International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 01-04-2019; Accepted: 03-05-2019
www.botanyjournals.com
Volume 4; Issue 4; July 2019; Page No. 64-70

Pharmacognostical evaluation of Spiny coriander (*Eryngium foetidum* L.): A traditional culinary and ethnomedicinal herb

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Abstract
Eryngium foetidum (Family: Apiaceae) is considered to be one the most commonly used ethno-medicinal plants found in certain parts of Asian and European countries. These plants are known to exhibit therapeutic properties that can be used in the treatment of various health ailments such as acute infections, fever, throat infection, pneumonia, tonsillitis, diarrhea, hypertension, epilepsy, asthma etc. The main aim of the present study was to evaluate the antioxidant, anti-inflammatory, anti-diabetic and anti-microbial activity of leaf extracts of *Eryngium foetidum*. Phytochemical screening of methanol (ME), ethanol (EE), methanol: water (HME) and ethanol: water (HEE) extract of leaf tissue of *E. foetidum* was performed. The total phenolic and flavonoid content was found to be 0.205± 0.0015 mg GAE/g and 0.0345 ± 0.0015 mg QE/g FWt respectively. *In vitro* antioxidant capacity of hydromethanolic extract of *E. foetidum* by linear regression analysis was expressed as IC_{50} values and found to be 131.94 µg/ml and 195.6 µg/ml for DPPH radical scavenging and H_2O_2 scavenging respectively. The IC_{50} values for *in vitro* anti-inflammatory activities of the hydromethanolic extract of *E. foetidum* were evaluated by the following assays: heat induced protein denaturation (IC_{50} = 127.57µg/ml) and RBC membrane stabilization (IC_{50} = 225.32µg/ml) at different concentrations using aspirin as control. Studies were also carried out to assess the hypoglycaemic potential by assaying the ability of the plant to inhibit alpha amylase (IC50 = 442.0 µg/ml) and glucose diffusion. Antimicrobial activity of the extracts was studied against common pathogens. The zone of inhibition was observed by agar well diffusion method.

Keywords: *E. foetidum,* anti-inflammatory, anti-diabetic, anti-microbial, alpha-amylase, phytochemicals

1. Introduction

Most of the developing countries have accepted traditional medical practice as an essential part of their culture. In earlier times, medicines were prepared from plants, used in the form of raw plant parts or in the processed form of crude extracts and mixtures. About 60% of the world's population still uses traditional medicine for primary health care. Thousands of plants are known for their medicinal uses in different communities. There are evidences of various Indian, European and Mediterranean communities working on herbs for over 4000 years as medicine. India has about 2.4% of the world's land with 8% of total biodiversity. It is one of the twelve mega-diversity hot spots of the world. The use of medicinal and aromatic plants is a valuable part in daily life despite the progress in recent medical and pharmaceutical activity. This progress is created on the belief that plants have an extensive ability for use as therapeutic medicine. The chemically active compounds present in the plants can be used as remedial agents in the treatment of infectious as well as chronic diseases. The medicinal significance of plants lies in the phytochemical components which produce a physiological and pathological effect on the human body. These phytochemicals are broadly classified as alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952) [1]. Medicinal plants are used to treat many ailments throughout the world and many of them are scientifically proven to possess biological activities like; anti-inflammatory, antihypertensive, anti-atherosclerosis, antidiabetic, anti-hyperglycemic, antibacterial and antifungal activities (Anwar *et al*., 2007) [2].

