

Phytochemical Constituents and Antimicrobial Potential of Essential oil from *Eryngium foetidum* plant (Sawtooth coriander) on Selected Clinical Isolates

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Abstract *Eryngium foetidum* L. has been used traditionally for the treatment of different ailments. This study was conducted to determine the phytochemical constituents and antimicrobial potential of essential oil from *Eryngium foetidum* L. (**Sawtooth coriander**) plant on selected clinical isolates using standard phytochemical and microbiological techniques. The methanolic extract of the plant was obtained using the maceration method, in 70% methanol as the organic solvent. The extract obtained was subjected to phytochemical screening. The essential oil was extracted using hydro-distillation and analyzed via the Kirby-Bauer agar well diffusion method for antimicrobial activity. Results revealed the presence of bioactive constituents such as anthraquinones, cardiac glycosides, flavonoids, saponins and tannins. Antimicrobial activity results obtained showed that the essential oil highest activity against *Pseudomonas aeruginosa* with zone of inhibition (ZOI) of 21.2 ± 0.4 mm, followed by *Escherichia coli* (18.2 ± 0.2 mm), *Staphylococcus aureus* (15.3 ± 0.2 mm). Antifungal activity was more potent against *Microsporum* species (23.4 ± 0.5 mm) than *Candida albicans* (16.2 ± 0.1 mm). Moreover, the minimum inhibitory concentration (MIC) for *P. aeruginosa*, *E. coli* and *S. aureus* was 6.25mg/ml and 25mg/ml and 50mg/ml respectively but *Microsporum* species. with 25mg/ml. The minimum bactericidal concentration (MBC) revealed *P. aeruginosa* with the lowest MBC of 25mg/ml, *E. coli* (50mg/ml), *S. aureus* (100mg/ml). The minimum fungicidal concentration (MFC) also presented *Microsporum* species. with 50mg/ml, and *Candida albicans* 100mg/ml respectively. The activity index of the essential oil at 100mg/ml against *C. albicans* was 1.07 and 1.20 compared to the antifungal drug fluconazole and ketoconazole respectively. The result of this research presents *Eryngium foetidum* as plant that contains a good number of bioactive compounds and its oil with high antimicrobial activities thus supporting the traditional usage of this plant. The results also suggest that *Eryngium foetidum* essential oil should be exploited as an alternative for treatment of infections especially skin infections associated with microorganisms highlighted in this study.

Keywords: Phytochemical, Essential oil, *Eryngium foetidum*, Antimicrobial

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1. Introduction

Medicinal plants have been used as traditional medicines and as ethnomedicine all over the world and have been the basis of treatment of various diseases, in which 80% of the world population still depends on [1]. The use of plants as novel, alternative therapeutic agents has a direct or indirect positive impact on human, non target and environmental health [2]. Antimicrobial resistance has been a growing threat to the effective treatment of infectious diseases caused by bacteria and fungi for several decades. It results in reduced efficacy of

antimicrobial agents, making the treatment of patients difficult, costly, and even impossible in some cases. Therefore, there is a growing and urgent need for alternatives that are commonly assessable, cost effective, and more effective against pathogenic microorganisms and their resistant strain. Currently, over 7,000 species of plants have been reported as being used in traditional medicine as a result of their bioactive compounds [3].

Plant essential oils are known to possess antimicrobial properties that have been recognized for many years, and their preparations have been used as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation and others [2].

Eryngium foetidum is commonly used as a traditional medicine in treatment of various ailments and diseases. According to Merghache *et al.* [4], they observed that the leaves of this plant have been used by most people from different parts of the world as an ethnomedical plant for treating kidney and bladder dysfunctions as well as for the treatment of a number of ailments such as fevers, chills, vomiting, burns, fevers, hypertension, headache, earache, stomachache, asthma, arthritis, scorpion stings, malaria and diarrhoea. The decoction and infusion from the leaves were prepared to cure fever, flu, diabetes, hypertension, constipation diuretic, anticonvulsant, cold, muscular pain in South East Asia [5].

