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P.L. Rajagopal
Professor, Department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

K. Premaletha
Professor and Principal, Department of Pharmaceutics, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

M.K. Preetha
Professor and Principal, College of Nursing, Academy of Medical Sciences, Pariyaram Medical College, Kannur, Kerala.

K.R. Sreejith
Asst.Professor,Department of Pharmaceutical Chemistry, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

VV Madhusoodanan
Senior Assistant in Administration, Kerala State Hospital Complex and Centre for Advanced Medical Services Limited, Pariyaram Medical College Hospital, Kannur, Kerala.

C. Sudhir Nambiar
Executive Director, Paithal Hill Resorts, Paithal Mala, Kannur, Kerala.

Correspondence:
P.L. Rajagopal
Professor, Department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

Antioxidant, antibacterial and antiinflammatory potential of *Eryngium foetidum* (L.)

P.L. Rajagopal, K. Premaletha, M.K. Preetha, K.R. Sreejith, VV Madhusoodanan, C. Sudhir Nambiar

Abstract
Antioxidant potential of the Methanolic extract of the plant were evaluated by DPPH method and found out that the plant possess potent antioxidant properties. Antibacterial properties were tested against eight human pathogens and the drug was found to be having an antibacterial action. The plant also shows significant Anti-inflammatory action by studies conducted by HRBC membrane stabilisation method.

Keywords: *Eryngium foetidum*, Disc diffussion method, DPPH, HRBC membrane stabilisation.

1. Introduction
Eryngium foetidum is a tropical perennial and annual herb in the family Apiaceae. It is native to Mexico and South America, but is cultivated world-wide (Ramcharan C. 1999). It is cultivated as a spice plant in India. It also possess a wide range of ethnomedicinal uses including treatment for burns, earache, fevers, hypertension, constipation, fits, asthma, stomach ache, worms, infertility complications, snake bites, diarrhoea and malaria. These findings suggest the need for further research of this herb and its products (Paul J H *et al*, 2011).

Antioxidants come up frequently in discussions about good health and preventing diseases. These powerful substances, which mostly come from the fresh fruits and vegetables that we eat, prohibits the oxidation of the molecules in the body. The benefits of antioxidants are very important to good health, if free radicals are left unchallenged, they can cause a wide range of illnesses and chronic diseases. Anti-oxidants are a type of compel compounds found in our diet that act as a protective shield for our body against certain diseases such as arterial and cardiac diseases, arthritis and also premature ageing along with several chronic diseases (Science Tech Entrepreneur, 2007).

The evolution of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Infectious diseases represent an important cause of morbidity and mortality among the general population in developing countries. Hence, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years due to the constant emergence of micro-organisms resistant to conventional antimicrobials (Sakagami Y, 2002). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al*, 1999). The major aim of the present study was to evaluate the antimicrobial activity of the leaves of *Eryngium foetidum* for treatment of manifestations caused by micro-organisms. Hence the methanol, Acetone and Ethyl acetate extract of the leaves were tested for their potential activity against microbial pathogens.

Inflammation is a pathophysiological response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma noxious chemical or microbial agents. It is the body response to inactive or destroys the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells (Tripathi K D, 2008).

The most commonly used drug for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers (Bennet P N, 2005). Hence it is necessary to find out an alternative remedy for the situation which in turn leads to the search of medicinally active plants with anti-inflammatory potentials. Hence in the present study an attempt has been carried out to screen the methanolic as well as aqueous extract of the leaves of *Eryngium foetidum* for its anti-inflammatory potential.

2. Materials and Method

2.1 Plant material and Preparation of the extract

The Fresh leaves of *Eryngium foetidum (L)* were collected from Paithalmala, a hill station at Kannur District of Kerala State. The plant material was identified and a voucher specimen bearing number EFL-245 was deposited in the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kerala. The leaves were dried and powdered mechanically and stored in an air tight container. The extraction was carried out by hot percolation method. The solvent used was methanol. The extract was concentrated to dryness under controlled temperature. A preliminary phytochemical screening has also been carried out for the detection of various chemical constituents (Kokate C K, 1999).