Eryngium foetidum, commonly known as Culantro or Mexican coriander is a tropical perennial herb belonging to the family *Apiaceae*. It is known to be native to Mexico and South America but also cultivated worldwide. It is vastly used in Thailand, India, Vietnam, Laos and other parts of Asia as a culinary herb (Singh *et al*., 2014) [3]. Ethnomedicinal use of the plant has been documented in ancient texts for the treatment of various diseases such as asthma, epilepsy, vomiting, cough, paralysis, arthritis, pneumonia, burns, hypertension, worms and convulsion in children (Honeychurch 1980 [4]; Mitchell and Ahmad, 2006 [5]; Roumy *et al*., 2007 [6]; Paul *et al*., 2011) [7]. The leaves are believed to be rich in calcium, protein, phosphorus, potassium and various dietary micronutrients that suggest the use in food fortification (Singh *et al*., 2013) [8]. The leaf extracts are used in rural India for treating hepatic issues (Yuhlung and Bhattacharyya, 2014) [9] and arthritis (Leishangthem and Sharma, 2014) [10]. The essential oil obtained from *E. foetidum* is used in perfumery and pharmaceutical industries (Lingaraju *et al*., 2016) [11]. Thus, the aim of the present investigation is to understand the in vitro potential for antioxidant, anti-inflammatory, anti-microbial and anti-diabetic activities of *E. foetidum* leaf extract in different solvents.

2. Materials and Methods

2.1 Collection and preparation of samples: The leaves of *E. foetidum* were collected from Imphal, Manipur, India in June 2017. The leaves were cleaned, rinsed in distilled water, sun dried and ground into a fine powder. The powdered samples were stored in air tight bottles at -20 °C

for further analysis. Crushed samples were extracted using three different solvent systems at 5% (w/v): distilled water (aqueous extract, HE), methanol (methanolic extract, ME), 1:1 water: methanol (hydromethanolic extract, HME) and 1:1 water: ethanol (hydroethanolic extract, HEE). The crude extracts were then centrifuged at 8000 rpm for 15 min. The supernatant obtained and stored at 4ºC until further use.

2.2 Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methodology to confirm the presence of phytoconstituents (Harborne, 1991; Khandelwal, 2009) [2, 13].

2.3 Fluorescent study of leaf powder

0.5 g of the dried leaf powder of *E. foetidum* were taken into clean and dry test tubes. To each tube, 5 ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid, 5% $FeCl_3$, 5% I_2, picric acid, 1N NaOH and 1N NaOH + methanol was added separately. All the tubes were shaken and allowed to stand for about 20-25 min. The solutions obtained were observed under visible and UV light for their characteristic colour reaction which was then compared with a standard colour chart and recorded (Chase and Pratt, 1949) [14].

2.4 Total phenolic and flavonoid contents

Total phenolic contents were estimated according to the spectrophotometric method of Slinkard and Singleton (1999) [15] and expressed in terms of gallic acid equivalence (mg of GAE/g of tissue). Aluminum chloride colorimetric method was used for determination of total flavonoids according to the method of Chang *et al*., (2002) [16] and expressed in terms of quercetin equivalence (mg of QE/g of tissue).

2.5 Determination of Antioxidant activity

2.5.1 DPPH radical scavenging activity

Procedure of Braca *et al*., (2002) [17] was used for determination of DPPH scavenging capacity of various fractions. A stock solution of ascorbic acid (1000 µg/ml) was diluted ranging from 10-100 µg/ml. 0.1 ml solution from different dilutions was pipetted out in respective tubes and was made up to 3 ml with DPPH (20 µg/ml). The tubes were incubated for 10 min at room temperature. The contents of each tube were mixed well and the absorbance was measured at 517 nm against a blank. The percentage inhibition of DPPH by the samples was calculated as follows:

$$\% \, Inhibition = \frac{(OD\ of\ control - OD\ of\ sample)}{OD\ of\ control}\ x\ 100 \text{ ---------- (Eq. 1)}$$

2.5.2 Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of the extracts was determined by using the method of Ruch *et al*., (1989) [18] with minor modification. Solutions of hydrogen peroxide (45mM) was prepared using 1M phosphate buffer (pH 7.4). The sample extracts of different concentration (100-500µg/ml) was added to hydrogen peroxide solution and incubated for 10 min. Absorbance was read at 230 nm against blank solution containing phosphate buffer only. Ascorbic acid was used as standard. The concentration of H_2O_2 in the assay medium was determined using a standard curve (y=0.1245x+0.3319, R^2=0.9365). H_2O_2 scavenging ability was expressed as IC_{50}. The percentage inhibition was calculated according to Eq. 1.

