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PROBING THE BIOACTIVITIES OF TWO *FOENICULUM VULGARE* SPECIES – *FOENICULUM VULGARE VAR. VULGARE* (BITTER FENNEL) AND *FOENICULUM VULGARE VAR. DULCE* (SWEET FENNEL)

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ABSTRACT

Foeniculum vulgare (fennel) has a long history of use as a key spice and medicinal plant. It's two varieties; *Foeniculum vulgare.* var. *vulgare* (FVV) and *Foeniculum vulgare* var. *ducle* (FVD) were studied to investigate the chemical composition, antidiabetic, antimicrobial, antioxidant and cytotoxic activities in aqueous seed extracts. Total phenolic contents were 63.76 ± 4.54 mg GAE/100g for FVD and for FVV was 56.95 ± 3.16 mg GAE/100g. Total flavonoid content in FVD and FVV were of 111.83 ± 9.51mg CE/100g (p<0.05) and 86.02 ± 6.81 mg CE/100g respectively. Antioxidant and antidiabetic activities of FVD were 51.14 ± 0.36% and 45.63 ± 0.013% (p<0.05), respectively and were higher than those of FVV (43.04 ± 0.48%, 25.57 ± 0.31%). Both varieties have no zone of inhibition against *E.coli* while zone of inhibition by FVV and FVD was 12mm and 13mm against *S. aureus* respectively. The % hemolysis of FVD was 1. 41 ± 0.34 while that of bitter fennel was 1.11 ± 0.44. The Fourier Transformed Infrared Spectroscopy showed the presence of alcohol, carboxylic, alkanes, ethers, amines, sulfates, ketones and anhydrides etc. The results have demonstrated that both species reflect the potential as a natural therapeutic alternative to synthetic drugs. However, further studies are warranted to explore its efficacy in animal trials.

Key-words: Antioxidant, Cytotoxicity, Antimicrobial, Antidiabetic, *Foeniculum vulgare*, Alpha amylase

INTRODUCTION

Medicinal plants have an advantageous pharmacological impact on human health. The fennel herb, *Foeniculum vulgare* Mill. (Apiaceae) is grown all over the tropical and temperate climates of the globe for its fragrant seeds, which are used as edible spices. With a rich history of diverse uses, it has two species; sweet fennel (*Foeniculum vulgare var. dulce*) and bitter fennel (*Foeniculum vulgare var. vulgare*). Sweet fennel, having a pleasant aroma is used in Mediterranean and Asian cuisines due to its sweet taste. Bitter fennel, having a bitter taste and aroma is part of therapeutic formulations in traditional medicines. *Foeniculum vulgare var. vulgare* (FVV) spreads wildly while *Foeniculum vulgare var. dulce* (FVD) is cultivated extensively (Sanli and OK, 2023).

Fennel contributes largely to improving human health as it is a complex mixture with abundant bioactive compounds. The primary ingredients of fennel essential oils fenchone, anethole and estragole strongly influence their flavor. While anethole has a sweet and estragole has a bitter note (Rather *et al*., 2016). Antioxidant activity is the potential of any compound to neutralize hazardous effects of reactive oxygen species and free radicals. Antidiabetic activity is the efficacy of any substance to reduce hyperglycemia and to manage diabetes. Ability of a substance to damage cells is known as cytotoxicity. The chemical components of fennel like coumarins, monoterpenids, triterpenoids, glycosides, phenylpropanoids, flavonoids, phenols, amino acids, carbohydrates, minerals, fiber, vitamins A, E, C, chavicol and essential oils are responsible for pharmacological attributes especially antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, antiseptic, anti-diabetic, anti-ulcer, diuretic, and cardio-protective traits (Zafar *et al*., 2023; Ncube and Gupta, 2025).