Various research has been carried out to determine the chemical components of the essential oil of this plant, a study by [6] reported that the leaf volatile oil contained high proportion of (E)-2-Dodecenal (28.43%) among other compounds and also confirmed the anti-oxidant activity of the plant's essential oil. Several pharmacological research has also confirmed the anti-diabetic [7], anti-inflammatory [1], anti-convulsant [8], anti-leishmanicidal [9], anti-helminthic [10], anti-plasmodial and anti-larvacidal [8,11] activity of the plant essential oil and crude extract. *Eryngium foetidum* contains various bioactive constituents which attributes to its antimicrobial activities. Not much is known about the antimicrobial properties of *E. foetidum* in Uyo, Nigeria. There is still a dearth of information on the antimicrobial activities of the essential oil and other extracts of this plant. This study was designed to determine the phytochemical properties and antimicrobial potentials of *E. foetidum* essential oil.

2. Materials and Methods

2.1. Collection of Plant Materials

Fresh aerial part (leaves, stems) of *E. foetidum* were collected from mature plants cultivated at Utang Street, Uyo Local Government Area, Akwa Ibom State, Nigeria. The samples were identified in the taxonomy laboratory of the Department of Botany and Ecological Studies, University of Uyo.

2.2. Sample Preparation and Extraction of Essential Oil

The extraction of essential oil from the plant was carried out in the laboratory of the Department of Pharmacognosy and Natural Medicine, University of Uyo, following the methods of [6]. The hydro-distillation method with a clavenger-type apparatus was used. Freshly collected aerial part of *E. foetidum* was sliced into tiny pieces, weighed using a triple beam balance and transferred into a 5000ml Clavenger flask containing 4000ml of water, enough to cover the plant material with space left for steam. The clavenger (fixed to a running tap) was tightly fixed with the flask and was subjected to boiling using an electric mantle. The set-up was heated to boil for 4 hours. The volatile oil was collected from the tapper using a sterile bottle with cover. The process took 4 hrs for complete extraction to allow the extraction of

oxygenated compounds which takes time to volatilize unlike the hydrocarbon compounds which volatilize more easily.

2.3. Extraction of Methanolic extract of aerial part of *E. foetidum*

Methanolic extraction from *E. foetidum* was performed following the methods of [12] and [13]. The aerial part of the plant was macerated with 70% methanol and was then decanted after 72hrs. The filtrate was concentrated in a water bath until the solvent totally evaporated.

2.4. Phytochemical Screening

The qualitative phytochemical screening of the crude extract of *E. foetidum* aerial part was carried out to determine the constituent bioactive compounds in line with the standard methods [12,13].

2.5. Clinical Isolates Used in the Study

Clinical isolates were collected from the culture collection of the Department of Pharmaceutical Microbiology and Biotechnology, University of Uyo. The bacterial isolates were selected to represent Gram-positive: *Staphylococcus aureus*, *Streptococcus pyogenes*; Gram-negative: *Escherichia coli*, *Pseudomonas aeruginosa*; one yeast; *Candida albicans*, and a mold - *Microsporum spp.* Cultures of these organisms were made from stock cultures, on nutrient agar slant for the bacteria at 37°C for 24hrs, and Sabouraud dextrose agar slant for fungi and yeast at room temperature for 48hrs before they were transferred to the fridge to control their growth.

2.6. Essential oil Incorporation

Incorporation of essential oil was carried out using the method of [14]. Since essential oil cannot dissolve in water or any broth media due to their nature. For the essential oil to obtain its full effectiveness when used for sensitivity test, it must be dissolved or emulsified with any emulsifying agent. In this case, Tween 80 was used to emulsify and evenly disperse the essential oil in the media used, allowing it to come in contact with the test microorganisms. The final tween 80 concentration used was 20% (v/v) which was prepared by dissolving 20ml of tween 80 in 80ml of water in a conical flask, the mixture was then sterilized in an autoclave at 121°C for 15 minutes.

