EVALUATION OF PHYTOCHEMICAL CONSTITUENTS OF THE LEAVES EXTRACT OF CHENOPODIUM ALBUM LINN
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ABSTRACT
Chenopodium album (L.), commonly known as Bathua or Goose foot is a fast growing vegetable plant that belongs to the family Chenopodiaceae. The plant contains essential oils, besides alkaloids, trigonelline and chenopodine. It is traditionally used as a curative medicine for various diseases. In the present study, aqueous, methanolic and ethanolic extracts of the leaves of Chenopodium album were analysed to detect the presence of various bio-active compounds, namely: proteins and amino acids, saponins, phenols, terpenoids, flavonoids, carbohydrates, alkaloids, glycosides, tannins and steroids using standard qualitative tests. There were variable results for alkaloid and carbohydrate tests with different methods. Tests for glycosides and tannins showed negative result for all the extracts. Mostly, tests for proteins and amino acids, saponin, phenol, terpenoids and flavonoids showed impressive result in all the extracts. Steroids were present in ethanolic and aqueous extracts, while it was absent in methanolic extraction. Thus, the result of the present comprehensive analysis demonstrates the presence of almost all the phytochemicals in the leaves of Chenopodium album which can be used in effective pharmacological actions.

Keywords: Bio-active compounds; Chenopodium album; Extracts; Phytochemical analysis.

1. INTRODUCTION
Plants play a key role for all living organisms and the use of plants for treating diseases is as old as the human species. In developed countries, traditional medicine having compounds derived from medicinal plants is used by about 80% of individuals1. In the Indian subcontinent, thousands of plants are known to have medicinal value and are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, folk medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs2. Also, the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.

The major remedy in traditional system of medicine are the herbal medicines which includes herbs, herbal materials, herbal preparations and finished herbal products containing parts of plants or other plant materials as active ingredients3 and are suitable for treating a wide range of infections and diseases. Through recent researches on herbal plants and medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine. Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries of Africa, Asia and some part of Europe4. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side effects, easily medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of India5.

A number of oxygen derived reactive oxygen species (ROS) are known to exist in plants. The importance of ROS and free radicals has attracted increasing attention of researchers involved in the field of medical science. Although the production of ROS is normal and is an essential process during cell metabolism to carry out important physiological functions6, yet, overproduction of ROS exerts oxidative damaging effects to cell components and molecules7. ROS including free radicals and non-free radicals with various forms of free oxygen species are
involved in hepatocellular injury and related disorders, chronic degenerative diseases, inflammation, atherosclerosis, cataract, rheumatism, ischemia and arthritis. Antioxidant has the ability to trap free radicals and thus can help prevent oxidative damage induced by free radicals. However, commercially available synthetic antioxidant agents like butylated hydroxyanisole (BHT) have been reported to be toxic to animals including human beings which have stimulated the interest of many researchers to search natural antioxidants. Naturally occurring antioxidants have been recognised as having the potential to reduce disease risk by increasing the antioxidant capacity of the plasma. It has been reported that antioxidant activity of plants might be due to their flavonoids and phenolic compounds.

Chenopodium album, commonly known as Bathua or Goose foot, belonging to the family Chenopodiaceae, is a fast growing vegetable plant. It has a worldwide distribution and contains about 250 species. In India it is represented by about 21 species and is usually found as a weed in Madhya Pradesh, Rajasthan, Punjab, Kashmir, Sikkim, Bengal and Mumbai. In Assam it is abundantly found in Dangtol, Bongaigaon (Assam Biodiversity Portal; updated on April 2018). Traditionally, it is used as a curative medicine for various diseases including hepatic ailments and is also known to be useful in abdominal pain, eye disease, throat troubles and cardiovascular disorders. Several flavonoids and alkaloids are reported to possess antioxidant and hepatoprotective properties. The medicinal property of this plant is mainly present in leaves and seeds. Therefore, the present investigation is taken up to know the phytochemicals present in the leaves of Chenopodium album.

2. MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIALS

The plant C. album was collected from local area of Guwahati and was authenticated (Ref. no. BSI/ERC/ Tech/2019/887) in Botanical Survey of India, Eastern Regional Centre, Shillong.

