

## STRUCTURAL CHARACTERIZATION, ANTIOXIDANT, ANTIDIABETIC AND ANTIMICROBIAL ACTIVITIES OF *TRACHYSPERMUM AMMI* AND *FOENICULUM VULGARE*

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**ABSTRACT:** The purpose of this study was to analyze the chemical composition, anti-diabetic, antioxidant and antibacterial properties of seeds of *Trachyspermum ammi* and *Foeniculum vulgare*. The flavonoid and phenolic contents among both plants were significantly varied ( $P < 0.05$ ). The methanolic extract of *T. ammi* and *F. vulgare* showed the highest scavenging ability of DPPH (60.0798% and 77.84431%) respectively. It was discovered that the growth of Gram-positive bacterial strains was significantly inhibited by *T. ammi* and *F. vulgare* with an estimated inhibition zone of 11 mm and 13 mm respectively, against *staphylococcus* strains. In case of gram-negative bacteria both plants showed no inhibition zone. Aqueous extract of *T. ammi* and ethyl acetate of *F. vulgare* showed maximum percentages for antidiabetic activity as determined by glycation inhibition assay. High Performance Liquid Chromatography showed that both plants contain gallic acid and quercetin as major chemical compounds. FTIR of *Trachyspermum ammi* showed the presence of compounds like alcohol, phenols, carboxylic acid, Sulfones, sulfonyl chlorides, sulfates, sulfonamides, primary and secondary amines and amides and aromatic compounds. Similarly, in *Foeniculum vulgare* compounds like alcohols, phenols, aldehyde, carboxylic acid, ether, ester, anhydrides and amines were identified.

**Key words :** Antioxidant ; Antibacterial ; Antidiabetic ; HPLC ; FTIR.

### INTRODUCTION

Nature tends to be an incredible indicator of the widespread phenomenon of interdependence. The demand for medicinal plants is high and their popularity is growing day by day (Yusupova *et al.*, 2023). Plants have medicinal value because of bioactive compounds that have significant physiological effects on the human body. Medicinal plants drug research employs a widespread approach that incorporates botanical, phytoconstituents, biochemical and molecular approaches (Gul *et al.*, 2023).

*Trachyspermum ammi* generally called Ajwain, is herbaceous plant in the Apiaceae family. Ajwain seeds were traditionally used in a variety of diseases and were widely administered by traditional healers. The dried seeds were also examined for their pharmacological properties due to their various chemical constituents (Noreen *et al.*, 2023). It is known to aid digestion, improve gastrointestinal discomfort and exhibit antimicrobial and anti-inflammatory effects. Additionally, they are known for their analgesic and respiratory health benefits, providing relief from coughs, asthma and bronchitis (Awais Hanif *et al.*, 2021).

Thymol, -terpinene and p-cymene are main phytochemicals found in extract made from *T. ammi* seeds. Its seeds are consumed in herbal medicine to treat gastrointestinal problems such as indigestion, flatulence and diarrhea as these have significant digestive and antimicrobial potential (Plaskova and Mlcek, 2023). Thymol and carvacrol, two components of ajwain extract, exhibit antimicrobial potential against a broad range of microorganisms. Because of the unique contribution of ajwain oil and extract components, it is a great alternative for food storage to avoid food deterioration caused by microbes (Islam *et al.*, 2023).

Fennel or *Foeniculum vulgare* (Apiaceae), is a well-known medicinal and sweet-smelling plant which is mainly used as a flavoring agent, digestive and hypertensive, as well as in the treatment of respiratory and digestive problems (Jadid *et al.*, 2023). Seeds of the plant are also used in bakery products, meat and in sea food recipes as flavourings (Goswami *et al.*, 2022). The main phytochemicals of this plant have been found as phenols and phenolic glycosides that are volatile in nature such as estragole and fenchone. The ability of Fennel to produce antimicrobial, antioxidant and antihyperglycemic effects has been demonstrated in various *in-vitro* and *in-vivo* bioactivities, proving the logic behind its various

pharmaceutical uses. The antioxidant activity of *F. vulgare* is due to phenolic compounds extracted from the plant (Sabzi-Nojadeh *et al.*, 2021).

The current study was designed to investigate the antibacterial, antioxidant and antidiabetic effects of different extracts of these plants, to develop plant based natural medicine for the various pathophysiological diseases.

## MATERIALS AND METHODS

**Preparation of Plant Extracts:** Powder samples of both plants were subjected to maceration. This was performed in three solvents viz. ethyl acetate, methanol and aqueous by using definite sample to solvent ratio. Solvents were placed on shaker for six hours. Extracts were separated by filtration and were placed on water bath for evaporation (Yaqoob *et al.*, 2023).

