



## **Comparative Study on Antibacterial and Selected Antioxidant Activities of Different *Eryngium foetidum* Extracts**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author RMUSKR designed the study and finalized the manuscript. Author DACKD performed the chemical tests, statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

*Eryngium foetidum* is an herb which is used as a medicinal and a spice plant in tropical regions. This plant is used in traditional medicine in treating conditions such as fever, chills, vomiting diarrhea, stomach aches, worms, pneumonia, flu, diabetes, constipation and malaria due to the presence of phytochemicals that is responsible for these activities. Further, it is also used as a food ingredient which is with health benefits in some countries. However, these claims have not been well proven by scientific studies. As such, this study focused on the investigation of antibacterial properties, total phenolic content and DPPH radical scavenging activity of different leaf extracts of *Eryngium foetidum*. Three different solvent extractions of leaves namely methanol, chloroform and water were studied. The methanol and chloroform extracts showed high antibacterial activity against gram positive bacteria (*Streptococcus pneumoniae*, *Listeria monocytogenes* and *Staphylococcus aureus*). Water extracts showed highest activity against gram negative microorganism (*Salmonella*). Phenolic compounds and antioxidant activity were high in the methanol extract, water extract and chloroform extract respectively. The results of the study have revealed the presence of phytochemical responsible for the above activities and possible use of *Eryngium foetidum* as a food source with health benefits.

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## 1. INTRODUCTION

Plant extracts have been used for medical purposes from ancient times. The possibility of using most of the medicinal plants for various treatments may be due to the chemical components depicting bioactive properties such as antioxidant and antimicrobial properties [1]. *Eryngium foetidum* of family Apiaceae is one such medicinal herb grown in the tropical regions which is believed to be an indigenous plant to tropical America and West Indies [2]. It is known by several local and common names such as Mexican coriander, Long coriander, Fit weed, Spirit weed [3]. In Sri Lanka it is called "Andu" in Sinhalese and "Katu kothimali" in Tamil language.

*Eryngium foetidum* is a biennial, pungently smelling tropical herb [2]. It is found grown in wet and moist conditions and in open banks and pastures [2]. The plant consists of a fleshy root, solitary and frequently branched stems with oblanceolate leaves (8-20 cm) with toothed margins that grow in a basal rosette pattern [2]. Long branched shoot bear whitish inflorescence and ovoid shaped fruits covered with rounded protrusions [2].

*Salmonella enteritica*, *Listeria monocytogenes*, *Shigella dysenteriae*, *Saphylococcus aureus*, *Escherichia coli* and *Pseudomonas pneumoniae* are some pathogenic bacteria. *Salmonella enteritica*, *Escherichia coli* and *Listeria monocytogenes* are food borne pathogenic bacteria causing gastroenteritis in humans. *Salmonella* is one of the major food and water borne pathogenic bacteria which is the causative agent of disease Salmonellosis [4,5]. *Escherichia coli* is a bacterium present in the intestinal tract of warm blooded animals as normal microflora. They are mostly nonpathogenic but some strains are pathogenic and may cause severe diarrhea and fever [6]. *Listeria monocytogenes* is an opportunistic pathogen causing the disease listeriosis [7]. *Saphylococcus aureus* has the ability to produce toxins that cause food poisoning and may cause gastrointestinal illnesses [8]. Pneumonia is a threatening pathogen that causes respiratory disorders.

Research has led to the identification and understanding of the potential mechanisms of biologically active components in food, which could improve health and possibly reduce the

risk of disease while enhancing the overall well-being [9]. Plants are always recognized as rich sources of such bioactive compounds called phytochemicals [10,11]. Plant based phenolic compounds are more important among them due to their antioxidant, anti-cancer and anti-inflammatory functions [12]. In the human body imbalance of free radicals may contribute to degenerative processes related to aging and diseases such as cancer, cardiovascular diseases, diabetes and neurodegenerative disorders [11]. The plant phytochemicals having antioxidant activity has the ability to act against these free radicals [11]. There is evidence of studies about such properties of *Eryngium foetidum*.

*Eryngium foetidum* is used in ethno medicine to treat conditions such as fever, hypertension, fit, asthma, stomach aches, worms, infertility complications, snake bites and malaria [2]. It is also used as a spice plant in the South East Asian countries as a substitute condiment for coriander (*Coriandrum sativum*) [2]. Therefore, it is of increasing importance as a food and culinary purposes. Besides its uses as medicinal and culinary purposes it is also used in the perfumery and cosmetics industries due its essential oil component [2].

