Pharmacognosical study of Cissus quadrangularis Linn. and Zingiber officinalis ROSC

J Viswanath, Chakrapani Cheekavolu, Renu Dixit, S Sankaraiah, P Leela and M Naresh Kumar

Abstract

Introduction: To identify the pharmacognosical study of purity and strength of extracts for Cissus quadrangularis Linn and Zingiber officinalis rosc.

Methodology: To identify the purity and strength of extracts moisture content, total ash, alcohol-soluble extractive, water-soluble extractive along with identify various test for Alkaloids, Carbohydrates, Proteins and Amino Acids, Phytosterol, Glycosides, Saponins, Flavonoids, Tannins and Phenolic compounds, Triterpenoids and Fixed Oils.

Results: Cissus quadrangularis Linn. of moisture content, total ash, alcohol-soluble extractive, water-soluble extractive values were 8.21%, 19.72%, 7.82% and 21.23%, with presence of Alkaloids, carbohydrates, steroids, phenolic and tannins with pH of 7 while Zingiber officinalis rosc. for moisture content, total ash, alcohol-soluble extractive, water-soluble extractive values were 10.21%, 5.12%, 8.18% and 12.34% with presence of carbohydrates, Saponins, Steroids, Starch, Protein and Amino Acids with pH of 6.

Conclusion: Cissus quadrangularis Linn. of water-soluble extractive values were high with presence of Alkaloids, carbohydrates, steroids, phenolic and tannins, while Zingiber officinalis rosc. of moisture content and water-soluble extractive values were high with presence of carbohydrates, Saponins, Steroids, Starch, Protein and Amino Acids.

Keywords: Cissus quadrangularis Linn, zingiber officinalis rosc, Pharmacognostic

Introduction

Medicinal plants form the major part of the raw materials used by the Ayurvedic practitioners. In most of the books dealing with the materiamedica of Ayurveda the correct identity of the botanical source has become very difficult on account of the synonyms and the use of vernacular names. For this a scientific investigation of the medicinal plants embodying proper identification of all source plants and correlating them properly to the drugs described in Ayurvedic literature is absolutely necessary. This can be possible only by the study of pharmacognosy.

Quality control of crude plant drugs is the other major problem faced by the Ayurvedic system of medicine. The therapeutic efficacy is absolutely dependent on the quality of the plant drug used. And if the plant drugs are adulterated the quality of the preparation cannot go up to the standard level. The identification of adulterants from crude plant drugs and powdered drugs is also essential. For this, various pharmacognostic standards may be applied to standardize and maintain the ‘quality control’ of the single plant drugs. Though pharmacognostic standards alone may not always be adequate to ensure their quality but can play a major role to standardize a plant drug.

Cissus quadrangularis has been prescribed in Ayurveda as an alternative, anthelmintic, dyspeptic, digestive, tonic, analgesic and in the treatment of irregular menstruation. In some parts of world, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various conditions. Cissus quadrangularis justifies its effectiveness in management of obesity and complications associated with metabolic disorders (1). As well as its antioxidant and free radical scavenging activity in vitro (2). Present study to identify the pharmacognosical study of purity and strength of extracts and identification test for Cissus quadrangularis Linn and Zingiber officinalis rosc.

Methodology

The study was carried out in Department of Dravyaguna, S.V Ayurvedic Medical College, Andhra Pradesh, India.
Tirupathi after obtain the institutional ethical committee approval in the period of June 2015 to June 2016.

**Identity, Purity and Strength [3-4]**

1. **Loss on drying at 105°C/Moisture content**

   10 gm of trial drug samples are placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of sample in a tarred evaporating dish is dried at 105°C for 5 hours and it is weighed. After drying tarred evaporating dish was allowed to cool in desiccators for 30 minutes and then weighed the remnant material.

   \[
   \text{The \% of Loss on drying} = \frac{\text{Difference in weight after heating}}{\text{Weight of sample taken}} \times 100
   \]

2. **Determination of Ash**

   1. **Determination of Total Ash**

   About 2.0g of powdered drugs was weighed and placed in three separate previously ignited and tarred silica crucibles. The samples were spread evenly and then ignite or incinerate it to a constant temperature not exceeding 450°C until it is white indicating the absence of carbon. The crucible then cooled in desiccators and final weighed. The results were then calculated the content of total ash in terms of percentage w/w of the air-dried drug.

