Comparative analysis of Phytochemicals presents in different extracts of Helicteres isora.L leaves

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ABSTRACT
Plant kingdoms possess an infinite source of active ingredients valuable in the management of many uncontrollable diseases. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Phytochemical techniques played a vital role in searching raw materials and resources for pharmaceutical industry. Phytochemical screening of various extracts of leaves of Helicteres isora.L was carried out by employing standard methods for conducting Qualitative phytochemical analysis for studying the presence of active compounds like Alkaloids, Tannins, Saponins, Glycosides, Phenols, Flavonoids, Sugars, Carbohydrates, Terpenoids, and Steroids. The crude extracts of the powdered leaves revealed the presence of saponins, steroids, alkaloids, terpenoids, flavonoids, cardiac glycosides. It equally confirms the bioactive components in the plant, thus agreeing with the potential therapeutic significance of the plant as a natural source of drug development.

Keywords: Phytochemical, Helicteres isora, alkaloids, therapeutic agents

INTRODUCTION:
From the first light of civilization medicinal plants are part and package of human society to fight diseases. They are commonly used in treating and preventing particular diseases. Medicinal plants are always playing a beneficial role in health care. In a protocol, it is estimated that worldwide 70-80% of people meet their primary healthcare needs predominantly by using herbal medicine. Recent research on phytochemicals is mainly focusing on health promotion, disease prevention and the development of therapeutic inventions. Phytochemistry is a branch of science that deals with the chemicals obtained from plants with desirable biological activities. It is estimated that today, plant materials are present in, or have provided the models for 50% of Western drugs. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments.

MATERIALS AND METHODS
Plant Material
The leaves of Helicteres isora L. were collected from Kolliimalai hills, Namakkal Dt, Tamil Nadu and authenticated by Rapinet Herbarium, St Joseph’s College, Trichy. The leaves were separated, washed well using clean water to remove adhering matter, dried under shade, finely powdered. Dried powdered materials were placed in the soxhlet thimble to obtain sequential extracts of different solvents ranging from non-polar to polar – Hexane, Ethyl acetate, Acetone, and distilled water by placing them in 250 ml round bottom flask. The materials were refluxed with each solvent for 12-14 hours at 40-70ºC. Extracts were collected and cooled at room temperature and poured in glass Petri dishes & then evaporated at 40ºC using hot air oven. Dried extracts were kept in desiccators for two days and stored at 5ºC in air tight containers.

Extract Yield
The calculation of the extraction yield was the weight percentage of the crude extract to the raw material (20g). The percent extraction yield was calculated as follows:

% Extraction yield = Weight of the plant extract/Weight of the initial sample x 100%

Qualitative Phytochemical screening
The Phytochemical screening of all four extracts was performed by the standard methods.

Test for alkaloids
a) Mayer’s Test- Test solution (1 ml) was taken in test tube and few drops of Mayer’s reagent (Potassium mercuric iodide solution) were added into it and cream color precipitate was observed.
b) Dragendorff’s Test - Test solution (1 ml) was taken in test tube and few drops of Dragendorff’s reagent (Potassium bismuth iodide solution) were added into it and observed for reddish brown precipitate.
c) Tannic acid Test- Test solution (1 ml) was taken in test tube and few drops of 10 % tannic acid solution was added to it and observed for buff coloration.

**Test for tannins**

a) FeCl3 Test- About 0.5 mg of dried powdered samples were boiled in 20 ml water in test tubes and filtered. A few drops of 0.1 % ferric chloride solution was added and observed for brownish green or blueblack coloration.

b) Gelatin Test- About 1 ml test solution was taken in a clean dried test tube and 1 % gelatin solution was added followed by 10 % sodium chloride solution and observed for white precipitate to form.

c) Vanillin hydrochloride Test- Test solution was treated with few drops of vanillin hydrochloride reagent and observed for purplish-red color.

**Test for cardiac glycosides**

a) Keller killiani Test- Test solution (1 ml) was taken in a test tube and 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it. Carefully added 0.5 ml of concentrated sulphuric acid by the side of the test tube and observed for blue color to appear in the acetic acid layer.

b) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

**Test for Steroids**

a) Liebermann Buchard test- Test solution of 1 ml was treated with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added from the sides of the test tube and observed for a brown ring at the junction of the two layers and green layer in upper layer.

