Phytochemical Screening of The Rhizome of Kaempferia Galanga

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ABSTRACT
Different extracts of Kaempferia galanga rhizome were screened for the presence of chemically active compounds by standard methods. The results revealed the presence of sterols, Triterpenoids and resins in petroleum ether extract, sterols, Triterpenoids, Flavanoids and resins in chloroform extract, Steroids, Triterpenoids, alkaloids, Flavanoids, carbohydrates, resins and proteins in methanolic extract. The water extract showed the presence of saponins, carbohydrates and proteins. However the tannin content was not detected from any of the rhizome extracts under sturdy, were commended. Further research on this plant leaves for possible isolation and characterization of the various chemical active substances.

Key Words: Kaempferia galanga, Screening, Triterpenoids, steroids, Flavanoids, Carbohydrates.

INTRODUCTION
Many medicinal plants are used in modern medicine where they occupy a very significance place as raw material for important drugs and plants used in traditional system of medicine in pharmaceutical houses are collected from wild sources. Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannins, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes.

Kaempferia galanga belonging to family Zingiberaceae is a genus of rhizomatous herbs distributed in the tropics and sub tropics of Asia and Africa. It is found throughout the plains of India and is cultivated for its aromatic rhizomes. Since the rhizomes of this plant contain volatile oil and other important compounds of enormous medicinal values, they are very demanding to the traditional health care practitioner. The rhizome of this plant has been used traditionally for the treatment of many ailment and few biological activities have proven its importance. The rhizome is rich in essential oils and is being used for the treatment of indigestion, cold, pectoral and abdominal pains, headache, expectorant, diuretic, carminative, stomachic, coughs, pectoral affections, stoppage of nasal blocks, asthma and hypertension.

The aim of this study was to determine the phytochemical properties various extracts of Kaempferia galanga rhizomes an important medicinal plant.

MATERIALS AND METHODS
Plant material
Rhizomes of Kaempferia galanga Linn were collected from Foundation for Revitalization of Local Health Tradition (FRLHT), Bangalore, in the month of March. It was identified and authenticated by Dr. Jawahar C Raveendran (FRLHT), Bangalore. The Identified and authenticated rhizomes were used for Phytochemical screening studies.

Extraction
Dried and coarsely powdered rhizomes of Kaempferia galanga (350g) were refluxed with petroleum ether (60-80°C) for Four hours. The extract was decanted off and fresh quantity of the petroleum ether was added again and refluxed for another 2 hours. The combined petroleum ether extracts were concentrated on water bath set at 40°C whereby a highly viscous greenish thick mass obtained. The defatted dried rhizomes were successively extracted with chloroform and methanol to obtained amorphous buff coloured powder and brownish yellow sticky mass whereby after concentration. Finally, marc was macerated with water for 1-2days, filtered and concentrated to obtained brownish yellow powder.

Thin layer chromatographic extaminaion of the extracts
Thin layer chromatographic plate (5 X 20 cm) 0.5mm thickness was prepared by usual method using silica gel G. the samples of 0.1% of all the extracts were dissolved in methanol separately and spotted manually using a capillary tube. The plate was developed in n-hexane: ethyl acetate (9:1) as solvent system. After development, on examination of the chromatogram under U.V. light and observed for the presence of spots.

Phytochemical Screening

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Table 1: Phytochemical constituents of Petroleum ether, Chloroform, methanol and water extracts of Kaempferia galanga rhizome

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Test</th>
<th>Pet ether extract</th>
<th>Chloroform extract</th>
<th>Methanolic extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sterols and triterpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Resins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Phytochemical Screening for all the extracts were performed using standard procedures.

1. Test for Sterols and Triterpenoids: 25mg chloroform and methanolic extracts were dissolved in chloroform, filtered and filtrate was tested for sterols and Triterpenoids.
   a. Salkowski’s Test: Few drops of concentrated sulphuric acid were added to chloroform solution and observed for Red colour in lower layer for sterols and golden yellow colour indicates the presence of Triterpenoids.
   b. Libermann Buchard test: Few drops of acetic anhydride were added to chloroform solution, shaken well. 1ml of concentrated sulphuric acid carefully added from sides of the test tube. A reddish brown coloration indicates the presence of sterols and Red ring indicates the presence of Triterpenoids.

2. Test for Alkaloids: 0.5g of extracts were diluted separately to 10ml with acid alcohol, boiled and filtered. To 5ml of the filtrate was added 2ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10ml of acetic acid. These were divided in to 3 portions.
   a. Dragendroff’s Test: (Potassium Bismuth Nitrate): Few drops of Dragendroff’s solution added to Chloroform solution, Reddish brown precipitate indicates the presence of alkaloids.
   b. Mayer’s Test: (Potassium Mercuric Iodide): Few drops of Mayer’s Reagent added to Chloroform solution, Creamy white precipitate indicated the presence of alkaloids.
   c. Wagner’s Test: (Iodine in Potassium Iodide): Few drops of Wagner’s solution added to chloroform solution, Brown precipitate indicate the presence of alkaloids.

