Research articles

In vitro antioxidant and antidiabetic properties of *Eryngium foetidum* Linn.

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ABSTRACT

Introduction and Aim: *Eryngium foetidum* Linn. belongs to the family Apiaceae, is an aromatic herbaceous plant with culinary importance, that is used worldwide as substitute for coriander. The aim of the present study was to extract the phytochemicals of *E. foetidum* leaves and evaluate the *in vitro* antioxidant and antidiabetic properties.

Materials and Methods: *E. foetidum* plants were collected, shade dried, coarsely powdered and defatted. The defatted material was subjected to Soxhlet extraction using methanol as solvent. The extract was subjected for phytochemical screening by qualitative analyses by UV-vis spectroscopy and TLC and RP-HPLC. Quantitative analyses of phenolics, flavonoids and saponins was carried out. The extract was evaluated for antioxidant activity by DPPH and ABTS radicals scavenging assays, and for antidiabetic activity by performing α-amylase inhibition and α-glucosidase inhibition assays.

Results: Results of the investigations suggest that *E. foetidum* leaves are rich source of phytochemicals like flavonoids and saponins. The phytochemicals present in the methanolic extract of *E. foetidum* leaves possess antioxidant and antidiabetic properties.

Conclusion: The present study reveals that *E. foetidum* leaves possess therapeutically important phytochemicals that could be further exploited as nutraceuticals considering the culinary importance of the plant.

Keywords: Phytochemicals; *Eryngium foetidum* Linn.; flavonoids; saponins; antioxidant activity; antidiabetic activity.

INTRODUCTION

Eryngium foetidum Linn., is an aromatic biennial herb, belonging to Apiaceae family, native to Central America and Mexico, introduced or cultivated in both tropics (1). It is widely known as Culantro or wild coriander, Mexican coriander or long coriander. It is a popular condiment widely used in continental cuisine as food flavoring and seasoning herb like soups, salads, sauces, salsa, noodles and curries among the ethnic populations (2, 3). *E. foetidum* grows wild and also now a days cultivated in all parts of the world for its culinary purposes. In Karnataka, *E. foetidum* grows in Hassan and Coorg Districts of the state as naturalized weed. It's common vernacular name in Kannada - Kadu Sambaru, in Hindi - Ban Dhaniya.

The plant *E. foetidum* is used as an ethno-medicinal plant for the treatment of various ailments. It is been used in the treatment of fever, ear pains, diarrhea, hypertension, oedema, sinusitis, snake or scorpion bite, and decoction prepared from leaves of *E. foetidum* is used in traditional medicine as a vulnerary, and hypotensive and for digestive troubles (2,4,5). The crushed leaves are placed in the ear to treat pain, and are used for the local treatment of arthritic processes (6). It has showed the topical anti-inflammatory activity and myeloperoxidase activity (7) and antimalarial and antibacterial activity (2, 3 and 8). Aerial parts have been reported to show anthelmintic activity and anti-convulsant activity (3, 4). Methanolic extract of irradiated *E. foetidum* fresh plantlets yielded phenolic compounds - coumarin, caffeic acid, p-coumaric acid, salicylic acid, benzoic acid, and apigenin (9). Commonly the plant extract is used for anti-inflammatory activity (10) and anti-microbial activity (3). The earlier studies focused on the extraction of leaf and other parts of the plant using solvents of different polarity (11). In view of the culinary and pharmacological properties of the present study has been carried out for the evaluation of antioxidant and antidiabetic properties of *E. foetidum* leaf extract that contains flavonoids and saponins under *in vitro* conditions.

MATERIALS AND METHODS

Collection of plant materials

E. foetidum plants were collected from different parts of Hassan and Coorg Districts of Karnataka state during the monsoon and post-monsoon seasons, identified and authenticated with the help of Flora of Karnataka (http://florakarnataka.ces.iisc.ac.in/).

Chemicals and Reagents

Chemicals and reagents were procured from either Sigma-Aldrich (USA) or HIMEDIA (Mumbai, India)

or Sisco Research Laboratories (Mumbai, India) and were of either analytical grade. Millipore (Milli-Q) water was used throughout all the investigations.

Preparation of leaf methanolic extract

Leaves of *E. foetidum* were excised, shade dried and coarsely powdered, defatted using n-hexane. The defatted material was subjected to Soxhlet extraction using methanol as solvent. The extract was concentrated using Rotary evaporator (Medica Instruments, Mumbai) and dried in the oven at 40°C. A greasy, viscous material, semi-solid in nature and aromatic in odor, soluble in methanol was obtained, stored in the air tight bottles at 4°C till further use.

