

Evaluation of Antibacterial and Antifungal Activity of *Foeniculum Vulgare* (Fennel) Extracts and Elemental Composition.

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ABSTRACT

OBJECTIVES: The objective of the current study was to assess the antibacterial and antifungal activity of *Foeniculum vulgare* (fennel) ethanolic extract, along with its elemental analysis with the aim of justifying its use as well as to ascertain its safety in relation to its potential benefit in medicine.

METHODS: Plant material was collected from District Mastung, Balochistan, Pakistan. Dried seeds and leaves were subjected to successive solvent extraction to obtain their ethanolic extracts. The antibacterial and antifungal activities were examined on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* by agar well diffusion method and the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using the broth micro-dilution method. The elemental composition was determined with Atomic Absorption Spectroscopy (AAS) for essential (e.g. potassium, calcium, magnesium) and heavy (e.g. lead, cadmium) elements. The SPSS was used for statistical analysis of antimicrobial efficacy and elemental data.

RESULTS: The ethanolic extracts showed potent antibacterial activity against *S. aureus* and *E. coli* with inhibition zones of 17 mm and 16 mm, respectively; the extracts did not, however, exhibit antifungal activity towards *C. albicans*. The moderate inhibitory properties were also reflected by MIC values presumable due to bioactive compounds such as anetholeic and phenolics. Elemental analysis identified potassium (12.5 mg/g), calcium (8.3 mg/g), and magnesium (5.7 mg/g) with trace amounts of iron (0.9 mg/g) and zinc (0.4 mg/g), and low amount of lead (0.05 mg/g) confirming the safe use of the plant.

CONCLUSION: Ethanolic extracts of *Foeniculum vulgare* exert strong antibacterial activity due to its bioactive compounds, and with the possession of beneficial elements with a low level of toxicants, it can be used as a safe natural antimicrobial agent. More studies should be conducted to make predictably of extraction for antimicrobial activity, especially to fungi.

INTRODUCTION.

Foeniculum vulgare which is commonly known as fennel, is an important medicinal and aromatic plant of the family Apiaceae (Rafieian et al., 2024). It has long been used in many cultures for its nutritional and therapeutic properties, such as digestive relief, anti-inflammatory and antimicrobial activity (Rafieian et al., 2024). Growing problem of antibiotic-resistant bacterial and fungal strains are now revealing them as promising sources of natural antimicrobial agents. Medicinal plants such as fennel, which contains bioactive compounds, have potentially good efficacy in this respect (Yadav et al., 2022).

The characteristic spicy fragrance of anethole, fenchone, limonene, and estragole phytochemicals are present in *Foeniculum vulgare*'s seeds, leaves, and fruits (Mehra et al., 2021). These compounds provide antioxidant, anti-inflammatory, and antimicrobial activity (Vella et al., 2024). Fennel is ethnobotanically used as a galactagogue and aids in the relief of menstrual discomfort and childbirth (Divya, 2022). Additionally, it is used to relieve gastrointestinal bloating, indigestion, and colic (Noreen et al., 2023).

Like calcium, magnesium, potassium, and zinc, fennel also contains these essential minerals which may further contribute towards fennel's safety profile (Samy et al., 2024). These minerals are also important to the human body as they support some metabolic processes and the body's immune system. *F. vulgare* grows in many different climatic zones, including the hot and dry semi-arid areas of Balochistan, Pakistan, where it has great economic importance (Shah et al., 2025). As of now, medicinal plants (MPs) still serve as the main form of health care in the developing world. The World Health Organization (WHO) estimates around 80 percent of the global population, particularly in developing countries, relies on alternative forms of medicine, particularly traditional herbal medicine to fulfill their health care needs (Maddheshiya et al., 2024).

The growing concern of antibiotic-resistant bacteria and pathogenic fungi poses a considerable danger to worldwide public health. There is a need to search for alternative compounds as antimicrobial agents are becoming less effective. Plants such as *F. vulgare* are well-known for their rich phytochemical profiles and for being used traditionally in medicine, attracting attention due to herbal medicine (Badgujar et al., 2014) conducted a comprehensive review of fennel's botany, photochemistry, and pharmacology, emphasizing its use in traditional medicine for digestive, endocrine, and respiratory disorders (Badgujar et al., 2014) presented a detailed review of the botany, photochemistry, and pharmacology of fennel related to its usage in traditional medicine covering digestive illnesses, endocrine, respiratory disorders.