**Test for Flavonoids**

a) Alkaline reagent test- About 1 ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow coloration which disappears on the addition of dilute HCl.

b) Lead acetate Test- Test solution (1 ml) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow colored precipitate.

**Test for Terpenoids**

a) Salkowski Test- Test solution (1 ml) was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

**Test for Proteins**

a) Ninhydrin Test- Test solutions were boiled with 0.2 % solution of ninhydrin and observed for violet color to appear.

**Test for reducing sugars**

a) Fehling Test- Test sample of 1 ml was taken into a clean and dried test tube and 0.5 ml of Fehling A and Fehling B solutions were added to it, boiled and observed for brick red coloration.

**Test for Carbohydrate:**

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

**Test for Saponins**

a) Froth test- Test solution (1 ml) was placed in a test tube containing water and shaken well and noted for a stable froth that persists for at least 2 min.

**RESULTS**

Yield of different extracts of Helicteres isora leaves. The yield of plant is mainly dependent on the type of solvent used in the extraction procedure. Table-1 represents the % yield of H.isora leaves in different solvents (non-polar to polar sequentially). The values were high in aqueous extract of leaves (14.37%). After the aqueous extract, Ethyl acetate extract has shown a little higher value yield of 10.5%. Among other solvents used for study, Acetone has shown least extraction yield of 0.44% whereas Hexane extract has shown moderate yield of 3.4%.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Yield of extracts/20 g of dried leaves %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>3.4</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Table 1: Extraction yield of different extracts of H.isora leaves
SCREENING OF DIFFERENT EXTRACTS OF LEAVES OF HELICTERES ISORA

The results of preliminary phytochemical analysis are shown in Table 2. The present study carried out on the leaf extracts revealed the presence of medicinally active constituents.

**Table 2. Results of preliminary phytochemical analysis**

<table>
<thead>
<tr>
<th>S.No</th>
<th>PHYTOCHEMICALS</th>
<th>Hexane</th>
<th>Ethyl acetate</th>
<th>Acetone</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Benedict’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Reducing sugars</td>
<td>Fehling’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Monosaccharides</td>
<td>Barfoed’s Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Hexose sugars</td>
<td>Seliwanoff’s Test</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Non Reducing sugars (starch)</td>
<td>Iodine Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Proteins</td>
<td>Ninhydrin Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>7</td>
<td>Amino acids Tyrosine</td>
<td>Xanthoproteic Test</td>
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<td>-</td>
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<tr>
<td>8</td>
<td>Steroids</td>
<td>Liebermann Buchard test</td>
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<td>+</td>
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<tr>
<td>9</td>
<td>Glycosides</td>
<td>Fehling’s solution Test</td>
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<td>+</td>
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<tr>
<td>10</td>
<td>Cardiac glycosides</td>
<td>Keller Killiani Test</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>11</td>
<td>Alkaloids</td>
<td>Dragendorff’s Test</td>
<td>+</td>
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<tr>
<td>12</td>
<td>Tannins</td>
<td>Ferric chloride Test</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>13</td>
<td>Phenolic compounds</td>
<td>Ferric chloride Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>14</td>
<td>Flavanoids</td>
<td>Lead acetate Test</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>15</td>
<td>Terpenoids</td>
<td>Salkowski Test</td>
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<td>+</td>
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<tr>
<td>16</td>
<td>Saponins</td>
<td>Froth Test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = present, - = absent

DISCUSSION

In the present study, qualitative analysis for all four extracts showed significant indication about the presence of primary and secondary metabolites. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of deadly diseases. As a matter of fact, many have been shown to have wonderful biological and pharmacological activities and likely to be used as chemotherapeutic agents. Plant phenolic compounds are currently of growing interest because of their anti oxidant properties in promoting health. In the present investigation, the leaf extract showed the presence of alkaloid indicates that the plant extracts could be used for the...
antifungal activity. Saponins, a special class of glycosides, have expectorant action which is very useful in the respiratory tract inflammation. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens. The presence of carbohydrates and reducing sugars in the plant seems to indicate the high energy content that could be exploited as a source of raw materials for pharmaceutical industries.

CONCLUSION
The preliminary phytochemical analysis revealed the presence of steroids, reducing sugars, terpenoids, alkaloids, flavonoids, cardiac glycosides, saponins and tannins in different extracts of leaves of Helicteres isora L. The study apparently highlighted the scientific basis for the possible use of Helicteres isora L. leaves in ethno-medication.

REFERENCES