QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN KUPPAIMENI CHOORANAM – A SIDDHA HERBAL FORMULATION

Dr.P.C.Berfin Flower¹, Dr.P.Sathish Kumar², Dr.T.Komalavalli³
¹PG scholar, ²Professor, ³Head of the department and Professor
Department of Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli (dt), Tamilnadu, India.

Abstract: Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Prevention and treatment are the basic aim of the Siddha system of medicine. The basic principle of the Siddha system of medicine is, “food itself is a medicine”. At the moment there are numerous scientific findings that support the potentiality of the Siddha system of medicine to treat various diseases. In recent times, there has been a noticeable increase in the utilization of traditional system of medicine. Therefore, it is imperative to subject Siddha drugs to modern scientific methods for characterization, and to further explore the essential bioactive components.

Aim: The aim of the present study is phytochemical analysis of the Siddha drug formulation of Kuppaimeni Chooranam.

Method: The extract was prepared with Kuppaimeni Chooranam to be a photochemical screening test for saponins, tannins, terpenoids, alkaloids, flavonoids, steroids, glycosides, carbohydrates, quinones and proteins.

Results and discussion: The qualitative and quantitative analysis of phytochemical screening of the siddha drug Kuppaimeni Chooranam shows the presence of protein, terpenoid, alkaloid, flavonoid, glycoside respectively in 140, 5, 76, 51, 44 mcg/100gm.

Conclusion: The phytochemical screening study for Kuppaimeni Chooranam shows the presence of protein, terpenoid, alkaloid, flavonoid, and glycoside, which are responsible for its biological activity. This evidence based data provide valuable information is helpful to standardization of Kuppaimeni Chooranam.

Keywords: Siddha system, Kuppaimeni Chooranam, Phytochemical, Standardization.

Introduction

The Tamil traditional medicinal system, the so called Siddha system of medicine, is an ancient indigenous practice the flourished and practiced for many centuries in Tamil Nadu, India. The basic principle of the Siddha system of medicine is, “food itself is a medicine” which was postulated by the great 18 sages called Siddhars. The million-year old Siddha literature indicates that this traditional medicinal system can cure many chronic diseases. At the moment there are numerous scientific findings that support the potentiality of the Siddha system of medicine to treat various diseases. However, there are many challenges and issues that need to be properly addressed to preserve this age-old indigenous health practice by conducting more research and development on the toxicity and potentiality of Siddha medicinal preparations. Due to the fact that people have been using Siddha medicine more frequently recently, it is imperative to subject Siddha drugs to modern scientific methods for characterization, and to further explore the essential bioactive components. The analysis of phytochemicals plays a crucial role in the discovery of novel sources of biologically active compounds such as carbohydrates, glycosides, steroids, tannins, saponins, alkaloids, flavonoids, proteins, phenols, and terpenoids. The primary objective of this investigation is to identify the phytochemical components present in Kuppaimeni Chooranam, a Siddha drug formulation which is used in the treatment of Bleeding hemorrhoids.

Materials and Methods

Preparation of Drug

The Kuppaimeni leaves are freshly collected and is purified according to the proper procedure. Adulterants are removed and the leaves are dried in shade. Purified raw drug is made into fine powder. Then it is filtered using pure white cloth. The drug was labelled as Kuppaimeni Chooranam.
Table 1. Ingredients of the Kuppaimeni Chooranam

<table>
<thead>
<tr>
<th>S.No</th>
<th>TAMIL NAME</th>
<th>BOTANICAL NAME</th>
<th>FAMILY</th>
<th>PARTS USED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KUPPAIMENI</td>
<td>Acalypha indica</td>
<td>Euphorbiaceae</td>
<td>Leaves</td>
</tr>
</tbody>
</table>

**Phytochemical screening:**

**Extract Preparation:**

100gm of powdered medicine was measured into a conical flask and 200ml of solvent such as acetone, methanol, benzene and water respectively were added and it is packed into soxhelt extractor, for 48hrs and labelled. Finally the extract was filtered with Watt man No.1 filter paper and the filtrate obtained was stored in airtight bottles. However, the extract was evaporated to dryness by heating in water bath to obtain semi solid mass. Dried extract was stored in refrigerator at 4°C for their future use in phytochemical analysis.

**Qualitative Analysis of Kuppaimeni Chooranam:**

1. **Test for Saponins**
   To a few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

2. **Test for Tannin**
   To substrate in water is added with 5% alcoholic ferric chloride. Dark blue colour shows presence of tannin.

3. **Test for Terpenoids**
   To a few mg of extract in chloroform, add conc. H₂SO₄. Presence of dark brown precipitate indicates the presence of terpenoids.

4. **Test for Phenol**
   To substrate in water is added with 5% alcoholic ferric chloride. Dark blue or green colour shows presence of phenol.

5. **Test for Steroids (Lieberman Burchard Test)**
   To a few mg of the extract 2 ml of chloroform is added in a dry test tube. Few drops of acetic acid is added, heated and few drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added. The green colour indicates the presence of steroid.

6. **Test for Quinones**
   To a few mg of extract, add few drops of concentrated sulphuric acid. Appearance of red colour shows the presence of quinone.

