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Bimal Dutta

Department of Botany, Sibsagar
Girls' College, Sivasagar- 785640

Bonita Mahanta

Department of Botany, Sibsagar
Girls' College, Sivasagar- 785640

Studies on secondary metabolites, total phenol and flavonoid contents of *Eupatorium cannabinum* L. in Assam, India

Bimal Dutta, Bonita Mahanta

Abstract

Eupatorium cannabinum L., an ethnobotanical plant of Deories of Assam, India, was screened for secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins and saponins, and total phenol and flavonoid contents were also studied. It was found that the methanol extract of leaves contains all the screened compounds. The total phenol and flavonoid contents were found 64.82 mg/g and 25.05 mg/g in terms of gallic acid and quercetin equivalent respectively. Findings of the study provided evidence that crude extract of the plant species contains medicinally important bioactive compounds and justifies the uses of the plant in the traditional medicine for the treatment of different diseases.

Keywords: *Eupatorium cannabinum* L., ethno-medicinal plants, secondary metabolites.

Introduction

Secondary metabolites, a group of bioactive substances, having diverse classes of organic compounds like alkaloids, terpenoids, phenols, flavonoids, tannins, saponins, etc., are produced through secondary metabolism in different plants. The medicinal value of plants lies in these chemical substances that have definite physiological action on the human body [1]. Phytochemical analysis of ethno-medicinal plants for secondary metabolites is an important area of fundamental research because of its relevance for the discovery of therapeutic agents and providing clues for new sources of bioactive compounds [2-3].

Assam, a state in the North-Eastern region of India, with a geographical area of 78, 438 sq. km. (88°25' – 96°0' E latitude and 24°5' – 28° 0' N longitudes) with hills and plains, is known for its rich flora and diverse forests and vegetations due to its unique topography, climate and altitude patterns. This region of India is also a homeland of people belonging to more than 100 ethnic tribes and sub-tribes accordingly has been endowed with rich indigenous knowledge [4]. *Eupatorium cannabinum* L. (Photoplate 1), belonging to family Asteraceae, a median sized shrub plant, is one of the important sacred plants among the Deories of Assam. Deori, one of the distinguished ethnic groups of Assam, are presently distributed in the upper Brahmaputra valley districts of Assam. Among the Deories, *E. cannabinum* L, the plant species is known as 'Kimaru' and used in different religious and socio-cultural activities including the ritual purification functions of dead. Moreover, the plant has several ethno-medicinal applications. An infusion (100 ml) prepared from the tender leaves is given orally to women once a day for five days in excess bleeding during menstruation period. Leaves and tender stem juice is applied to cuts and bruises to stop bleeding. It is also used as a detoxifying herb for fever, colds, flu and other viral infections [5]. However, the plant is less known to the literature of Indian Medicinal Plants [6-11]. While, in the line of phytochemical studies on this plant species, a few works have been reported from abroad [12-13], however, from this region works have been found to be scanty. The present study was undertaken for screening of secondary metabolites such as alkaloids, terpenoids, phenols, flavonoids, tannins, saponins and to study the total phenol and flavonoid contents.

Materials and Methods

Collection of Plant Material

Fresh leaves of the plant species were collected from the homestead garden. Taxonomic identification was carried out with the help of regional floras and monographs [14-15], and also compared with the herbarium present in the North East Institute of Science and Technology,

Correspondence

Bimal Dutta

Department of Botany, Sibsagar
Girls' College, Sivasagar- 785640

Jorhat, Assam and India. Voucher specimen is deposited in the Herbarium of Sibsagar Girls' College, Sivasagar, Assam and India.

Preparation of Extract of Plant Material

The leaves were washed with running tap water, leaf material was then air dried under shade and after complete drying the material was grained in mixer. Plant extract was prepared using methanol as extracting solvent. 100g of the dried and powdered plant material (leaf) was extracted with 400ml of ethanol at 65°C for 2 days using Soxhlet extraction method. After filtering and evaporating to dryness, the crude methanolic extract was obtained.

Phytochemical Screening for secondary metabolites

Chemical tests were carried out qualitatively on the extract following standard procedures to identify the phytochemical constituents [16-17].

1. Test for alkaloids

Dragendroff's test: In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

Mayer's test: To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicates the presence of alkaloids.

2. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Shinoda test: To the test solution, a few drops of concentrated HCl and a few pieces of magnesium turning were added. Development of a pink or magenta red colour indicated the presence of flavonoids.

3. Test for phenolic compounds

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

4. Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicates the presence of tannin.

5. Test for terpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicates the presence of steroids. Formation of reddish brown colour of interface after addition of conc. sulphuric acid to the side carefully (without shaking) indicates the presence of terpenoids.

6. Test for saponins

Foam test: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously then some drops of olive oil were added. The formation of stable foam was taken as an indication for the presence of saponins.

Determination of total phenol and flavonoid content

Folin-Ciocalteu method, as described by Nabavi *et al.* [18], was used for phenol content determination. Briefly, 0.5 ml (1mg/ml) of plant extract was diluted to 5 ml with distilled water. Folin-Ciocalteu reagent 5ml (1:1 diluted with distilled water) was added and mixed thoroughly. After five minutes 5 ml of 10% Na₂CO₃ solution was added. The solution was warmed for one minute, and then cooled. The absorbance of the reaction mixtures was measured at 760 nm with UV-Visible spectrophotometer. Total phenol content was calculated as gallic acid equivalent from a calibration curve. The standard curve was prepared by 10, 25, 50, 100, 200 and 250 µg/ml solutions of gallic acid in methanol: water (50:50 v/v). Total phenol value is expressed in terms of gallic acid equivalent as mg/g of dry mass.