Some studies have already explored the pharmacological properties, bioactivities and phytochemistry in the medicinal context. However, the use of *Eryngium foetidum* in food industry against food borne microbial pathogens are limited.

The objective of this study was to investigate the antibacterial activity against six selected above mentioned food borne pathogens and to determine selected antioxidant activities of different leaf extracts of *Eryngium foetidum*.

## 2. MATERIALS AND METHODOLOGY

### 2.1 Preparation of Extracts

Fresh leaves of *Eryngium foetidum* were harvested from the plants which were previously identified and grown at the Sbaragamuwa University of Sri Lanka, washed and dried in air until the leaves were crushable. They were ground using a blender until fine particles were obtained. Then 9.00 g each of the macerated samples were mixed with 200 ml of different

extracts and were kept in a shaker at 100 rpm for 6 hours and then left still overnight in the dark. Three different extracts namely chloroform, methanol and water were obtained. The three different mixtures were then filtered using cotton wool. The aqueous extract was directly obtained whereas the chloroform and methanol extracts were evaporated using the rotary evaporator and the crude was obtained. They were preserved in the refrigerator until used for testing.

## 2.2 Antibacterial Properties

### 2.2.1 Preparation of microbial cultures

Six bacteria, obtained from the culture collection of Medical Research Institute (MRI) of Sri Lanka, used in this study *Salmonella enteritica*, *Listeria monocytogenes*, *Shigella dysentery*, *Saphylococcus aureus*, *Escherichia coli* and *Pseudomonas pheumoniae* were separately enriched by culturing for 24 hours at 37°C in nutrient broth media. The culture plates were prepared using nutrient agar medium.

### 2.2.2 Antibacterial activity assays

Prepared cultures of *Salmonella enteritica*, *Listeria monocytogenes*, *Shigella dysentery*, *Saphylococcus aureus*, *Escherichia coli* and *Pseudomonas pheumoniae* were used for this assay. Disk diffusion method was used to determine the antibacterial activity. 1 mg/ mL stock solutions of the extracted crude samples of methanol and chloroform were prepared by dissolving in 30% by volume of dimethyl sulfoxide (DMSO) and 70% of volume by distilled water. These dissolved samples were used in antimicrobial assays. 5 mm diameter sterile filter paper disks were soaked in 20 µl of each prepared sample and left in the laminar flow for 30 minutes until dry. These disks were placed on the cultured plate after inoculating 100 µl of each bacterium on nutrient agar plates. Then they were incubated at 37°C for 24 hours. After incubation clear zone of inhibition were measured. Thereby antimicrobial activity of different extracts of *Eryngium foetidum* against the tested pathogenic bacteria was evaluated using those measurements.

### 2.3 Total Phenolic Content (TPC)

The TPC was determined according to a previously described method [13], using Folin-

Ciocalteu reagent with some modifications. A stock solution of 1 mg/mL was prepared by dissolving 5 mg of extract in 150 µL of Dimethyl sulfoxide (DMSO) solvent and 4850 µL of distilled water. Then, 250 µL of the stock solution was mixed with 2 mL of Folin–Ciocalteu reagent which was diluted ten times and 1.5 mL of 10% sodium carbonate solution was added to the mixture and was left for incubation at room temperature for 30 minutes. The absorbance was read at 760 nm using UV Visible Spectrometer. Gallic acid was used as the standard antioxidant.

### 2.4 DPPH Radical Scavenging Activity

DPPH radical scavenging assay was performed according to a previously described method [14] with some modifications. A reaction volume of 10 ml was obtained by mixing 5 mL of methanol, 2 mL of sample extracts of different concentrations (2000, 1000, 500, 250, 125, 62.5 mg/mL) and 3 mL of 0.1 mM DPPH radical and was left for incubation at room temperature for 30 minutes. The absorbance was read at 517 nm using UV Visible Spectrometer. Ascorbic acid was used as the standard antioxidant.

### 2.5 Statistical Analysis

Data obtained in the study were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the difference among treatment means.  $P < 0.05$  was regarded as significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Antimicrobial Activity