2.6 Determination of *in vitro* anti-inflammatory activity

2.6.1 Inhibition of heat induced protein denaturation

The anti-inflammatory activity was determined using inhibition of albumin denaturation with slight modifications (Sakat *et al*., 2010) [19]. The extracts of different concentration (100-500 µg/ml) were treated with 1% aqueous solution of bovine serum albumin. The pH was maintained by the addition of few drops of 1N HCl. The sample extracts were incubated at 37°C for 15 min and then heated at 51°C for 15mins. The extracts were cooled, and its turbidity was measured at 660 nm. Aspirin was used as standard. The percentage inhibition of protein denaturation was calculated according to Eq.1. IC_{50} was calculated from y=0.0778x+5.7976, R^2=0.9742.

2.6.2 RBC membrane stabilization activity

The test was performed using the method of Azeem *et al*., (2010) [20]. Extracts at different concentration (100-500 µg/ml) were mixed with 1ml of 1M phosphate buffer (pH 7.4), 2ml of hyposaline and 0.5 ml of RBC suspension. Diclofenac sodium was used as the standard drug. The mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant was used to estimate the hemoglobin content spectroscopically at 560 nm. The percentage inhibition of the protein denaturation was calculated using Eq.1. IC_{50} was calculated from y=0.1183x+33.117, R^2=0.5326.

2.7 Evaluation of hypoglycaemic activity using various *in* vitro methods

2.7.1 Alpha-amylase inhibition assay

Alpha amylase activity can be measured by the determination of reducing groups arising from hydrolysis of soluble starch by isolated pancreatic α-amylase. The reduction of 3, 5-dinitrosalicylic acid to nitro-aminosalicylic acid produces a red colour, which was measured spectrophotometrically at 540 nm. Inhibition of starch hydrolysis by an α-amylase inhibitor results in diminished absorbance at 540 nm compared to control and standard drug (Acarbose). 1 ml of the plant extract was pre-incubated with 1 ml of the enzyme solution for 30 min and then 1ml of 1% w/v starch solution was added. This was further incubated at 37 °C for 10 min. The reaction was arrested by heating the contents with 1 ml dinitrosalicylic acid (DNSA) reagent in boiling water bath for 5 min. blank containing only the buffer solution was used. The absorbance was measured at 540 nm against a blank comprising of only buffer solution. Acarbose was used as positive control at the concentration of 500 µg/ml. The percentage inhibition was calculated according to Eq. 1.

2.7.2 In vitro inhibition of glucose diffusion activity

The inhibition of glucose movement by *E. foetidum* alcoholic extracts was determined using the method proposed by Edwards *et al*., (1987) [21]. The model consisted the use of a dialysis tube into which 2 ml of plant extract in 1% CMC and 1 ml of 0.15 M NaCl containing 0.22 M D-glucose was added. The dialysis tube was sealed at each end

and placed in a 50 ml beaker containing 45 ml of 0.15M NaCl. The tubes were placed on an orbital shaker at room temperature. The movement of glucose into the external solution was monitored at set time intervals.

2.8 Screening of anti-microbial activity
Agar well diffusion assay the anti-bacterial activity was carried out by employing 48h cultures of *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis.* The activity of the methanolic, ethanolic and the aqueous of *E. foetidum* was determined using agar well diffusion method. The medium was sterilized by autoclaving at 120ºC, 15lb/in [2] for 15 min. About 20 ml of the agar medium was poured into the sterilized petriplate and allowed to solidify. 300 µl of the respective test cultures were swabbed on the top of the solidified media and allowed to dry. A well of 6 mm diameter was punched with the help of sterile cork borer. Ciprofloxacin (50 µg/ml) was used as a standard drug and was placed in the well along with the concentrated sample extracts. After 24 h of incubation at 37ºC, zone of inhibition (ZOI) was observed and diameter measured.

