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Research Article

A PHYTOCHEMICAL STUDY ON *EUPATORIUM GLANDULOSUM*

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ABSTRACT

Objective: *Eupatorium glandulosum* is a useful medicinal plant belongs to the family Asteraceae. It is traditionally used to treat various diseases such as wound healing, antioxidant, and antiproliferative. The present study was aimed to investigate the physicochemical and phytochemical properties of various extracts of aerial parts of *E. glandulosum.*

Methods: The *E. glandulosum* plant materials were extracted using the solvents alcohol, ethyl acetate, and chloroform by Soxhlet method. The extracts were screened for physiochemical constants, preliminary phytochemical analysis for carbohydrates, glycosides, alkaloids, flavonoids, phenols, tannins, and saponins. The quantitative phytochemical analysis was carried out for total flavonoid and total phenols using standard procedures.

Results: The physiochemical constituents such as total ash, acid-insoluble ash, and water-soluble ash were found to be 14.25% (w/w), 5% (w/w), and 7.30% (w/w), respectively. The preliminary phytochemical screening confirmed the presence of carbohydrates, glycosides, alkaloids, flavonoids, phenols, tannins, and saponins. The flavonoid content of the plant extracts was found to be in the descending order ethyl acetate >alcohol >chloroform and the phenolic content was found to be alcohol >ethyl acetate >chloroform.

Conclusion: The result showed the presence of phytochemical constituents and higher values of phenolic and flavonoid content make the plant useful for the formulation of the different drugs for human uses for treating various diseases.

Keywords: Asteraceae, Carbohydrates, Flavonoid content, Proximate values, Saponins.

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INTRODUCTION

In recent years, the popularity of the herbal medicines is increasing day by day. The developing countries, especially India, provide two-third of the plants used in the modern system of medicine and also the economic importance of medicinal plants is much more in these countries. Treatment with medicinal plants is safe because there is very less side effect compared to the allopathic medicines. The benefit of herbal medicines is that these treatments are compatible with nature. The traditional people only prefer the herbals as a primary treatment for cure many diseases and they have experienced that some disease is a cure without any side effect with the medicinal plants rather than other treatments. Most of the drugs are formulated with the medicinal plants not show any side effect or reactions. This is the reason that herbal treatment is accepted all across the world. Nowadays, the medicinal plants are used for many drug formulations or development either non-pharmacopoeial, pharmacopoeial, or synthetic drugs because they are considered as a rich resource of ingredients as well as nutrition [1].

Phytoconstituents are the bioactive compound present in the plant. It is responsible for medicinal and biological as well as the toxic activities of plants. The phytochemicals located or concentrated mainly in the leaves, stem, flowers, roots, bark, fruits, and seeds depending on the family of the species. Hence, many phytochemicals or secondary metabolites are used as a lead compound for a new medicine or the active pharmaceutical ingredients in the commercial purpose. Hence, studying the phytochemical constituents helps to reveal the usage of plants [2].

Eupatorium glandulosum is in different parts of world popularly known as cat weed, Nilgiri weed, goat weed, or Mexican devil [3]. It is occasionally a perennial, simple, opposite, subentire, glabrous, decussate, and deltoid-ovate-shaped dark green leaves with purple underneath. White-colored flowers are seen from February to May [3]. In folklore, the leaves of the *E. glandulosum* used as a stimulant, astringent, and thermogenic [4]. The extract of plant possesses the antiproliferative activity and highest antioxidative activities [3]. Conventionally, the leaves are used to treat wounds because it has a broad spectrum of inhibitory activity against both Gram-negative and positive bacteria. The present study involved the screening of the plant to confirm the presence of a large number of secondary metabolites [5].

METHODS

Plant material

The aerial parts of *E. glandulosum* are freshly collected from Ooty (Nilgiri hills), Tamil Nadu, India, in the month of August 2017. The plant was identified by Dr. M. N. Naganandini, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru, India. Further, the plant material was dried under shade, coarsely powdered then passed through 40 meshes and stored in a well-closed container until further use.

Method of extraction

The coarsely powdered *E. glandulosum* plant material was extracted with alcohol, ethyl acetate, and chloroform separately using Soxhlet apparatus for 48 h. The process was repeated for 4 times and the filtrates were combined. The solutions were concentrated using rotary evaporator under reduced pressure and stored in desiccator.