Preliminary phytochemical screening

Leaf methanolic extract of *E. foetidum* was subjected for the preliminary phytochemical screening tests to find the presence of the active chemical constituents using standard procedures (11, 12).

Thin layer chromatography (TLC) analysis

Leaf methanolic extract of *E. foetidum* was subjected for thin layer chromatography using silica gel G and mobile phase. For flavonoids, methanol: ethyl acetate: chloroform (1:1:8) was used as solvent and detected under UV-light exposure at 25-260nm. For saponins chloroform: ethanol: water (60: 40: 5 v/v) was used as solvent and 5% vanillin-sulphuric acid was used as spraying reagent.

UV-visible spectroscopy analysis

Leaf methanolic extract of *E. foetidum* was subjected for UV-Visible spectroscopy analysis (13), scanned through 200 to 800 nm wavelength range using UV-Vis spectrophotometer (Systronics, India). The characteristics peaks were detected. The peak values of the UV-Vis analysis were recorded.

RP-HPLC analysis

Leaf methanolic extract of *E. foetidum* was subjected for reverse phase high performance liquid chromatography (RP-HPLC) analysis using Agilent 1260 system equipped with quaternary pump and diode array detector (DAD). Separation was carried out in a Zorbax SB-C18 reverse phase column (5 mm, 4.6×250 mm), methanol: water (35:65) used as mobile phase, flow rate was monitored at 1 ml/min, column temperature was maintained at 35° C, and analytes in the effluent were detected at 330 nm for flavonoids and at 210 nm for saponins.

Determination of total phenolics and flavonoids

Total phenolic content of the extract was determined using Folin–Ciocalteu reagent, absorbance was measured at 670 nm, and a calibration curve was generated using the gallic acid standard. Total phenolic content was expressed as gallic acid equivalent (GAE) mg/g tissue extract (14). Total flavonoids content was determined by the aluminum chloride colorimetric method, absorbance was measured at 510 nm, and a calibration curve was generated by using the catechin standard (15). Total flavonoid content was expressed as catechin equivalents (CE mg/g tissue extract).

Determination of total saponin content

Total saponin content of the extract was determined using vanillin-sulphuric acid reagent method (16) *Quillaza* bark saponin was used as standard, absorbance was measured at 540 nm using UV-Visible spectrophotometer (Systronics, India). Total saponin content was expressed as *Quillaza* bark saponin equivalent (QSE) mg/g tissue extract.

DPPH radical scavenging assay

Leaf extract of *E. foetidum* containing saponins was subjected for 1, 1-diphenyl-2 picryl hydrazyl (DPPH) radical scavenging assay (17). Gallic acid was used as the positive control. The per cent (%) inhibition of absorbance (scavenging activity) was calculated using the following formula,

$$\% \text{ scavenging activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

ABTS radical inhibition assay

Leaf extract of *E. foetidum* containing saponins was subjected for 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay (18). Gallic acid was used as the positive control. The per cent (%) inhibition of absorbance (scavenging activity) was calculated using the following formula,

$$\% \text{ scavenging activity} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

α-amylase ((EC 3.2.1.1) inhibition assay

Leaf extract of *E. foetidum* containing saponins was subjected for α-amylase inhibition assay (19) using pancreatic α-amylase (EC 3.2.1.1) and 2-chloro-4-nitrophenol-α-D-maltotrioside (CNP-G3) as substrate. The amount of product 2-chloro-4-nitrophenol formed was measured at 405 nm using a microplate reader (BioRad, Germany). Acarbose was used as positive control. Inhibition per cent (%) of α

amylase activity was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

The concentration of the extract required to inhibit 50% of α-amylase activity under the assay conditions was defined as the IC_{50} value.

α-glucosidase (EC 3.2.1.20) inhibition assay

Leaf methanolic extract of *E. foetidum* containing saponins was subjected for α- glucosidase (EC 3.2.1.20) inhibition assay using *Saccharomyces cerevisiae* α-glucosidase (Merck-Millipore formerly Sigma-Aldrich) and p-nitrophenyl-α-D-glucopyrano side as substrate (20). The amount of product p-nitrophenol released was measured at 405 nm using a microplate reader (BioRad, Germany). Acarbose was used as positive control.