(Martins et al., 2019) tested the fennel essential oil as an antifungal agent against food spoilage fungi, *Aspergillus niger*, and *Fusarium oxysporum*. The minimum inhibitory concentrations (MIC) of *A. niger* and *F. oxysporum* were 750 and 250 following sectors respectively, which indicated their possible usage as food additive. (Agarwal et al., 2017) studied methanolic and hexane extracts of seed of five fennel genotypes cultivated in India and found variability between genotypes with regards to their antimicrobial activity. The inhibitory activity of methanolic extract of the plant against *S. aureus* was stronger than hexane extract due to high amount of phenolic content.

According to (Rather et al., 2016) that the various extracts of fennel, especially bark, have considerable antifungal activity against the strain *Candida albicans*. The essential oil inhibited mycelial growth of *Sclerotinia sclerotiorum*, a characteristic that makes it a prospective alternative of bio-fungicide (Adel et al., 2019). Some studies involved comparison of fennel seeds extracts of Egypt and China, where antioxidant and antibacterial activities were found higher in Egyptian extract, due to the higher amount of phenols contained.

As the results of the work carried out by (Beyazen et al., 2017) reveal, extracts of fennel leaves show significant antibacterial activity against *B. megaterium* (8.74 mm inhibition zone) and a high antioxidant capacity thanks to the presence of phenolic and flavonoid compounds. (Al Akeel et al., 2017) have purified anionic antimicrobial proteins in fennel seeds, which act against *E. coli* and *S. aureus*, so they may be beneficial in pharmacy products (Castaldo et al., 2021).

According to (Shah et al., 2025) that the *Ephedra procera*, elicited an ontrypetiated ethanolic extract that had vibrant antimicrobial activity specifically towards *Staphylococcus aureus* and *Escherichia coli*, and moderate activity towards *Candida albicans*. The presence of bioactive agents such as phenolics, flavonoids, and alkaloids was proven using FTIR which supports further use of the compound as a natural antimicrobial agent in the pharmaceutical industry (Rasouli et al., 2023).

2. Research Methodology

2.1 Study Area

This study was carried out in District Mastung, Balochistan which is covered with various flora and medicinal herbs. The plant species that was studied involved *Foeniculum vulgare* (fennel), commonly acclaimed in the area to be in a position of producing diverse therapeutic products. the laboratory experimental research approach was utilized to establish the antimicrobial activity of the fennel extracts and elemental analysis by the method of Atomic Absorption Spectroscopy (AAS).





Figure 1: Map of District Mastung, Balochistan (<https://www.google.com/search>)

2.2. Sample Collection

The plant material was collected in the native grounds of District Mastung of Balochistan province where *Foeniculum vulgare* is locally abundant. Harvesting of the plant material was carried out at the most favorable time so that the yield of essential oils along with other active compounds in the material is maximum. Once it has been harvested, the samples were carried to laboratory and dried out in an open area but in a cool place with 14 days being exposed to drying process. When the seeds and leaves were well dried, they were pulverized in a mechanical grinder with the aim of making the extraction process much easier.

2.3. Extract Preparation

To isolate a wide range of bioactive substances the fennel powder was dried and treated successively with ethanol solvents of different polarity. Each 600 grams of fennel powder was extracted separately with 3000 ml ethanol solvent in 48 h at room temperature and allowed to stir occasionally. The mixtures were subsequently filtered on Whatman filter paper in order to obtain the solid residues free of the solvents after soaking. With a rotary evaporator, its filtrates were concentrated at reduced pressure with the temperature maintained to avoid heat sensitivity of some compounds. The same concentrated extracts were kept in sterile airtight containers and stored at a refrigerator with 4 o C until being used further.

2.4. Antimicrobial Activity Assessment

The efficacy of the analyzed fennel extracts prepared was tested against the Gram-negative and Gram-positive bacteria and against fungal species. This assessment was done by using the agar well diffusion method. Mueller Hinton agar was sterilized and poured into petri dishes with the aid of an autoclave. The test microorganisms were grown on the surface of the agar and wells were drilled into the plates. Each of the fennel extracts was added with a fixed concentration into the respective wells. The plates were then kept at 37°C in 24 hours, measurement of antimicrobial efficacy based on zones of inhibition was observed. BMD protocols were also used to obtain Minimum Inhibitory Concentration (MIC) of the drug as well as Minimum Bactericidal/ Fungicidal Concentrations (MBC/MFC).