   3. **Determination of Extractable Matter in water and alcohol**

   About 4.0g of coarsely powdered air dried samples, was accurately weighed in three glass stopped conical flask and macerated with 100ml of the solvent (Water, Methanol, Ethanol, Hydro alcoholic, Ethyl acetate, Chloroform, Benzene and Hexane) specified for the plant material concerned for 6 hours, shaking frequently and then allowed to stand for 18 hours. Filtering was done by whatman paper, taking care not to lose any solvent, and then transfer 25 ml of filtrate to tarred flat bottomed shallow dish. The extracted matter was dried at 105°C for 6 hours, cooled in desiccators for 30 minutes and then weighed. The percentage extractable matter was calculated.

**Preliminary Phytochemical Study [5]**

The formulations were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The pharmacological actions of crude drugs are determined by the nature of their constituents. The phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used.

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them

**I. Tests for Alkaloids**

1. **Dragondroff’s Test:** To 1 ml of the extract, 1 ml of Dragondroff's reagent was added; formation of orange red precipitate indicated the presence of alkaloids.

2. **Wagner's Test:** To 1 ml of the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicated the presence of alkaloids.

3. **Mayer's Test:** To1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of alkaloids.

4. **Hager's Test:** To1ml of the extract, 3 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

**II. Test for Carbohydrates**

1. **Molisch Test:** To 2 ml of the extract, 1 ml of α-naphthol solution and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

2. **Fehling's Test:** To 1ml of the extract, equal quantities of Fehling's solution A and B were added, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.

3. **Benedict's Test:** To 5ml of Benedict’s reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

**III. Tests for Proteins and Amino Acids**

1. **Biuret Test:** To 1 ml of the extract, 1ml of 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins.

2. **Xanthoprotein Test:** To 1 ml of the extract, 1ml of concentrated nitric acid was added. A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsequently added; orange colour indicated the presence of aromatic amino acids.

3. **Lead Acetate Test:** To the extract, 1ml of lead acetate solution is added. Formation of a white precipitate indicated the presence of proteins.

4. **Ninhydrin Test:** Two drops of freshly prepared 0.2% ninhydrinreagent were added to the extract solution and it was then heated. Development of blue colour revealed the presence of proteins, peptides or amino acids.

**IV. Tests for Phytosterol**

1. **Libermann Burchard Test:** The extract was dissolved in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution turned red, then blue and finally bluish green, indicated the presence of steroids.

2. **Salkowski Test:** Dissolve the extract in chloroform and equal volume of concentrate sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.
V. Tests of Glycosides
1. **Legal Test:** The extract was dissolved in pyridine and sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour showed the presence of glycosides.

2. **Baljet Test:** To 1 ml of the test extract 1 ml sodium picrate solution was added and the yellow to orange colour revealed the presence of glycosides.

3. **Borntrager’s Test:** A few ml of dil. HCl was added to 1 ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1 ml of ammonia. The formation of red colour showed the presence of anthraquinone glycosides.

4. **Keller Killiani Test:** The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue, confirmed the presence of glycosides.

VI. Test for Saponins
1. About 1 ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. 1cm layer of foam indicated the presence of saponins.

VII. Test for Flavonoids
1. **Shinoda Test:** To 1 ml of the extract, magnesium turnings were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of red colour showed the presence of flavanoids.

VIII. Test for Tannins and Phenolic compounds
1. To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.
2. To the extract, potassium dichromate solution was added, formation of a precipitate showed the presence of tannins and phenolic compounds.

IX. Test for Triterpenoids
1. Two or three granules of tin metal in 2 ml thionyl chloride solution were dissolved. 1ml of the extract was then added into the test tube. The formation of a pink colour indicated the presence of triterpenoids.