Inhibition per cent (%) of α-glucosidase activity was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

RESULTS

The results of qualitative analysis for phytochemicals revealed the presence of active principles like phenolic compounds, flavonoids, tannins, alkaloids, saponins and steroids in leaf methanolic extract of *E. foetidum*. Methanol that has the high polarity was found to extract the maximum active principles. The dry weight of extract was found to be 1.1197 g. Results of the UV-visible spectroscopy and chromatography analyses (TLC and RP-HPLC) confirm the presence of both flavonoids and saponins in the leaf methanolic extract of *E. foetidum*. Results of UV-visible spectroscopy analysis showed the absorption peaks at 200 – 230 nm and 270-330 nm (Fig. 1). Thin layer chromatography (TLC) analysis showed the presence of flavonoids and saponins (Fig. 2 a & b), this was also confirmed further by reverse-phase HPLC analysis confirmed the presence of both flavonoids (330 nm) and saponins in the leaf extract of *E. foetidum* (Fig. 3 a & b).

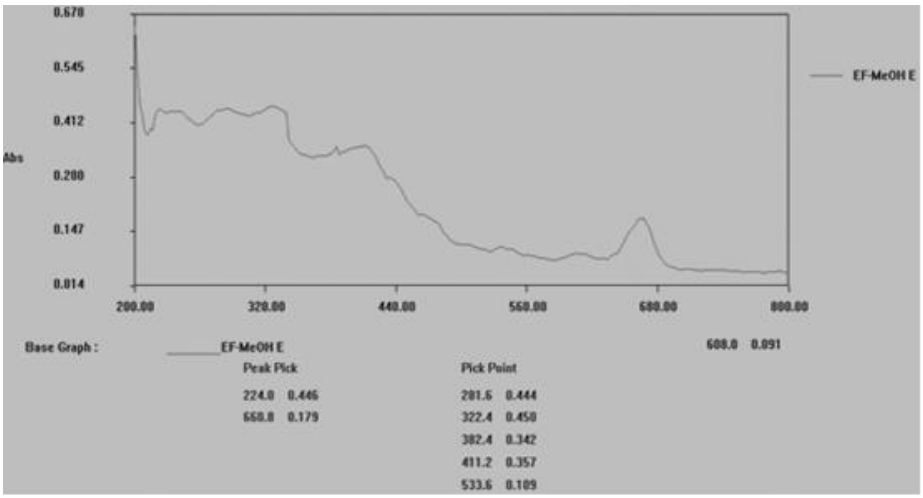

Fig. 1: UV-visible spectrum of *E. foetidum* leaf extract scanned through 200 nm to 800 nm

