Preliminary phytochemical studies in whole plant dry sample extracts of *H. Verticillata* grown in unpolluted and polluted water sources were carried out to determine the presence or absence of possible secondary metabolites using various solvents (petroleum ether, chloroform, ethyl alcohol, methanol, and water). Among the phytochemicals tested, flavonoids and phenols are not reported in all the extracts of *H. Verticillata* from unpolluted and polluted water sources. The steroids and tannins are present in the petroleum ether and chloroform extracts of plant samples from unpolluted and polluted water sources. Presence of alkaloids in water, chloroform, ethyl acetate and methanol extract (except petroleum ether) of both unpolluted and polluted plant samples was noted. Saponins are present only in water and petroleum ether extracts of both unpolluted and polluted plant samples. The results of the qualitative preliminary phytochemical screening of whole plant extracts of *H. Verticillata* plants from unpolluted and polluted water sources exhibited the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins depend upon the polarity of solvent and solubility level of phytocomponents.

1. Introduction

Phytochemicals are defined as bioactive, non-nutrient plant compounds found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases. The medicinal values of plants exist in their phytocomponents which produce a definite physiological action on the human body. Preliminary phytochemical screening reports, generally revealed the presence of alkaloids, tannins, cardiac glycosides and saponins in the methanol leaf extracts of...
Mitracarpus scaber (Abere et al. 2007); presence of alkaloid, catachin, coumarin, steroid, phenols, amino acid, sugar, protein and xanthoprotein in the ethanolic extract of Alpinu calcarata and Hiptage benghalensis leaves (Thirumurugan et al., 2008); presence of glycosides, mucilage, carbohydrates, tannins, alkaloids and reducing sugars in the hexane, methanol and the aqueous extracts of Tridax procumbens leaf (Kiran et al. (2008); and presence of iridoids, flavonoids and triterpenes in methanol extract of Mussanda pupescens leaf (Vidyakalshmi et al., 2008).

Ethanol extract of Rubia cordifolia leaf showed positive results for alkaloids, cardiac glycosides, tannins, flavonoids, and phenols and negative for saponins, volatile oils, anthraquinones and cyanogenic glycosides and very trace for steroids (Kannan et al., 2009); the ethanolic extracts of Costus mexicanus leaf showed the presence of phytosterols, saponins, glycosides and phenolic compounds (Sheba et al., 2009). The occurrence of coumarines, flavonoids, sterols and triterpenoids was reported by Kalaskar et al. (2010) in petroleum ether, ethanol, methanol and aqueous extract of Ficus carica leaf samples. Ethanolic leaf extract of Pistia stratiotes (aquatic weed) revealed the presence of anthraquinone, glycosides, cardiac glycosides, flavonoids, steroids and tannins (Khan et al., 2011). The present study was carried out to determine the phytocomponents present in the H. verticillata plant grown in polluted and unpolluted water conditions.

2. Materials And Method:

2.1 Study Area And Plant Materials Used

The selected plant H. verticillata belongs to the family Hydrocharitaceae was collected from polluted and unpolluted water bodies in Asaripallam, Agastheeswaram Taluk, Kanyakumari District, Tamil Nadu, India (Elevation about 460 meters (Mean Sea Level) and used for preliminary phytochemical screening.

2.2 Preparation of whole plant dry powder

The selected plant was collected and dried separately at room temperature (30±2°C) for about two weeks to get a constant weight. The dried plant materials (as whole plant) were ground to powder by mechanical device and stored for further biochemical analysis.

2.3 Preparation Of Plant Extracts

The whole plant dry powder samples were extracted with different solvents such as chloroform, methanol, ethyl acetate, petroleum ether and water at 20 % (w/v) level in a soxhlet apparatus. The extracts were concentrated and used for qualitative phytochemical analysis.

3. Qualitative Phytochemical Analysis

Preliminary phytochemical screening of different solvent extracts of H. verticillata was carried out separately following the methods of Herborne (1984), Trease and Evans (1987) and Kolkate et al. (1995).

