





## Research Article

# Comparative Study of Antioxidant, Antidiabetic, Cytotoxic Potentials, and Phytochemicals of Fenugreek (*Trigonella foenum-graecum*) and Ginger (*Zingiber officinale*)

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*Trigonella foenum-graecum* and *Zingiber officinale* are used as traditional medicinal plants for the treatment of infectious and inflammatory diseases. However, a comparative analysis and bioactivities of *T. foenum-graecum* and *Z. officinale* lack some necessary information for therapeutic purposes. This study was designed to evaluate the biochemical characterizations and biological efficacy of *T. foenum-graecum* and *Z. officinale* as antioxidant, antidiabetic, anti-amnesic, and cytotoxic agents. Antioxidant activity was determined by DPPH free radical scavenging assay. Antidiabetic potentials were evaluated by glycation, alpha-amylase, and acetylcholinesterase inhibition assays. We performed biochemical characterization through analyses of high-performance liquid chromatography (HPLC) and FTIR (Fourier transform infrared spectroscopy). Results revealed that total phenolic contents (TPCs) (g GAE/100 g) of *T. foenum-graecum* and *Z. officinale* were  $5.74 \pm 0.81$  g and  $6.15 \pm 0.06$  g, respectively, and total flavonoid contents (TFCs) varied from  $1.51 \pm 0.58$  g CE/100 g to  $17.54 \pm 0.58$ . DPPH scavenging potentials of *T. foenum-graecum* and *Z. officinale* extract were 50.27% and 88.82%, respectively. Antiglycation potentials of *T. foenum-graecum* and *Z. officinale* showed a maximum activity at 16–29% and 96%. Alpha-amylase and alpha-glucosidase inhibition ranged from 9.43–24.95 and 10.52–27.89 and 54.97%, respectively. All the test samples of *T. foenum-graecum* and *Z. officinale* showed acetylcholinesterase inhibition potential at 0.37–46.88%. HPLC analysis of *T. foenum-graecum* revealed the presence of quercetin, gallic acid, caffeic acid, vanillic acid, syringic acid, and cumeric acid, while *Z. officinale* revealed the quercetin, gallic acid, vanillic acid, benzoic acid, chlorogenic acid, p.Coumaric acid, ferulic acid, and cinnamic acid. FTIR analysis revealed the presence of aldehydes, ketones, aromatic compounds, amines, and carbonyl groups in *T. foenum-graecum*, while alcohol, alkane, alkene, ketone, amine, and ether are bioactives present in the methanolic extract of *Z. officinale*. It was concluded that a comparative analysis of *T. foenum-graecum* and *Z. officinale* showed that *Z. officinale* showed higher therapeutic effects.

## 1. Introduction

Different synthetic drugs and chemical compounds have been used for the treatment of infectious diseases. However, the application of phytomedicines has increased attention recently due to their therapeutic advantages over allopathic

medicines like bioavailability, high solubility, and fewer side effects [1]. The extensive use of plant-derived medicines and medicinal plants in traditional cultures globally has enhanced the incorporation of phytochemicals into contemporary products for disease treatment and health promotion [2]. Medicinal plants contain secondary metabolites, also

known as phytochemicals, which encompass a large variety of natural products, including phenolics, flavonoids, alkaloids, glycosides, saponins, steroids, and tannins [2].

*T. foenum-graecum* is used as a traditional medicinal plant around the world due to its diverse nature of phytoconstituents, such as steroids, saponins, diosgenin, gito-genin, glycosides, hydrocarbons, amino acids, and gingerol [3]. Along with it, *Z. officinale* is also used as a phytomedicine for the treatment of infectious and metabolic diseases [4]. *Z. officinale* is a rich source of bioactive compounds, electrolytes, vitamins, volatile oily components, gingerols, heptanoids, alkaloids, sulphates, steroidal derivatives, and glycosides isolated [5]. Due to the presence of these bioactive compounds and secondary metabolites, *T. foenum-graecum* and *Z. officinale* are potentially employed for the treatment of inflammatory and infectious diseases.

