



Research Article

ANALYSIS OF PHYTOCHEMICALS FROM *EUPATORIUM ODORATUM* FLOWER

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ABSTRACT

The *Eupatorium odoratum* is included in sun flower family Asteraceae has many properties as anti-bacterial, anti-mycobacterial properties. It also contains many phytochemicals. It used as tea in many places. An ointment prepared from the leaves of *Eupatorium odoratum* is has been shown to promote the healing of soft tissue wounds by enhancing the proliferation and migration of fibroblast. It has been identified that the presence of antioxidants and other bioactive compounds might have helped the people to use the leaves and roots of this plant as natural medicine. It used as medicinal as well as ornamental plant. Successive extracts from dried flowers of *Eupatorium odoratum* were investigated for the presence of phytochemicals. Methanol extract was used for the phytochemical and antibacterial studies. Aqueous extracts of *Eupatoriumodoratum* was used for the insecticidal and larvicidal activity. As a result it was found that the plant flowers have insecticidal and larvicidal activity and a slight range of antibacterial activity. Aqueous extract exhibited the presence of enzyme polyphenol oxidase. The extract was subjected to ammonium sulfate precipitation and dialysis to precipitate proteins. It was then eluted out using ion exchange chromatography and partially purified. The PPO have a wide range of applications, it is an important natural component in dye industry, it is good for bioremediation as it cleaves phenol rings of many of the petroleum compounds which cause soil pollution.

KEYWORDS: *Eupatorium odoratum* flower, Phytochemical analysis, Antibacterial, Insecticidal and Larvicidal activity, Polyphenol oxidase.

INTRODUCTION

Eupatorium odoratum Linn is a shrub of genus of *Eupatorium* (Compositae). It is also known as *Chromolaenaodorata*. It is a shrub belonging to the Astereceae family. The *Eupatorium odoratum* leaf extract has diverse biological activities like inhibition of the growth of some bacteria to enhancement of haemostasis and blood coagulation,^[1] anti-inflammatory, astringent, diuretic, and hepatotropic activities.^[2,3] Phytochemical analysis revealed the presence of many secondary metabolites. These are mainly responsible for the activities of *Eupatorium odoratum* like antimicrobial, enhancement of antibiotic activity, antioxidant, anti-inflammatory, etc.^[4,5] Mishra et al.,^[6] analysed the phytochemicals from ethanol and chloroform extracts of *Eupatoriumodoratum*. Nisha Rani, et al., 2006^[7] reported an anionic peroxidase isoenzyme having a pI of 3.5 and was purified from *Eupatorium odoratum*.

Herbal medicines are gaining a great importance in recent times. *Eupatorium odoratum* was used as a traditional medicinal plant for centuries. The young leaves of *Eupatorium odoratum* are used to treat skin wounds. The studies of Heiss et al ^[8] identified phytoprostane compound chromomoric acid c-l which is a strong inducer of transcription

factor NFE2L2 (Nrf2), a master regulator of gene with defensive, anti-inflammatory & detoxifying functions.

Amatya et al^[9] evaluated the antioxidant activities of ethanolic extracts of leaf, stem, root and defatted flower parts. The study revealed good antioxidant activities in both leaf and flower extracts. This study suggests that leaf and flower parts of *Eupatorium odoratum* is could be pharmaceutically exploited. The flowers of *Eupatorium odoratum* exhibited four flavanones. Compound 1 exhibited moderate antimycobacterial activity against *Mycobacterium tuberculosis*.^[10] A wide range of chemicals compound have been isolated from *Eupatorium odoratum* and is used extensively for the treatment of several diseases like diarrhea, diuretic activity, wound healing, antimycobacterial activity and insect repellent properties^[11]. Harun et al,^[12] studied the cytotoxic effects and mechanism of action of *Eupatorium odoratum* extracts on MCF-7 and Vero cell lines. Their studies suggested that acetone and ethyl acetate extracts of *Eupatorium odoratum* induce cell death through induction of autophagy and hold potential for development as potential anticancer drugs.

MATERIALS AND METHODS

Preparation of *Eupatorium* extract

Flowers of *Eupatorium odoratum* were collected from local areas of Thrissur. The flowers were dried in shade and pulverized into fine powder. The flowers were defatted using hexane and was used for further studies. Extraction of *Eupatorium* flower powder was performed using solvents methanol, water and 0.1 M Tris-HCl (pH 8.0) buffer under constant stirring at room temperature. The extracts obtained were concentrated. Most of the solvents were removed by evaporation. The extract obtained was stored at 4°C for future use. The extract preparations were done at Department of Biochemistry, St. Mary's College Thrissur. The buffer extract was subjected to 80% ammonium sulphate precipitation.