### Antioxidant activity

**TPC:** The phenolic contents of a 96-well plate were determined using the Folin-Ciocalteu reagent. 200  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> solution, 250  $\mu$ L of test samples and 50  $\mu$ L of diluted reagent (10%; 50  $\mu$ L) were added. The absorbance was measured at 750 nm following a two-hour incubation period. The results were expressed in mg GAE/100g (Nawaz *et al.*, 2023).

**TFC:** The flavonoid contents were determined by the AlCl<sub>3</sub> colorimetric method. In a 96-well plate, add the test sample (38  $\mu$ L each), 9.5  $\mu$ L NaNO<sub>2</sub>, and 156  $\mu$ L aqueous sample for 10 minutes. After incubating the solution for 5 minutes with 9.5  $\mu$ L of NaNO<sub>2</sub>, 19  $\mu$ L of 10% AlCl<sub>3</sub> was added and it was again incubated for approximately 5 minutes. The absorbance was taken at 510 nm. The readings were compared to a standard curve and results were expressed as mgCE/ 100g (Nawaz *et al.*, 2023).

**DPPH:** Antioxidant potential was assessed by the ability to scavenge DPPH. After adding 2.5  $\mu$ L of plant extract and covering along with the aluminum foil for 35 minutes, 250  $\mu$ L of DPPH mixture (0.004 mg DPPH in 100 mL methanol) was added. Using a spectrophotometer, the absorbance was measured at 517 nm. The calculations were taken in triplicates.

The following formula was used to determine the % DPPH scavenging capability:

$$[A(\text{control}) - A(\text{sample}) / A(\text{control})] \times 100,$$

Where A (control) and A (sample) are the absorbance of the control and sample. Plotting percentage inhibition against concentration curve gave the IC<sub>50</sub> (half-maximal inhibitory concentration (Nawaz *et al.*, 2023).

**Antibacterial Activity:** Antibacterial activity was assessed by using the Agar well diffusion method, Sample was prepared in Eppendorf tube by mixing 5mg

ethyl acetate extract in 1mL DMSO solution. Agar solution was prepared by adding 2.66g agar in 70mL water into two separate flasks. Agar solution and petri plates were autoclaved. Temperature of agar solution was cool down to 40°C and 100 $\mu$ L bacterial strains, *staphylococcus aureus* gram+ive in one flask and *E. coli* gram-ive in another flask was added. Then, two petri plates in laminar air flow were filled with bacteria-containing agar solution, and the solution was kept to solidify. After that, three wells were formed in gel by using tip (1mL) of micropipette and 100 $\mu$ L of both samples were poured onto the two wells in both plates and third well was filled with ciprofloxacin as positive control. For 18 hours, plates were incubated in incubator. Diameter of inhibition zone was then measured (Barrahi *et al.*, 2020).

### Antidiabetic activity

**Antiglycation potential:** A reaction solution comprising 10 mg of bovine serum albumin (BSA) and 100 mg of D-glucose was added to 67 mM sodium phosphate buffer (pH 7.2) and placed at 37 °C to measure the antiglycation potential. 150  $\mu$ L of the plant sample and 1 ml of the above reaction solution were placed in an eppendroff, where they were incubated for two days. Incubated solution and 200  $\mu$ L of distilled water were added to a 96-well plate. The absorbance was measured at 440 nm for the emission wavelength and 370 nm for the excitation wavelength using a spectrophotometer (BioTek, Winooski, VT, USA). For the control a blank solution of D-glucose was used. Metformin was used a reference substance (Ali *et al.*, 2022).

Calculation of % inhibition:

$$\% \text{ inhibition} = [A_{(440 \text{ nm})} / A_{(370 \text{ nm})} - A_{(440 \text{ nm})}] \times 100$$

**Alpha-Amylase Inhibition Assay:** Prepared 0.1 % alpha amylase solution (1g alpha amylase + 10 ml phosphate buffer solution). 10  $\mu$ L of bacterial alpha amylase and 30  $\mu$ L of plant sample were added in 96 well plate and incubated for 10 minutes. 40  $\mu$ L of one percent (1%) starch solution was added and incubated for 30 min. Before measuring the absorbance at 580 nm in comparison to a control, each well was filled with 20  $\mu$ L of 1M HCl and 75  $\mu$ L of iodine solution (Nawaz *et al.*, 2023). It was calculated by using this formula:

$$\% \text{ inhibition} = 1 - A(\text{control}) / A(\text{sample}) \times 100$$

### Structural Analysis:

**Fourier Transform Infrared Spectroscopy (FTIR):** For structural characterization, FTIR was used. The sample was bireduced in a chloroauric solution. Later that, it was centrifuged at 10,000 rpm for 15 minutes. The formed pellets were rinsed thrice by using 20 mL deionized water to wash out unbound proteins or enzymes. The materials were dehydrated and processed

in a potassium bromide pellet Mill. The analysis was performed using a thermo Nicolet model 6700 in diffuse reflectance mode at a resolution of 4 cm. Detailed scan of 512 were conducted to get the intentional suitable signal/noise ratio (Mirniyam *et al.*, 2022).