3. Test for Saponins:
   a. Foam Test: To 0.5gm of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.
   b. Haemolysis Test: To 2ml of 1.8% Nacl solution taken in two test tubes, 2ml of distilled water added to one of the test tube and 2ml of 1.0% extract was added to another test tube, 5 drops of blood was added to each test tube and gently mixed the contents and observed under microscope. If haemolysis observed in the test tube containing the extract, it indicates the presence of saponins.

4. Test for Tannins:
   a. Ferric Chloride Test: About 0.5gm of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.
   b. Gelatin Test: Few ml of 1% solution of gelatin in 10% Sodium chloride was added to the above extract and observed for white precipitate indicates the presence of Tannins.

5. Test for Flavonoids: Three methods were used to test for flavonoids. First, few drops of 1% neutral ferric chloride to a portion of an aqueous filtrate of the extract. A blackish green coloration that produced on standing indicates the presence of flavonoids. Second, A few drops of 10% lead acetate solution were added to a portion of the extract. A yellow precipitates indicates the presence of flavonoids. Third, a portion of the extract was dissolved in the methanol, to this a small piece of magnesium ribbon was added, one ml of concentrated Hydrochloric added from the side of the test tube. A magenta colour indicates the presence of flavonoids.

6. Test for carbohydrates: 100mg of methanolic and water extracts were dissolved in little quantity of distilled water and filtered. The filtrate was used to test the presence of carbohydrates. There are four methods were used to test for carbohydrates.
   a. Fehling’s test: The filtrate was hydrolyzed with dil Hcl, neutralized with alkali and heated with Fehling’s solution A and B. The formation of Red precipitates indicates the presence of reducing sugars.
   b. Barfoed’s test: Few ml of Barfoed’s reagent was added to the filtrate and boiled in a water bath. The formation of Reddish precipitates indicates the presence of Monosaccharide.
   c. Molisch’s test: Few ml of Molisch’s reagent were added to the filtrate and concentrated sulphuric acid was added along the sides of the test tube. The formation of Reddish violet ring indicates the presence of Carbohydrates.
d. Benedict’s Test: Few ml of Benedict’s reagent was added to the filtrate and heated. A reddish orange precipitate indicates the presence of reducing sugars.

7. Test for Resins: Two methods were used to test for resins. First, 0.5gm of the extract was diluted to 10 ml with water and shaken for 5 minutes. The formation of turbidity indicates the presence of Resins. Second, the methanolic extract was dissolved in 5 to 10ml of acetic anhydride by gentle heating and cooled. To this, 0.5ml of Sulphuric acid was added. The formation of Bright purplish red colour changes to violet colour indicates the presence of resins.

8. Test for Proteins: Two methods were used to test for proteins. First, few ml of 0.1% of copper sulphate solution was added to the aqueous solution of extract containing 10% sodium hydroxide. The formation of pink or purple colour indicates the presence of proteins. Second, freshly prepared Ninhydrin reagent was added to the aqueous solution of the extract and boiled for few minutes, allowed to cool. The formation of violet or purple colour indicates the presence of proteins.

RESULTS
The phytochemical screening analysis of all the extracts of Kaempferia galanga rhizome shows that the petroleum ether, chloroform and methanolic extracts showed the presence of sterols and triterpenoids, flavonoids and resins. Methanolic extract showed the presence of alkaloids and water extracts showed the presence of saponins. The chloroform and methanolic extract showed the presence of flavonoids. The methanolic and water extracts showed the presence of carbohydrates and proteins. The thin layer chromatography revealed that the clearly visible spots has been identified in the methanolic extract of the rhizome when compared with the standard Ethyl-p-methoxy cinnamate.

DISCUSSION
The phytochemical screening analysis of the Kaempferia galanga rhizome extract has showed the presence of different constituents in the extracts. The sterols and triterpenoids were found to be present in petroleum ether, chloroform and methanolic extracts. The alkaloid content was identified in methanolic extract, Saponin content was mainly present in water extract and Tannins was totally absent in all the extracts. The flavonoid content was found to be present in chloroform and methanolic extract, Carbohydrates was found to be present in methanol and extracts, Resins was found to be present in Petroleum ether, Chloroform and Methanolic extracts, and proteins was found to be present in methanolic and water extracts.

CONCLUSION
It has been concluded that the rhizome extracts of the Kaempferia galanga (Zingiberaceae) showed the presence of sterols, triterpenoids, alkaloids, saponins, flavonoids, carbohydrates and proteins.

REFERENCES