Phytochemical investigation of the extracts of *Eichhornia crassipes* and its solvent fractionates

P.Jayanthi, P.Lalitha*, and Shubashini.K.Sripathi

Department of Chemistry, Avinashilingam Deemed University for Women, Coimbatore, Tamilnadu, India

Received on: 05-12-2010; Revised on: 14-01-2011; Accepted on:09-03-2011

**ABSTRACT**

Petroleum ether, acetone, ethyl acetate, aqueous, acid extracts and the chloroform and ethanol fractionates of aqueous extracts of *Eichhornia crassipes* were screened for the presence of phytochemicals by standard procedures. Presence of sterols was observed in acetone and aqueous extract. Petroleum ether and aqueous extracts tested positive for flavonoids. Terpenoids were present in the acetone extract. Proteins were present in aqueous extract and carbohydrates in acid extract. Presence of alkaloids, quinones and anthocyanins was noted in aqueous and acid extract. Anthraquinones were found to be present in all extracts except petroleum ether and phenols were present in ethyl acetate extract. Ethanol fractionate showed the presence of proteins and terpenoids which separated from aqueous extract. The results obtained provide a motivation that this plant which poses major threat to the environment and economy could be utilised in an efficient means.

**Key words:** *Eichhornia crassipes*, water hyacinth, solvent fractionation

**INTRODUCTION**

Plants synthesize a wide variety of chemical compounds, which can be sorted by their chemical, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolic process of building and maintaining of living cells. Secondary metabolites do not seem to be vital to the immediate survival of the organism that produces them and are not an essential part of the process of building and maintaining living cells [18] whereas these carry out a number of protective functions in the human body. Plant secondary metabolites can boost the immune system, protect the body from free radicals, kill pathogenic germs and much more. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [4].

The water hyacinth, *Eichhornia crassipes* (Mart.) Solms, is a tropical species belonging to the picerealweed family (Pontederiaceae) [2]. It is a free floating aquatic plant well known for its production abilities and removal of pollutants from water. It can quickly grow to very high densities (over 60kg m$^{-2}$); thereby completely clogging water bodies, which in turn may have negative effects on the environment, human health and economic development [3,5].

On the other hand, when looked from a resource angle, it appears to be a valuable resource with several unique properties. As a result, research activity concerning control (especially biocidal control) and utilization (especially wastewater treatment or phytoremediation) of water hyacinth has boomed up in the last few decades. This study focuses on the preliminary screening of the phytochemicals present in various extracts of water hyacinth which may aid in the identification and isolation of compounds which may be of use to the public.

**MATERIALS AND METHODS:**

**Plant collection:**
Six tonnes of fresh water hyacinth was collected from Singanallur boat house, Coimbatore, Tamilnadu. The root portion was cut off and the plant was washed thoroughly to free from debris. The leaves and shoot portion were shade dried for 20 days. The dried plant material was sliced, ground coarsely and stored for further use.

**Preparation of extracts:**
Water hyacinth (1500g) was defatted twice with petroleum ether (5L) for 6 hours and then refluxed twice with ethanolic KOH (4.5L) for 6 hours. The extract was desolvatised under reduced pressure and the residue was extracted thrice with acetone under reflux for 1 hour. The acetone extracts were pooled and concentrated to get acetone extract.

Water hyacinth (140kg) was extracted successively with ethyl acetate (800L) to get the ethyl acetate extract. The plant residue was then refluxed with water (800L) twice for 6 hours to get the aqueous extract. A small portion (1500g) of the plant residue was extracted with 1:1 hydrochloric acid (3L) for 6 hours to get the acid extract.

The aqueous extract was fractionated with chloroform and ethanol. The various solvent extracts and fractionates of aqueous extract were screened for the presence of different phytochemicals [6, 9, 20].

**Phytochemical tests:**

**Test for Alkaloids**

Mayer’s test
A fraction of extract was treated with Mayer’s test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

Wagner’s test
A fraction of extract was treated with Wagner’s reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

Hager’s test
A few ml of extract was treated with Hager’s reagent (saturated aqueous solution of picric acid) and observed for the formation of prominent yellow precipitate.

**Test for Flavonoids**

Nasiol test
A small amount of extract was treated with aqueous NaOH and HCl observed for the formation of yellow orange colour.

H$_2$SO$_4$ test
A fraction of the extract was treated with concentrated H$_2$SO$_4$ and observed for the formation of orange colour.

**Test for Sterols**

Liebermann-Burchard test
Extract (1ml) was treated with chloroform, acetic anhydride and drops of H$_2$SO$_4$ was added and observed for the formation of dark pink or red colour.