2.7. Preparation of Essential oil Stock Concentration for Antimicrobial Screening

The preparation of stock concentration of the essential oil extract was carried out using the method adopted from [14]. A stock concentration of 100mg/ml was prepared by dissolving 1ml of the essential oil extract in 9ml of 20% tween 80 (this 9ml of 20% tween 80 was added in bits and mixed properly with the oil for proper dissolution) in a McCadney bottle, smaller concentrations were prepared by carrying out a two-fold serial dilution; 50mg/ml

concentration was prepared by taking 5ml of the stock solution and dissolving it in 5ml of 20% tween 80, and similar dilutions were carried out to achieve lower concentrations (25mg/ml, 12.5mg/ml). The positive control drugs used were Gentamate (8mg/ml), levofloxacin (50mg/ml) for bacteria and fluconazole (5mg/ml), ketoconazole (20mg/ml) for fungi and the negative control used was 20% tween 80.

2.8. Preparation of Microorganism for Antimicrobial Screening

A loopful of the test organism was taken from their respective agar slants and sub-cultured in nutrient broth for bacteria and Sabouraud dextrose broth for fungi. The subculture test-tubes were incubated for 24hrs at 37°C for bacteria and for 48hrs at 30°C for the fungi. The obtained test isolates in the broth were adjusted to 0.5MacFarl and standard using normal saline to obtain microbial population density of 1.5×10^8 CFU/ml as well as population density of 10^8 Cfu/ml for fungi.

2.9. Antimicrobial Sensitivity Assay (Agar well diffusion assay)

Agar well diffusion assay method for antimicrobial sensitivity was adopted from [15] with some modifications. A loopful of the test organism was taken from the stock culture and sub-cultured in Nutrient broth for bacteria and Sabouraud dextrose broth for fungi and yeast and were incubated at 37°C for 24hrs and 30°C for 24 – 48hrs for bacteria and fungi respectively. About 15ml of freshly prepared Mueller-Hinton agar was poured into properly labelled 12-well sterile Petri dishes (4 plates for bacteria, 2 plates for fungi and 6 plates for control). The media plates were allowed to completely solidify, and then swabbed with a 0.1ml of the sub-cultured test organisms all over the surface of the media. Using sterile cork borer of 5mm diameter, four (4) wells were bored into the seeded agar plates according to the number of concentration used, and these wells were inoculated with a 0.3ml of different concentrations (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the extracted essential oil. The control plates had three wells bored (two wells for positive control and one well for negative control). A 0.3ml of the control antibiotic drugs and 0.3ml of the negative control were introduced into their respective wells. Gentamate, levofloxacin (Standard antibacterial drugs) and Ketoconazole, Fluconazole (Standard antifungal drug) were used as positive controls while 20% tween 80 was used as the negative control. All the plates were incubated at 37°C for 24hrs for the bacteria and 30°C for 48hrs for the fungi and yeast. Antimicrobial activities of the essential oil extracts were evaluated by measuring the zone of growth inhibition against the test microorganisms using a transparent ruler calibrated in millimetre and the results recorded. The experiments were performed in duplicates to ascertain the results obtained.

2.10. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil was determined using the broth dilution method adopted from [15] Six (6) concentrations of the essential oil extract (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) were used for this test, with positive and negative controls employing a 4ml two-fold serial dilution. Nutrient broth and Sabouraud dextrose broth were used for bacteria and fungi respectively. For each organism, eight (8) sterile tubes, each containing 2ml of broth media, 100mg/ml stock concentration of essential oil was prepared. 2ml of the stock concentration was taken and added into the first tube containing 2ml of the broth media, mixed properly using a two-fold serial dilution. Two (2) milliliters was transferred from the first tube to the second tube and mixed, and subsequent transferred from the second tube to the third tube and mixed. This step was repeated until the sixth (6) tube and 2ml was discarded from the last tube. Six (6) concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) were obtained. Overnight broth sub-culture of each organism in nutrient broth (bacteria) and Sabouraud dextrose broth (fungi and yeast) were prepared and 0.1ml was added to respective test tubes. A test tube containing only broth media and test organism was used as a positive control while a test tube containing only blank culture broth was used as a negative control. All the tubes were incubated at 37°C for 24 hours for bacteria and 30°C for 48hrs for fungi and yeast. After incubation, MIC was considered as the lowest concentration in which no bacterial growth occurred in the test tube. The experiments were replicated twice to ascertain the results obtained.