#### High-Performance Liquid Chromatography (HPLC):

*Trachyspermum ammi* and *Foeniculum vulgare* were hydrolyzed and their dried seed powder were used. 20 mL of ethanol with 1 g/L BHT and 0.5 g of dry material were combined to make a combination. 10 mL of 1M hydrochloric acid was then added. After thorough mixing, sonication was done for 15 min. After injecting sample, readings were taken at 280 nm. Different compounds were identified using their retention periods (Mirniyam *et al.*, 2022).

**Statistical Analysis:** The data underwent ONE WAY ANOVA analysis, which compares more than two population means at the time (Montgomery, 2019).

## RESULTS

**Antioxidant activity:** Table 1 shows comparative analysis of antioxidant potential of *T. ammi* and *F. vulgare* among their different activities. Values given as (mean  $\pm$  SD) or as a percentage of measurements taken in triplicate. Gallic acid equivalents per milligram (mg) of total phenolic contents (TPC) in 100 g of dry weight. The number of total flavonoids (TFC) in g of catechin equivalents per 100 g of dry weight. The ratio of 2,2

diphenyl 1-picrylhydrazyl's capacity to scavenge free radicals. In *T. ammi*, highest total phenolic contents (TPC) were observed in ethyl acetate extract while in *F. vulgare*, highest total phenolic contents were observed in methanolic extract. Highest total flavonoids contents (TFC) were observed in aqueous extract in *T. ammi* while in *F. vulgare* it was observed in methanol extract. Ethyl acetate extract of both plants showed the highest DPPH radical scavenging activity.

**Antibacterial activity:** With a positive control displaying a 39mm inhibitory zone, the antibacterial activity of *Trachyspermum ammi* and *Foeniculum vulgare* was assessed against *Escherichia coli* and *Staphylococcus aureus* as evident in Table 2. Both plant extracts showed no zone of inhibition against *E. coli*, whereas *T. ammi* demonstrated an 11mm inhibition zone against *S. aureus*, and *F. vulgare* exhibited a slightly larger inhibition zone of 13mm against the same bacterial strain.

**Antidiabetic Potential:** The antidiabetic potential of *T. ammi* and *F. vulgare* extracts was evaluated for antiglycation and  $\alpha$ -amylase inhibition activities as depicted in table 3. Results showed that aqueous extract of *T. ammi* exhibited significant antiglycation activity (70.26%) while in *F. vulgare*, ethyl acetate extract showed highest antiglycation activity (81.54%). *F. vulgare* methanol extract showed higher  $\alpha$ -amylase inhibition (72.97%) than *T. ammi* (30.73%).

**Table 1: Antioxidant potential of *T. ammi* and *F. vulgare***

Extracts	<i>T. ammi</i>			<i>F. vulgare</i>		
	TPC (GAE mg/g)	TFC (mg/g)	DPPH %	TPC (GAE mg/g)	TFC (mg/g)	DPPH %
Ethyl acetate	605.482 $\pm$ 3.83	7.14545 $\pm$ 6.54	60.079	636.272 $\pm$ 1.90	141.509 $\pm$ 1.37	77.844
Methanol	438.114 $\pm$ 9.79	86.4182 $\pm$ 4.94	42.115	669.167 $\pm$ 8.03	648.661 $\pm$ 042.46	70.758
Aqueous	302.939 $\pm$ 4.59	215.9394 $\pm$ 0.37	16.96	399.43 $\pm$ 14.97	541.1147 $\pm$ 11.47	63.37

**Table 2: Antibacterial activity of *Trachyspermum ammi* and *Foeniculum vulgare*.**

Bacterial Strains	Plant Extract	Positive Control	Inhibition zone
<i>Escherichia coli</i>	<i>Trachyspermum ammi</i>	39mm	None
	<i>Foeniculum vulgare</i>		None
<i>Staphylococcus Aureus</i>	<i>Trachyspermum ammi</i>	39mm	11mm
	<i>Foeniculum vulgare</i>		13mm

**Table 3: Antidiabetic potential of *T. ammi* and *F. vulgare***

Extract	<i>T. ammi</i>		<i>F. vulgare</i>	
	Antiglycation %	$\alpha$ -amylase inhibition %	Antiglycation %	$\alpha$ -amylase inhibition %
Ethyl acetate	61.009	30.33	81.54	70.85
Methanol	23.11	30.73	75.92	72.97
Aqueous	70.26	30.39	81.425	67.8
Control	76.57		76.57	