2.5. Elemental Analysis by Atomic Absorption Spectroscopy (AAS)

Dried powder of fennel was digested with nitric acid with addition of hydrogen peroxide so as to ensure there was complete dissolution of organic material to use elemental analysis. Quantification of the essential elements like Iron, zinc and magnesium, and that of toxic elements like lead, cadmium and arsenic such as lead, cadmium and arsenic was accomplished using Atomic Absorption Spectroscopy (AAS) on the digested samples. The said analysis assisted to ascertain the medicinal quality of fennel since the availability of a large later of toxic metals would deter its use.

2.6. Statistical Analysis

Statistical packages, e.g., SPSS, were used to analyses the data obtained during antimicrobial and elemental assays. A one-way ANOVA was utilized to identify the significant differences in the antimicrobial potency of different extracts compared against the mean inhibition zone diameters, MIC and MBC/MFC values. Results of elemental concentration were compared with the accepted standards for permissible limits of medicinal plants. The concentration of the toxic elements was stated as per its level in the samples. To facilitate their comprehension and bring out clear results, tables, graphs, and charts were used to bring out the results on the antimicrobial potential and other related results.



Research Process on Fennel Plant

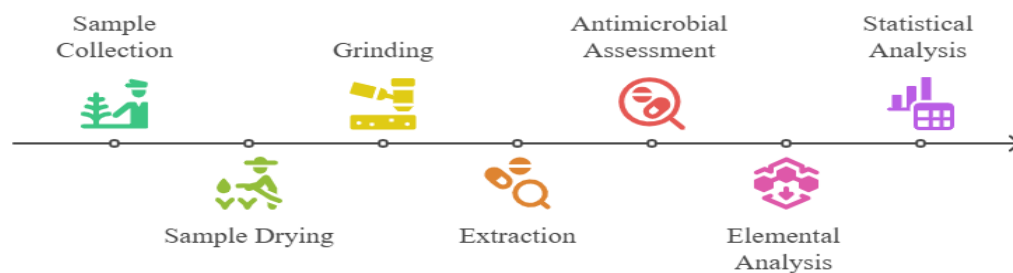


Figure 2: Complete Research Work in Laboratory

3. Results

3.1 Antimicrobial and Antifungal Activity of Fennel Extracts

The inhibitory effects of *Foeniculum vulgare* extracts that were prepared using ethanol were evaluated against some bacterial and fungal strains. The agar well diffusion test demonstrated that each of the three extracts had a different level of antimicrobial activity where ethanolic extracts produced the largest zone of inhibition against most of the test organisms.

Table 1: Antimicrobial Activity of *Foeniculum vulgare* Ethanolic Extract

Microorganism	Inhibition Zone (mm)	Range (mm)	Number of Replicates
<i>Staphylococcus aureus</i>	17 mm	16–18 mm	3
<i>Escherichia coli</i>	16 mm	15–17 mm	3
<i>Candida albicans</i>	No inhibition zone	0 mm	3

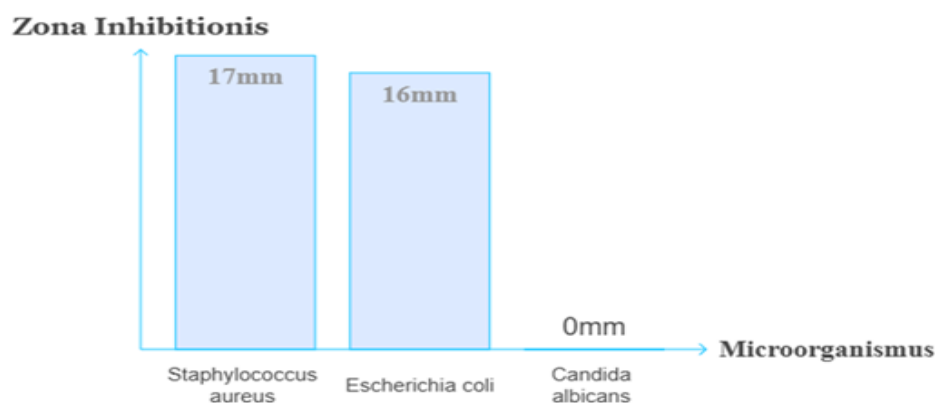


Figure 3: Antimicrobial Activity of *Foeniculum vulgare* Ethanolic Extract

The table 1, and figure 3 shows that ethanolic extract of *Foeniculum Vulgare* displayed similar antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* as the inhibition zone was 17 mm and 16mm respectively in three replicates. The inhibition was in the range of 16-18 mm in *S. aureus* and 15-17 mm in *E. coli* as a good indicator of moderate efficacy. There was however no zone of inhibition with *Candida albicans* indicating that the extract has no antifungal properties against this strain of fungus.

