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Original Article

In Vitro Antioxidant and Anti-Proliferative Activities in *Eryngium Foetidum* L

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ABSTRACT

The present communication is aimed at exploring the *in vitro* antioxidant and antiproliferative activities using *Eryngium foetidum* L. leaves. The essential oil, saponin and methanolic extracts from the leaves of the plant were investigated. The antioxidant potential of the leaf was determined using DPPH assay. The leaf samples showed high antioxidant potential as evidenced by their high IC_{50} values against standard Ascorbic acid. The cytotoxic efficacy of the leaf was elucidated by MTT assay against HeLa, A-549 and PC-3 cell lines. The cytotoxic effect of the essential oil against the cell lines tested were significantly different at $p<0.05$ levels and the highest CTC_{50} value was recorded against PC-3 cell lines (42.5 µg/ml), whereas the saponin and the methanolic extract failed to evoke significant antiproliferative response. The reporting of *in vitro* antioxidant and cytotoxic activities in the leaf essential oil and saponin are maiden attempts.

Key words: *Eryngium foetidum*, essential oil, saponin, methonolic extract, PC-3 cell line, MTT assay, DPPH assay.

1. INTRODUCTION

India being one of the richest repositories of biodiversity includes nearly 7000 species of medicinal herbs with explicit and wide usage in traditional medicine for over centuries [1]. These plants form the origin and source of drugs to more than 80% of the population to sustain their essential primary health care [2]. However records on ancient healing does not account for standardized studies of the medicinal plants in terms of chemo-pharmacology and toxicology.

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Hence the utility of such medicinal herbs becomes limited in modern medicine unless specific protocols are validated for isolation, characterization and elucidation of bioactivity in them. It is therefore imperative to conduct a thorough research to understand the underlying bioactive principles in these herbs which could serve as valuable aid in drug designing and pharmacological kinetics.

Keeping in view of the above aspects the present investigation has been directed to elucidate the bioactivities in *Eryngium foetidum* L. The plant is an aromatic and medicinal plant belonging to the family Apiaceae. It is commonly known by the name culantro or wild coriander and is a popular condiment widely used in continental cuisine like soups, salads, sauces, salsa, sofrito, noodles and curries among the ethnic populations [3]. The plant contains aldehyde rich essential oils valuable to perfumery industry [4, 5]. The essential oil of the plant is also an essential ingredient of drug formulation to treat arthritis and skin diseases (NEIST, Imphal and CSIR). The scientific validations for the medicinal attributes of the plant have been obtained for its analgesic and anti-inflammatory [6, 7] anti diabetic [8], antioxidant [9] and anticlastogenic activities [10]. The anti-helminthic property of the plant (Eryngial) against parasitic trypanosome *Strongyloides stercoralis* has been patented [11] .The plant is being used in formulation for skin whitening under Japanese patent [12].

It has been recorded that the triterpenoid saponins from the plants exhibit antioxidant and anticarcinogenic activity due to their complex structure and chemical heterogeneity [13, 14]. The same is true of essential oils which are known to possess high levels of free radical scavenging activity preventing oxidative damage and inflammation owing to their mosaic combination of terpene constituents. Since reactive oxygen species activate signal pathways contributing to tumor development, the essential oils are considered to be active against cancer cells [15]. Therefore, during the present investigation, a pioneer study has been carried out to extract the essential oil and triterpenoid saponin from the leaves of *E. foetidum* and to subsequently test their potential for the *in vitro* antioxidant and anti-proliferative activities. The above tests were also carried out using the methanolic extract of the leaf in order to obtain a comparative profile (bioactivity of the individual bioactive components and the total plant extract) and to determine the synergistic bioactive effects of the plant.

2. MATERIAL AND METHODS

Collection of plants: The plants of *E.foetidum* were collected from Hassan, Karnataka in the months June and July, identified and preserved at Regional Research Institute, Bangalore.

Methanolic extract: The leaves of *E. foetidum* were excised, shade dried and coarsely powdered. 20g of dried plant material was extracted with 250 ml of methanol with continuous agitation at room temperature for 48 h, filtered and centrifuged at 5000g for 15 min. The supernatant collected was evaporated to obtain the methanolic extract which was stored in air tight bottles at 4^0C till further use.