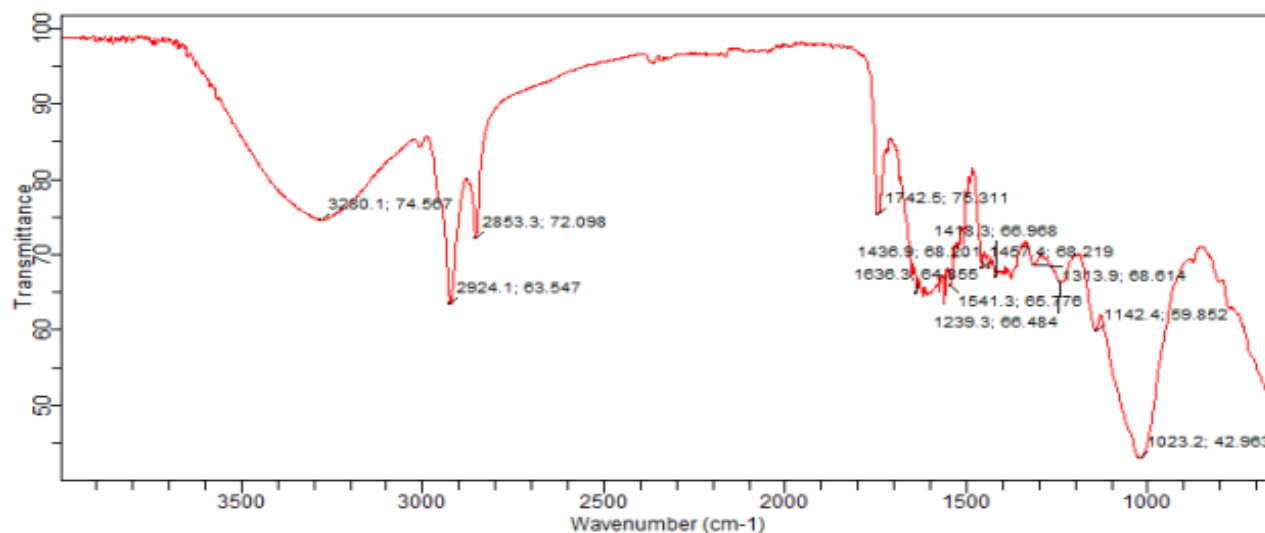
## Structural Characterization

### FTIR Analysis

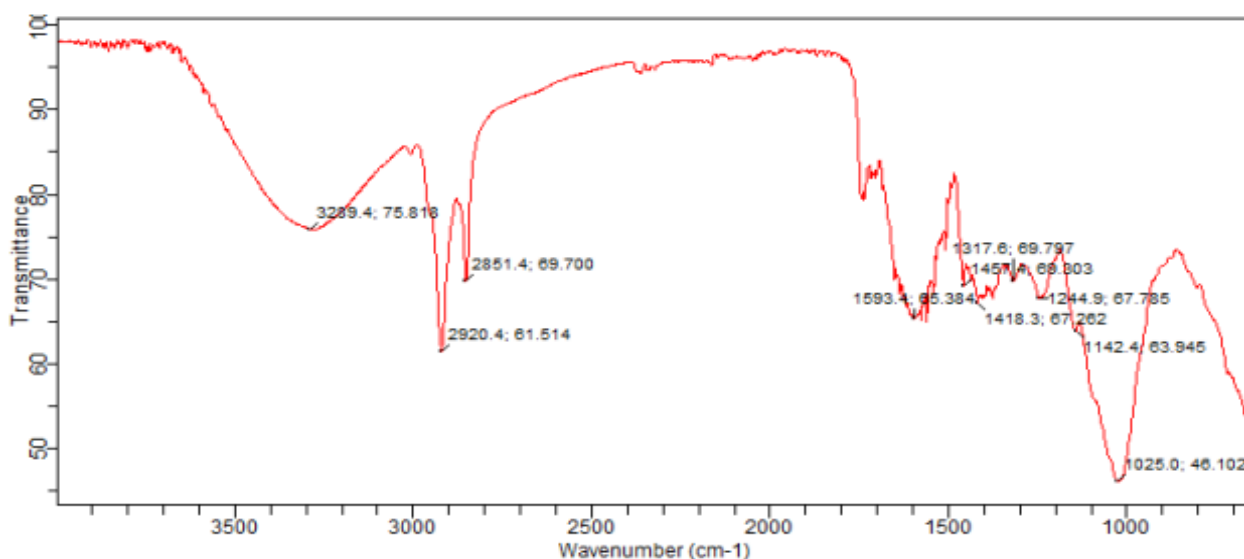
**FTIR spectra of *Trachyspermum ammi*:** The FTIR analysis of *T. ammi* plant revealed characteristic peaks indicating the presence of various functional groups and compounds. Peaks at 3280.1 cm<sup>-1</sup> represented O-H and N-H stretching in alcohols, phenols, carboxylic acids and amines. Other peaks highlighted C-H stretching in alkane, aldehyde and carboxylic acid groups (2924.1 cm<sup>-1</sup>, 2853.3 cm<sup>-1</sup>), C=O stretching in esters (1742.5 cm<sup>-1</sup>) and C=C stretching in alkene and amide compounds (1636.3 cm<sup>-1</sup>, 1541.3 cm<sup>-1</sup>). Furthermore, peaks at lower wavenumbers revealed the existence of several functional groups in compounds comprising

