon.

Percentage of Total ash = \frac{\text{weight of the ash}}{\text{weight of the sample taken}} x 100

**Determination of acid insoluble ash**
Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure: Added to the ash 15 to 25 ml of the hydrochloric acid and boiled for 10 minutes, covering the dish with a watch glass to prevent sputtering. Allowed to cool and filtered and contents of the dish through the ash less filter paper. Washed the filter paper in hot water until the washings are free from hydrochloric acid, as tested by silver nitrate solution and returned it to the dish. Evaporated carefully on the water bath and ignited in the muffle furnace at 550° C ± 25° C for 1 hour. The dish was allowed to cool in the desiccators and weighted. Percentage of Acid insoluble ash = \frac{\text{weight of the acid insoluble residue}}{\text{weight of the sample taken}} x 100

**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 15min at a temperature not exceeding 450°C in a muffle furnace. Difference in weight of ash and weight of water.

**The preliminary phytochemical screening Test**
The preliminary phytochemical screening test was carried out for each extracts of the samples as per the standard procedure. 

**Preparation of Extract**
Each extraction was taken by dissolving the 4 gram of respective sample with 40 ml of distilled water and heat it in water bath at 60 C. Then filtered and used the filtrates for testing Preliminary Phytochemicals.

1. **Detection of alkaloids**
Extract was dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test
2 ml of extract was treated with few drops of Mayers’ reagent, formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test
2 ml of filtrate was treated with Wagner’s reagent. Formation of brown reddish precipitate indicates the presence of alkaloids.

2. **Detection of carbohydrate**
Extract was dissolved individually in 5 ml of distilled water and filtered. The filtrates were used for test the presence of carbohydrates.

Molisch's test
2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of carbohydrates.

Benedict’s test
Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. **Detection of Glycosides**

Liebermann’s test
2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet colour change into blue and green indicates presence of Glycosides.
4. Detection of Saponins

**Froth test**
Extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimeter layer of foam indicates the presence of Saponins.

**Foam test**
0.5 gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of Saponins.

5. Detection of phytosterols

**Salkowski's test**
Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow colour indicates the presence of triterpenes.

6. Detection of phenols

**Ferric Chloride test:** 2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. Detection of tannins

**Gelatin test**
To the extracts, 1% of gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of phenols.

8. Detection of flavonoids

**Alkaline reagent test**
Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow colour then on addition of diluted hydrochloric acid it becomes colourless, it indicates the presence of flavonoids.

**Lead acetate test**
Extract was treated with few drops of lead acetate solution, yellow colour precipitate indicates presence of flavonoids.

9. Detection of Proteins and Aminoacids

a) Xanthoproteic Test: The extract were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25 % ninhydrine reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

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.

11. Detection of gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

12. Detection of Quinones

Extract was treated with concentrated HCL and observed for the formation of yellow precipitate or yellow discolouration.

RESULT

Organoleptic Characters

Organoleptic evaluation of three Siddha formulations were carried out using traditional and standard techniques. And Organoleptic Characters of TKC, PPC and MSC were tabulated in Table:1.

<table>
<thead>
<tr>
<th>S.n.</th>
<th>Organoleptic Characters</th>
<th>TKC</th>
<th>PPC</th>
<th>MSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Appearance</td>
<td>Fine Powder</td>
<td>Fine Powder</td>
<td>Fine Powder</td>
</tr>
<tr>
<td>2</td>
<td>Color</td>
<td>Dark brown</td>
<td>Mud brown</td>
<td>Cream white</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Pungent</td>
<td>Astringent</td>
<td>Astringent</td>
</tr>
<tr>
<td>4</td>
<td>Odour</td>
<td>Pleasant</td>
<td>Pleasant</td>
<td>Pleasant</td>
</tr>
</tbody>
</table>

Physiochemical parameters

Three samples were screened for, total ash value, acid insoluble ash, water soluble ash to estimate the quality of study drugs. And the results were tabulated in Table:2.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Samples</th>
<th>Ash value</th>
<th>Acid insoluble ash</th>
<th>Water soluble ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thirikaduku chooranam</td>
<td>7.50%</td>
<td>2.45%</td>
<td>4.54%</td>
</tr>
<tr>
<td>2</td>
<td>Parangi pattai chooranam</td>
<td>6.85%</td>
<td>2.0%</td>
<td>4.25%</td>
</tr>
<tr>
<td>3</td>
<td>Masikai chooranam</td>
<td>4.15%</td>
<td>1.25%</td>
<td>2.90%</td>
</tr>
</tbody>
</table>
Phytochemical screening of TKC, PPC and MSC

The Preliminary phytochemical studies of Trikadugu chooranam, Parangipattai chooranam and Masikkai chooranam were done using standard procedures.\textsuperscript{[10,11]} Each sample was tested for 12 phytochemicals ie alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids, diterpenes, gum and mucilage and quinones. The results were presented in Table:3.

Table 3: Results of Phytochemical screening of TKC, PPC and MSC.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Phytochemicals</th>
<th>Test Name</th>
<th>Trikadugu chooranam</th>
<th>Parangipattai chooranam</th>
<th>Masikkai chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>Mayer’s test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>Wagner’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>Libermann Burchard’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>Froth test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phytosterols</td>
<td>Salkowski’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>Gelatin test</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>Alkaline Reagent test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Protein and amino acids</td>
<td>Xanthoproteic test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Diterpenes</td>
<td>Copper acetate test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Gum and mucilage</td>
<td>Extract + alcohol</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Quinones</td>
<td>NAOH +Extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present. - = Absent

DISCUSSION AND CONCLUSION

Pharmacology screening of Zingiber officinale revealed its hepatoprotective, nephro-protective, anti-oxidant, antifungal, anti-bacterial, larvicidal, anti-inflammatory, analgesic, anti-diarrheal activity scientifically now a day.s.\textsuperscript{[1,12]} But TKC is indicated for above mentioned activity in siddha literature very long ago and it has been prescribed for various conditions by the siddha and Ayurveda physicians since more than 100 years.

Anita mural et al, Smilax zeylanica possess hepatoprotective activity in animal model.\textsuperscript{[13]} In this study, PPC showed the presence of alkaloids, carbohydrates, saponins, phytosterols, phenols, tannins, flavonoids, these phytochemicals are responsible for the hepatoprotective activity.

Subin mary et al, Quercus infectoria was evaluated against gram negative stain pseudomonas aeruginosa. In this study result revealed that masikkai possess tannin, Tannins mainly contribute to the antimicrobial activity.\textsuperscript{[7]} In siddha literature masikkai chooranam is indicated for dysentery, diarrhea, any bleeding due to various reasons. And Masikkai chooranam is practiced by the Siddha physicians in Government siddha hospitals since more than 100 years.

The results of physiochemical analysis of samples were found within normal limits. It proves the safety of the drug to use as internal medicine. Samples possess the major phytochemicals compounds like that Flavonoids, Tannins, Steroids, Protein, Terpenoids, Alkaloids, Carbohydrate, sugar and Phenols and these are responsible for efficacy of the drug to control and prevent the diseases indicated.

ACKNOWLEDGEMENT

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Conflict of Interest: Nil.

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