

Original Research Article

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## Biochemical Characterization of *Eupatorium glandulosum* Plant Extracts

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### ABSTRACT

The present study was undertaken to identify the phytochemicals present in *Eupatorium glandulosum* of leaf, stem, flower and root. The use of natural plant part resources for applying ecofriendly finish for textiles has opened up new avenues in this area of research. *Eupatorium glandulosum* (*Ageratina adenophora*) belongs to the family Asteraceae. Asteraceae is one of the largest families of vascular plants, distributed over most of the earth and in almost all habitats and comprises of nearly 1200 species distributed mainly in the tropical regions of Americas, Europe, Africa, and Asia. Phenolics possess a wide spectrum of biochemical activities such as antimicrobial and antibacterial activity. The different plant parts of *Eupatorium glandulosum* was prepared using two solvents viz., 70 % ethyl alcohol and distilled water. Extracts were characterized qualitatively for the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids and quantitatively for its Total Phenolic Content (TPC) was determined by Folin-Ciocalteu reagent assay method. The yield of *Eupatorium glandulosum* leaf, stem, flower and root using 70% ethanol solvent was found to be higher than the distilled water. Results of phytochemical screening revealed the presence of alkaloids in all the plant part extracts *Eupatorium glandulosum*. However, flavonoids, tannins, saponins and terpenoids were present in some plant parts. Irrespective of the solvent Total Phenolic Content (TPC) of the leaf extract was higher compared to the other plant parts. Hence, such compounds contribute to the antimicrobial and antioxidant activity and can be used for eco-friendly processing and finishing of the textile material for functional performance.

#### Keywords

*Eupatorium glandulosum*,  
Phenolic content  
and Phytochemical

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### Introduction

Phytochemicals are the chemicals produced by various parts of the plants. These bioactive constituents of plants include terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. (Santhi and Sengottuvel, 2016) There are about eight thousand naturally occurring plants containing a wide spectrum of phenolics performing biochemical activities

such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression (Marinova *et al.*, 2005).

*Ageratina adenophora* (Spreng) (Syn. *Eupatorium glandulosum*) belongs to the Family Asteraceae. Asteraceae is one of the largest families of vascular plants, distributed over a major part of the earth and in almost all habitats. The genus *Eupatorium* belongs to the

*Eupatorieae*, one of the 13 tribes of the Asteraceae, and comprises of nearly 1200 species distributed mainly in the tropical regions of America, Europe, Africa and Asia. *Eupatorium glandulosum* is commonly called Goat weed or Crofton weed (Balaiyeswarya, 2013)

*Eupatorium glandulosum* is a perennial herbaceous shrub that may grow to 1 or 2 metres (3.3 or 6.6 ft) height. It has opposite trowel-shaped serrated leaves that are 6–10 cm (2.4–3.9 in) long by 3–6 cm (1.2–2.4 in) width. The small compound flowers occur in late spring and summer and are found in clusters at the end of branches.

Each flower head is up to 0.5 cm in the diameter and creamy white. They are followed by a small brown seed with a white feathery parachute. (Vasanthi and Gopalakrishnan, 2013) few species of *Eupatorium* are known for their medicinal value and are used in pain life, influenza and inflammation. *Eupatorium* is a widely grown weed in the Western Ghats of Karnataka. Therefore, the study was carried to characterize phytochemical components of *Eupatorium glandulosum*.

## Materials and Methods

Samples of *Eupatorium glandulosum* was collected from the field of University of Agricultural Sciences, Dharwad and the different parts (leaf, stem, flower and root) were segregated for further use.

## Chemicals used

Analytical reagent (AR) grade ammonia, chloroform, ethyl alcohol, hydrochloric acid, sulfuric acid, ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, lead acetate, and gelatin. sodium nitrite, Folin-Ciocalteu reagent, Dragendorff's reagent, Wagners reagent and Gallic acid.

## Preparation of extracts

Different plant parts of *Eupatorium* were washed separately and dried at  $40\pm 2^\circ\text{C}$  in hot air oven. Powdered in a laboratory blender and stored in a refrigerator. Extraction process was optimized by two solvents viz., 70 % ethyl alcohol and distilled water. Two gram of weighed powder was extracted with 25ml of each solvent, incubated under agitation at  $25^\circ\text{C}$  for 3hrs. Centrifuged at 5000 rpm at  $5^\circ\text{C}$  for 10 min. the supernatant obtained was separated and residue was re-extracted with fresh 25ml of solvents, the process was repeated and the supernatants were pooled, and the extract obtained was measured and filtered using Whatman filter paper no. 40 (125 mm). Extracts were stored under refrigeration and further analysis of phytochemicals took place within a week.