The fennel seeds facilitate digestion and absorption in the gastrointestinal tract and modulate nervous, respiratory, renal, reproductive and hormonal systems. Fennel is utilized in treating a variety of ailments including irritable bowel syndrome, abdominal cramps, infantile colic, leucorrhoea, arthritis, diarrhoea, insomnia and constipation (Rafieian *et al*., 2024). Dermatological manifestations such as acne, puffiness and pigmented rashes, irritation are effectively reduced by fennel seeds. Fennel seeds alleviate tumours by inducing apoptosis in tumour cells through modulation of autocrine and paracrine signalling pathways (Misra *et al*., 2025).

Owing to its immense application in the pharmaceutical industry, fennel oldest and yet most important medicinal plant is acknowledged Worldwide. A review of the literature highlights several bioactive potentials

(Noreen *et al*., 2023). However, limited research is available regarding the comparative bio-analytical investigation of both bitter fennel and sweet fennel seeds. Therefore, current research was undertaken to evaluate phytochemistry, antidiabetic, antimicrobial, antioxidant and cytotoxic efficacies of FVD and FVV seeds.

MATERIALS AND METHODS

Sample Preparation

Foeniculum vulgare var. *vulgare* (FVV) and *Foeniculum vulgare.* var. *Ducle* (FVD) seeds were dried in shade and ground into fine powder. Aqueous extracts were prepared by heating in a microwave with very little radioactivity (low power: 100 W) for 30 seconds. This cycle was repeated thrice with an interval of three minutes. After filtration, the semisolid sample was made at ambient temperature. The extracts were then put into falcon tubes and stored in the refrigerator (Nawaz *et al*., 2023).

Antioxidant Profile

Total phenolic content (TPC) was measured with the Folin-Ciocalteu (FC) reagent. Test samples (125 µL), 100 µL Na_2CO_3 and 25 µL (10%) diluted FC reagent were mixed and incubated for 2 hours. Absorbance at 765 nm was taken to calculate TPC as mg gallic acid equivalents (GAE)/100g. The aluminium chloride colorimeter method was used to evaluate the TFC (total flavonoid content) and expressed in mg catechin equivalents (CE)/100g. Briefly, test samples (138 µL), $NaNO_2$ (9.5 mL) and distilled water (156 µL) were incubated (10 minutes). Absorbance at 510 nm was noted. The antioxidant capacity of samples (S) was measured by its DPPH (2,2-diphenyl-l-picrylhydrazyl) scavenging potential. DPPH solution (250 µL) was added to 2.5 µL plant extract and incubated for 35 minutes. Butylated hydroxytoluene was a positive control (C). Absorbance (A) at 517nm was used to calculate antiradical potential as: [C (A) - S (A)/C (A)]/100 (Jabeen *et al*., 2023).

Antidiabetic Activity

The starch-iodine assay was performed to evaluate the antidiabetic potential of extract samples (S). Test sample (30 µL) and catalyst (10 µL) alpha-amylase (Source: *Bacillus sp*.; E.C.3.2.1.1) were mixed, kept at room temperature (10 minutes) and 40 µL 1% starch solution was added for 30 minutes further incubation. After the addition of 1 M HCl (20µL) and 75 µL iodine solution, the absorbance (580 nm) was measured (Jabeen *et al*., 2023) with acarbose as a positive control (C). % inhibition = [1– A (C) /A (S)] × 100

Cytotoxicity Activity

Hemolytic assay was employed as described by Nawaz *et al*., (2023). Human blood was centrifuged (5 minutes) and washed thrice with PBS (phosphate buffer saline). Red blood cells suspension (180 µL) and 20 µL test sample (TS) were mixed and centrifuged. The 100 µL supernatant and PBS (900 µL) mixed. Triton X-100 and PBS were taken as +ve (CP) and –ve (CN) controls respectively. The absorbance (576 nm) was used to calculate cytotoxic potential: [A (TS) – A (CN)/A (CP)] × 100.

Antibacterial Activity

The antibacterial potential against two bacterial strains; *S. aureus* (gram +ve) and *E. coli* (gram –ve) was checked by well diffusion method. As a positive control, ciprofloxacin was used. After 16-18 hours of incubation at 35-37°C, the diameter of the zone of inhibition (mm) was calculated (Nawaz *et al*., 2023).

