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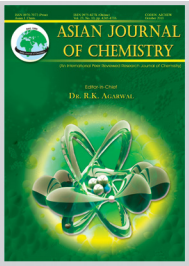

Phytochemical Investigation and Screening of Antibacterial Activity of Extracts from Leaves of *Eupatorium odoratum* Linn.

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Eupatorium odoratum Linn. is found in the tribal area of Koraput district and extensively used traditionally by the tribal people as anthelmintic, antibacterial, antifungal and wound healing agent. The present study is an attempt to preliminary investigation of phytochemical constituents and to explore the antibacterial activity of different extracts of leaves of plant *Eupatorium odoratum* using petroleum ether, ethanol and chloroform as solvents. Various doses of extracts were screened for phytochemical constituent and evaluated for their antibacterial activities on human pathogens like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonela typhi*. Tests for alkaloids and tannins were positive for all extracts. Tests for saponins, proteins, amino acids and anthraquinones glycoside were negative for all extracts. All extracts showed antibacterial activity at 2 mg/mL concentration. The activities are compared with the chloramphenicol. All the doses of petroleum ether, ethanol and chloroform extracts of *Eupatorium odoratum* showed lesser antibacterial activity than the standard drug. When the dose of the extract is increased, a gradual increase in antibacterial activity was observed. The petroleum ether and chloroform extracts exhibited weak antibacterial activity. The ethanol extract showed good inhibitory action against all the tested microorganisms, as compared to the other two extract. The data verified as statistically significant by using one way ANOVA at 5 % level of significance ($p < 0.05$).

Key Words: *Eupatorium odoratum*, Asteraceae, Antibacterial, Chloramphenicol.

INTRODUCTION

During the last two decades, various medicinal plants have been studied for their possible antimicrobial activity to discover new antimicrobial agents capable of resolving problems such as the development of drug resistance in pathogenic microorganisms as well as the side effects of some present antibiotics. Infectious diseases are the second leading cause of deaths worldwide. Treatment of infections continues to be problematic in modern time because of the severe side effects of some drugs and the growing resistance to antimicrobial agents. Hence, search for newer, safer and more potent antimicrobials is a pressing need. Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and the environment[1].

Eupatorium odoratum Linn. (Asteraceae) is also commonly called as Christmas bush (English). The plant is mostly perennial herb or shrub, sometimes climber. Leaves are opposite. There is 15 to 25 tubular florets per head, white, lavender or purple colour, cylindrical glandular often hairy. It is a scrambling shrub. The seeds are brownish gray to black achene, 4 mm long with a pale brown pappus 5 or 6 mm long[2,3].

It is used as a traditional medicine in Indonesia. The young leaves are crushed and the resulting liquid can be used to treat skin wounds. In herbal medicine, the leafy extracts with salt are used as a gargle for sore throats and colds. Extracts of Christmas bush have been shown to inhibit or kill *Neisseria gonorrhoeae* (the organism that causes gonorrhoea) *in vitro*[4]. In the southern part of Nigeria, the leaves are used for wound dressing, skin infections, antimicrobial and to stop bleeding[5]. The literature survey reveals that there are no reports on the antibacterial activity of the leafy extracts of *Eupatorium odoratum*. This prompted us to investigate the antibacterial activity of *Eupatorium odoratum* leaves extracts.

EXPERIMENTAL

All the chemicals were procured from different suppliers. Chloramphenicol (Micro Lab. Ltd., Goa), Petroleum Ether AR (60-80 ºC, Thomas Baker Chemical Pvt. Ltd.), chloroform GR (Loba Chemicals), ethanol AR (Merck Pvt.

Ltd. Mumbai) and dimethyl sulfoxide (DMSO) which was used as a control.

The leaves of *Eupatorium odoratum* Linn. (Family: asteraceae) were collected from local area of Koraput district (India) in the month of June 2008. The plant was identified and authenticated by the Biju Pattnayak Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Odisha (Letter no. MR08/DBT/115, date, 12.06.2008). The leaves were shade dried under normal environmental condition. The dried leaves were powered and stored in a closed container for further use.