fluoride, alcohols, ethers, esters, carboxylic acids and amines, including C-O, C-N, S=O and C-X.

**FTIR spectra of *Foeniculum vulgare*:** *F. vulgare*'s FTIR study showed distinctive peaks that were associated with a number of functional groups and chemicals. Notably, peaks at 2920.4 cm<sup>-1</sup> and 2851.4 cm<sup>-1</sup> represented C-H stretching in alkane, aldehyde and carboxylic acid groups and those at 3289.4 cm<sup>-1</sup> suggested O-H stretching in alcohols, phenols and carboxylic acids. Additionally, lower wavenumber peaks (e.g., 1317.6 cm<sup>-1</sup>, 1244.9 cm<sup>-1</sup>, 1142.4 cm<sup>-1</sup>, 1025.0 cm<sup>-1</sup>) indicated various functional groups such as C-N, S=O, C-O and C-X in amines, sulfones, sulfates, alcohols, ethers, esters, carboxylic acids and fluoride containing compound. Additionally, peaks at 1593.4 cm<sup>-1</sup> suggested the presence of aromatic compounds.



FTIR spectra of *Trachyspermum ammi* seeds.



FTIR spectra of *Foeniculum vulgare* seeds.

Table 4: Structural analysis of *T.ammi* and *F. vulgare* by FTIR.