## Phytochemical analysis

The chemical tests described by Ajayi *et al.*, (2011), Raaman (2006), Rahul *et al.*, (2010) and Jyoti *et al.*, (2014) were adopted for qualitative phytochemical screening of *Eupatorium* plant part extracts for the identification of various chemical constituents like alkaloids, flavonoids, tannins, saponins, and terpenoids, the details of which are listed below:

## Test for tannins and phenolic compounds

Ferric chloride test: One milliliter of extract was separately stirred with 10 mL of distilled water and then filtered. A few drops of 5 percent  $\text{FeCl}_3$  were added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of tannins.

Gelatin test: Two milliliters of a 1 percent solution of gelatin containing 10 percent NaCl was added to 1 mL of the extract. White

precipitate indicates the presence of phenolic compounds.

**Lead acetate test:** Three milliliters of a 10 percent lead acetate solution was added to 1 mL of extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

### **Test for flavonoids**

**Ammonia test:** A few drops of a 1 percent NH<sub>3</sub> solution was added to 1 mL of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

**Sodium hydroxide test:** A few drops of a 20 percent NaOH solution was added to 1 mL of extract. When HCl is added, the yellow color of the extract turns to a colorless solution that indicates the presence of flavonoids.

### **Test for alkaloids**

**Dragendorff test:** To 1 mL of extract, a few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicates a positive test.

**Wagner test:** A few drops of Wagner's reagent were added by the side of the test tube to 1 mL of extract. A reddish brown precipitate confirms the test as positive.

### **Test for saponins**

For the foam test, about 1 mL of the sample extract was boiled in 20 mL of distilled water in a water bath and filtered; 10 mL of the filtrate was mixed with 5 mL of distilled water and mixed vigorously for 15 minutes to form a stable persistent froth.

The presence of froth after 5 minutes was taken as an indication of the presence of saponins.

### **Test for terpenoids**

To perform the Salkowski test, 1 mL of each extract was mixed with 0.5 mL of chloroform, and 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added carefully to form a layer. The reddish brown coloration of the interface showed positive results for the presence of terpenoids.

### **Quantitative estimation of phenolics**

Total phenolic content (TPC) in the extracts was determined using the Folin-Ciocalteu assay method (Singleton and Rossi 1965) with little modification using gallic acid as the reference standard.

All the solvent extracts were diluted to appropriate volumes and were mixed with 2 mL of a 10 percent Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was incubated at room temperature for 3 minutes, and 100  $\mu$ L of Folin-Ciocalteu reagent was added to the mixture.

The resulting solution was incubated for 90 minutes at room temperature in the dark, and the absorbance was measured at 765 nm using the Ultraviolet-Visible (UV-Vis) spectrophotometer (Jyoti and Giridhar, 2016).

## **Results and Discussion**

### **Yield of extracts**

The yield of ethanol extracts of *Eupatorium* leaf (43 ml of extract/50 ml of solvent) was found to be higher than the distilled water (41 ml of extract/50 ml of solvent). *Eupatorium* flower exhibited higher yield in ethanol solvent (42 ml of extract/50 ml of solvent) followed by distilled water (40 ml of extract/50 ml of solvent) extracts (Fig. 1). On the other hand, 37 ml of yield was found in ethanol extract of the root and the stem extract exhibited 30-35 ml yield of extracts in both the solvents.

**Table.1** Phytochemical screening of *Eupatorium glandulosum* plant part extracts

Phytochemical group	Chemical tests	Extracts of <i>Eupatorium glandulosum</i> plant parts							
		Distilled water				70% ethanol			
		Leaf	Stem	Flower	Root	Leaf	Stem	Flower	Root
Alkaloids	Dragendorff's	+++	+++	+++	+++	++	+++	+++	+++
	Wagners	-	-	-	-	-	+++	+++	+++
Flavonoids	Ammonia	-	-	-	-	-	+++	+++	++
	Sodium hydroxide	-	+++	-	+++	-	++	+	+++
Phenolic compound and tannins	Ferric chloride	+++	-	-	-	+++	-	+++	-
	Gelatin	-	-	-	-	+++	+++	+++	+++
	Lead acetate	+++	+++	+++	+++	-	+++	++	++
Saponin	Foam	-	-	+++	+	-	-	+	+
Terpenoids	Salkowski	+++	+	+++	+	-	+++	-	++