Structural Analysis - Fourier Transform Infrared Spectroscopy (FTIR)

The Bruker Tensor 27 FTIR spectrometer was used to conduct the FTIR analysis. The samples were finely powdered with potassium bromide with compression dye to form pellets for measurements in the 400-4000 cm^{-1} range (Hafeez *et al*., 2023). For baseline corrections, a background measurement was done to remove interferences from instrument signals, environmental gases and sample solvent. This background spectrum was used to deduct such unwanted signaling from sample spectrum.

Statistical analysis

Every assessment was done in triplicate. Using Minitab statistical software version 17, the T-test was used for data analysis with a level of significance $p<0.05$. Results are presented as mean ± S.E.

RESULTS

Microwave-assisted extraction technique was used for the preparation of aqueous extracts from seeds. The percentage yields were 20.82% and 19.4% for FVD and FVV species respectively.

Antioxidant Profile

Antioxidant activity was measured by using an aqueous extract of *Foeniculum vulgare Mill*. var. *vulgare* and *Foeniculum vulgare Mill*. var. *Ducle* (Table 1). Almost analogous TPC were observed FVD and FVV samples. While TFC was significantly higher in FVD than in FVV. Anti-radical activity was comparable among both species.

Antidiabetic Activity

The antidiabetic activity was measured by the potential of plant extracts in restriction of carbohydrate hydrolyzing enzyme alpha amylase. FVD was more potent than FVV as it showed significant ($p < 0.05$) enzyme inhibition (Table 1).

Cytotoxicity Activity

Toxicity of extracts towards erythrocytes was determined as percent hemolysis (breakdown). Both the species were non-toxic as minimal damage (1.11 ± 0.44 to 1.41 ± 0.34%) was observed as compared to standard Triton X-100 (Table 1).

Antibacterial Activity

Aqueous extract of both species was used to assess antimicrobial activity against *Escherichia coli* and *Staphylococcus* isolates (Fig. 1, Table 2). FVD and FVV had slight activity against *S. aureus*.

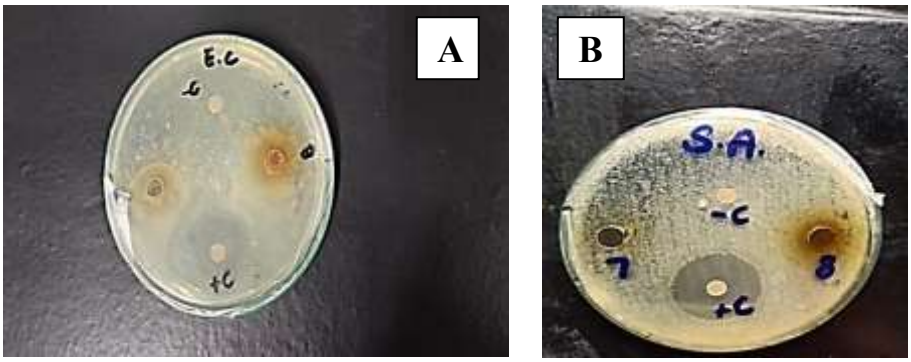

Fig.1. (A) Growth inhibition - *E.* coli, (B) Growth inhibition - *S. aureus*

Table 1. Antioxidant, antidiabetic and cytotoxic profile

Extract	TPC	TFC	DPPH	Antidiabetic Profile	Cytotoxic Profile
FVD	63.76 ± 4.54	111.83 ± 9.51*	51.14 ± 0.36	45.63 ± 0.013*	1.41 ± 0.34
FVV	56.95 ± 3.16	86.02 ± 6.81	43.04 ± 0.48	25.57 ± 0.31	1.11 ± 0.44
Control	-	-	89.63 ± 0.05	81.47 ± 2.78	94.07 ± 0.00

Data presented in mean percentage ± S.E. * Significant at $p < 0.05$. FVD: *Foeniculum vulgare.* var. *Ducle*, FVV: *Foeniculum vulgare* var. *vulgare*, TPC: mg GAE/100g, TFC mg CE/100g, DPPH: percentage (%), Controls: DPPH assay: ascorbic acid, anti-diabetic (alpha-amylase inhibitory) assay: acarbose, cytotoxic assay: Triton X-100