2.11. Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

The tubes with no visible growth in the MIC test were selected and samples were used to determine the MBC and MFC. Following homogenization, a loopful of each suspension was cultured on nutrient agar for bacteria and Sabouraud dextrose agar for fungi. This culture was incubated aerobically at 37°C for 24hrs for bacteria and 48h at 30°C for fungi and yeast. The MBC and MFC were estimated from the culture medium in which no visible microbial growth was recorded upon examination. The tests were performed in duplicates.

3. Results

3.1. Results of Qualitative Phytochemical Screening of aerial part of *E. foetidum*

The result of the phytochemical screening of the methanolic crude extract from the aerial part of *E. foetidum* is shown in Table 1 below. The results revealed the presence of phytochemicals such as anthraquinones, cardiac glycosides, flavonoids, saponins, and tannins, while the test for alkaloids proved the absence of alkaloids in the extract.

Table 1. Qualitative phytochemical screening of aerial part of *E. foetidum*

Phytochemical	Test	Crude extract
Alkaloids	Dragendroff's test	-
Anthraquinones	Condensed anthraquinones test	-
	Free anthraquinones test	+
Cardiac Glycoside	Salkowski's test	+
	Liebermann-Burchard test	+
	Keller-kiliani test	+
Flavonoids	Magnesium metal test	-
	Sodium hydroxide test	+
Saponins test	Frothing test	+
Tannin test	Ferric chloride test	+

Key: (+) = Present, (-) = Absent

3.2. Antimicrobial Activity of Essential oil of aerial part of *E. foetidum* and Control Antibiotics on Selected Microbial Isolates

The result of the antimicrobial activity of the essential oil extract of *E. foetidum* aerial part against test isolates is presented in Table 2. The results obtained revealed that the antibacterial activity *E. foetidum* essential oil was more effective against *P. aeruginosa* with a zone of inhibition 20.5 ± 0.5 mm, followed by *Escherichia coli* 18.2 ± 0.2 mm, *S. aureus* 15.3 ± 0.2 mm all tested with 100 mg/ml concentration of the essential oil. The result also revealed the resistance of *S. pyogenes* to the essential oil at all concentrations used. The antifungal activity study proved *Microsporum spp.* as the most susceptible, with zone of inhibition of 23.4 ± 0.5 mm, followed by *Candida albicans* with zone of inhibition of 16.2 ± 0.2 mm when tested with 100 mg/ml concentration of the plant's essential oil.

Table 2. Antimicrobial Activity of essential oil of aerial part of *Eyngium foetidum* and control antibiotics on selected microbial isolates