Test for alkaloids (Wagner’s reagent test): To 1 ml of the extract, a few drops of Wagner’s reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids (Shinoda test): To 1 ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.
Test for carbohydrates (Fehling’s test): 5 ml of Fehling’s solution was added to 2 ml of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Test for tannins and phenols (Lead acetate test): In a test tube containing about 5 ml of extracts, 1 ml of 10 % lead acetate solution was added. Formation of yellow precipitate shows the presence of tannins.

Test for steroids (Salkowski’s test): To 2 ml of the extract, equal volume of conc. H₂SO₄ was added carefully along the sides of the tubes. The upper layer turned red and the lower layer turned yellow with green fluorescence, indicating the presence of steroids in the extract.

Test for glycosides: To 1 ml of the test extract, 1 ml of sodium picrate solution was added. The colour change from yellow to orange reveals the presence of glycosides.

Test of saponins: To 5 ml of the filtrate, a drop of 5 % sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed which shows the presence of saponins.

Preliminary phytochemical studies in different whole plant dry sample extracts of *H. verticillata* grown in unpolluted and polluted water sources were carried out and the results are presented in Table-1. Among the phytochemicals tested, flavonoids and phenols are absent (not detected) in all the extracts of *H. verticillata* whole plant samples. The phyto components steroids and tannins are noted only in the petroleum ether and chloroform extracts of *H. verticillata* whole plant samples. But alkaloids found in all extracts (water, chloroform, ethyl acetate and methanol extract) of *H. verticillata*. Similarly Saponins is present only in water and petroleum ether extracts of *H. verticillata* samples.

Table –1: Preliminary phytochemical screening in the extract of *Hydrilla verticillata* whole plant samples collected from unpolluted and polluted water sources.

<table>
<thead>
<tr>
<th>Whole plant solvent extracts used</th>
<th>Habitat of whole plant samples collected</th>
<th>Phytochemicals screened in <em>H. verticillata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Petroleum ether</td>
<td>UPWS</td>
<td>Alkaloids Flavonoids Phenols Saponins Steroids Tannins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- - - + + +</td>
</tr>
<tr>
<td></td>
<td>PWS</td>
<td></td>
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<tr>
<td>2. Chloroform</td>
<td>UPWS</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>+ - - + +</td>
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<td></td>
<td>PWS</td>
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<tr>
<td>3. Ethyl acetate</td>
<td>UPWS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ - - - -</td>
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<td></td>
<td>PWS</td>
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</tr>
<tr>
<td>4. Methanol</td>
<td>UPWS</td>
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<td></td>
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<td>+ - - - -</td>
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<td>PWS</td>
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<tr>
<td>5. Water</td>
<td>UPWS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ - - + -</td>
</tr>
<tr>
<td></td>
<td>PWS</td>
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</tr>
</tbody>
</table>

UPWS-Unpolluted Water Source;  PWS-Polluted Water Source;

The plant produced secondary metabolites in response to stress (Keeling and Bohlmann 2006). These plant based secondary metabolites, have been subsequently exploited by human beings for their beneficial role in a diverse array of applications (Balandrin *et al.*, 1985). Often, plants secondary metabolites may be referred to as plant natural products, which have illicit effects on other organisms.
4. Findings & Discussion

The results of present study showed that the plant parts of *H. verticillata* having rich primary and secondary metabolites such as alkaloids, flavonoids, phenols, saponins, steroids, tannins can be used as industrial raw materials. These metabolites are further used for biosynthesis of bioactive compounds.

Preliminary phytochemical screening of plants is very useful for the determination of the active constituents in different solvent extracts. Among the phytochemicals tested, flavonoids and phenols are not detected in all the extracts of *H. verticillata* samples while steroids and tannins are observed in petroleum ether and chloroform extracts of plant samples present in water, chloroform, ethyl acetate and methanol extract (except petroleum ether) were also noted and the saponins are present only in water and petroleum ether extracts of *H. verticillata* samples.

The results of the qualitative primary phytochemical screening of whole plant extracts of *H. verticillata* plants from unpolluted and polluted water sources exhibited the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins depend upon the polarity of solvent and solubility level of phytocomponents.

5. References