Leaf essential oil: The leaves were shade dried and hydrodistilled in Clavenger-type apparatus for 4 h. The essential oil was collected and dried over anhydrous Na_2SO_4 and stored in refrigerator until further use.

Saponin: About 20g of coarsely powdered leaves of *E. foetidum* were used for the extraction of saponin according to the procedure elaborated by Obadani and Ochuko, 2001.[16]

The saponin thus obtained was loaded on a silica gel column (mesh size 60-120) and eluted with chloroform followed by chloroform: methanol with increasing polarity. The fractions were collected at regular intervals and subjected to TLC on 0.25 mm Silica gel

60 F254(Merck) precoated aluminium plates. The chloroform:methanol:water (7:2:1) was used as developing solvent system and detection was carried out using Vanillin reagent (15g vanillin in 250 ml ethanol+2.5 ml con. Sulfuric acid) or p-anisaldehyde reagent (0.5ml p-anisaldehyde in 50 ml glacial acetic acid+1 ml con. Sulfuric acid). Fractions with similar Rf values were pooled, concentrated and used for bioactivity.

DPPH radical scavenging assay: The ability of the samples (the leaf essential oil, methanolic extract and saponin) to scavenge synthetic free radical 1, 1-diphenyl 2-picryldihydrazyl (DPPH) was determined by the method described by Bondet *et al*, 1997 [17] and Hsu *et al*, 2007 [18] .Different concentrations of the leaf essential oil, methanolic extract and saponin (10-50µg/ml) in DMSO were added to 5 ml of methanolic DPPH (0.1mM) solution. The mixture was shaken vigorously and incubated in the dark at room temperature for 20 minutes and the absorbance read at 517nm against a blank (methanol). Ascorbic acid (1mg/ml) was used as the reference standard (positive control). The radical scavenging activity of the samples was determined from a calibration curve. The tests were run in triplicates and averaged. The DPPH radical scavenging activity was calculated as follows:

DPPH Radical-scavenging activity = $[(A_{control} - A_{test}) / A_{control}] \times 100$

Where, $A_{control}$ and A_{test} are the absorbance of the control (methanolic DPPH) and the sample (methanolic DPPH + samples) respectively.

The concentration of the samples at which 50% inhibition (IC_{50}) occurred was calculated from the graph.

All the results obtained were expressed as means ± SD. Analysis of variance (ANOVA) was used to determine the significant differences for multiple comparisons which was completed using the Tukey Honestly Significant Different (HSD) test at $\alpha = 0.05$. All of these were carried out using MATLAB 7.5.0 (R2007b).

MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay

Cell lines and Culture medium: HeLa (Cervix carcinoma), A-549 (Lung carcinoma) and PC-3 (prostrate carcinoma) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. The stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until 75-80% confluence was achieved. TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) was used to dissociate the cells. The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates.

Preparation of samples: The samples of *E. foetidum* viz. methanolic extract, leaf essential oil and crude saponin were dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

MTT assay: After trypsinization of the monolayer cell culture, the cell count was adjusted to 1.0 x 10^5 cells/ml using Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. 0.1 ml of the diluted cell suspension was seeded to each well of the 96 well microtitre plate. After 24 h incubation, the monolayer was washed with DMEM medium and 100 µl of various concentrations of methanolic extract, leaf essential oil and saponin extract were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2

atmosphere and tracked through microscopic examination carried out at 24 h interval. After 72 h, the extracts and the essential oil in the wells were discarded and 50 µl of MTT (5mg/ml) in Phosphate Buffer Solution (PBS) was added to each well. The plates were gently shaken and incubated for 3 h at 37^{o} C in 5% CO_2 atmosphere. The supernatant was removed; followed by the addition of 100 µl of propanol and subsequent gentle agitation to solubilize the formazan formed. The absorbance was read using a microplate reader at a wavelength of 540 nm (Francis and Rita, 1986).The percentage growth inhibition was determined using the formula:

% growth inhibition= 100- (mean OD of sample/mean OD of control) X 100

All the assays were done in triplicates and expressed as means ± SD. Analysis of variance (ANOVA) was used to determine the significant differences for multiple comparisons which was completed using the Tukey's Honestly Significant Different (HSD) test at $\alpha = 0.05$ in SPSS version 20.