Table 2. Antibacterial activity

	Zone of inhibition (mm)	
Treatments	***E. coli***	***S. aureus***
FVD	00	13
FVV	00	12
Ciprofloxacin (positive control)	27*	28*

*p <0.05, average zone diameter is based on triplicate plates. FVD: *Foeniculum vulgare.* var. *Ducle*, FVV: *Foeniculum vulgare* var. *vulgare*

Table 3. Chemical Characterization

Foeniculum vulgare Mill. var. *Ducle*			*Foeniculum vulgare Mill.* var. *vulgare*		
Wave number (cm^{-1})	**Functional group**	**Type of bond**	**Wave number (cm^{-1})**	**Functional group**	**Type of bond**
3274.5	Alcohol, H-bonded, Primary and Secondary amines and amides	O-H N-H	3854.1 3801.9 3747.8 3691.9 3649.1	Alcohol	O-H
2920.4	Alkanes, Carboxylic acid,	C-H O-H	3268.9	H-bonded, Carboxylic acid, Primary and secondary amines and amides	O-H N-H
2849.5	Aldehyde, Carboxylic acid	C-H O-H	2918.5	Alcohol, Carboxylic acid, amine salt, Alkane	O-H N-H C-H
2163.7	Allenes, ketenes, isocyanates, isothiocyanates, Alkyne	X=C=Y C≡C	2849.5	Carboxylic acid, Alcohol, Alkane, Amine salt	O-H, C-H, N-H
1735.1	Aldehyde, Ester	C=O	2163.7	Thiocyanate, Alkyne, Allenes, ketenes, isocyanates, isothiocyanates	S-C≡N C≡C X=C=Y
1319.5	Sulfates, Amines	C-X S=O C-N	2111.5	Alkyne, Allenes, ketenes, isocyanates, isothiocyanates	C≡C X=C=Y
1589.7	Amines, amides,	N-H C=C	1735.1	Ester, Aldehyde	C=O
1418.3	Carboxylic acid, Alcohol	O-H	1593.4	Aromatic, Primary and secondary amines and amides	C=C N-H
1243.1	Sulfates, Amides, ketone, ethers, anhydrides	C-X S=O C-O	1412.7	Nitro, Alkanes	N=O C-H
1019.4	Amines, ethers, anhydrides, esters, alcohols	C-X C-N C-O	1321.8	Sulfates, sulfonamides	S=O
-	-	-	1243.1	Alcohols, amides, ethers, esters, anhydrides	C-O C-N
-	-	-	1008.2	Alcohol, ethers, esters, carboxylic acids,	C-X C-O

Fourier Transform infrared spectroscopy (FTIR)

The results of structural characterization are summarized in Fig. 2, 3 and Table 3.