Preparation of solvent extracts: For extraction, about 1.5 Kg of powdered leaves were used for each solvent. The solvents are petroleum ether, chloroform and ethanol. For each solvent, 50 cycles were run. Each extract was filtered and concentrated by distilling of the solvent to obtain the crude extract, followed by drying with rotary evaporator. On successive solvent extraction of leaves of *Eupatorium odoratum* resulted in separation of constituents of different polarity range in different solvent extracts. The percentage yields of petroleum ether, chloroform and ethanol were found to be 0.555, 1.052 and 0.855 % respectively.

Phytochemical screening: Chemical tests were carried out for all the extracts for the qualitative determination of phytochemical constituents[6-8]. Total phenolic content was determined using Folin-Ciocalteau reagent[9]. Total phenol value was expressed as mg/g gallic acid equivalent.

Test organisms: Various Gram-positive and Gram-negative bacteria including both standard and clinical isolates were used as test strains. The Gram-positive bacteria like *Staphylococcus aureus* (NCTC 3761) and *Bacillus subtilis* (NCTC 5677) were isolated from patients in Institute of Microbial Technology (IMTECH), Chandigarh, India. The Gram-negative bacteria like *Salmonella typhi* (NCTC 8201) and *Escherichia coli* (NCTC 9001) were laboratory strains, obtained from National Institute of Immunology (NII), New Delhi, India. The organisms were maintained on soybean casein digest agar (SCDA) and transferred onto fresh slants on a regular basis.

Antibacterial activity: The antibacterial activity of the crude extracts was investigated against 4 bacterial strains by the paper disk diffusion technique[10]. Each extract was redissolved in dimethyl sulphoxide to make a 100 mg/mL solution and then filtered. From this solution, 40-µL aliquots were transferred onto blank paper disks (6 mm diameter)[11]. Dried disks were placed onto Mueller Hinton agar medium (Merck) previously inoculated with a bacterial suspension (*ca.* 108 CFU/mL) and incubated at (35 ± 1) ºC for 24 h. Plates were then examined for the presence of growth inhibition zones and diameters were measured, using chloramphenicol (30 µg/mL) used as positive control. A disk loaded with 40 µL DMSO served as the negative control. The experiments were carried out five times and the results are presented as mean ± standard deviation.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of each extract was determined for each micro-organism. The minimum inhibitory concentration of each extract was determined employing different concentrations of the extract by the paper disk diffusion technique. The reconstituted extract was diluted to give concentrations of 100, 150, 200, 250, 300, 350 and 400 µg/mL. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC[11].

Statistical analysis: The data on biological studies were reported as mean ± standard deviation (n = 5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. P values < 0.05 were considered significant[12].

RESULTS AND DISCUSSION

Table-1 shows the phytochemicals detected in *Eupatorium odoratum* leafy extracts. Tests for alkaloids and tannins were positive in all extracts. Tests for saponins, proteins, amino acids and anthraquinone glycosides were negative in all extracts. Triterpenoids, sterols & steroids, flavones & flavonoids are present in petroleum ether and chloroform extracts but absent in ethanolic extracts. Cardiac glycosides are present in ethanolic extracts but absent in petroleum ether and chloroform extracts. Gum mucilage is present in petroleum ether and ethanol extracts but absent in chloroform extracts. The phytochemicals detected in the extracts are playing a major role to possess medicinal properties.

TABLE-1
PHYTOCHEMICALS IN LEAFY EXTRACTS OF *EUPATORIUM ODORATUM*

Phytochemicals	Pet. Ether extract	Chloroform extract	Ethanol extract
Alkaloids	+	+	+
Cardiac glycosides	-	-	+
Anthraquinone glycosides	-	-	-
Gums	+	-	+
Mucilage	+	-	+
Proteins	-	-	-
Amino acids	-	-	-
Tanins	+	+	+
Phenolic compounds	+	+	+
Triterpenoids	+	+	-
Steroids & sterols	+	+	-
Saponins	-	-	-
Flavones & flavonoids	+	+	-