3. RESULTS AND DISCUSSION

The radical scavenging abilities of the essential oil, methanolic extract and the saponin samples were determined using the DPPH radical scavenging assay. High DPPH radical scavenging potential confers superior antioxidant activity to the samples. The highest percentage of inhibition of DPPH radicals was observed in the methanolic extract and essential oil closely followed by the saponin (Table1). The essential oil, saponin and methanolic extract recorded an IC_{50} of 22.14µg/ml, 23.79µg/ml and 22.00 µg/ml respectively as against an IC_{50} of 19.07µg/ml for the standard ascorbic acid indicating high antioxidant property in all the samples tested (Fig.1). All samples showed significantly different ($p<0.05$) antioxidant activity for the assay.

The essential oil, methanolic extract and saponin demonstrated varying levels of dose dependent cytotoxicities against HeLa, A549 and PC3 cell lines in MTT antiproliferative assay. The comparative levels of cytotoxicities of the extracts against the various cell lines have been represented in Fig.2. The cytotoxic effects of the essential oil against the cell lines were significantly different at $p<0.05$ levels, the highest CTC_{50} values recorded against PC3 cell lines (42.5 µg/ml) (Table 2). The saponin evoked least response for Hela and PC3 cell lines, but a moderate response was observed against the A549 cell lines at higher dosages. The methanolic extract generated a low cytotoxic response for Hela and PC3 cells but a significant inhibitory effect against A549 cell lines. Going by the American National Cancer Institute, the LC_{50} limit to consider for a crude extract promising for further purification to isolate biologically active (toxic) compounds should be lower than 30µg/ml [19]. It has also been stated that oils and extracts from plants presenting LC_{50} values below 1000 µg/ml are known to contain physiologically active principles [20].

A number of prior reports have indicated a possible significant role of reactive oxygen species being responsible for stimulating growth related responses triggering tumorigenesis and subsequent malignancy [21, 22, 23]. It has been also established that the bioactive principles in plants like phenolics, flavonoids, terpenoids, saponins, ascorbic acid and essential oils are potent inhibitors of reactive oxygen species thereby acting as anti inflammatory, anticarcinogenic and anti proliferative agents [24, 25, 26]. A high phenolic, flavonoid and ascorbic acid content has already been reported in *E.foetidum* [27, 28] and could be the stemming factor for the high antioxidant properties being presently observed in the extracts and leaf essential oil of *E.foetidum*. Earlier investigations have also recorded the antioxidant potential of *E. foetidum* [29, 30, 31] and

species of *Eryngium* viz. *E.creticum* and *E.maritimum* [32, 33] .However the antioxidant properties have been recorded for the first time in the saponin extract and essential oil of *E.foetidum*.

In traditional medicine, the decoction of *E.foetidum* leaves has been documented as an effective remedy against malignant prostate adenoma [34]. This goes in accordance with our study as the essential oil from leaves was found to be highly cytotoxic against the PC-3 cells (CTC_{50} - 42.5µg/ml). However further systematic investigations are obligatory to understand the pharmacology and toxicity of the essential oil of *E.foetidum*. The saponin extract failed to generate adequate response against any of the cell lines thereby instigating intensive standardization of protocols for isolation and its purification. The *in vitro* cytotoxicity studies of methanolic extract, saponin and essential oil of *E.foetidum* against various carcinogenic cell lines have also been a maiden endeavor.

Table 1: DPPH radical scavenging activity of leaf essential oil, saponin and methanolic extract with ascorbic acid (standard).

Samples	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)*
Ascorbic acid	10	14.945	19.07±0.02
	20	57.126	
	30	95.627	
	40	96.572	
	50	97.653	
Leaf essential oil	10	9.845	22.14±0.04
	20	45.812	
	30	90.291	
	40	94.951	
	50	96.674	
Saponin	10	11.162	23.79±0.01
	20	29.095	
	30	91.81	
	40	95.154	
	50	95.863	
Methanolic extract	10	9.845	22.00±0.01
	20	46.826	
	30	91	
	40	94.343	
	50	95.56	

Data are expressed as mean ± SD. *Values statistically differ at P > 0.05 as measured by Tukey HSD test.