3. Results and Discussion
3.1 Phytochemical screening
Phytochemicals, produced by the plants through primary or secondary metabolism play an important role main role in the growth and also defense against competitor, pathogen and predator. The photochemical screening of the leaves extracts of *E. foetidum* were investigated respectively in aqueous (HE), methanol (ME), ethanol (EE), methanol: water (1:1, HME) and ethanol: water (1:1, HEE). The investigation revealed the presence of alkaloids, glycosides, saponin, terpenoids, phenol, flavonoid, protein and amino acid, carbohydrates, tannins in variable concentrations (Table 1).

Table 1: Phytochemical investigation of *E. foetidum* in various solvent systems.

Sl No.	Phytochemical analyzed	Test Performed			Result		
			HE	ME	HME	EE	HEE
1	Alkaloids	Mayer's test	-	+	-	+ +	-
		Wagner's test	+ +	+	+ +	+	+
		Dragendroff's test	+ + +	+	+	+	+
2	Glycosides	Keller-Killiani's test	-	+	+ +	+	+ +
3	Saponin	Foam test	+ +	+	+ +	+	+
4	Terpenoids	Salkowski's test	+ +	-	+ + +	+	+ + +
5	Phenols	Ferric chloride test	+ +	+	+ +	+	+ +
6	Flavonoids	Lead acetate test	+ + +	+	+ +	+	+ +
7	Protein and amino acid	Xanthoproteic test	-	+	-	+	-
		Ninhydrin test	-	+	+ + +	+ +	+
8	Carbohydrate	Molish test	+ +	+	+	+	+
		Benedict's test	+	+	+	+	+
		Fehling test	+ +	+	+ + +	+	+ +

-indicate absence, + denotes average, ++ means abundance, + + + means more abundance of phytochemicals.
*HE-aqueous extract, ME- methanol extract, EE- ethanol extract, HME- water: methanol extract, HEE- water: ethanol extract.

3.2 Fluorescence analysis
This is one of the pharmacognostic procedures useful in the identification of genuine samples and recognizing contaminants. The fluorescent studies of dried leaf powdered of *E. foetidum* with different chemical agents were observed under visible and UV light and the results are as tabulated (Table 2).

Table 2: Fluorescence analysis of *Eryngium foetidum.*

Sl No.	Experiment	UV Light (365nm)	Visible light
1	Distilled Water	Colorless	Light green
2	Acetone	Orange	Greenish brown
3	Ethanol	Orange	Green
4	Benzene	Pale orange	Brown
5	Chloroform	Orange	Greenish brown
6	Diethyl ether	Orange	Green
7	Methanol	Orange	Light green
8	GAA	Orange	Light green
9	Conc. H_2SO_4	Dark green	Brown
10	HCl	Black	Brown
11	HNO_3	Dark green	Dark green
12	5% $FeCl_3$	Colorless	Light green
13	5% I_2	Black	Brown
14	1N NaOH	Green	Brown
15	1N NaOH + Methanol	Fluorescence green	Brown
16	Picric acid	Greenish brown	Dark green

3.3 Total phenolic and flavonoid contents
Plant phenolics are secondary metabolites formed biologically by the pentose phosphate, shikimate and phenylpropanoid pathways. Phenolic compounds which are

from medicinal herbs and dietary plants comprises of phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, etc. Several bioactivities of phenolic compounds are liable for their chemo-preventive properties such as antioxidant, anti-allergenic, anti-atherogenic, anticarcinogenic, antimutagenic and anti-inflammatory effects (Naji *et al*., 2017 [22]; Alisha *et al*., 2018 [23]; Dsouza *et al*., 2018 [24]; Hana *et al*., 2019 [25]; Shediwah *et al*., 2019) [26]. The plants that are rich in polyphenols maintain carbohydrate metabolism, lipid digestion, dyslipidemia, insulin resistance and enhance tissue digestion. The total phenol of *E. foetidum* was found to be highest in methanol: water extract (HME) i.e., 0.205± 0.0015 GAE/g fresh weight tissue (Table 3). The flavonoid concentration was found to be highest in methanol extract i.e., 0.0345 ± 0.0015 mg QE/g fresh weight tissue (Table 3). Hence, HME was used for all further assays.