**Test for Terpenoids**

Liebermann-Burchard test
Extract (1ml) was treated with chloroform, acetic anhydride and drops of H$_2$SO$_4$ was added and observed for the formation of dark green colour.

**Test for Anthraquinones**

Borntrager’s test
About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. In the reaction vessel, the solution became reddish brown. Five ml of 5% sodium nitrite solution was added and the mixture was allowed to stand for 5 minutes. Five ml of 10% Alkaline ferric chloride solution was added and observed for the formation of reddish brown precipitate.

**Test for Anthocyanin**

NaOH test
A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour.

**Proteins**

Ninhydrin test (Aqueous)
The extract was treated with aqueous ninhydrin and observed for the presence of blue colour indicating the presence of amino acid or purple colour indicating the presence of protein.

Ninhydrin (acetone)
Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed for the formation of purple colour.
Table 1: Preliminary phytochemical screening of the extracts and solvent fractionates of aqueous and anti-cancer activity [21]. Anthocyanins exhibit important anti-oxidant and anti-inflammatory activity [17]. Flavanoids show anti allergic, anti-inflammatory, anti-microbial activity [19]. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [18]. Phytochemicals may protect humans from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [10]. Different phytochemicals may protect humans from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [10].

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>PEE</th>
<th>AcEt</th>
<th>EAE</th>
<th>AqF</th>
<th>AcidF</th>
<th>CHClF</th>
<th>EtOHF</th>
<th>AqF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Bioactive compounds</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>AqF</td>
</tr>
</tbody>
</table>

Key: + Indicates presence; - indicates absence

Biuret test
The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

Test for Phenols
Ferric chloride test
The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Liebermann's test
The extract was heated with sodium nitrite, added H₂SO₄ solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Quinones
A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Carbohydrates
Molisch's test for Carbohydrates
Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Fehling's test for free reducing sugar
About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

RESULTS AND DISCUSSION:
The results obtained in the present investigation for the extracts viz; petroleum ether(PEE), acetone(AcEt), ethyl acetate(EAE), aqueous(AqF) and acid(AcidF) and the fractions of aqueous extract i.e chloroform(CHClF), ethanol(EtOHF), and aqueous(AqF) are summarised in table 1. The preliminary phytochemical tests result indicates the presence of phenol, flavonoids, alkaloids, terpenoids, sterols, tannins, carbohydrates, anthraquinone, anthocya-nins, and proteins in different extracts. The presence of wide range of phytochemical constituent indicates that the plant could be used in a multitude of ways which may be beneficiary to the population.

Triterpenoids are a large class of natural isoprenoids present in higher plants, which exhibit a wide range of pharmacological activities [7]. Steroids and isoprenoid phytochemicals have been observed to promote nitrogen retention in osteoporotic and in animals with wasting illness [11]. Anthraquinones are a main source of natural dye, which are gaining importance due to the environmental pollution caused by synthetic dyes [11]. Carbohydrate is reported to have numerous roles in living things, such as the storage and transport of energy (starch, glycogen) and structural components (cellulose in plants, chitin in animals). Additionally carbohydrates and their derivatives play major roles in the working process of the immune system, fertilization, pathogenesis, blood clotting and development [14].

Lata et al. [12] demonstrated the presence of few secondary metabolites in Eichhornia crassipes except saponins in aqueous extract which was prepared by soaking the dried, powdered plant material for 12 hours. Kandukuri et al. [8] reported the presence of alkaloids, phenols, steroids, tannins and triterpenoids in the methanol extract which was prepared by soxhlet extraction whereas the authors have reported the absence of flavonoids in water hyacinth. Ndbubisi et al. [16] revealed the presence of saponin, glycoside and anthraquinone but absence of alkaloid in the chloroform extract of water hyacinth. Dubey et al. [13] reported the presence of alkaloids in the aqueous extract of water hyacinth.

Our present study involves a detailed phytochemical investigation of bulk quantities of water hyacinth. As a preliminary step, phytochemical screening of different extracts was carried out. Fractionation makes it possible to isolate more than two components in a mixture in a single run. This property sets it apart from other separation techniques. Hence solvent fractionation was employed for the aqueous extract. The ethanol fractionate of aqueous extract showed that flavonoids, terpenoids and proteins which were present in aqueous extract, after fractionation was found in ethanol fractionate.

Bulk isolation of compounds based on the results of phytochemical screening is under progress in our laboratory. Preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmaco-logically active chemical compounds.

ACKNOWLEDGEMENT:
The financial support of DRDO-LSRB is acknowledged and the authors thank Avinashilingam Deemed University for providing necessary facilities to carry out this work.

REFERENCES:

Source of support: DRDO-LSRB, Conflict of interest: None Declared