+ = Present and - = Absent

The inhibitory effects of petroleum ether, ethanol and chloroform extracts of *Eupatorium odoratum* against different test organisms are shown in Table-2. The extracts exhibited significant antibacterial activity (growth inhibition zone diameters ranging from 6 to 22 mm) against both Gram-positive and Gram-negative bacteria. The results indicate that the ethanolic extract was more potent than the other two extracts against all the four micro-organism. The order of antibacterial activity was ethanol extract > chloroform extract > pet-ether extract. The activity revealed the concentration dependence nature of the different extracts. Among different organisms, *Bacillus subtilis* is found to be more sensitive to ethanolic and chloroform extracts while *Escherichia coli* is sensitive to petroleum ether extract. The minimum inhibitory concentration for each extract was also determined using the disk diffusion technique and the results are presented in Table-3. It was interesting to note that the *Bacillus subtilis* strain was the most sensitive species to the investigated extract, with the MIC_{90}

TABLE-2
ANTIBACTERIAL ACTIVITY OF LEAVES EXTRACTS OF *EUPATORIUM ODORATUM*

Groups	Treatments	*Bacillus subtilis* (X ± S.D.)	*Staphylococcus aureus* (X ± S.D.)	*Escherichia coli* (X ± S.D.)	*Salmonella typhi* (X ± S.D.)
1	Vehicle (DMSO)	-	-	-	-
2	Chloramphenicol (30 µg/mL)	27.1 ± 0.05	25.08 ± 0.05	22.04 ± 1.12	22.1 ± 0.5
3	PE (2 mg/mL)	7.8 ± 0.17	8.5 ± 0.08	8.9 ± 0.11	6.3 ± 0.11
4	PE (5 mg/mL)	8.2 ± 0.27	9.2 ± 0.11	9.4 ± 0.23	6.9 ± 0.08
5	PE (10 mg/mL)	9.2 ± 0.15	9.7 ± 0.08	10.16 ± 0.41	7.6 ± 0.23
6	CE (2 mg/mL)	9.6 ± 0.08	8.6 ± 0.17	8.5 ± 0.02	7.5 ± 0.08
7	CE (5 mg/mL)	11.1 ± 0.23	9.2 ± 0.08	9.4 ± 1.12	8.5 ± 0.2
8	CE (10 mg/mL)	12.7 ± 0.32	9.9 ± 0.11	9.9 ± 0.51	9.1 ± 0.11
9	EE (2 mg/mL)	14.1 ± 0.63	10.1 ± 0.4	12.2 ± 0.09	8.6 ± 0.11
10	EE (5 mg/mL)	17.4 ± 0.62	13.06 ± 1.11	15.4 ± 0.52	9.1 ± 0.11
11	EE (10 mg/mL)	21.9 ± 1.51	19.12 ± 0.39	20.2 ± 0.43	9.7 ± 0.08
ANOVA data		df	F	P-value	F *crit*
		39	1.1555	0.03401	2.8662

Each data is expressed as zone of inhibition (mm) for 72 h of study. Each value is represented as mean ± standard deviation (n = 5). Standard error mean < 0.872. Data are found to be significant by testing through one-way ANOVA at 5 % level of significance ($p < 0.05$). PE–petroleum ether extracts, CE–chloroform extracts and EE –Ethanol extracts

against these isolates being 100 µg/mL. By employing one-way ANOVA, all data were verified and found to be statistically significant at 5 % level of significant ($p < 0.05$).

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (µg/mL) OF DIFFERENT EXTRACTS OF *EUPATORIUM ODORATUM*

Microorganism	Minimum Inhibitory Concentration (µg/mL)		
	PE	CE	EE
Gram-positive bacteria			
Staphylococcus aureus	200	200	200
Bacillus subtilis	150	100	100
Gram-negative bacteria			
Salmonella typhi	400	200	200
Escherichia coli	250	200	150

PE–Petroleum ether extracts, CE–Chloroform extracts and EE– Ethanol extracts

Conclusion

It is concluded that the ethanolic extract showed most potent antibacterial activity. The results of this study support the use of these plants for human and animal disease therapy and reinforce the importance of the ethno botanical approach as a potential source of bioactive substances. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant, which are responsible for antibacterial activity and to establish the effectiveness and pharmacological rationale for the use of *Eupatorium odoratum* as an antibacterial drug.

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