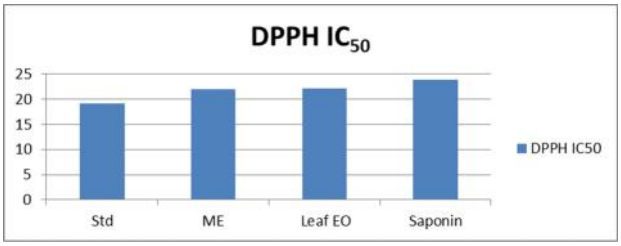

Fig 1: Radical scavenging activity (% inhibition) and IC_{50} values of methanolic extract, leaf essential oil and saponin with ascorbic acid as std in DPPH assay.

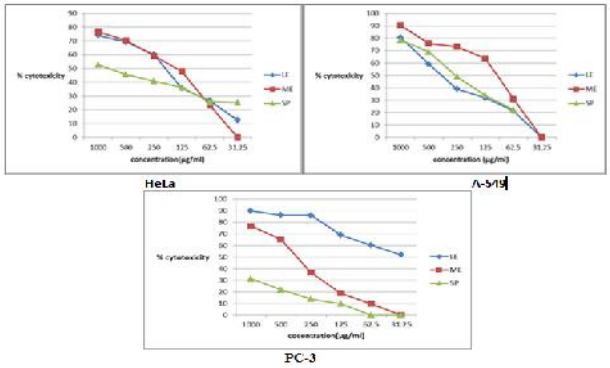

Fig 2: *In vitro* cytotoxicity of leaf essential oil, methanolic extract and saponin against HeLa, A-549 and PC-3 cell lines respectively

[LE- leaf essential oil; ME- Methanolic extract; SP- Saponin]

Table 2: CTC_{50} values of leaf essential oil, methanolic extract and saponin against various cell lines

Sample	CTC_{50} (µg/ml)		
	HeLa	**A-549**	**PC-3**
Leaf essential oil	203.33±1.9[a]	393.33±1.3[b]	42.5±0.08[c]
Methanolic extract	340.00±1.4[a]	103.33±1.9[b]	363.33±1.8[a]
Saponin	810.00±1.2[a]	263.33±1.3[b]	1000±1.6[c]

Data are expressed as mean ± SD. *Values statistically differ at P > 0.05 within the row as measured by Tukey HSD test.

4. CONCLUSION

This work is an attempt to provide strong evidence for the bioassay guided pharmacological activities in *E.foetidum*. The antioxidant potential of extracts and essential oil of *E.foetidum* proves its possible usage in therapeutics as a natural antioxidant. The above study also provides preliminary data for the use of essential oil and the methanolic extract in further confirmatory studies as anti-cancer drugs against respective cell lines.