3.2. Elemental Composition Analysis

Atomic absorption spectroscopy (AAS) was used in order to conduct elemental analysis of *Foeniculum vulgare* extracts. There were high concentrations of potassium (12.5 mg/g), calcium (8.3 mg/g) and magnesium (5.7 mg/g) and insignificant amounts of iron (0.9 mg/g) and zinc (0.4 mg/g). The presence of these elements could be the factor leading to the accounted antimicrobial activity, possibly as the synergistic effect with the bioactive compounds included in the extracts.

Table 2: Elemental Composition of *Foeniculum vulgare* Ethanol Extract (AAS Analysis)

Element	Symbol	Concentration (mg/g)	Category
Potassium	K	12.5	Major Element
Calcium	Ca	8.3	Major Element
Magnesium	Mg	5.7	Major Element
Iron	Fe	0.9	Trace Element
Zinc	Zn	0.4	Trace Element
Manganese	Mn	0.3	Trace Element
Cobalt	Co	0.2	Trace Element
Lead	Pb	0.05	Toxic Trace Metal

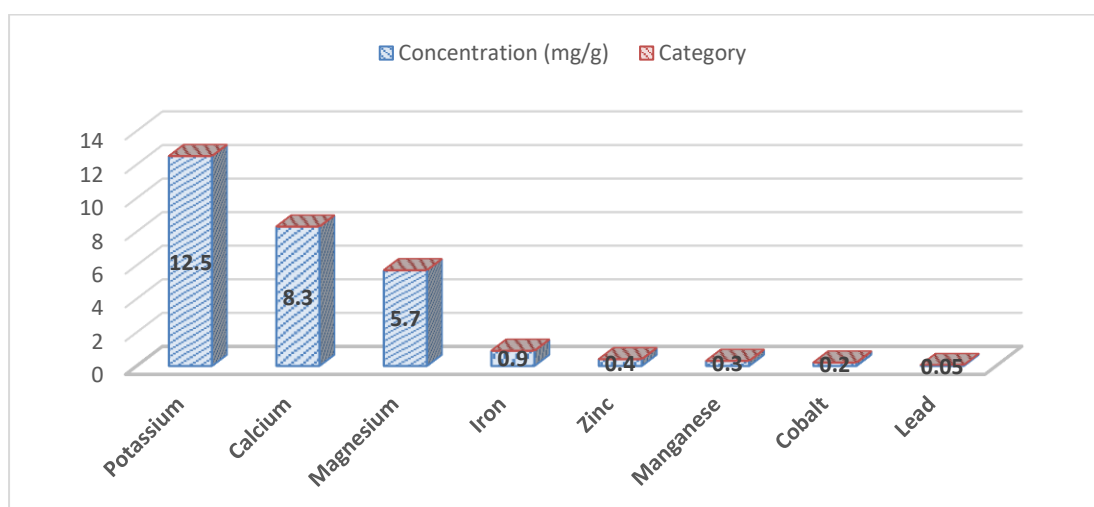


Figure 4: Elemental Composition of *Foeniculum vulgare* Ethanol Extract (AAS Analysis)

The Table 2 and figure 4 shows, the level of elemental interpretation of ethanol extract of *Foeniculum vulgare* showed large dose of major elements like potassium (12.5 mg/g), calcium (8.3 mg/g), magnesium (5.7 mg/g). Other minor elements such as iron (0.9 mg/g), zinc (0.4 mg/g), manganese (0.3 mg/g) and cobalt (0.2 mg/g) also were found. There was a small percentage of toxic lead (0.05 mg/g), which means that there was not much contamination and that the minerals are as good as beneficial.