Table 3: Quantitative analysis of total phenols and total flavonoids of *E. foetidum*

Sample extract	Total Phenols (mg GAE/g Fwt)	Total Flavonoids (mg QE/g Fwt)
Water (WE)	0.173 ± 0.07	0.0067 ± 0.00
Ethanol (EE)	0.075 ± 0.003	0.0331 ± 0.001
Methanol (ME)	0.163 ± 0.007	0.0345 ±0.0015
Ethanol: water (HEE)	0.203 ± 0.004	0.0087 ±0.0001
Methanol: water (HME)	0.205± 0.0015	0.006 ±0.0001

*GAE- Gallic acid equivalents; QE- Quercetin equivalents, Fwt- Fresh weight tissue

3.4 *In vitro* antioxidant activity

Total antioxidant activity of the various extracts was determined by calculating the percentage inhibition against standard ascorbic acid (Table 4). It was found that of all the extracts aqueous fraction of methanol and ethanol of *E. foetidum* showed highest scavenging activity against DPPH i.e., at a concentration of 250 µg/ml *E. foetidum* was found to be 81.6 % (HME) and 82.7% (HEE). IC_{50} was found to be 122.4 µg/ml (ascorbic acid, y=0.3459x+7.659, R^2=0.9316), and 131.94 µg/ml (HME) and 162.56 µg/ml (HEE) for *E. foetidum.* This reveals that HME and HEE of *E. foetidum* consist of bioactive compounds having anti-oxidant activity that has the ability to decrease DPPH concentration.

The hydrogen peroxide scavenging activity was determined by calculating the percentage inhibition of various extracts against standard ascorbic acid (Table 4). The IC_{50} was found to be 195.6 ± 10.2 µg/ml (HME) and 315.7 ± 15.8 µg/ml (HEE). The IC_{50} for standard ascorbate was found to be higher than the HME and HEE, 400.55 µg/ml (y=0.1245x+0.3319, R^2=0.9365) indicating that HME and HEE has better scavenging potential.

Table 4: DPPH and H_2O_2 radical scavenging activity of *E. foetidum* and Ascorbic acid.

	Assay	Conc. (µg/ml)	% inhibition				
			Control	Extracts of *E. foetidum*			
				ME	EE	HME	HEE
In vitro antioxidant scavenging activity	DPPH	50	22.95 ± 1.2	13.1± 2.2	21.3 ± 3.5	43.7 ± 2.1	38.3 ± 8.
		100	49.2 ± 5.6	34.4 ± 1.9	45.7 ± 14.2	80.3 ± 8.2	39.8 ± 7.3
		150	70.2 ± 9.4	46.2 ± 5.9	66.8 ± 9.8	81.1 ± 9.3	43.9 ± 6.4
		200	80.60 ± 10.3	45.6 ± 7.8	72.6 ± 11.4	81.4 ± 7.3	82.2 ± 9.1
		250	82.24 ± 9.6	40.2 ± 8.3	98.74 ± 9.9	81.6 ± 6.8	82.7 ± 11.4
		IC50	122.4 ± 12.1	237 ± 15.1	359.7 ± 19.3	131.9 ± 12.5	162.5 ± 10.0
	H_2O_2	50	8.04 ± 1.1	15.05 ± 5.7	12.05 ± 1.2	27.3 ± 2.9	20.1 ± 2.3
		100	24.47 ± 3.4	25.65 ± 6.4	23.33 ± 4.1	44.5 ± 8.7	37.3 ± 1.7
		150	48.95 ± 9.8	45.03 ± 9.9	41.66 ± 5.2	64.8 ± 12.1	39.2 ± 4.7
		200	50.52 ± 8.6	61.9 ± 12.1	55.05 ± 6.6	71.6 ± 8.7	55.2 ± 9.8
		250	56.77 ± 12.5	66.1 ± 14.4	54.16 ± 3.2	81.2 ± 7.9	63.4 ± 11.1
		IC50	400.5 ± 18.7	353.1±11.4	410.2 ± 12.2	195.6 ± 10.2	315.7 ± 15.8