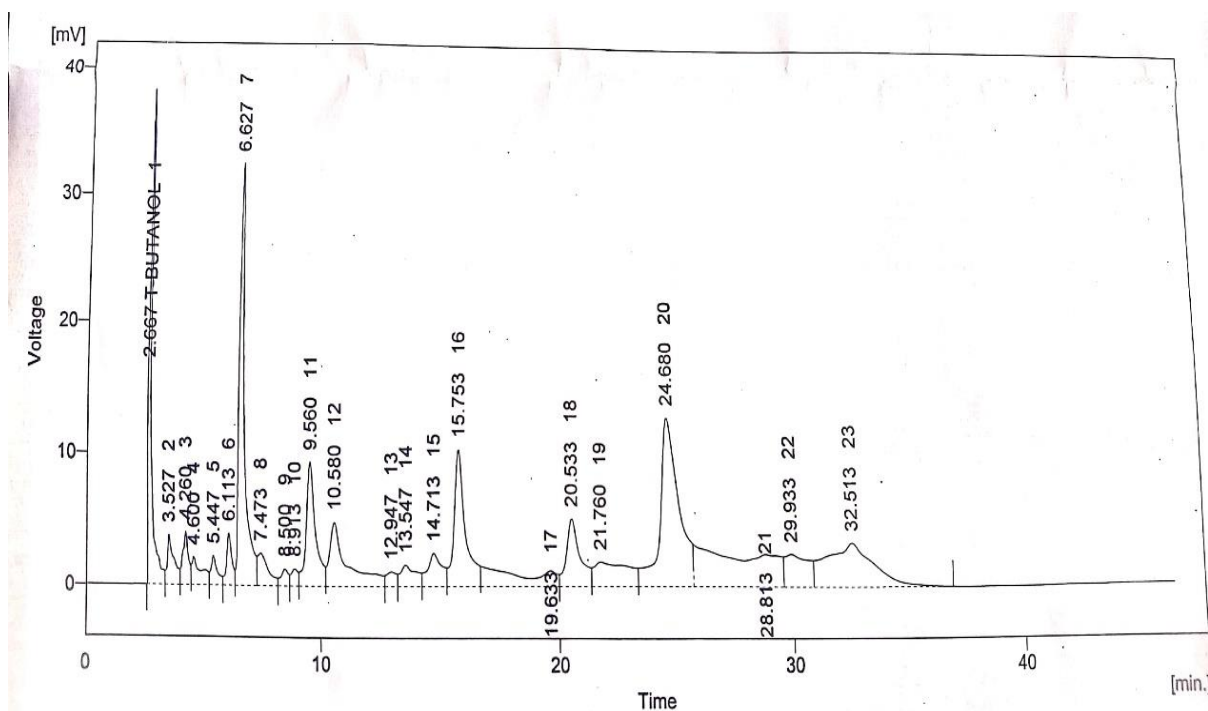
<i>Trachyspermum ammi</i>			<i>Foeniculum vulgare</i>		
Characteristic peak	functional group	Compound	Characteristic peak	functional group	Compounds
3280.1	O-H O-H N-H stretched	Alcohols, phenols bounded Carboxylic acid Primary, secondary Amines & Amines	3289.4	O-H	Alcohols, phenols, H bounded
2924.1	C-H stretched	Alkane	2920.4	O-H N-H stretched	Alcohols, phenols, carboxylic acids Primary, secondary Amines & Amines
2853.3	O-H C-H stretched	Carboxylic acid Alkane	2851.4	C-H stretched	Alkane
1742.5	C-H O-H C=O	Aldehyde Carboxylic acid Ester	1593.4	O-H C-H stretched	Alcohols, phenols, carboxylic acids Alkane
1436.9	C-H bend	-CH <sub>3</sub>	1317.6	C-H O-H C=C	Aldehyde Carboxylic acid Aromatic
1636.3	C=C C=O N-H bend	Alkene Amide Primary, secondary Amines & Amines	1457.4	N-H C-N S=O C-X N=O	Primary, secondary Amines & Amines Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride Nitro (R-NO <sub>2</sub> )
1541.3	C=C N=O	Aromatic Nitro (R-NO <sub>2</sub> )	1418.3	C-H bend	-CH <sub>3</sub>
1418.3	N=O C-H bend N=O	Nitro (R-NO <sub>2</sub> ) -CH <sub>3</sub> Nitro (R-NO <sub>2</sub> )	1244.9	N=O C-O S=O C-N C-X	Nitro (R-NO <sub>2</sub> ) Alcohols, esters, carboxylic acid, ethers anhydrides Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride
1239.3	C-O C-X S=O	Alcohols, ethers, carboxylic acid, esters, anhydrides Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride	1142.4	C-O S=O C-X C-N	Alcohols, esters, ethers carboxylic acid, anhydrides Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride
1313.9	C-N S=O C-X	Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides fluoride	1025.0	C-O C-X C-N	Alcohols, ethers, carboxylic acid, esters, anhydrides Amines Fluoride
1142.	C-O S=O C-X C-N	Alcohols, ethers, carboxylic acid, esters, anhydrides Amines Sulfones, sulfonyl chloride, sulfates. Sulfonamides Fluoride			
1023.2	C-O C-X C-N	Alcohols, ethers, carboxylic acid, esters, anhydrides Amines Fluoride			

### HPLC Analysis

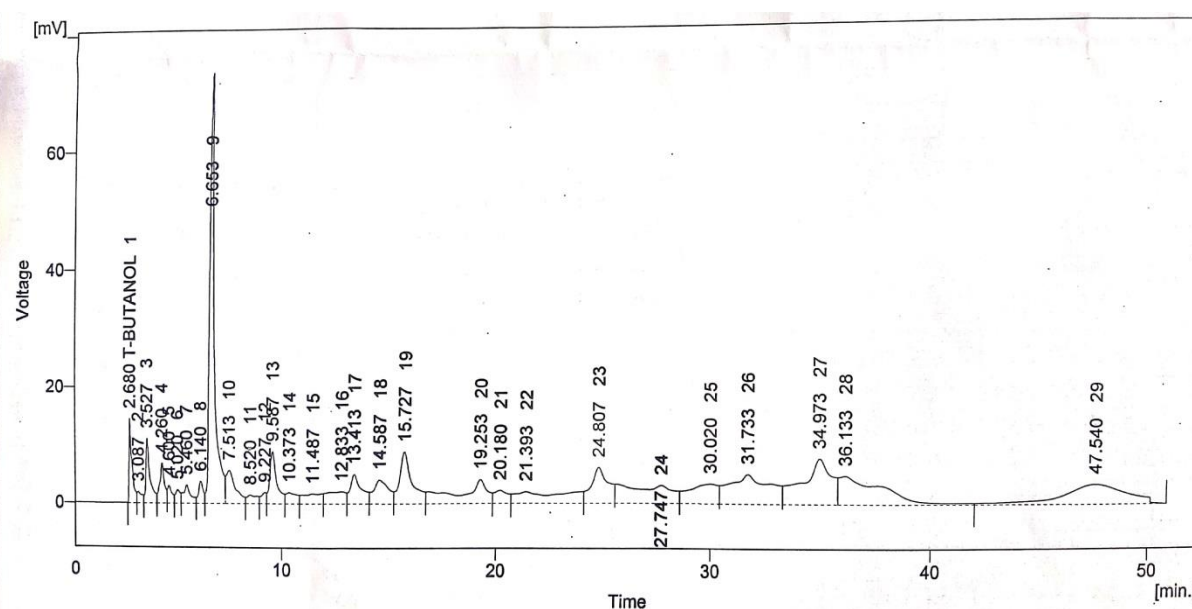
**HPLC Spectra of *T. ammi*:** Quercetin (18.2105 ppm), Gallic acid (2.5154 ppm), Caffeic acid (1.4546 ppm), Vanillic acid (4.4648 ppm), Benzoic acid (11.0046 ppm), Chlorogenic acid (26.3468 ppm), M-coumaric acid (2.4507 ppm), Ferulic acid (13.4992 ppm) and Cinnamic acid (25.0542 ppm) are significant compounds found in