Phytochemical analysis^[13]

Methanolic extracts were used to carry out phytochemical studies. The analysis of extracts had been carried out at Department of Biochemistry, St. Mary's College Thrissur.

Detection of phytosterols (Liebermann-Burchard's test)

The extract (5mg) was dissolved in 2ml of acetic anhydride and one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. The formation of blue green color indicated the presence of triterpenoid or phytosteroids.

Detection of tannins (Ferric chloride test)

The extract (5mg) was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicated the presence of tannins.

Detection of phenols (Lead acetate test)

The extract (5mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenol.

Detection of flavonoids

An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Detection of coumarins

10% NaOH (1ml) was added to 1 ml of plant extract formation of yellow color indicated the presence of coumarins.

Detection of saponin

Distilled water 2 ml was added of each plant extract and shaken in graduated cylinder for 15 minutes lengthwise. Formation of 1 cm foam indicates the presence of saponins.

Detection of Quinone

Concentrated sulphuric acid was added to the plant extract in the ratio 1:1. Formation of red color indicated the presence of Quinone.

Detection of cardiac glycosides

Glacial acetic acid (2 ml) and few drops of concentrated sulphuric acid were added carefully to 0.5 ml of extract. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated presence of cardiac glycosides

Detection of terpenoid

Chloroform (2ml) and concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red brown color at the interface indicated the presence of terpenoids.

Detection of acids

Plant extract 0.5 ml was treated with sodium bicarbonate solution. Formation of effervescence indicated presence of acids.

Detection of anthraquinones

Few drops of 2% HCL were added to 0.5 ml of root extract. Appearances of red color precipitate indicate presence of anthraquinone.

Detection of steroids and phytosteroids

To 0.5 ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Antibacterial activity analysis of *Eupatorium odoratum* flower extract

The agar diffusion method is usually used to check the sensitivity of antibiotics against microorganisms. The activity of *Eupatorium odoratum* flower extract was tested against six microorganisms (*E.coli*, Staphylococcus, Streptococcus, Bacillus, Proteus, Pseudomonas, and Klebsiella). 100ml of nutrient broth was inoculated with the test organisms and incubated at 37°C over night. 0.1ml of culture were swabbed over six plates containing solidified sterile nutrient agar medium and allowed to dry. Required number of wells was cut upon each plate with equal distributions using a sterile gel puncture and the agar plugs were removed carefully. In each wells methanol extracted samples (0.083g/ml of DMSO) of 10µl, 15µl, and 20µl were loaded. It was incubated over night at 37°C. The diameter of zone of inhibition was measured

Insecticidal and larvicidal activity

White mealy bugs and third instar mosquito larvae were collected and kept in 10 ml of aqueous extract of flower. 10 ml of water was taken as control. The insects and larvae were kept separately and

monitored for several hours to calculate the mortality rate contributed by the flower extract.

Detecting the presence of polyphenol oxidase

Polyphenol oxidase catalyses oxidation of diphenols to produce quinones leads to browning of fruits and plants. 0.5 ml of tannic acid was added to the plant extract prepared in Tris-HCl buffer and incubated at room temperature for 30 min. The increase in absorbance was noted. The increase in enzyme activity gradually made an increase the browning. These increases were detected using spectrum scanning. 1mg/ml of tannic acid were used. The extract without tannic acid was used as control.

Confirmatory test for the presence of polyphenol oxidase

Resorcinol showed to be noncompetitive-type inhibitor for polyphenol oxidase. Resorcinol was added to the buffer extract of flower while extraction. 1g flower of *Eupatorium odoratum* was weighed and added into 25 ml of distilled water, into this 0.5g of resorcinol was added. It was extracted at room temperature for 5 hours of time. Buffer extract without resorcinol was kept as control. Spectrum of both extractions were compared for detecting the presence of polyphenol oxidase.

