PRELIMINARY PHYTOCHEMICAL ANALYSIS OF HERBAL PLANT HYGROPHILA AURICULATA
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Running Title: Phytochemical analysis of Hygrophyla auriculata

Abstract:
The study of phytochemicals in plants has great significance in understanding their various medicinal properties. Flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, quinones, proteins, cardiac glycosides of the whole plant extract of selected medicinal plant, Hygrophyla auriculata was performed. The plant was collected from paddy fields near Chengalpattu, Tamil Nadu, India. Ethyl acetate was used as solvent. Alkaloids and proteins were present in abundant amounts whereas tannins and quinones were absent. Other phytochemicals, namely, saponins, flavonoids, terpinoids, steroids and cardio glycosides were present in moderate amounts. This knowledge could be of great value for ascertaining the medicinal role of this plant.

Keywords: Phytochemicals, Hygrophyla auriculata, Ethyl acetate, flavonoids, Terpenoids, Saponins.

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INTRODUCTION:
The use of medicinal plants for their curative properties is a time tested experience of humans. Some of the phytochemicals like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids have therapeutic values [1, 2, 3, 4, 5]. Knowledge of these phytoconstituents is very helpful in drug discovery and new drug molecule formulation [6, 7, 8]. The present study reports the phytochemicals present in one herbal plant namely, Hygrophyla auriculata.

The plant is a known for its medicinal value. The diuretic effect of this plant was studied by Hussain et al, 2009; Preethi et al, 201 [9, 10]. Vijaya Kumar et al, 2006, have reported the curative effect of this plant on streptozotocin induced oxidative stress in rats [11]. The toxicity of this plant was also reported [12]. The plant has hepatoprotective and antioxidant activities [13]. The role of the plant on paracetamol and thioacetamide intoxication in animals was studied by Singh and Handa, 1995. [14]

A related plant H. spinosa was also studied for its antibacterial, anti-inflammatory and antipyretic role by Patra et al, 2009 [15]. Eazhisavallabhi et al, 2012, has reported preliminary phytochemical analysis and antibacterial role of H. spinosa [16]. In the present work, qualitative phytochemical analysis of ethyl acetate extracts of Hygrophyla auriculata was carried out.

METHODS:
Collection of Samples
The medicinal plants used for the experiment were whole plants of collected from the nearby Chengalpattu in Tamil Nadu, India.

Preparation of Extracts:
500 grams of dried powder of Hygrophyla auriculata whole plant was packed in separate round bottom flask for sample extraction using ethyl acetate as solvent. The extraction was conducted by 750 ml of the solvent for a period of 72 hours. At the end of the extraction the solvent were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phytochemical Analysis
The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature [16, 17, 18].

Test for Alkaloids
The extract of the crude dry leaf powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer’s reagent, one portion was treated with equal amount of Dragendorff’s reagent and the third portion was treated with equal amount of Wagner’s reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

Test for Saponins
About 0.5 g of the plant leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for Tannins
About 0.5 g of plant leaf extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for Steroids
2 ml of acetic anhydride was added to 2 ml of plant leaf extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Flavonoids
2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones.

Test for Anthraquinones
About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for Cardiac Glycosides
0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins
To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution
was added. A violet colour indicated the presence of peptide linkage of the molecule.

**Test for Tri-Terpenoids**

5ml of each extract was added to 2ml of chloroform and 3ml of con. H2SO4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

**RESULTS AND DISCUSSION:**

The various phytochemicals present in the ethyl acetate extraction of the plant, *Hygrophila auriculata* is indicated in Table 1.

<table>
<thead>
<tr>
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<th>Contents</th>
<th><em>Hygrophyla auriculata</em></th>
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<tr>
<td>1.</td>
<td>Tannins</td>
<td>-</td>
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<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Proteins</td>
<td>+++</td>
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<tr>
<td>4.</td>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Saponin</td>
<td>+</td>
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<tr>
<td>6.</td>
<td>Terpenoid</td>
<td>+</td>
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<tr>
<td>7.</td>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Cardio glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

*+- Present, -+-Absent

The present day medicines owe their origin to plant products and plant extracts. In all the three Indian systems of medicine, Ayurveda, Siddha and Unani, most of the medicines are produces by herbal plants or their derivatives. All the traditional systems of medicine all over the world hinge on herbal medicine. The range of treatment by the herbs spans from common cold to cancer. The present day medicine is crippled due to the massive side effects they produce and poor affordability. It is high time to search for medicines which are natural, with less or no side effects and are available at cheap cost [20]. The plants are bestowed with many important phyto chemicals which are responsible for their therapeutic role. The present study reports the presence of some phyto chemicals in the ethyl acetate extract of plant *Hygrophila auriculata*. This plant finds its place in the treatment of a wide range of ailments. Asolkar et al., 1992 have reported the alcoholic extract of this plant was spasmylic and hypotensive [21]. It was observed that alkaloids and proteins were present in abundance whereas tannins and quinones were conspicuous by their absence. Saponins, Terpinods, Steroids, Flavonoids and Cardioglycosides were present in small amounts. Ghani et al., 2003 has reported the presence of essential oils in the root of this plant [22]. Palmitic, stearic, oleic and linoleic acid were reported to be present in the seed oil of this plant (Rastogi and Mehrotra, 1993). [23]

**CONCLUSION:**

The presence of alkaloids in large amounts indicates that *Hygrophila auriculata* owes it medicinal properties due to the presence alkaloids.

**COMPETING INTERESTS**

This is to inform that no conflict of interest exist among the author.

**REFERENCES:**