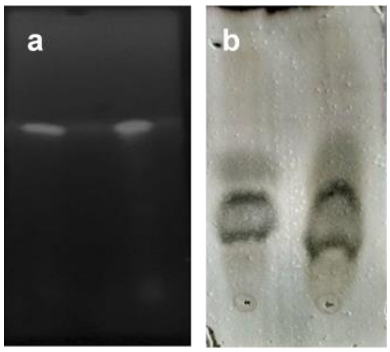

Fig. 2: TLC separation of *E. foetidum* leaf extract (a) Flavonoids (b) Saponins

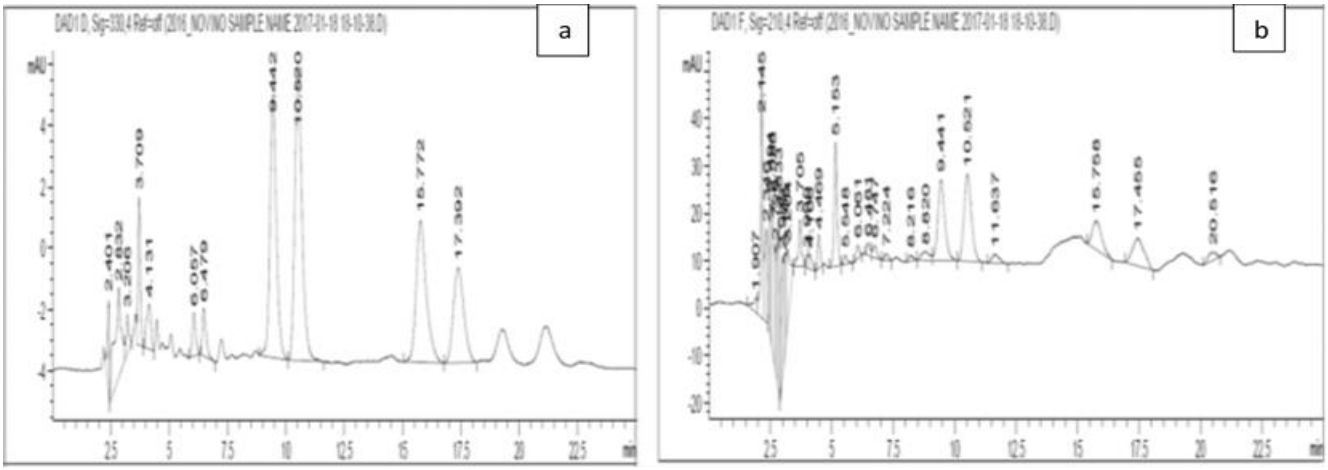

Fig. 3: RP-HPLC analysis of *E. foetidum* leaf extract
(a) for flavonoids at 330 nm; (b) for saponins at 210 nm.

Results of the quantitative analyses suggest that, *E. foetidum* leaf extract is a rich source of polyphenols, flavonoids and saponins (Table 1).

Table 1: Total phenolics, flavonoids and saponin content in *E. foetidum* leaf extract

Phytochemical in the leaf extract	Quantity determined
Total phenolics	378 mg GAE/g
Total flavonoids	196 mg GAE/g
Total saponins	292 mg QSE/g

Total phenolic content of extract was 378 mg GAE/g**,** total flavonoid content was 196 mg GAE/g, and the total saponin content of was 292 mg QSE/g leaf extract was the concentration of total saponin (Table 1).

DPPH radical scavenging activity of E. foetidum leaf extract

The free radical scavenging activity of *E. foetidum* leaf extract was evaluated (5-500 µg/ml) by DPPH radical scavenging assay, and results are depicted in Table 2. DPPH radical scavenging activity of *E. foetidum* leaf extract was observed to be increased with increasing concentration and showed 94.2% inhibition at 500 µg/ml. The $_{IC50}$ value for *E. foetidum* leaf extract was determined as 31.45 µg/ml, while positive control gallic acid showed 97.38% inhibition at 5 µg/ml and its IC_{50} value was found to be 1.14 µg/ml (Table 2).

Table 2: DPPH inhibitory activity of different concentrations of *E. foetidum* leaf extract

Test sample	Concentration tested (µg/ml)	% inhibition	IC_{50} (µg/ml)
***E. foetidum* leaf extract containing flavonoids**	5	32.8±0.16	31.45
	25	46.6±1.62	
	50	68.8±2.18	
	100	89.6±2.42	
	500	94.2±1.96	
Gallic acid	0.5	28.42±1.42	1.14
	1.5	62.82±3.08	
	2.5	83.46±3.44	
	5	97.38±2.72	

ABTS radical scavenging activity of E. foetidum leaf extract

The free radical scavenging activity of *E. foetidum* leaf extract was evaluated (5-500 µg/ml) by ABTS radical scavenging assay, and results are depicted in Table 3. The free radical scavenging activity of *E. foetidum* leaf extract was evaluated (5-500 µg/ml) by ABTS radical scavenging assay, and results are depicted in Table 3. ABTS radical scavenging activity of *E. foetidum* leaf extract was observed to be increased with increasing concentration and showed 90.44% inhibition at 500 µg/ml. The $_{IC50}$ value for *E. foetidum* leaf extract was determined as 40.82 µg/ml, while positive control gallic acid showed 84.42% inhibition at 5 µg/ml and its IC_{50} value was found to be 1.32 µg/ml (Table 3).

Table 3: ABTS radical inhibitory activity of *E. foetidum* leaf extract

Test sample	Concentration tested (μg/ml)	% inhibition	IC_{50} (μg/ml)
***E. foetidum* leaf extract containing flavonoids**	5	22.62±1.72	**40.82**
	25	40.32±1.88	
	50	62.16±2.24	
	100	79.82±2.18	
	500	90.44±2.96	
Gallic acid	0.5	22.38±2.14	**1.32**
	1.0	38.16±1.72	
	1.5	56.54±2.16	
	2.0	68.88±2.76	
	2.5	84.42±2.98	

Effect of E. foetidum leaf extract on α-amylase activity

Leaf extract *E. foetidum* (5-500 μg/ml) was evaluated for α-amylase inhibitory activity and, the results could be extrapolated as the antidiabetic property/potential. The percentage of inhibition displayed by *E. foetidum* leaf extract at different concentrations are depicted in Table 4. *E. foetidum* leaf extract was found to have inhibitory effects on α-amylase in a dose dependent manner with maximum 78.62% inhibition at 500 μg/ml, and the IC_{50} value was 88.64 μg/ml. While the positive control acarbose (1 μg/ml) has shown significant inhibition of enzyme activity (92.44%), with IC_{50} value 0.48 μg/ml (Table 4).