2.2 Antioxidant studies

Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) free radical

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine. The ability to scavenge the free radical, DPPH was measured in the absorbance at 517 nm.

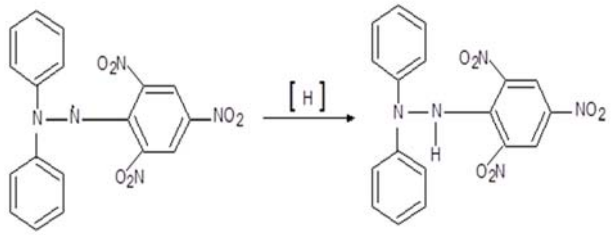

Fig 1: Reduction of DPPH free radical.

To the 1ml of various concentrations of methanolic extract in a test tube, 1ml of solution of DPPH 0.1 mM (0.39 mg in 10ml methanol) was added to the test tube. An equal amount of ethanol and DPPH were added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate (Sreejayan N *et al.*, 1996).

2.3 Antibacterial studies

The dried and powdered leaves were subjected to cold extraction using methanol, ethylacetate and acetone. After 20-25 days the extracts were filtered and used for antibacterial activity. The Bacterial Stains were supplied by the department of Microbiology, Academy of Pharmaceutical Sciences, Pariyaram, Kerala and also from the Microbiology department of Karaveli College of Pharmacy, Mangalore, Karnatalka. Two Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and six Gram negative (*Escherchia coli Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Shigella boydii* and *Vibrio cholera)* bacterial stains were used in the evaluation. The organisms were sub cultured on Muller Hinton Agar medium and incubated at 37 ^{O}C for 24 hours and stored at 4 ^{O}C in the refrigeration to maintain stock culture (Prakash SK, 2006).

2.4 Antibacterial assay

The assay was carried out by using Disc diffusion method. Plates were prepared by using 20ml of sterile Muller Hinton Agar. The tested cultures were applied on top of the soldified media and allowed to dry for 15-20 minutes. The concentrated crude extract was dissolved in Dimethyl sulphoxide. The test was conducted at three different concentration of the crude extract like 50, 75 and 100 micro litter per disc with three replicates. The loading disc were placed on the surface of the medium and left for 30minutes at room temperature for compound diffusion. Zone of inhibition was recorded in millimetres and the experiment was repeated in three replicates (Bauer RK, 1996).

2.5 Antiinflammatory Studies

The leaves were dried under shade and finally powdered. The powder was transferred to soxhlet extractor and subjected to extraction with methanol and Double distilled water. After extraction, the solvents were distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity. (Gandidasan. R, 1991) Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution. The blood was centrifuged at 4000 rpm and packed cells were washed with isosaline and a 10% v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration, 1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacin was used as the reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated by using the formula;

%Protection = 100 – (OD sample /OD Control) x 100

(Where OD is Optical Density)

3. Results and Discussion

3.1 Phytochemical screening: Phytochemical screening of the leaves indicated the presence of flavonoids, polyphenolic compounds, saponin, Phytosteroids and triterpenoids in the methanolic fraction.

3.2 Antioxidant Studies

DPPH is a potent scavenger for any other radicals, due to the easiness in following the procedure; violet colour of DPPH faints into the yellow colour of its reduced congener, with a high shift in the visible spectra (Ionita P, 2005). The phytochemical screening revealed mainly the presence of flavonoids and polyphenolic compounds. The flavonoids are well documented to have potent antioxidant and free radical scavenging activity (Raj Narayanan *et al*, 2001) In addition to this polyphenolic compounds are proven good natural antioxidant (Rao M, 1999). The methanoic extract of the leaves have shown a remarkable antioxidant effect may be due to the presence of the above said active principles.