Proximate value analysis

Determination of ash values

The ash values help to determine the purity and quality of the crude drug. The ash values of medicinal plants were determined using standard methods. After ignition of the plant materials, this measures total ash, acid-insoluble ash, and water-soluble ash [6]. For the

determination of various ash values, the *E. glandulosum* subjected for standard methods.

Total ash

In a tarred silica dish, 2 g of accurately weighed powdered drug was taken and incinerated at a temperature not exceeding 450°C until free from carbon. The ash was cooled and weighed [6].

Alcohol-insoluble ash

The ash obtained after incineration (total ash) was boiled for 5 min with 25 ml of dilute hydrochloric acid. The solution was filtered through ashless filter paper and collected insoluble matters were washed with hot water and ignited until getting constant weight. Calculate the percentage of the alcohol-insoluble ash was with the reference to the air-dried drug [6].

Water-insoluble ash

The ash obtained as a total ash was boiled 5 min with 25 ml of water and filtered through ashless filter paper and collected insoluble matters were washed with hot water and ignited in a crucible at a temperature not exceeding 450°C. The weight of the recently obtained ash is subtracted from the weight of total ash. The content of water-soluble ash with reference to the dried drug was calculated [6].

Determination of extractive values

Extractive values give an idea about the nature of the chemical constituents present in the plant drug. The alcohol and water were used as a solvent for the determination of extractive values [6].

Water-soluble extractive values

In a conical flask, 5 g of the powdered drug was taken and added 100 ml of water. The flask was closed with the help of a cotton plug or stopper and the mixture was kept 24 h, shaking is done frequently during the first few hours in a specific interval. After the time period (24 h), the mixture was filtered rapidly through using Whatman filter paper. The filtrate was collected and discards the upper solid content. Weighed the empty tarred flat-bottomed shallow dish and weight was noted down. Evaporate 25 ml of 5% solution of and weighed. Calculated the percentage yield of w/w of water-soluble extractive with reference to the air-dried drug [6].

Alcohol-soluble extractive values

In a conical flask, 5 g of the powdered drug was taken and added 100 ml of alcohol. The flask was closed with the help of a cotton plug or stopper and mixture was kept 24 h, shaking is done frequently during the first 6 h in a specific interval. After the time period (24 h), the mixture was filtered rapidly through using Whatman filter paper and precaution was taken against excessive loss of alcohol. The filtrate was collected and discards the upper solid content. Weighed the empty tarred flat-bottomed shallow dish and weight was noted down. Evaporate 25 ml of 5% solution of drug to dryness in an evaporating dish. The drug mixture was dried at 105°C and cooled and weighed. The calculation is done by the percentage of w/w of alcohol-soluble extractive with reference to the air-dried drug [6].

Determination of moisture content

Without preliminary drying, 5 g of the drug was placed in a tarred evaporating dish and dried at 105°C for 5 h and weigh. Continue the weighing and drying at 1 h interval until the difference between two corresponding weights not more than 0.25%. After drying for 30 min and after cooling for 30 min in desiccators, two constant weights are reached and it does not show more than 0.01 g difference [6-8].

Preliminary qualitative phytochemical analysis

The alcohol, ethyl acetate, and chloroform extracts were subjected to the preliminary phytochemical screening for the detection of the major phytoconstituents such as alkaloid, amino acid, carbohydrates, flavonoids, cardiac glycoside, saponins, mucilage, tannins, phenolic compound, and proteins [9-13].

Test for alkaloids

Required quantity (10 mg) of all three plant extracts was taken separately and dissolved it in 2% HCl, and filtered and the clear solution was used for the study.

Mayer's test

A small quantity of the extract was taken and treated with Mayer's reagent which was observed for the cream-colored precipitate.

Dragendorff's test

The test solution was treated with Dragendorff's reagent and observed for reddish-orange precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent and the observed color was reddish-brown precipitate.

Test for flavonoids

Aqueous NaOH test

The test fraction of extract was treated with a drop of 1 N NaOH solution and observed for yellow-orange color.

Sulfuric acid test

The fraction of extract was taken and treated with concentrated sulfuric acid which was observed for the orange color.