Table 4: α-amylase inhibitory activity of *E. foetidum* leaf extract

Test sample	Concentration tested (μg/ml)	% inhibition	IC_{50} (μg/ml)
***E. foetidum* leaf extract containing flavonoids**	5	10.28±1.24	**88.64**
	25	24.32±1.38	
	50	35.46±1.68	
	100	57.82±2.12	
	500	78.62±1.98	
Acarbose	0.1	22.64±2.28	**0.48**
	0.5	52.86±2.72	
	1.0	92.44±3.46	

Effect of E. foetidum leaf extract on α-glucosidase activity

Leaf extract *E. foetidum* (5-500 μg/ml) was evaluated for α-glucosidase inhibitory activity and, the results could be extrapolated as the antidiabetic property/potential. The percentage of inhibition displayed by *E. foetidum* leaf extract at different concentrations are depicted in Table 5. *E. foetidum* leaf extract was found to have inhibitory effects on α-glucosidase in a dose dependent manner with maximum 62.82% inhibition at 500 μg/ml, and the IC_{50} value of 312.4 μg/ml. While the positive control acarbose (2 μg/ml) has shown significant inhibition of enzyme activity (88.14%), and IC_{50} value 0.82 μg/ml (Table 5).

Table 5: α-glucosidase inhibitory activity of *E. foetidum* leaf extract

Test sample	Concentration tested(μg/ml)	% inhibition	IC_{50} (μg/ml)
***E. foetidum* leaf extract containing flavonoids**	5	10.28±1.22	**312.4**
	25	16.12±1.64	
	50	22.34±1.82	
	100	32.42±2.16	
	500	62.82±2.88	
Acarbose	0.5	42.62±2.28	**0.82**
	1	58.28±1.92	
	2	88.14±2.16	

DISCUSSION

Results of the present study suggest that the phytochemicals of *Eryngium foetidum* L. possess antioxidant and antidiabetic properties. The findings for the present study are in agreement with the earlier studies (21). Distress during the day-today life results in the increased generation of free radicals of both oxygen (ROS) and nitrogen (RNS), which may exhaust antioxidant defenses thus leading to the oxidative stress leading to oxidative damage of vital biochemicals (22). One of the strategies for alleviating the oxidative damage is the usage of natural antioxidants. During the present study it has been found that, *E. foetidum* phytochemical fractions present in the leaf extract possess significant antioxidant activity as confirmed by both DPPH and ABTS radicals savaging activities comparing with positive control gallic acid (Tables 2 & 3). The antioxidant potential of *E. foetidum* leaf extract could be attributed to the presence of flavonoids.

The best therapeutic approach that has been suggested for diabetes is to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes in the digestive organs (23). During the present study, *E. foetidum* leaf extract showed significant inhibition of both α-amylase and α-glucosidase activities as compared to positive control acarbose (Tables 4 & 5). Slowing of carbohydrate absorption is associated with improved glycemic control, and so α-glucosidase inhibitors (AGIs) have been developed to delay intestinal absorption of carbohydrates (24). Results of the present study suggest that, *E. foetidum* leaf extract inhibits both α-amylase and α-glucosidase activities in a dose dependent manner at a concentration of 500 µg/ml (Table 4 & 5), From the results of the present study it could be concluded that, *E. foetidum* leaf extract may interfere with transit, digestion or absorption of carbohydrates like starch, sucrose and maltose, and may delay carbohydrate digestion and causing decreased rate of glucose absorption into blood stream. The α-amylase and α-glucosidase inhibition activity by *E. foetidum* leaf extract could be attributed to the presence of inhibitory substances like saponins in the extract for both the enzymes. Similar findings of *in vitro* antioxidant and antidiabetic potential of phytochemical extracts of other medicinal plants were also reported (25).

CONCLUSION

Leaves of *Eryngium foetidum* Linn. are the rich source of valuable phytochemicals such as flavonoids and saponins with antioxidant and antidiabetic properties. Further studies are warranted for the isolation, purification and characterization and also to explore the therapeutic potentials of these flavonoids and saponins of *Eryngium foetidum* as nutraceuticals.

CONFLICT OF INTEREST: None

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