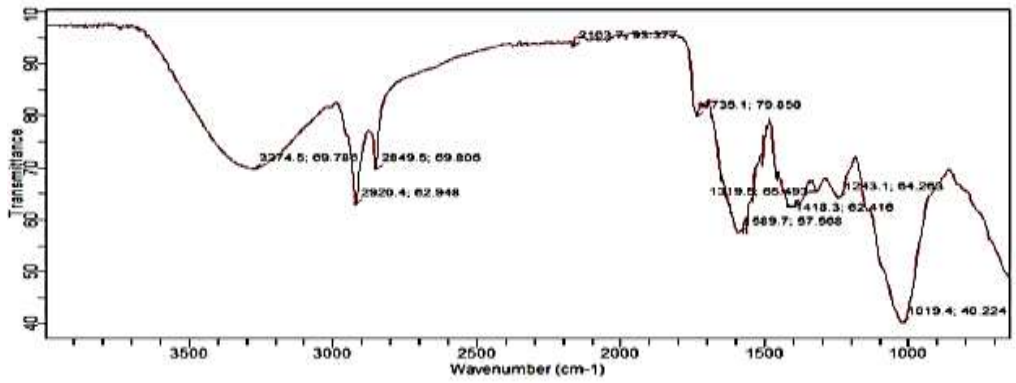

Fig.2. FTIR spectra of *Foeniculum vulgare Mill var. Ducle*

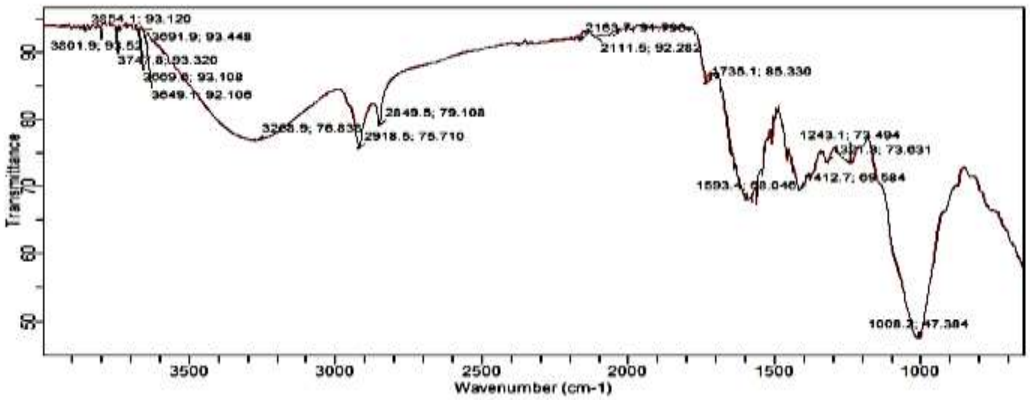

Fig.3. FTIR spectra of *Foeniculum vulgare Mill* var. *vulgare*

DISCUSSION

Antioxidant activity

The calculation of percentage yield in plant extraction is critical for several reasons. It indicates process efficiency, gaps to optimize the process and validation of the process with literature values. The percentage yields of FVD and FVV seed extracts were 20.82% and 19.4% respectively. Similar yield (17% aqueous extract) is reported by Zahi *et al*., (2025). While Khammassi *et al*. (2023) have stated contradictory results. They obtained a 9.7% yield of *Foeniculum vulgare* seeds methanolic extract.

The total phenolic content of FVD and FVV was observed to be 63.76 mg GAE/100g and 56.95 mg GAE/100g respectively. The total flavonoid content was 111.83 mgCE/100g and 86.02 mgCE/100g in both FVD and FVV varieties respectively. TFC in FVD was significantly higher (p<0.05) than TFC in FVV species. Variable figures are previously documented in the literature. Zahi *et al*., (2025) observed 196.6 ± 8 (µg gallic acid /10 mg extract dry weight) phenolic contents in butanol extracts of fennel seeds. Similarly, in their study, butanol extract exhibited 7.63 ± 0.05 (µg quercetin/10 mg extract dry weight) flavonoid contents. In another research, Abdellaoui *et al*, (2020) observed that the TPC of bitter fennel seeds was 222.24 mg GAE/100g. In current research, the anti-radical potential (DPPH radical scavenging potential) of *Foeniculum vulgare Mill*. var. *ducle* (sweet fennel) and *Foeniculum vulgare Mill*. var. *vulgare* (bitter fennel) were 51.14% and 43.04% respectively. Contrary to this, Karamat *et al*., (2024) observed 77.84% antioxidant activity of fennel. *vulgare*. Diverse extraction methods and solvents may be the reason for such contradictory results.

Regarding the anti-diabetic profile, significantly higher (p<0.05) enzyme inhibition was shown by FVD as compared to FVV. While positive control showed 81.47% inhibition. Earlier, Abu-Zaiton *et al*. (2015) studied *in vitro* alpha-amylase inhibition activity of *Foeniculum vulgare*. Alpha amylase inhibition was 82.43% by methanolic seed extract. Recently, methanol, ethyl acetate and aqueous samples of *Foeniculum vulgare* seeds showed 67.8 – 72.97 % inhibition of alpha amylase (Karamat *et al*., 2024).