Partial purification of polyphenol oxidase

The *Eupatorium odoratum* is dried flower extract was prepared in 0.1M Tris-HCl buffer pH 8.0 containing 0.1% ascorbic acid. The supernatant of the extract was subjected to 80% ammonium sulphate precipitation and was dialysed. After dialysis the sample was centrifuged and the supernatant was loaded on to DEAE-cellulose ion exchange column. The column was equilibrated with 0.1M phosphate buffer pH 7.5. The bound protein was eluted by changing the pH. Batch elution of flower extract at varying pH (7.0 - 8.0) was performed for the purification of PPO. The eluted protein was measured at 280 nm. The PPO activity was confirmed by the catechol assay.

RESULTS AND DISCUSSION

Analysis of secondary metabolites from *Eupatorium odoratum* flower extract

The methanol extract of *Eupatorium odoratum* flower gave positive results for Tannin, Phytosterols or Triterpenoids, Flavanoids, Coumarins, Quinones, Cardiac Glycosides, Terpenoids, Steroids, Acids, and Phenols. Anthraquinones gave negative result in phytochemical analysis of methanol extract. Similar to leaves, flowers are also rich source of secondary plant metabolites. Qualitative analysis showed that *Eupatorium odoratum* flower is rich in quinones. Table below shows the presence of secondary metabolites in *Eupatorium odoratum* flower.

Phytochemical analysis of *Eupatorium odoratum* flower extract in summarised form:

Phytochemical test	Methanol extract
a) tannin	+
b) phytosterols	+
c) coumarin	+
d) saponins	-
e) quinone	++
f) cardiacglycosides	+
g) terpenoids	+
h) anthraquinone	-
i) steroids	+
j) acids	+
k) phenol	+
l) flavanoid	+

Antibacterial activity of flower extract of *Eupatorium odoratum*

The antibacterial activity of methanol extract of *Eupatorium odoratum* flower was studied using agar diffusion method. Out of 7 (*E.coli*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Proteus*, *Pseudomonas*, and *Klebsiella*) microorganisms were tested 2 of them (*Staphylococcus* and *Bacillus*) showed zone of inhibition. The obtained zone of inhibition was measured. Figure 1 indicates the antibacterial activity of *Eupatorium odoratum* flower. The maximum zone was obtained for *Staphylococcus* and a minimum for *Bacillus*. Other tested organisms are not showing any antibacterial activity. *Eupatorium odoratum* is flowers exhibit less antimicrobial activity compared to the leaf extracts. These results are in agreement with the already available data.

Insecticidal and larvicidal activity

The aqueous extract of *Eupatorium odoratum* flower showed insecticidal activity against white mealybug and larvicidal activity against mosquito larvae. Among the five bugs used, one of the white mealy bug were died in 3hours, two of them were in 4½ hours and another two of them were died within 5 hours of time. Mortality was completed in 5 hours. In the case of mosquito larvae two were died in 30 minutes one was in 40 minutes and the last two were in 45 minutes of time. The bugs and larvae in distilled water which has been used as a control were not showing any mortality rate compared to those in the extract. This data sheds light to use this source as a means of larvicidal and insecticidal activity. Figure 2 represents the insecticidal and larvicidal activity of *Eupatorium odoratum* flower. In the war against organic pesticides we can point out this plant as a potent source as a biological pest control device.

Detection of polyphenol oxidase

The methanolic extract of flower was pale yellow in colour while buffer extract was dark in color. This observation suspected the presence of an active enzyme helping in the darkening process. Therefore to

confirm the presence of polyphenol oxidase, the enzyme responsible in the browning process, the extract was incubated with tannic acid, which is a polyphenol and the more amount of substrate can increase the reaction rate, the product formed was measured by noting the spectrum of the reaction with and without tannic acid. The increase in browning of the tannic acid added extract indicated the presence of polyphenol oxidase. The following figures 3a and 3b shows peak obtained by spectrum scanning.

Comformatory test for the presence of polyphenol oxidase

Resorcinol is an inhibitor of polyphenoloxidase. The addition of resorcinol found to inhibit polyphenoloxidase activity. Resorcinol was added to the extract of flower while extraction. It was detected by the decrease in browning of resorcinol added extract. Spectrum of both extractions was compared for detecting the presence of polyphenol oxidase. PPO is an enzyme having wide industrial applications. This flower is a new source of PPO.

Partial purification of PPO

The flower extract in 0.1M tris-HCl buffer was subjected to 80% ammonium sulfate precipitation followed by dialysis. The dialyzed sample was loaded on to DEAE cellulose for purifying the protein. The protein was purified using a pH gradient of pH 7-8 using 0.1M phosphate buffer. The purified proteins were collected and analyzed for the presence of PPO. Figure 4 indicates the purification profile of PPO using ion exchange chromatography. The presence of PPO was confirmed using catechol assay. The PPO was eluted from the fractions having pH 7.5. The fractions having catechol oxidizing activity were collected for further work. Figure 5 shows the picture of *Eupatoriumodoratum* and its flower chosen for the study.