X. Test for Fixed Oils
1. **Spot Test:** A small quantity of extract was pressed between two filter papers. Oil stains on paper indicated the presence of fixed oils.
2. **Saponification Test:** To 1 ml of the extract few drops of 0.5 N alcoholic potassium hydroxide was added along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. The formation of soap or partial neutralization indicated the presence of fixed oils.

## Results

### Table 1: Purity and Strength analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cissus quadrangularis Linn.</th>
<th>Zingiber officinale Rosc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign matter</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Moisture content</td>
<td>8.21%</td>
<td>10.21%</td>
</tr>
<tr>
<td>Total ash</td>
<td>19.72%</td>
<td>5.12%</td>
</tr>
<tr>
<td>Alcohol – soluble extractive</td>
<td>7.82%</td>
<td>8.18%</td>
</tr>
<tr>
<td>Water – soluble extractive</td>
<td>21.23%</td>
<td>12.34%</td>
</tr>
</tbody>
</table>

### Table 2: Identity phytochemical Analysis

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>TEST</th>
<th>Cissus quadrangularis Linn.</th>
<th>Zingiber officinale Rosc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>Phenolic compounds and tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>Protein and Amino Acids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>VI</td>
<td>Test for flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIII</td>
<td>Starch</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IX</td>
<td>Acid test (P&lt;0.05)</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Results

The fresh stem of *Cissus quadrangularis* Linn. And *Zingiber officinale* Rosc. was collected from the surroundings of Tirupati shade dried to fine powder. These physico chemical properties of *Cissus quadrangularis* Linn. In the parameters of moisture content, total ash, alcohol-soluble extractive, water- soluble extractive values were 8.21%, 19.72%, 7.82% and 21.23% respectively and the parameters of *Zingiber officinale* Rosc. form moisture content, total ash, alcohol-soluble extractive, water- soluble extractive values were 10.21%, 5.12%, 8.18% and 12.34% respectively (Table-1). The phytochemical screening was done and it revealed that *Cissus quadrangularis* Linn contained alkaloids, carbohydrates, steroids, phenolic compounds and tannins. In the same way, *Zingiber officinale* Rosc. Contained carbohydrates, saponins, steroids, starch, protein and aminoacids (Table-2).

Discussion

*Cissus quadrangularis* have high contents of ascorbic acid, carotene, anabolic steroidal substances, and calcium. The stem contains two asymmetric tetracyclic triterpenoids, and two steroidal principles. β-sitosterol, δ-aminirin, δ-amyrone, and flavanoids (quercetin) having different potential metabolic and physiological effects has also been reported. It is potent fracture healing property and antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastroprotective, cholinergic activity as well as useful effects on cardiovascular diseases. Earlier Phytochemical studies of *Cissus quadrangularis* have shown the presence of flavanoids.
triterpenoids, Vitamin C, stilbene derivatives and many others, e.g. resveratrol, piceatannol, pallidalolphenocissin and phytoesters. Efficacy of Cissus quadrangularis made remodeling of bones have been reported and acts by stimulation of metabolism and increased uptake of the minerals calcium, sulphur and also builds up the chemical composition of the fractured bone namely its mucopolysaccharides, collagen, calcium, phosphorus and others as well as its functional efficiency. Methanol extract Cissus quadrangularis of analgesic, anti-inflammatory effect due to flavonoids especially luteolin and by β-sitosterol. Calcium oxalate, carotene, tetraterpenoids, β-sitosterol, amyrin and anabolic ketosteroids, which are responsible for acceleration of healing and possess anti-inflammatory and analgesic activity in present study Cissus quadrangularis and Zingiber officinale Rosc. Water-soluble extractive values were high with presence of Alkaloids, carbohydrates, steroids, phenolic and tannins and further experiment need to do for various mechanisms.

Conclusion

Cissus quadrangularis Linn. of water-soluble extractive values were high with presence of Alkaloids, carbohydrates, steroids, phenolic and tannins. while Zingiber officinale Rosc of moisture content and water-soluble extractive values were high with presence of carbohydrates, Saponins, Steroids, Starch, Protein and Amino Acids.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

References