Both test samples were non-toxic as the aqueous extract of FVD (sweet fennel) showed 1.41% hemolysis and the aqueous extract of FVV (bitter fennel) showed 1.11% hemolysis of RBCs. While positive control showed 94.07% hemolytic activity. The current results are supported by the previous studies. The methanolic extract of *F. vulgare* exhibited *in vitro* cytoprotective effects against healthy human blood cells (Anka *et al*., 2020). *F. vulgare* seed extract significantly inhibited hemolysis RBC (9.67±0.3%) compared to the reference drug (Cherbal *et al*., 2023). Lutviani *et al*., (2023) stated that ethanolic leaf extracts of *Foeniculum vulgare* had antihemolytic activity.

In the case of *staphylococcus aureus*, both plants FVD and FVV showed antimicrobial activity with growth inhibition zones of 13 mm and 12 mm respectively while the control had a 28 mm inhibition zone. Similarly, in case of *E. coli* both plants showed no antimicrobial activity and the control gave the inhibition zone of 27 mm. Lack of antibacterial action against *E. coli* can be due to several reasons. Susceptibility of tested strain against phytoconstituents, incubation time, pH, temperature, inadequate plant sample preparation (as some compounds may degrade or loss activity, during preparation, storage or testing phase) are some of the factors that can explain results of this assay.

In a study by Barrahi *et al*. (2020), the inhibition zones of aqueous extract of *Foeniculum vulgare Mill*. against *Escherichia coli* and *Streptococcus aureus* were 8 mm and 20 mm, respectively. While in current study, inhibitory

zone of fennel against *E.coli* was 0 mm while inhibitory zone against *S. aureus* was 13 mm. In another study, Kaveh *et al*. (2023) observed that the inhibition zone of methanol extract of fennel seeds against *Streptococcus aureus* was 7mm.

The powdered form of seeds of *Foeniculum vulgare Mill var. Ducle* IR spectra showed 10 peaks. The possible functional groups present include alcohol, carboxylic acid, alkanes, ethers, primary and secondary amines, sulfones, aldehydes, ketones and anhydrides. The wavenumbers were: 3274.5cm^{-1}, 2920.4cm^{-1}, 2849.5cm^{-1}, 2163.7cm^{-1}, 1735.1 cm^{-1}, 1589.7cm^{-1}, 1319.5cm^{-1}, 1418.3cm^{-1}, 1243.1cm^{-1}, 1019.5cm^{-1}. The FT-IR analysis done by various researchers has confirmed that functional groups like alcohols, alkynes, aromatic amines, carboxylic acids, and aldehydes are present in the crude extracts of *Foeniculum vulgare Mill*. var.*ducle.* When aqueous extracts of this plant were analyzed by Chen *et al.* (2022) it was revealed that alcoholic, phenolic hydroxyl groups and aromatic rings in excessive quantity are present that's why the plant is reported to possess the highest values of antioxidant activity.

Singh *et al*., (2024) studied the IR spectrum of fennel extract. Different peaks were shown at FTIR spectra which were associated with stretching vibrations between different functional groups.

Peak at 1407 cm-1 is due to bending of C-H bonds and at 1014 cm-1 peak indicates C-O extending. Both peaks are indicative of esters. The C= O stretching vibrations arise due to amide groups and exhibited peak at 1650 cm-1. Their FTIR spectrum showed a peak at 2932 cm-1 that is mostly associated with the extending vibrations in alkane compounds with C-H bonds. Similarly, another peak was present at 3178 cm-1 that is commonly associated with the presence of nitrogenous compounds. The presence of phenols and flavonoids was revealed by a peak at 3416 cm-1 due to hydrogen-bonded hydroxyl groups.

Conclusion

The results have demonstrated that both species reflect the potential as a natural therapeutic alternative to synthetic drugs, with *Foeniculum vulgare var. dulce* slightly better in biochemical evaluation than *Foeniculum vulgare var. vulgare*. It is concluded that the data have contributed to the available inferences about fennel's medicinal characteristics. However, further studies are warranted to explore its efficacy in animal trials.

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