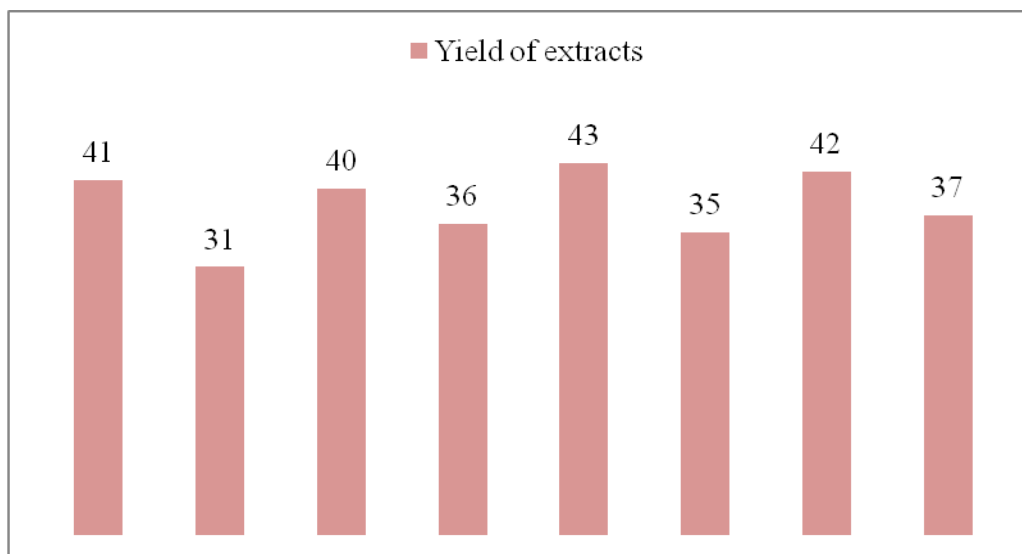
(+) = Presence of compound, (-) = Absence of compound, (++) = Dark colour and (+++) = Dark colour with precipitate

**Table.2** Total Phenolic Content (TPC) of different solvent extracts of *Eupatorium glandulosum* plant part extracts

Sl. No	Solvent extracts	Total Phenolic Content mg/g (GAE)*			
		<i>Eupatorium glandulosum</i>			
		Leaf	Stem	Flower	Root
1	Distilled water/aqueous	55.93 (±3.25)	15.18 (±0.13)	48.42 (±0.20)	15.12 (±0.23)
2	70% Ethanol	70.32 (±1.92)	15.53 (±1.15)	50.39 (±0.95)	25.55 (±1.74)

\*GAE= Gallic Acid Equivalent  
Data are mean ± standard deviation of duplicate determinations

**Fig.1** Yield of extracts



### **Phytochemical screening of *Eupatorium G.* plant part extracts**

Table 1 depicts the results of phytochemical screening of plant extracts of *Eupatorium*. From the table it was observed that all the plant part extracts of *Eupatorium* exhibits positive results for alkaloids as proved by Dragendorff's test in both distilled water and ethanol solvents. In ethanol solvent except leaves all the plant part extracts depicts the positive results for alkaloids by Wagner's test.

The presence of flavonoids was positively proved by ammonia and sodium hydroxide test in ethanol extracts of stem, flower and root of *Eupatorium* and in aqueous extract of stem and root was positively proved by sodium hydroxide test.

All the extracts of *Eupatorium* leaf, stem, flower and root indicated the presence of tannins as proven by lead acetate test except in ethanol extract of leaf. Whereas ethanol extract of all the plant parts showed positive results with gelatin test. However aqueous extract of leaf and ethanol extracts of leaf and flower showed the presence of tannins using ferric chloride test.

In both the solvents, flower and root extracts showed the presence of saponins through foam test and no saponins was present in the leaf and stem extracts. In aqueous extract of all the parts of *Eupatorium* proved the presence of terpenoids with salkowski test and terpenoids was present in ethanol extract of stem and root.

### **Total phenolic content of different solvent extracts of *Eupatorium glandulosum* plant part**

Total Phenolic Content (TPC) of solvent extracts of *Eupatorium* plant part is described in Table 2. In general, TPC was found to be

high in ethanol extracts compared to extracts in distilled water, probably due to the affinity of the substances with the employed solvent. Phenolic substances have a higher affinity with polar solvents, such as ethanol and ethyl acetate (Tiwari *et al.*, 2011 and Sideney *et al.*, 2015). Whereas, in both the solvent and total phenolic compound of leaf extracts (70.32mg/g and 50.39mg/g) was found to be high followed by flower and root extracts. On the other hand, ethanol (50.39 mg/g) extract of *Eupatorium* flower yielded higher amount of TPC compared to distilled water (48.42 mg/g). Further, *Eupatorium* stem and root exhibited minimum TPC using aqueous against the phenolic concentration of ethanol solvents.

Alkaloids were found to be present in all the extracts of *Eupatorium* plant parts. Flavonoids were present in ethanolic extracts of stem, flower and root. All the extracts of *Eupatorium* leaf, stem, flower and root indicated the presence of tannins as proved by lead acetate test except ethanol extract of leaves. Ethanolic extract of all the plant parts depicted the presence of tannins as proven by gelatin test and only aqueous extract of leaf and ethanol extract of leaf and stem showed the presence of tannins by ferric chloride test.

Positive results for saponins and terpenoids were obtained in all the plant parts of *Eupatorium* except ethanol extract of leaf and flower. TPC was high in aqueous and ethanol extract of leaf followed by flower, root and stem.

The phenolic components perform varied functions that can be made use of the textile finishing. *Eupatorium* is a weed and hence the source can be used for eco-friendly processing and finishing of the textile material for functional performance. Studies on the biosafety of utilizing such natural sources also need to be explored.

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