7. **Test for Glycosides**
   Substance is treated with anthrone and concentrated sulphuric acid. On heating over a water bath, the appearance of green colour shows the presence of glycoside.

8. **Test for Carbohydrates**
   To the sample solution, added few drops of α-naphthol and 2-3 ml conc. H₂SO₄. The appearance of reddish violet or purple ring at the junction of two liquids indicates the presence of Carbohydrates.

9. **Test for Alkaloids (Dragendorff’s Test)**
   Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff’s reagent were added. The presence of orange red precipitates indicates the presence of alkaloids.

10. **Test for Flavonoid**
    To the substance in alcohol add 10% NaOH or ammonia. A dark yellow colour indicates the presence of flavonoid.
11. Test for Proteins (Biuret test)
To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

METHODOLOGY

PHYTOCHEMICAL QUANTITATIVE ANALYSIS

Quantitative Estimation of flavonoids
Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Glycoside
Take 10ml of the extract and 10ml of Baljet’s reagent (95ml 1% picric acid+ 5ml of 10 % aqueous sodium hydroxide) are taken and allowed to stand for one hour. Then dilute the solution with 20ml distilled water and mix. Read the intensity of the colour obtained against blank at 495nm using a spectrophotometer. The difference between test and control is taken for calculation. Standard graph can be prepared using standard digitoxin.

Quantitative Estimation of Alkaloids
To 1ml of Methanolic extract add 5 ml pH 4.7 phosphate Buffer and 5 ml BCG solution then shake the mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalent.

Quantitative Estimation of Terpenoid
Total terpenoid content was determined by the method of Ghorai et al (2012)17. To 1 mL of the plant extract, 3 mL of chloroform was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 μl of concentrated sulfuric acid (H2SO4) was added. Then it was incubated at room temperature for 1.5h-2h in dark condition and during incubation a reddish brown precipitate was formed. Then carefully and gently, all supernatant of reaction mixture was decanted without disturbing the precipitation. 3 mL of 95% (v/v) methanol was added and vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 538 nm using UV/visible spectrophotometer.

Quantitative Estimation of total Proteins
Protein content was estimated by the method of Lowry et al. [14]. 1 ml of sample was mixed with 0.5 ml of 0.1 N sodium hydroxide and 5 ml of alkaline copper reagent. The mixture was incubated in room temperature for 30 minutes. Folin– Ciocalteau reagent, 0.5 ml was added and incubated again for 10 minutes at room temperature. The absorbance was read at 660 nm against a reagent blank. The estimation was done in triplicates and the results were expressed mcg/g sample

Table- 2: Qualitative analysis of Kuppaimeni Chooranam

<table>
<thead>
<tr>
<th>Test name</th>
<th>Kuppaimeni Chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>Absent</td>
</tr>
<tr>
<td>Tannins</td>
<td>Absent</td>
</tr>
<tr>
<td>Phenols</td>
<td>Absent</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Absent</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Absent</td>
</tr>
<tr>
<td>Quinones</td>
<td>Absent</td>
</tr>
<tr>
<td>Protein</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 3: Quantitative analysis of Kuppaimeni Chooranam

<table>
<thead>
<tr>
<th>Test</th>
<th>OD Value 1</th>
<th>OD Value 2</th>
<th>Mean Value OD</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.671</td>
<td>0.682</td>
<td>0.676</td>
<td>140 mcg/100mg</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>0.141</td>
<td>0.148</td>
<td>0.145</td>
<td>5 mcg/100mg</td>
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<tr>
<td>Alkaloid</td>
<td>0.232</td>
<td>0.238</td>
<td>0.235</td>
<td>76 mcg/100mg</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>0.228</td>
<td>0.221</td>
<td>0.224</td>
<td>51 mcg/100mg</td>
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<tr>
<td>Glycoside</td>
<td>0.568</td>
<td>0.573</td>
<td>0.570</td>
<td>44 mcg/100mg</td>
</tr>
</tbody>
</table>

Figure 1: Quantitative analysis of Kuppaimeni Chooranam

Discussion

The extract was prepared with Kuppaimeni Chooranam drug was to being photochemical screening test for saponins, tannins, terpenoids, alkaloids, flavonoids, steroids, glycosides, carbohydrates, quinones and proteins. The qualitative analysis of phytochemical screening of siddha drug Kuppaimeni Chooranam shows the presence of protein, terpenoid, alkaloid, flavonoid, glycosides. The quantitative analysis of Kuppaimeni Chooranam contains protein, terpenoid, alkaloid, flavonoid, glycosides respectively in 140, 5, 76, 51, and 44 mcg/100 gm. These phytochemicals show a range of pharmacological activities, such as, anti-bacterial, anti-inflammatory, and anti-oxidant activities.

Conclusion

The qualitative analysis of phytochemical screening of siddha drug Kuppaimeni Chooranam shows the presence of protein, terpenoid, alkaloid, flavonoid, glycosides. The quantitative analysis of Kuppaimeni Chooranam contains protein, terpenoid, alkaloid, flavonoid, glycosides respectively in 140, 5, 76, 51, and 44 mcg/100 gm. This study revealed that this preparation has important phytochemical constituents with various medicinal properties. However, taking into consideration, safety aspects, toxicity and isolation of active compounds and phytochemicals, further studies need to be carried out to unravel the search for bioactivity.

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