Shinoda's test

The fraction of extract was treated with a piece of magnesium turnings and few drops of concentrated hydrochloric acid. This was slightly heated and observed for the formation of a dark pink color.

Test for tannins and phenolics

Ferric chloride test

The fraction of extract was treated with 5% ferric chloride solution and observed for deep blue color.

Lead acetate test

The fraction of extract was treated with lead acetate solution and observed for white precipitate.

Test for amino acids

Millon's test

To the test solution, 2 ml of Millon's reagent was added and observed for white precipitate.

Ninhydrin test

To the test solution, ninhydrin solution was added, boiled, and observed for the formation of violet color.

Test for carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used for check the existence of carbohydrates.

Molisch's test

Filtrates are tested in a test tube with two drops of alcoholic α-naphthol solution and analyzed at the junction of two liquids for the violet ring.

Benedict's test

Filtrates were treated and heated gently with Benedict's reagent. For the reduction of sugars, the orange-red precipitate was observed.

Fehling's test

Dil. HCl was used to hydrolyze the filtrates and neutralized with alkali and warmed with Fehling's A and B solutions. Red precipitate for the reduction of sugars was found.

Test for glycosides

Keller–Killiani test

About 0.5 g of extract was shaken with 5 ml of distilled water. To the sample solution, 2 ml of glacial acetic acid holding few drops of ferric chloride was added and then 1 ml of sulfuric acid along the side of the test tubes. The formation of a brown ring was observed at the liquid interface.

Legal test

The test solution was treated with pyridine and then added alkaline nitroprusside solution. The appearance of red blood color was observed.

Test for proteins

Heat test

Test solution was heated on a water bath and observed for the coagulation proteins.

Test with trichloroacetic acid

To the test solution, the trichloroacetic acid was added and precipitate formation was observed.

Biuret test

Two milliliters of the test solution was added to 2 ml of biuret reagent and the presence of protein is indicated for the violet color.

Test for saponins

Forth formations test placed 2 ml solution of extract in a test tube containing water. Shake well and observed for froth formation.

Quantitative estimation of phytoconstituents

Determination of total phenolic content

The total phenolic content of plant extracts was determined using the colorimetric methods. The plant extract (1 ml) was mixed with Folin–Ciocalteu's reagent (1 ml). Saturated sodium carbonate solution (1 ml) was added to the mixture after 3 min. The volume was made up to 10 ml using distilled water. The reaction kept 90 min in a dark place and after which absorbance was taken at 725 nm. Gallic acid was used as a standard solution for constructing the standard curve [14,15].

Determination of flavonoid content

Aluminum chloride colorimetric assay was used for the determination of total flavonoid content. The plant extract (1 ml) and distilled water (4 ml) were taken into the 10 ml volumetric flask. The 5% sodium nitrate (0.3 ml) was added to the flask and 5 min later 10% aluminum chloride (0.3) was mixed with it. After 5 min, the solutions treated with 1 M sodium hydroxide (2 ml) and make up the volume with water. Quercetin was used as a standard solution for constructing a standard curve in the same manner as described earlier. Absorbance was taken at 510 nm in the ultraviolet/visible spectrophotometer [16].

Table 1: Physicochemical constants determination

S. No.	Parameters determined	Percentage (w/w)
1.	Total ash	14.25
2.	Acid-insoluble ash	5.00
3.	Water-soluble ash	7.30
4.	Alcohol-soluble extractive	16.00
5.	Water-soluble extractive	24.00
6.	Moisture content	10.00

RESULTS

Plant material and extraction

The yield of alcohol, ethyl acetate, and chloroform extract of aerial parts of *E. glandulosum* was found to be 19.32 (%) w/w, 10.85 (%) w/w, and 7.24 (%) w/w, respectively.

Physicochemical constants determination

The physicochemical constants such as ash values and extractive values were determined by standard procedure. The total ash, acid-insoluble ash, and water-soluble ash were 14.25% w/w, 5% w/w, and 7.30% w/w, respectively. Higher percentage yield of water-soluble extractive (24% w/w) and alcohol-soluble extractive 16% w/w values were observed. The moisture content of the drug material found to be 10% w/w. The results are shown in Table 1.