Test Organism	100mg/ml Mean±SD (mm)	50mg/ml Mean±SD(mm)	25mg/ml Mean±SD (mm)	12.5mg/ml Mean±SD (mm)	Gentalate (8mg/ml) Mean±SD (mm)	Levofloxacin (50mg/ml) Mean±SD (mm)	Fluconazole (5mg/ml) Mean±SD (mm)	Ketoconazole (20mg/ml) Mean±SD (mm)	Tween 80 (20%)
<i>Escherichia coli</i>	18.2±0.2	16.2±0.1	16.3±0.3	15.1±0.2	32.3±0.5	39.3±0.2	NT	NT	NA
<i>P. aeruginosa</i>	21.2±0.4	20.5±0.5	19.2±0.4	18.1±0.3	35.2±0.2	38.2±0.2	NT	NT	NA
<i>S. aureus</i>	15.3±0.2	11.5±0.4	NA	NA	28.0±0.1	35.3±0.4	NT	NT	NA
<i>S. pyoyenes</i>	NA	NA	NA	NA	26.2±0.3	30.1±0.2	NT	NT	NA
<i>C. albicans</i>	16.2±0.1	13.5±0.5	11.3±0.3	NA	NT	NT	13.4±0.3	15.2±0.3	NA
<i>Microsporum spp.</i>	23.4±0.5	20.0±0.2	19.1±0.2	17.3±0.1	NT	NT	30.1±0.2	33.2±0.3	NA

N/B : Values are means of duplicate cultures and standard deviation.

Key: NA = No Activity; NT = Not Tested; SD = Standard Deviation; mg = Milligram; ml = Millilitre; Resistant = (0 – 10mm); Intermediate = (11 – 13mm); Sensitive = 14mm and above.

Table 3. Minimum inhibitory concentration (MIC) of essential oil extract from aerial part of *E. foetidum* on selected test isolates

Test Isolates	Concentration (mg/ml)						MIC
	100	50	25	12.5	6.25	3.125	
<i>E. coli</i>	-	-	-	+	+	+	25mg/ml
<i>P. aeruginosa</i>	-	-	-	-	-	+	6.25 mg/ml
<i>S. aureus</i>	-	-	+	+	+	+	50mg/ml
<i>S. pyogenes</i>	+	+	+	+	+	+	NA
<i>C. albicans</i>	-	-	+	+	+	+	50mg/ml
<i>Microsporum spp.</i>	-	-	-	+	+	+	25mg/ml

Key: (+) = Growth; (-) = No growth

3.3. Minimum Inhibitory Concentration (MIC) of Essential oil Extract from Aerial Part of *E. foetidum* on Selected Test Isolates

The result of the minimum inhibitory concentration of essential oil extract of *Eryngium foetidum* on the test isolates is presented in Table 3. The result revealed that the essential oil extract of *E. foetidum* had MIC value of 25mg/ml for *E. coli* and *Microsporum spp.* For *P. aeruginosa*, an MIC of 6.25mg/ml was recorded, followed by *S. aureus* and *C. albicans* with an MIC value of 50mg/ml respectively. No activity was recorded with *Streptococcus pyogenes*.

3.4. Minimum Bactericidal and Fungicidal Concentration of Essential oil Extract of *E. foetidum* on Test Isolates

The result of the minimum bactericidal/fungicidal concentrations (MBC/MFC) of the essential oil extract is presented in Table 4 below. The result showed *P. aeruginosa* to have the least MBC value of 25mg/ml, followed by *E. coli* with 50mg/ml. *Staphylococcus aureus* had the highest value MBC value of 100mg/ml. No activity was observed with *S. pyogenes*. However, *Microsporum spp.* and *Candida albicans* have MFC values of 50mg/ml and 100mg/ml respectively for essential oil extract of *E. foetidum*.

Table 4. Minimum Bactericidal and Fungicidal Concentration of Essential oil Extract of *E. foetidum* on test isolates

Test Isolates	Concentration (mg/ml)						
	100	50	25	12.5	6.25	3.125	MBC/MFC
<i>E. coli</i>	-	-	+	+	+	+	50mg/ml
<i>P. aeruginosa</i>	-	-	-	+	+	+	25 mg/ml
<i>S. aureus</i>	-	+	+	+	+	+	100mg/ml
<i>C. albicans</i>	-	+	+	+	+	+	100mg/ml
<i>Microsporium spp.</i>	-	-	+	+	+	+	50mg/ml