Firstly, the leaves of the collected plant were separated and washed carefully with water and were allowed to shadow dry at room temperature. The dried leaves were then ground using a mechanical grinder into coarse powder which was stored in an airtight container and kept in a cool, dark and dry place until further analysis commenced.

2.2 PREPARATION OF THE SOLVENTS USED IN THE EXTRACTION PROCEDURES

2.2.1 Ethanolic extract: About 80g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 400ml of ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. Then it was filtered through Whatman filter paper. The filtrates so obtained were allowed to evaporate under normal temperature. Gummy concentrate of greenish and brownish colour was obtained which was designated as ethanolic extract of C. album, dried in vacuum evaporator and stored at 4°C until further investigation.

2.2.2 Methanolic extract: About 80g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 400ml of methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. Then it was filtered through Whatman filter paper. The filtrates so obtained were allowed to evaporate under normal temperature. Gummy concentrate of greenish colour was obtained which was designated as methanolic extract of C. album, dried in vacuum evaporator and stored at 4°C until further investigation.
2.2.3 **Aqueous extract:** About 20g of powdered material was dissolved in 200ml of distilled water and kept for a period of 3 days with occasional stirring. The mixture was filtered using Whatman filter paper and the filtrate was allowed to dry by freezing in a high vacuum. A dark brownish coloured aqueous extract was obtained that was stored at 4°C until further investigation.

2.3 **PHYTOCHEMICAL ANALYSIS**

The phytochemical analysis of all the extracts for the detection of some important secondary metabolites was carried out using standard conventional procedures.

2.3.1 **Test for Alkaloids**

0.2g of the extracts were dissolved individually in dilute Hydrochloric acid (5ml) and filtered.

a) **Mayer’s Test:** Filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

b) **Wagner’s Test:** Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

c) **Hager’s Test:** Filtrates were treated with Hager’s reagent (saturated Picric acid solution). Presence of alkaloids is confirmed by the formation of yellow coloured precipitate.

2.3.2 **Test for Carbohydrates**

0.2g of the extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch’s Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

b) **Benedict’s Test:** Filtrates were treated with Benedict’s reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.

c) **Fehling’s Test:** Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling’s A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.3.3 **Test for Proteins and Amino acids**

a) **Biuret Test:** 0.2g of the extracts were individually treated with 2ml of 10% NaOH solution and 2-3 drops of 1% CuSO₄ solution and were mixed together. Violet or purple colour confirmed the presence of proteins.

b) **Ninhydrin Test:** To the extracts, 0.25% w/v ninhydrin reagent were added individually and boiled for few minutes. Formation of blue colour indicated the presence of amino acids.

2.3.4 **Test for Glycosides (10% NaOH Test)**

1.2g of the extracts were individually hydrolyzed by 10ml of 1% HCl solution and neutralized with 10% of NaOH solution. A few drops of Fehling’s solution A and B were added. The formation of red precipitate indicated the presence of glycosides.

2.3.5 **Test for Flavonoids (Alkaline Reagent Test)**

0.2g of the extracts were taken individually and dissolved in diluted NaOH and 1M of HCl (5ml) was added. A yellow solution that turns to colourless indicated the presence of flavonoids.

2.3.6 **Test for Tannins (Ferric Chloride Test)**

0.2g of the extracts were mixed individually with 10ml of distilled water and heated. The mixtures were filtered and to each filtrate 5% (w/v) solution of ferric chloride were added. Formation of dark green solution indicated the presence of tannins.
2.3.7 Test for Saponins (Froth test)
0.2g of the extracts were individually diluted with distilled water to 20ml and was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

2.3.8 Test for Phenols (Ferric Chloride Test)
0.2g of the extracts were individually dissolved in a mixture of water and ethanol and a few drops (3-4) of ferric chloride solution were added. Formation of red, blue, green or purple colouration indicated the presence of phenols.

2.3.9 Test for Terpenoids (Salkowski’s Test)
0.2g of the extracts were mixed separately with 2ml of chloroform and concentrated sulphuric acid (3ml) and then added carefully to form a layer. A formation of reddish brown colouration of the solution at inert face indicated the presence of terpenes.