CONCLUSION

The flowers of *Eupatorium odoratum* shows larvicidal, insecticidal and a slight ranges of anti-bacterial activities. We also detected the presence of an enzyme polyphenol oxidase (PPO) and partially purified the enzyme from flowers. The polyphenol oxidase has a number of applications in industries. PPO can used in dye industry as a coloring agent, it also used in food industry. It is the reason behind the coloring of black tea and cocoa. PPO is also can used to cleave the phenol ring thus it can used to degrade petroleum compounds that cause soil pollution and so it is applicable in preventing bioremediation also.

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Study photographs

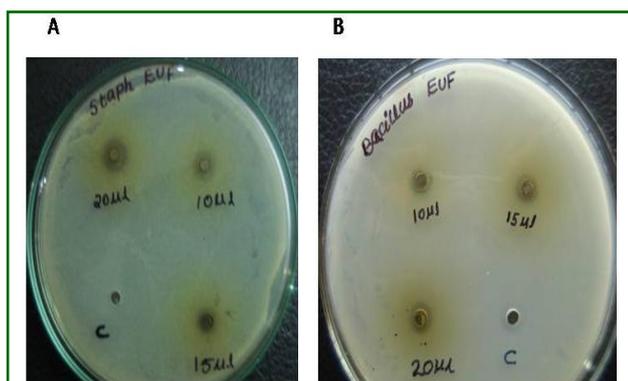


Fig 1. Antibacterial activity of *E. odoratum* flower extracts. (a) Zone of inhibition against Staphylococcus (b) Bacillus

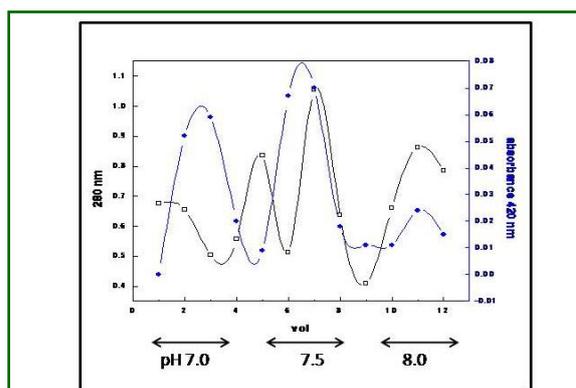


Fig 4 Purification profile of PPO on DEAE cellulose ion exchange chromatography

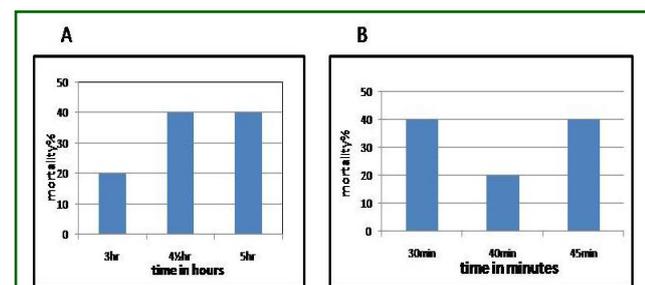


Fig 2 (A) Insecticidal activity of *E.odoratum* flower extract (B) Larvicidal activity



Fig 5. *Eupatorium odoratum* bearing flower

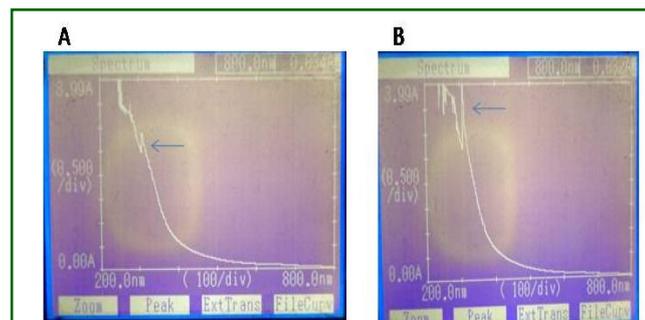


Fig 3 a) Extract without tannic acid, b) Extract with tannic acid. Arrow indicates the formation of quinone due to the action of PPO.