3.5 *In vitro* anti-inflammatory activity: Denaturation of proteins involves the disruption of bonding interaction of secondary and tertiary structures but not the primary structure. It was found that at a concentration of 1000 µg/ml the hydromethanolic extract of *E. foetidum* showed maximum inhibition of 79.2%. Aspirin, used as a standard anti-inflammatory drug was able to inhibit protein denaturation at 82.5% at the same concentration (Table 5). IC_{50} calculated and was found to be 570.0 µg/ml (Aspirin; y=0.0778x+5.7976, R^2=0.9742); and 127.7 µg/ml (*E. foetidum; y*=0.07062x+41.071, R^2=0.4961).

Membrane stabilization is a process in which the integrity of the membranes of the erythrocyte and lysozyme is maintained by stabilizing these membranes with the help of anti-inflammatory drugs (Mizushima *et al*., 1968) [27]. The structure and function of the erythrocyte membrane resembles the lysosomal membrane and hence the stabilizing effect produced by the drugs on these membranes might be similar (Akinpelu *et al*., 2015) [28]. It was found that the hydromethanolic extract of *E. foetidum* at a concentration of 500 µg/ml showed maximum inhibition of 66.7% while aspirin provided membrane stabilization activity of 81.1%. IC_{50} calculated and was found to be 143.0 µg/ml (Aspirin; y = 0.118x+33.117; R^2 = 0.5126); and 225.3 µg/ml (*E. foetidum;* y = 0.1037x+26.634; R^2= 0.5576).

Table 5: *In vitro* anti-inflammatory and anti-diabetic activity of *E. foetidum.*

	Assay	Control			*E. foetidum*	
		Conc.(µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)
In vitro anti-inflammatory activity	Heat induced protein denaturation	200	23.2 ± 2.1	570.0 ± 20.4	36.84 ± 4.8	127.7 ± 9.8
		400	45.6 ± 9.8		48.2 ± 11.1	
		600	53.7 ± 12.1		56.06 ± 9.6	
		800	65.2 ± 10.8		69.8 ± 4.5	
		1000	82.5 ± 8.4		79.2 ± 9.6	
	RBC membrane stabilization	100	72.2 ± 7.8	143.0 ± 16.3	58.1 ± 6.9	225.3 ± 12.8
		200	73.1 ± 8.9		59.9 ± 8.6	
		300	74.5 ± 12.4		64.0 ± 7.3	
		400	74.5 ± 11.6		66.5 ± 9.8	
		500	81.1 ± 10.3		66.7 ± 6.6	
In vitro anti-diabetic activity	Amylase inhibitory action	100	15.5 ± 3.5	379.7 ± 12.9	21.2 ± 3.2	442.0 ± 20.3
		200	25.2 ± 2.1		43.5 ± 4.4	
		300	36.1 ± 5.2		33.3 ± 8.1	
		400	54.6 ± 7.1		37.4 ± 7.3	
		500	66.6 ± 5.6		57.4 ± 9.1	
		500	76.3 ± 8.6		89.5 ± 5.9	

3.6 *In vitro* anti-diabetic activity

Diabetes mellitus is a metabolic disorder of glucose metabolism that brings about changes in the carbohydrate, protein and fat metabolism leading to microvascular and macro vascular changes resulting in secondary complications (Lloyd *et al*., 1995) [29]. In type 1 diabetes mellitus, excess secretion of insulin from the β-cells of pancreas takes place while in type 2, a dysfunction of the β-cells is seen (Heise *et al*., 2004) [30]. α-amylase, an enzyme found in pancreatic juice and saliva breaks down starch into glucose and maltose (Afifi *et al*., 2008) [31] thereby leading to hyperglycemia. Inhibitors of α-amylase has the ability to delay the hydrolysis of polysaccharides in the small intestine and diminishes the spike in blood glucose (Kwon *et al*., 2007) [32]. This assay was conducted to determine the ability of the alcoholic extracts of *E. foetidum* to inhibit the α-amylase activity. Acarbose was used as a standard anti-diabetic drug. The IC_{50} of *E. foetidum* were found to be 442.0 µg/ml while the control drug acarbose exhibited IC_{50} of 379.71µg/ml (Table 5). Our results showed that the hydromethanolic extract of *E. foetidum* possess strong antidiabetic activity. This shows the extract could be a good alternative to other medicines used for treating diabetes.