Colorimetric aluminum chloride method, as described by Nabavi *et al.* [18], was used for flavonoid content determination. Briefly, 0.5 ml (1mg/ml) of plant extract in methanol was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The solution was vigorously shaken and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 5, 10, 25, 50, 80 and 100µg/ml in methanol. Total flavonoid value is expressed in terms of quercetin equivalent as mg/g of dry mass.

Results and Discussion

The study was carried out on the methyl alcoholic extract of leaves revealed the presence of medicinally active secondary metabolites. The result is summarized Table 1. All the studied compounds i.e., Alkaloids, flavonoids, terpenoids, phenols, tannins and saponins were found present.

Various experiments have been demonstrated that phenolic compounds such as flavonoids, phenolic acids, etc. are potential antioxidant and antioxidant activity of these compounds is due to their ability to scavenge free radicals. Accumulation of free radicals can cause pathological conditions such as asthma, arthritis, inflammation, neuro-degeneration, heart disease, aging effect, etc. [19]. Additionally, phenolic compounds act as (i) metal chelators, (ii) antimutagens and anticarcinogens, (iii) antimicrobial agents [20]. The growth of many fungi, yeasts and bacteria was inhibited by tannins [21]. Further, tannins and terpenoids are attributed for analgesic and anti-inflammatory activities. Apart from these, tannins contribute property of astringency i.e., faster the healing of wounds and inflamed mucous membrane [22]. Saponins, present in plants, have been suggested as possible anti-carcinogens. The proposed mechanisms of anti-carcinogenic properties of saponins include direct cytotoxicity, immune modulator effects. Likewise, alkaloids are a diverse group of secondary metabolites found to have antimicrobial activities by inhibiting DNA topoisomerase [23]. Total amount of phenol and flavonoid contents were calculated from gallic acid ($y = 0.011x - 0.066$, $R^2 = 0.999$) and quercetin ($y = 0.030x - 0.077$, $R^2 = 0.999$) standard curves (Figure 1 & 2). The total phenol and flavonoid contents in methanol extract were found 64.82 mg/g and 25.05 mg/g in terms of gallic acid and quercetin equivalent respectively (Table 2). The phenol and flavonoid contents were well confirmed with qualitative investigations. Although, the total amount of flavonoid content (25.05 mg/g) found to be moderate, but, amount of the total

phenol content (64.82 mg/g) is comparatively higher which is encouraging and indicates the antioxidant potential of crude extract.

Conclusion

The plant species screened for secondary metabolites and the total phenol and flavonoid contents seemed to have the potency to act as a source of drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. It is also suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of the plant species.

Table 1: Secondary metabolites constituents in the methyl alcoholic extract of leaves of *E. cannabinum* L.

Secondary metabolites	Chemical tests	Indication: '+' for presence, '-' for absence
Alkaloids	Dragendroff's test	+
	Mayer's test	+
Flavonoids	Alkaline test	+
	Shinoda test	+
Phenols	Ferric chloride test	+
Tannins	Lead acetate test	+
Terpenoids	Salkowski test	+
Saponins	Foam test	+

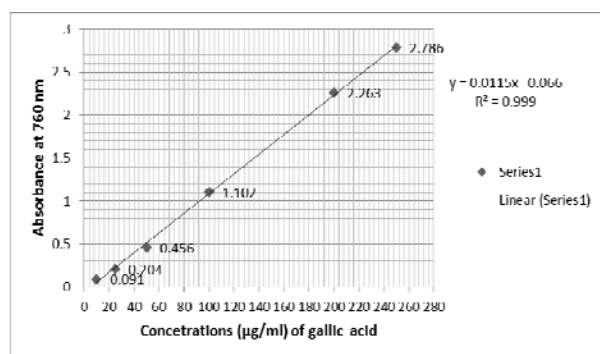


Fig 1: Standard calibration curve of gallic acid for the determination of total flavonoid content.

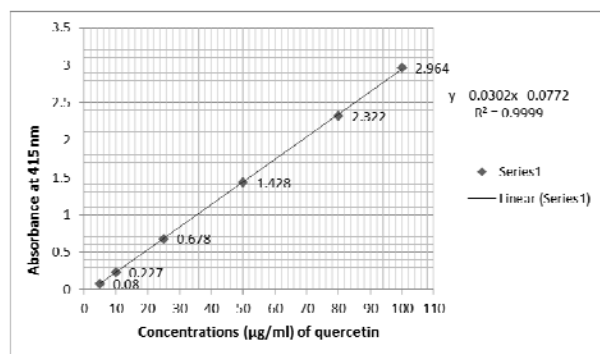


Fig 2: Standard calibration curve of quercetin for the determination of total phenol content.

Table 2: Total amount of phenol and flavonoid contents of *E. cannabinum* L.

Plant part/ Extract name	Total phenol (in mg/g, gallic acid equivalent)	Total flavonoid (in mg/g, quercetin equivalent)
Leaves/ Methanol extract	64.82	25.05



Photoplate 1: *Eupatorium cannabinum* L.

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