the HPLC analysis of *T. ammi*. The retention time and peak areas for both discussed plants are given in table 5.

**HPLC Spectra of *F. vulgare*:** Quercetin (7.6471 ppm), Gallic acid (3.9292 ppm), Caffeic acid (5.9190 ppm), Vanillic acid (11.4260 ppm), Benzoic acid (20.7617 ppm), Chlorogenic acid (27.4675 ppm), M-coumaric acid (1.1256 ppm) and Cinnamic acid (11.4084 ppm) were the main chemicals for *F. vulgare* found in HPLC.



HPLC Spectra of *T. ammi*



HPLC Spectra of *F. vulgare*

Table 5: Structural analysis of *T.ammi* and *F. vulgare* by HPLC.

<i>Trachyspermum ammi</i>				<i>Foeniculum vulgare</i>			
Compound name	Retention time (min)	Peak area (mV.s)	Amount (ppm)	Compound name	Retention time (min)	Peak area (mV.s)	Amount (ppm)
Quercetin	7.6	343.594	18.2105	Quercetin	2.680	144.280	7.6471
Gallic acid	4.260	69.871	2.5154	Gallic acid	4.260	109.142	3.9292
Caffeic acid	12.947	31.620	1.4546	Caffeic acid	12.833	128.673	5.9190
Vanillic acid	13.547	72.021	4.4648	Vanillic acid	13.413	184.290	11.4260
Benzoic acid	14.713	103.817	11.0046	Benzoic acid	14.587	196.855	20.7617
Chlorogenic acid	15.753	337.779	26.3468	Chlorogenic acid	15.727	352.147	27.4675
M-coumeric acid	20.553	204.225	2.4507	M-coumeric acid	20.180	93.799	1.1256
Ferulic acid	24.680	715.833	13.4992	Cinnamic acid	24.607	325.952	11.4084
Cinnamic acid	24.680	715.833	25.0542				

## DISCUSSION

In our study, we assessed the phytochemical composition and bioactive properties of *Trachyspermum ammi* and *Foeniculum vulgare*. Regarding total phenolic content (TPC), Dutta *et al.* (2021) reported varying yields across extraction solvents for *T. ammi*, with methanol demonstrating the highest extraction yield ( $325 \pm 14.6$  to  $1860 \pm 31.2$  mg GAE/100g). In contrast, Oueslati *et al.* (2016) found the aqueous extract of *T. ammi* to exhibit the highest TPC (162.17 mg GAE/g DW), while our study showed the ethyl acetate extract yielding the highest TPC ( $605.482 \pm 3.83$  mg GAE/100g). TFC content in *T. ammi* ranged from 5.94 mg QE g<sup>-1</sup>DW in the study of Mirniyam *et al.* (2021) to 797.17 mg QE ml<sup>-1</sup>. Eabed *et al.* (2018) reported TPC of  $7.78 \pm 0.12$  mg GAE/g dw in *F. vulgare*, contrasting with our study where the ethyl acetate extract displayed a TPC of  $669.167 \pm 8.03$  mg GAE/100g. TFC values for *F. vulgare* ranged from 5.17 to 16.42 mg QE/gDE (Khammassi *et al.*, 2022), while our methanolic extract showed the highest TFC ( $648.661 \pm 42.46$  mg QE/g). Bajpai *et al.* (2015) ethanolic seed extract of *Trachyspermum ammi* (ESETA) showed the antioxidant capacity as the inhibition of DPPH radical by 73.41% while our ethyl acetate extract showed 60.079%. Mata *et al.* (2007) observed DPPH radical scavenging activity for Fennel water extract giving maximum value  $48.0 \pm 0.1$  while our study showed 77.844% DPPH radical scavenging activity of ethyl acetate extract.