Table 1: Effect of alcoholic extract of *Eryngium foetidum* leaves on DPPH scavenging

Sl.No.	Conc.in µg/ml	Methanolic extract		Ascorbic acid (Standard)	
		Absorbance	% Scavenging.	Absorbance	% Scavenging.
1	5	0.776	2.67	0.715	8.45
2	10	0.709	9.65	0.619	29.77
3	20	0.699	15.67	0.533	44.67
4	40	0.539	34.66	0.079	59.22
5	80	0.405	51.16	0.067	79.14
6	100	0.319	71.44	0.053	88.65
7	200	0.217	89.33	0.044	90.88
8	400	0.109	91.44	0.032	92.93
9	800	0.101	93.32	0.022	99.75
10	Control	0.917		0.899	

3.3 Antibacterial Studies

The leaf extract of the plant inhibited *Pseudomonas aeruginosa* and *Staphylococcus aureus* at concentrations 75 and 100 micrograms per mille litre respectively. The methanol extract was effective against organism *Staphylococcus aureus.* In the present study ethylacetate, acetone and methanol extracts were showed significant zone of inhibition against *Enterococcus faecalis, Salmonella paratyphi* and *Shigella boydii* but other gram negative bacteria were less inhibited. Results are also showing that the crude drug extract possess a remarkable antibacterial action against the gram positive bacteria. Results also reveal the fact that the extracts at 75 litre per disc dose were more potent in their antibacterial activity.

Table 2: Antibacterial activity of *Eryngium foetidum* leaves

Microorganisms	Control	Ethylacetate			Methanol			Acetone		
		A	B	C	A	B	C	A	B	C
Salmonella typhii	---------	-	20	-	-	21	20	-	21	21
Salmonella paratyphi	---------	17	18	18	18	23	22	19	20	21
Escherichia coli	---------	14	15	17	19	16	20	21	22	22
Vibrio cholera	---------	16	23	21	22	21	27	19	22	23
Pseudomonas aeruginosa	---------	16	17	18	20	20	19	12	18	19
Staphylococcus aureus	---------	20	22	24	23	22	24	22	22	25
Enterococcus faecalis	---------	19	21	22	22	22	25	19	19	29
Shigella boydi	---------	12	12	20	13	14	16	12	13	20

*Zone of inhibition is expressed in mm/diameter

*A, B, C=50, 75, 100 microlitre respectively

3.4 Anti-inflammatory Studies

The erythrocyte membrane is an analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes; hence HRBC membrane stabilisation method was selected for the *in vitro* evaluation of anti-inflammatory property. The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale *et al.,* 2008). The major mechanism of action of non-steroidal drugs are by inhibiting these lysosomal enzymes or by means of stabilizing the lysosomal membrane. (Rajendran Vadivu, 2008). It was observed from the table 3 that the methanolic extract shows significant anti-inflammatory activity at the concentration of 400 micro gram/ml which is comparable to the standard drug, indomethacin. The anti-inflammatory activity of the alcoholic and aqueous extracts were concentration dependent, with the increasing concentration the activity was also increased. The alcoholic extract of the leaves of *Eryngium foetidum* has significant anti-inflammatory activity in comparison to the aqueous extract of the leaves of the same plant. The methanolic extract of the leaves exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. Further the compound isolation, purification and characterization of the

constituent which is responsible for this anti-inflammatory activity, has to be evaluated for the usage of *Eryngium foetidum* as an anti-inflammatory agent.

Table 3: Anti-inflammatory activity of *Eryngium foetidum* leaves

SL. No.	Concentration mg/ml	Anti-inflammatory activity		
		Methanolic Extract	Aqueous extract	Indomethacin (Standard Drug)
1	Control	----------	----------	----------
2	100	69.6± 0.05	66.1 ± 0.09	89.22± 0.09
3	250	74.5 ± 0.07	70.7 ± 0.07	90.44 ± 0.06
4	300	81.9 ± 0.04	77.4 ± 0.05	99.25 ± 0.08
5	350	99.4 ± 0.05	79.7 ± 0.09	104.29 ± 0.06
6	400	111.4± 0.08	83.4 ± 0.01	119.20± 0.05

* Values are expressed as SEM of 3 readings

4. Conclusion

The leaves of *Eryngium foetidum* where subjected to preliminary phytochemical screening, and which revealed the presence of various chemical principles that have got various role as an antibacterial, anthelmintic and anti-inflammatory agents. Further clarifications on the identification and characterisation of these principles in the in vivo analysis have to be evaluated.

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