According to the results all three different types of extracts namely; methanol, chloroform and water obtained from the leaves of *Eryngium foetidum* shows some antibacterial activity against all tested microbial pathogens. The activity of methanolic extract ranges from 6.33-9.92 mm showing highest activity against *Streptococcus pneumoniae*. The chloroform extract ranges from 6.33 -9.75 mm which too showed highest activity against *Streptococcus pneumoniae*. However, the water extract showed a lesser activity compared to the above extracts and the results ranged from 6.00-8.11 mm, water extract showed highest activity against *Salmonella typhimurium*. Comparing the three extracts, a significant difference was observed

only against *Salmonella typhimurium*. There was no significant difference among the extracts for the other tested microorganisms. However, the lowest activity was observed against *Salmonella typhimurium* and *Shigella Dysentery* in all three extracts. This shows that these two microorganisms are comparatively resistant to the *Eryngium foetidum* extracts. Studies show evidence that *Eryngium foetidum* shows weak activity when applied in concentration of 1 mg/mL [2]. In a previous study, extracts from aerial parts of the plants show negative antimicrobial activity when screened broadly [15]. However, that study itself reveals that the compound present in *Eryngium foetidum* called, E-2-Dodecenal when extracted in pure form shows bactericidal effect against all growth stages of *Salmonella choleraesuis*.

As per the results of the present study, methanol and chloroform extracts of *Eryngium foetidum* shows a higher antibacterial activity against gram positive bacteria (*Streptococcus pneumoniae*, *Listeria monocytogenes* and *Staphylococcus aureus*) compared to the gram negative bacteria tested. In contrast, water extracts shows highest activity against *Salmonella* which is a gram negative microorganism. Literature reveals that *Eryngium foetidum* has been used to treat conditions such as fevers, chills, vomiting, diarrhea, pneumonia, flu, diabetes, constipation and malaria [11]. Therefore, the results of this study is in compliance with most of the above conditions since according to the study the best activity is shown against pneumonia and pathogens like *Salmonella* which causes diseases like vomiting and diarrhea.

### 3.2 Total Polyphenolic Content

The results show that there is a significant difference ( $p < 0.05$ ) between the TPC of different leaf extracts of *Eryngium foetidum*. The highest mean TPC  $7.8 \pm 0.00$  was observed for the methanol extract while the lowest  $0.07 \pm 0.00$  was recorded for the chloroform extract. The water extract showed a mean TPC value of  $3.72 \pm 0.02$ . The results agree with the fact that polar compounds do not dissolve in non-polar solvents; chloroform is considered as a non-polar compound, since the phenolic compounds shows polarity the reason for the less amount of TPC levels in the chloroform may be due the above reason, so that the amount and kind of compounds extracted depends on the polarity of the solvent.

Antioxidant activities of plant extracts are related to their phenolics, ascorbic acid and carotenoids [11]. Polyphenolic compounds are believed to have biological activities such as antioxidant activities. Antioxidants play a vital role in managing oxidative stress, which is a condition that arises due to the imbalance of free radical generation and degradation within the body which may lead to many degenerative diseases. According to the results of this study, methanol and water extracts of *Eryngium foetidum* leaves shows a considerable amount of phenolic compounds. Therefore, the study shows that *Eryngium foetidum* has the potential to be used for the prevention and management of oxidative stress associated diseases.

### 3.3 DPPH Radical Scavenging Activity

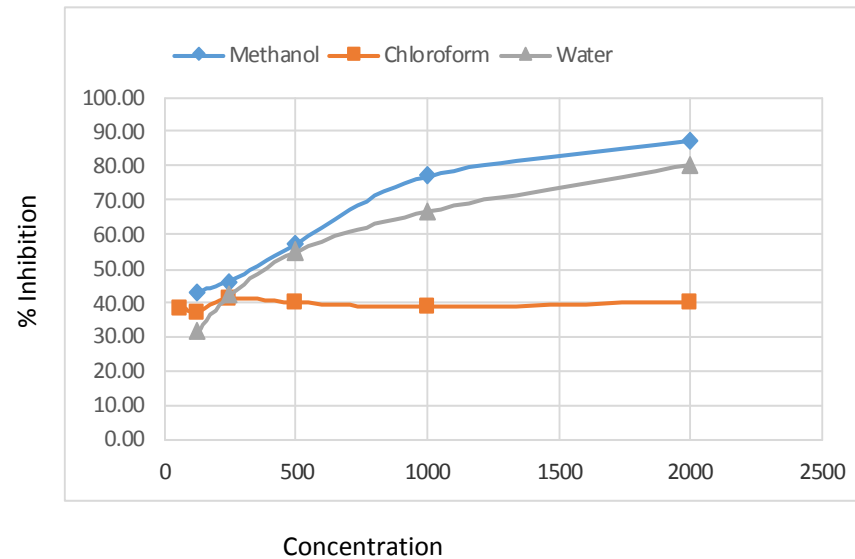
The DPPH radical scavenging activity of the different extracts of *Eryngium foetidum* leaves showed a significant difference ( $p < 0.05$ ) among the water and the methanol extracts. The chloroform extract did not show any activity. The DPPH radical scavenging activity followed the descending order methanol > water > chloroform; where chloroform did not show activity or any dose response. This variation among the three extracts may be due to the differences in the type and amounts of phytochemicals released from plant leaves into the type of solvent due to the changes in polarity.