The cells present in human body requires energy to perform certain metabolic function that sustain life. This energy is provided by glucose, a simple carbohydrate that acts as a primary fuel in brain muscles and other physical organs for the production of energy. It serves as building block for glycoproteins and glycolipids. Abnormal level of glucose in the body might lead to certain complication which could be life threatening. Thus, the glucose levels need to be closely regulated. Glucose uptake transport systems are ubiquitous in animal cells as they play an important role in transportation of glucose molecules across the cell membrane. The study of glucose uptake by the body cells is very important as it helps in the identification of certain diseases and metabolic disorders such as myocardial ischemia, diabetes mellitus and cancer (Yamamoto *et al*., 2011) [33]. In this test, the alcoholic extracts of *E. foetidum* was used to determine the possible anti-diabetic activity. The result revealed the poor inhibition of the uptake of glucose (Table 6).

Table 6: Effect of *E. foetidum* on the movement of glucose out of dialysis tube over 25h incubation period.

		Erynigium foetidum	
Time	Control	HME	HEE
1 h	0.0385 ± 0.01	0.183 ± 0.014	0.247 ± 0.007
2 h	0.028 ± 0.008	0.172 ± 0.003	0.278 ± 0.003
5 h	0.057 ± 0.013	0.346 ± 0.021	0.261 ± 0.00
6 h	0.155 ± 0.042	0.455 ± 0.003	0.275 ± 0.007
25 h	0.63 ± 0.18	1.639 ± 0.014	1.165 ± 0.14

3.7 Anti - microbial activity

The bioactive metabolites synthesized via plant secondary metabolic pathways are responsible for diverse therapeutic properties. Chemical or synthetic drugs derived from these bioactives are used for treatment of infections by inhibiting and/or stopping microbial growth via disruption of synthesis of microbial nucleic acids, proteins and cell walls (Randhir *et al*., 2004) [38]. The present study was carried out to determine the anti-microbial activity of various plant extracts i.e., ethanol, methanol and their aqueous fractions of *E. foetidum*. The values obtained were compared with Ciprofloxacin as control. Anti-bacterial activity of methanol, ethanol and aqueous extracts of *E. foetidum* leaf was identified against *B. subtilis, S. aureus* and *E. coli* (Table 7).

Table 7: Antimicrobial activity of extract of *E. foetidum*. The zone of inhibition is expressed in mm.

	Zone of inhibition (mm)				
Strain	Ciprofloxacin	EE	ME	HEE	HME
E. coli	33 ± 8	23 ± 4	19 ± 6	6 ± 1	2 ± 0.5
B. subtilis	25 ± 7	11 ± 2	12 ± 5	7 ± 2	7 ± 1
S. aureus	23 ± 8	13 ± 4	11 ± 4	12 ± 4	9 ± 2

4. Conclusions

The present study was conducted to understand the efficiency and limitations of the bioactives isolated from *E. foetidum* that would increase its scope in the therapeutic field. Pharmacognostical and physicochemical parameters proved that the hydromethanolic extract of *E. foetidum* possessed antioxidant, anti-inflammatory, anti-diabetic and

anti-microbial activity. These tests provide a scientific authentication to the traditional claims of its use in the treatment of various inflammatory diseases by folkloric medicine. However, these results should be confirmed by *in vivo* models and clinical trials for their effective utilization as therapeutic agents.

5. Acknowledgement
The authors are grateful to Mount Carmel College, Autonomous, Bengaluru, India for provision of facilities needed for conducting this project.

6. References

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