5. REFERENCES

1. Hill AF. In: Economic Botany: A Text Book of Useful Plants and Plant Products, second ed.,McGraw Hill Book Company, Inc., New York, 1989: 560 pp.
2. FAO, Trade in Medicinal Plants, Economic and Social Department, Food and Agriculture Organization of the United Nations, Rome, Italy, 2004.
3. Chowdhury JU, Nandi NC & Yusuf M. Chemical constituents of essential oil of the leaves of *Erygium foetidum* from Banglades. Bangladesh Journal of Scientific and Industrial Research 2007; 42: 347-352.
4. Wong KC, Feng MC, Sam TW and Tan GL. Composition of the leaf oil of *Eryngium foetidum* L. from Cuba, Journal of Essential Oil Research 1994; 6: 369-374.
5. Pino JA, Rosado A and Fuentes B. Composition of the root and leaf essential oils of *Eryngium foetidum* L. Journal of Essential Oil Research 1997; 9: 467-468.
6. Saenz MT, Fernandez MA, Garcia MD. Anti-inflammatory and analgesic properties from leaves of *Eryngium foetidum* L.(Apiaceae), Phytotherapy Research 1997; 11(5): 380-383.
7. Mekhora C, Muangnoi C, Chingsuwanrote P, Dawilai S, Svasti S, Chasri K, Tuntipopipat S. *Eryngium foetidum* suppresses inflammatory mediators produced by macrophages, Asian Pacific Journal Cancer Prevention 2012; 13: 653-664.
8. Mahabir D and Gulliford MC. Use of medicinal plants for diabetes in Trinidad and Tobago. Pan American Journal of Public Health, 1997; 1(3):174-179.
9. Singh S, Singh DR, Banu S, Salim KM. 2012. Determination of bioactivities and antioxidant activity in *Eryngium foetidum* L.-A traditional culinary and medicinal herb, Proceedings of Natlional Academy of Sciences,India - Sec B: Biol Sci. 2012; 83(3):453-460.
10. Promkum C, Butryee C, Tuntipopipat S, Kupradinun P. Anticlastogenic effect of *Eryngium foetidum* L. assessed by erythrocyte micronucleus assay, Asian Pacific Journal of Cancer Prevention 2012; 13: 3343-334.
11. Forbes WM & Steglich C. 2009. Methods of treating infectious diseases, Patent Application Number-20090047342.
12. Paul JH, Seaforth CE and Tikasingh T. 2011. *Eryngium foetidum* L. –A review. Fitoterapia 2011; 2(3):302-8.
13. Awasare S , Bhujbal S, Rabindrananda. *In vitro* cytotoxic activity of novel oleanane type of triterpenoid saponin from stem bark of *Manilkara zapota* Linn. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(4): 183-188.
14. Han LT, Fang Y, Li MM, Yang HB, and Huang F. 2013. The Antitumor Effects of Triterpenoid Saponins from the Anemone flaccida and the Underlying Mechanism, Evidence-Based Complementary and Alternative Medicine, 2013. doi:10.1155/2013/517931.
15. Bayala B, Imaël HN, Bassole, Scifo R, Gnoula C, Morel L, Lobaccaro JMA, Simpore J· Anticancer activity of essential oils and their chemical components - a review, Am J Cancer Res 2014; 4(6): 591–607.
16. Obadoni BO, Ochuko PO. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, Global Journal of Pure and Applied Sciences 2001; 8: 203-208.
17. Bondet V, Brand-Williams W and Berset C. Kinetics and mechanisms of antioxidant activity

using the DPPH• free radical method. Food Science and Technology 1997; 30: 609-615.

18. Hsu CY, Chan YP, Chang J. Antioxidant activity of extract from *Polygonum cuspidatum*. Biological Research 2007; 40: 13-21.
19. Suffness M, Pezzuto JM. Methods in Plant Biochemistry: Assays for Bioactivity Academic Press, London 1990; 6:71–133.
20. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45:31-34.
21. Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat Res 2001; 477:7–21.
22. Hassan LE, Ahamed MK, Majid ASA, Baharetha HM, MuslimNS, Nassar ZD and Majid AMSA. Correlation of antiangiogenic, antioxidant and cytotoxic activities of some Sudanese medicinal plants with phenolic and flavonoid contents ,BMC Complementary and Alternative Medicine 2014; 14:406.
23. Galati G, O'Brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radic Biol Med 2004; 37(3):287–303.
24. Liang C, Ding Y, Nguyen HT. Oleanane-type triterpenoids from Panax stipuleanatus and their anticancer activities, Bioorganic & Medicinal Chemistry Letters 2010; 20(23): 7110–7115.
25. Thara Saraswathi KJ, Chandrika R, Jayalakshmi NR and Mythri B. Ecotypic variations in Indian populations of *Eryngium foetidum* L. International Journal of Current Research and Review 2014; 6(4):1-6.
26. Aswathy PM and Saj OP. Carminative, phytochemical and antioxidant potentialities of the leaf extracts of *Eryngium foetidum L.* (Apiaceae), World journal of pharmacy and pharmaceutical sciences 2014; 3(6): 2269-2280.
27. Farhan H, Malli F, Rammal H, Hijazi A, Bassal A, Ajouz N and Badran B. Phytochemical screening and antioxidant activity of Lebanese *Eryngium creticum* L.Asian Pacific Journal of Tropical Biomedicin 2012; S1217-S1220.
28. Meot-Duros L, Floch GL and Magné C. Radical scavenging, antioxidant and antimicrobial activities of halophytic species, Journal of Ethnopharmacology 2008; 116(2): 258-262.
29. Emmanuel N. Ethno medicines used for treatment of prostatic disease in Foumban, Cameroon, African Journal of Pharmacy and Pharmacology, 2010; 4(11): 793-805.
30. Francis D and Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods 1986; 89: 271-277.
31. Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis:NADPH oxidase as target for cancer therapy. Cancer Lett, 2008; 266(1):37–52.

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