However, previous studies show evidence of considerable antioxidant activity for chloroform extracts of *Eryngium foetidum*. In a previous study it has been found that the *Eryngium foetidum* leaves are a rich source of ascorbic acid [11]. According to above study, the highest ascorbic acid amount was recorded in methanol extract and lowest in chloroform extracts and that was correlated with the antioxidant activity. This study is also in compliance with the finding of the above as methanol extract showed the highest antioxidant activity and chloroform showed the lowest antioxidant activity. In contrast that study shows higher antioxidant values compared to the present study. This may be due to the fact that the composition of the plant phytochemicals may vary according to the different geographical locations [2]. However, the IC 50 values of methanolic extracts of the present study (IC 50 = 272.43  $\mu\text{g/ml}$ ) and the previous study of Singh et al. [11] (IC 50 = 248.4  $\mu\text{g/ml}$ ) shows a similarity. The Graph 1 shows the dose response curve for the three types of extracts.

**Table 1. Antimicrobial activity of different extracts against selected microorganisms**

	Inhibition zone in mm					
	<i>Streptococcus pneumoniae</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>	<i>Shigella dysentery</i>	<i>Escherichia coli</i>
Methanol	9.92±1.3 <sup>a</sup>	7.33±0.67 <sup>a</sup>	7.00±0.33 <sup>a</sup>	6.44±1.07 <sup>b</sup>	6.33±0.71 <sup>a</sup>	6.83±0.93 <sup>a</sup>
Chloroform	9.75±2.47 <sup>a</sup>	7.33±0.33 <sup>a</sup>	8.17±1.76 <sup>a</sup>	6.50±0.44 <sup>b</sup>	6.33±0.00 <sup>a</sup>	6.78±0.42 <sup>a</sup>
Water	6.50±0.24 <sup>a</sup>	7.56±1.26 <sup>a</sup>	6.50±0.17 <sup>a</sup>	8.11±0.63 <sup>a</sup>	6.00±0.24 <sup>a</sup>	6.61±0.35 <sup>a</sup>

Data represented as mean ± SE(n=3). Mean value in a column superscripted by different letters are significantly different at p<0.05



**Graph 1. Dose response curve for the three types of extracts**

**Table 2. Total polyphenolic content of different extracts of *Eryngium foetidum* leaves**

Extract	mg gallic acid equivalents/g of leaves
Methanol	7.8±0.00 <sup>b</sup>
Chloroform	0.07±0.00 <sup>c</sup>
Water	3.72±0.02 <sup>a</sup>

Data represent as mean ± SE (n=3). Mean values in a column superscripted by different letters are significantly different at p<0.05

**Table 3. DPPH radical scavenging activity of different extracts of *Eryngium foetidum* leaves**

Extract	IC 50 µg/mL	mg ascorbic acid/g of leaves
Methanol	272.43 <sup>b</sup>	17.1
Chloroform	No activity	-
Water	555.50 <sup>a</sup>	34.56

Values in a column superscripted by different letters are significantly different at p<0.05

Free radicals are believed to be involved in the development of many non-communicable diseases such as diabetes, cardiovascular diseases and inflammatory diseases [16]. According to the results the methanolic and water extracts shows DPPH Radical scavenging activity and shows a potential of using *Eryngium foetidum* as a food source in prevention and management of oxidative stress associated diseases.

#### 4. CONCLUSION

*Eryngium foetidum* leaves were found to be having antibacterial effects and antioxidant activity, but depends on different type of solvents used. Methanol and chloroform extracts showed high antibacterial activity against gram positive bacteria (*Streptococcus pneumoniae*, *Listeria monocytogenes* and *Staphylococcus aureus*). Water extracts showed highest activity against *Salmonella* which is a gram negative microorganism. The order of presence of phenolic compounds and antioxidant activity were highest in methanol extract, then in water extract and lowest chloroform extract. The study recommends that *Eryngium foetidum* leaves can be incorporated as a food source and can be promoted as a food having functional properties. The experimental findings substantiate with the traditional uses of *Eryngium foetidum* as a medicinal herb. Further investigations are

required in the potential of using *Eryngium foetidum* leaves as a main ingredient in developing functional foods and their links with health effects in addressing oxidative stress related diseases.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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