Key: (+) = Growth, (-) = No growth, NA = No activity

4. Discussion

The aerial part of the plant *Eryngium foetidum* is greatly fortified with phytochemicals as revealed in methanolic crude extract analyzed in this study. The presence of phytochemicals such as anthraquinone, cardiac glycosides, flavonoids, saponins and tannins in *Eryngium foetidum* supports this fact. There was no alkaloid isolated in the study. This agrees with the research of [16], which had also confirmed the presence of anthraquinone, cardiac glycosides, flavonoids and tannins; and also confirmed the absence of alkaloids from the leaf extract of *Eryngium foetidum*. Other research by [17] also confirmed the presence of tannins, saponins, flavonoids and anthraquinone from the *Eryngium foetidum* plant extract. The presence of these bioactive components is of medical importance and attributed to antimicrobial activities [18,19]. Anthraquinones are known to serve as laxatives, antimicrobials, anti-inflammatory agents. They also have other therapeutic uses in the management of constipation, arthritis, multiple sclerosis and cancer [20]. Cardiac glycoside on the other hand, is of pharmaceutical importance in the treatment of cardiac failure or congestive heart failure in which the heart cannot pump sufficient blood to maintain body needs [21].

The antimicrobial activity of essential oil extract from *Eryngium foetidum* was observed against four (4) bacteria *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, a dermatophyte, *Microsporium* species and a yeast - *Candida albicans*. A minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) recorded for *P. aeruginosa* showed the lowest MIC value of 6.25mg/ml and MBC value of 25mg/ml. *Escherichia coli* had an MIC value of 25mg/ml and MBC value of 50mg/ml as the most susceptible bacteria. *Staphylococcus aureus* being reported as moderately susceptible is of great therapeutics values. The exhibition of antimicrobial potentials against pathogenic bacteria has high medical advantages in view of public health implications of these organisms especially with reported antibiotic resistance among some of these organisms [19].

There is no baseline information on the antimicrobial potentials of essential oil from *Eryngium foetidum* plant as described in the present work, especially in Nigeria. This study provides a baseline data on the antimicrobial potentials of essential oil from *Eryngium foetidum* plant in Nigeria.

The results of this study shows that *Pseudomonas aeruginosa* was the most sensitive with zone of inhibition of 21.2 ± 0.4 mm, followed by *E. coli* (18.2 ± 0.2 mm), and

S. aureus (15.3 ± 0.2 mm) when all tested with 100mg/ml concentration of the plant essential oil. The essential oil also exhibited high antifungal activities when tested against *Microsporium spp.*, producing a large zones of inhibition value of 23.4 ± 0.5 mm, and *Candida albicans* having a zone of inhibition value of 16.2 ± 0.1 mm. These results were higher than the zone of inhibition of the control drug fluconazole, which had zone of inhibition value of 13.4 ± 0.3 mm. This finding is again of great medicinal value. Similar studies have also reported the antimicrobial activities of the crude extract of *Eryngium foetidum*. For instance, the sensitivity test results in the work of [17] confirms the antimicrobial activity of the ethylacetate extract of *E. foetidum* against *Pseudomonas aeruginosa* with a zone of inhibition of 28mm, *S. aureus* (25mm), *Candida albicans* (18mm) and *E. coli* (17mm), with MIC values of 3.12µg/ml, 3.12µg/ml, 1.56µg/ml and 50µg/ml respectively.

The *in-vitro* antibacterial activity of *Eryngium foetidum* essential oil extract might not be enough to justify its potential, there is need for *in-vivo* studies of the antibacterial activities to be evaluated.

5. Conclusion

The methanolic extract of *Eryngium foetidum* contains a good number of bioactive compounds such as anthraquinones, cardiac glycosides, flavonoids, saponins, and tannins which are of medicinal importance. This study has further supported the traditional claims for its used in ethno-medicine. The essential oil of *Eryngium foetidum* has high antibacterial and antifungal activities when compared some commercially available controls on selected clinical isolates. It is major break through which forms baseline information for the plant to be further exploited and used as an alternative in the production of antimicrobial drugs for the treatment of several human infections.

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