Assessing the antimicrobial activity, Javed *et al.* (2012) found significant inhibition of various bacterial strains by *T. ammi* extracts, with our study demonstrating 11mm inhibition against *Staphylococcus aureus*. Mahboubi *et al.* (2011) observed 21.9-24.1 mm inhibition of *S. aureus* by Fennel oil extract, whereas our study exhibited a diameter of 13mm. In another study by

Anwar *et al.* (2009) Fennel essential oils exhibited considerable anti-microbial activity against *Escherichia coli* 14± 1 while in our study no activity was shown. For  $\alpha$ -glucosidase inhibition, Kaskoos (2019) reported values ranging from  $19.70 \pm 0.67$  to  $75.63 \pm 0.24\%$ , while our study demonstrated 61% inhibition by the ethyl acetate extract. Similarly, for  $\alpha$ -amylase inhibition, Ardeshtarijani *et al.* (2019) found a 55% inhibition by methanolic extract, contrasting with our study where the ethyl acetate extract displayed 30.73% inhibition. Godavari *et al.* (2018) showed  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition by Fennel seed extracts, whereas our study demonstrated antiglycation of 81.54% by the ethyl acetate extract and  $\alpha$ -amylase inhibition of 72.97% by the methanolic extract.

Our study's FTIR analysis supports the previous research. Functional groups identified include alcohol, aldehyde, amine, nitro and alcohol, consistent with Singh *et al.* (2017). Similar findings were observed at peaks of 1142.4 cm<sup>-1</sup>, 2853.3 cm<sup>-1</sup>, 3280.1 cm<sup>-1</sup>, 1541.3 cm<sup>-1</sup>, and 1023.2 cm<sup>-1</sup> in our study. Sahu *et al.* (2015) observed C-H stretching in alkane at 2987.84 cm<sup>-1</sup>, similar to our findings at 2924.1 cm<sup>-1</sup>. Khan *et al.* (2020) described peaks ranging between 3009 to 2836 cm<sup>-1</sup>, corresponding to aldehyde, consistent with our observations. Peaks attributed to O-H stretching and C=O align with our study's strong band at 1742.5 cm<sup>-1</sup> and 1636.3 cm<sup>-1</sup>. Arasu *et al.* (2021) observed C-O stretch at 1317.22 cm<sup>-1</sup> and 1414.22 cm<sup>-1</sup>, similar to our findings at 1239.3 cm<sup>-1</sup> and 1418.3 cm<sup>-1</sup>. Additionally, Kulkarni *et al.* (2014) identified various functional groups at different peaks, showing our observations, validating the FTIR analysis consistency. Our FTIR analysis differed slightly from Hussein *et al.* (2016) results, with peaks observed at 1418.3 cm<sup>-1</sup> for -CH<sub>3</sub> and Nitro (R-NO<sub>2</sub>), 1244.9 cm<sup>-1</sup> for ethers, esters, carboxylic acid and 1025.0 cm<sup>-1</sup> for carboxylic acid, anhydrides, amines and fluoride, among others.

Salami *et al.* (2016) identified polyphenolic components in Fennel, including Quercetin, 1,5-dicaffeoylquinic acid, rosmarinic acids, chlorogenic acid, caffeic acid, p-coumaric acid, rutin and ferulic acid, consistent with our findings. Satti *et al.* (2009) reported a retention time (RT) of 13.767 for benzoic acid and 16.650 for chlorogenic acid, which aligns closely with our findings of 14.533 for benzoic acid and 15.673 for chlorogenic acid. However, syringic acid was not identified in previous studies. Additionally, our study identified phenolic acids and flavonoids in *T. ammi*, further supporting its phytochemical diversity.

**Conclusion:** *T. ammi* and *F. vulgare*, which have a variety of therapeutic characteristics, are used as traditional medicines all over the world and are also utilized as phytomedicine to treat bacterial and viral infections as well as for the cure of diabetes. Quercetin, gallic acid, caffeic acid, vanillic acid, syringic acid, cumeric acid, benzoic acid, chlorogenic acid, p.coumaric acid, ferulic acid and cinnamic acid were all present in *T. ammi* and *F. vulgare* plants, which both showed more powerful medicinal effects. This study cleared the path for efficient and affordable *in-vitro* bioassays of several plants' active constituents. *T. ammi* and *F. vulgare* could be employed as dietary supplements because of their great potential for further research in both animal and human studies. Additionally, these therapeutic plants have the potential to be significant natural antioxidant and antibacterial agent sources. In order to uncover more public health benefits and therapeutic applications in the medical field, the bioactive elements of these plants must also be isolated, purified and studied.

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