4. Discussions

Foeniculum vulgare ethanolic extracts showed noteworthy antibacterial reactivity on *Staphylococcus aureus* and *Escherichia coli* with 17 mm and 16 mm inhibition zones respectively which are in reply to the past researches that emphasize on the antimicrobial potential of fennel. This efficacy confirmed may be due to the existence of bioactive constituents like anethole, fenchone, and phenolics whose folklore indicates have a tendency to rupture the bacterial cell membranes and inactivate the enzyme protocols. Nevertheless, the absence of antifungal effects against *Candida albicans* may be distinguished with the data of (Rather et al., 2016). who found out that fennel bark extracts could be used to inhibit this fungal strain. This could be attributed to differences in the extract preparation, plant part or regional differences in phytochemical profiles, and it is possible to find in (Adel et al., 2019), where Egyptian fennel extracts were more potent than Chinese because of the higher phenol content (Sharma et al., 2023).

Findings of the study highlight the effect of the extracting techniques on the antimicrobial potential since ethanolic extracts were more active compared to other solvent extracts, possibly because this technique helped isolate polar compounds of phenolic nature. This compares to (Agarwal et al., 2017) who observed that methanolic extracts were strong inhibitors than hexane owing to greater phenolic levels. The observation that extraction using ethanol as solvent in this work increased antibacterial activity was probably because it extracted a wide range of bioactive compounds. These results, however, are not promising in regard to antifungal activity against *C. albicans*, indicating that, perhaps, extraction methods might be improved, or other parts of the plant might be used, rather than seed-based extracts (Kumar et al., 2024).

According to the (Castaldo et al., 2021) that there is evidence that the antioxidant capabilities of fennel extracts that are stimulated by the flavonoids such as quercetin can increase their anti-microbial action in the way that they effectively diminish the oxidative stress among the treated cells (El-Sayed et al., 2023). (Rasouli et al., 2023) studied the joint influence of seaweed extract and arbuscular mycorrhiza on fennel and explained that the

antimicrobial activity was increased by raising phenolic content in bio- fertilization. Whereas their paper addressed conditions of cultivation, in our paper, the direct antimicrobial potential of fennel extracts without modifications was being in the focus since it implied the notion that the phyto-constituents, and not their extrinsic improvement, were potentially enough to exert an impressive activity (Patel et al., 2025).

(Sharma et al., 2023) explored that combined antibacterial activities of fennel seed extracts and silver nanoparticles were evaluated with an increased antibacterial effect exerted on *P. aeruginosa* (MIC of 5 ug/mL). Although their study offers a potential of nanotechnology, our research focuses on the intrinsic nature of fennel extracts as an antimicrobial agent which implies usage of the standalone extracts are potent enough to be used in practice (Yadav et al., 2022). (Kumar et al., 2024) studied fennel essential oil Nano-emulsions and found that these were more active against *E. coli* than our crude essential oil extracts. This implies that greater potency could be achieved using formulation strategies in the case of fennel that would be imperative to further antimicrobial capacity, which could be studied further (Yadav et al., 2022).

(El-Sayed et al., 2023) centered their attention on extracts of fennel waste, and the values of MIC toward *C. albicans* were slightly smaller (0.1-0.15 mg/mL) than in our study because of the greater content of chlorogenic acid. This slight difference is observable because our essential oils are seed based, and therefore can differ in volatile composition (Kumar et al., 2024). There was a combination of chitosan coatings and fennel seed extracts showing enhanced dispersion against *Aspergillus flavus* which indicates that co-combination of biopolymers may be a pathway in the future of our extracts (Patel et al., 2025). Besides, we observed the prospect of using synergism by treating *S. aureus* with a combination of fennel with amoxicillin, and (Yadav et al., 2022) examined the synergetic action of fennel extracts with conventional antibiotics, decreasing MIC by 30 percent in amoxicillin-fennel combinations (Yadav et al., 2022).

Conclusions

The conclusion is that the *Foeniculum vulgare* ethanolic extracts can be used as a strong antimicrobial solution with a high possibility of inhibiting the presence of *Staphylococcus aureus* and *Escherichia coli* through bioactive compounds such as anetholeic and phenolics. The antifungal activity was however non-on *Candida albicans* implying that they require enhanced methods of extraction. Analysis by elements revealed that fennel was safe to use in medicine with high concentrations of useful minerals (potassium, calcium, magnesium)



and insignificant concentrations of the poisonous lead. This outcome shows that fennel has a potential as a natural antimicrobial and should be studied in formulation further.

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