2.3.10 Test for Sterols (Salkowski’s Test)
The extracts were individually treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of red colour in lower layer indicated the presence of sterols.

3. RESULTS
The leaves of Chenopodium album were screened for the presence or absence of different phytoconstituents like alkaloids, carbohydrates, flavonoids, phenols, terpenoids, saponins, glycosides, tannins, sterols, proteins and amino acids by different extraction methods such as aqueous, methanolic and ethanolic extraction. Test for sterols were positive in ethanolic and aqueous extracts while glycosides and tannins were found to be absent in all the extracts (Table 1)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant Constituents</th>
<th>Tests performed</th>
<th>Name of the Extracts used</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>Aqueous</td>
</tr>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
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<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
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<td>Hager’s test</td>
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<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
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<td></td>
<td></td>
<td>Benedict’s test</td>
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<td>Fehling’s test</td>
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<td>3.</td>
<td>Proteins and amino acids</td>
<td>Biuret test</td>
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<td></td>
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<td>Ninhydrin test</td>
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<tr>
<td>4.</td>
<td>Glycosides</td>
<td>10% NaOH test</td>
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<td>5.</td>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>+</td>
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<tr>
<td>6.</td>
<td>Tannins</td>
<td>Ferric Chloride test</td>
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<td>7.</td>
<td>Saponins</td>
<td>Froth test</td>
<td>+</td>
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<td>8.</td>
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<td>9.</td>
<td>Terpenoids</td>
<td>Salkowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Sterols</td>
<td>Salkowski’s test</td>
<td>+</td>
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</tbody>
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(+)= present, (-)= absent

Different phytochemical tests were performed by distinctive reagents. Mayer’s test, Wagner’s test and Hager’s test were performed for the detection of alkaloids. Ethanolic and aqueous extracts showed positive result in Mayer’s test while Wagner’s test and Hager’s test revealed negative result in all the extracts. Benedict’s test and Molisch’s test revealed positive result when detected
for the presence of carbohydrates while Fehling’s test showed negative result for carbohydrate in all the extracts. Ninhydrin test showed positive result in all the extracts when detected for the presence of amino acids. Biuret test revealed positive result for protein in ethanolic extract while methanolic and aqueous extracts were found to be negative in proteins. The other phytoconstituents like saponin, phenol, terpenoids and flavonoids showed positive result in all the extracts.

4. DISCUSSION

Results of the different phytochemical tests performed on the leaves extract of C. album reveals that the plant is rich in various phytochemical constituents which are responsible for both pharmacological and toxic activities in plants. Alkaloids, which are a class of nitrogenous organic compounds of plant origin, were found to be present in the ethanolic and aqueous extracts when detected with Mayer’s test. Although, Wagner’s test for alkaloids was found to be negative for all extracts. On the contrary, [26] and [1] reported the presence of alkaloids in Wagner’s test. Carbohydrates, which are a large group of organic compounds, were found to be present in both Benedict’s test and Molisch’s test. Similar results were reported by [26] and [19]. Ninhydrin test revealed the presence of amino acids in all the extractsin the present investigation while, [1] reported the absence of amino acids in methanolic extract of Ninhydrin test. Proteins were found to be absent in methanolic and aqueous extracts of Biuret test which was also reported by [1]. Tests for other phyto constituents like saponin, phenol, terpenoids and flavonoids showed positive result in all the extracts. Similar findings were also reported by some researchers, [26] and [1]. Tests for glycosides and tannins showed negative result in all the extracts while some researchers reported the presence of glycosides and tannins [26], [1] and [19]. Sterols were found to be present in ethanolic and aqueous extracts only. The differences in results may be due to differences in area of plant collection, choice of solvent and method of extraction.

5. CONCLUSION

The present investigation reveals that the plant C. album has quite a number of chemical constituents which may be responsible for the many pharmacological actions. These metabolites are said to be useful to a plant itself but can be toxic to animals, including man. Their presence in the plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance.

6. ACKNOWLEDGEMENT

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