Preliminary phytochemical analysis

The phytochemical analysis of three different solvent extracts of *E. glandulosum* showed various compounds. All extracts revealed the presence of secondary metabolites. The ethyl acetate extract and alcohol extract showed the presence of alkaloids, carbohydrates, flavonoids, cardiac glycoside, a phenolic compound, saponins, and tannins. The chloroform extract showed the presence of carbohydrates, flavonoids, phenolic compounds, cardiac glycoside, and mucilage. The absence of some phytochemicals in the extracts may be due to the seasonal variation or geographical location of the plant. Time of collection of the plant also affects the active constituents. This is shown in Table 2.

Determination of total phenolic content

The total phenolic content was measured by Folin–Ciocalteu reagent, showed good phenolic content in all alcohol, ethyl acetate, and chloroform extracts and the alcohol extract shows more phenolic content than other two extracts. The results are shown in Table 3.

Determination of total flavonoid content

The total flavonoid content was determined by the aluminum chloride spectrophotometric method. Ethyl acetate extract of *E. glandulosum* containing the highest flavonoid content than alcohol and chloroform extracts. This is shown in Table 4.

DISCUSSION

The phytochemicals present in the plant extracts highly responsible for biological activity. The phytochemical analysis of extracts using different solvents showed various chemical constituents in *E. glandulosum*. All extracts revealed the presence of secondary metabolites. The alcohol extract showed the presence of alkaloids, flavonoids, saponins, tannins, and the phenolic compound. The ethyl acetate extracts showed

Table 2: Preliminary phytochemical screening

S. No.	Phytochemical constituents	Alcoholic extract	Ethyl acetate extract	Chloroform extract
1.	Alkaloids	+	+	–
2.	Amino acid	–	–	–
3.	Carbohydrates	+	+	+
4.	Flavonoids	+	+	+
5.	Glycosides	+	+	+
6.	Saponins	+	+	–
7.	Tannins	+	+	+
8.	Phenolic compound	+	+	+
9.	Proteins	–	–	–

+: Positive, –: Negative

Table 3: Determination of phenolic content

S. No.	Extracts	Phenolic content (µg of GAE/mg of extract)
1.	Alcohol	0.780
2.	Ethyl acetate	0.268
3.	Chloroform	0.147

GAE: Gallic acid equivalent

Table 4: Determination of flavonoid content

S. No.	Extract	Flavonoid content (µg RUE/mg of the extract)
1.	Alcohol	138.93
2.	Ethyl acetate	290.29
3.	Chloroform	20.51

RUE: Rutin equivalent

the presence of alkaloid, flavonoid, saponins, tannins, and phenolic compound and the chloroform extract showed the presence of flavonoids, tannins, and the phenolic compound. Alcoholic extract does not contain proteins and amino acids, nor does the extract of ethyl acetate contain proteins, amino acids, carbohydrates and glycosides. The alkaloid, amino acids, saponins, glycoside, and proteins are absent in the chloroform extract. The absence of some phytochemicals may be due to the seasonal variation or geographical location of the plant. Time of collection of the plant also affects the active constituents. All alcohol, ethyl acetate, and chloroform extracts exhibited good phenolic and flavonoid contents. The alcohol extract showed more phenolic content (0.78% w/w) than other two extracts. Ethyl acetate extract of *E. glandulosum* containing highest flavonoid content (290.29% w/w) than alcohol (138.93% w/w) and chloroform (20.51% w/w) extracts.

CONCLUSION

The phytochemical screening of alcohol, ethyl acetate, and chloroform extracts of the aerial parts of the *E. glandulosum* has revealed the presence of a mixture of phytochemicals. The phytochemical screening showed that the maximum presence of phytoconstituents in both alcohol and ethyl acetate extract compared to the chloroform extract. The three extracts showed good values of phenolic content and flavonoid content. The presence of phytochemical constituents and higher values of phenolic and flavonoid content make the plant useful for the formulation of the different drugs for human uses for treating various diseases. The obtained results explained the presence of possible phytoconstituents, potential usefulness and justified the traditional uses.

AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article Miss Silpa performed the experiment and collected the data; Hamsalakshmi helps in writing and designing the manuscript. Dr. J Suresh proofread the whole manuscript and suggested the necessary changes.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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