Ginger (*Z. officinale*) is well known for its potential against several diseases like cancers, hepatocellular carcinoma [6], diabetes mellitus [7], osteoarthritis, and myocardial infarction [8]. *Z. officinale* is also used for bacterial infections, boosts immunity, and enhances gastrointestinal functions [9]. Several studies revealed *Z. officinale* exhibited high potential in reducing oxidative stress and thus minimized the production of free radical species in living tissues [10]. It also activates the different genes responsible for suppressing tumor function, thus exhibiting anticancerous potential [11, 12].

*T. foenum-graecum* is used as a medicinal plant for the treatment of diabetes mellitus [13], rheumatic arthritis, injuries, muscular weakness, throat infections, hypertension [14], neurological disorders, and cardiac diseases [15]. Several studies showed the androgenic and anabolic effects of *T. foenum-graecum* in human reproductive physiology. The hydrolysates proteins obtained from the rhizome extract *T. foenum-graecum* showed anticancer potential as they minimized the levels of reactive oxygen species. Hydrolysates are a rich source of protein and are also used for the treatment of colorectal cancers [16].

Keeping in view of the literature update, we hypothesized that a comparative analysis of extracts of *T. foenum-graecum* leaf and *Z. officinale* rhizome might add sufficient knowledge to the scientific data. The biological potential of *T. foenum-graecum* leaf and *Z. officinale* rhizome lack the necessary information for therapeutic applications. However, this type of comparative study approach was not reported in the literature before. This study aimed to evaluate *in vitro* antioxidant, antidiabetic, and anti-amnesic activities of *T. foenum-graecum* leaf and *Z. officinale* rhizome. In addition, we performed biochemical characterization through high-performance liquid chromatography (HPLC) and FTIR (Fourier transform infrared spectroscopy) for the identification of bioactive compounds and functional groups.

## 2. Materials and Methods

**2.1. Plant Materials.** *T. foenum-graecum* (fenugreek) leaves and *Z. officinale* rhizome samples were bought from the vendor market of Faisalabad and were identified by the Department of Botany, University of Agriculture Faisalabad [17].

**2.2. Preparations of Extracts.** Extracts of ground rhizomes and leaves were prepared by using *n*-hexane, ethanol, and methanol, along with water extract. The first extraction was carried out by maceration method in methanol for three consecutive days with repeated filtrations. The semisolid final extract was dissolved in water and then fractionated into ethanol and *n*-hexane solvents [18].

**2.3. Total Phenolic Content (TPC).** Total phenolic contents were measured by following the Folin-Ciocalteu method [18]. In this method, the reaction mixture was prepared by dissolving the 1.58 mL distilled water into 20  $\mu$ L test samples. Then, 3 mL Na<sub>2</sub>CO<sub>3</sub> (1% w/v) was mixed into the reaction mixture, and incubation was carried out at 25°C for 10 minutes. At wavelength 750 nm, the blue color compound was produced, which showed the highest absorption, and it indicated the number of phenolics present in test samples.

**2.4. Total Flavonoid Content (TFC).** Total flavonoid contents were measured by following the aluminium trichloride (AlCl<sub>3</sub>) colorimetric method [19]. In this method, plant extract (50  $\mu$ L) was mixed with 160  $\mu$ L of NaNO<sub>2</sub>, along with 1.26 mL of distilled water, and incubation was carried out at 25°C for 10 minutes. Then, 1 mL of NaOH and 10% 300  $\mu$ L of AlCl<sub>3</sub> were added to the reaction mixture. The absorbance was measured at 510 nm, which indicated the number of flavonoids present in test samples.

**2.5. DPPH Radical Scavenging Assay.** The antioxidant potential of extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Extracts and 5 mL DPPH were mixed together and were kept at 25°C temperature for 30 minutes. Butylated hydroxytoluene was used as a standard [20]. The antioxidant activity was calculated by using the following formula:

$$\% \text{DPPH inhibition} = 100 \times \left[ \frac{\text{A Blank} - \text{A Sample}}{\text{A Blank}} \right] \quad (1)$$

### 2.6. Antidiabetic Activity

**2.6.1. Glycation Inhibition Assay.** Test samples, D-glucopyranose (100.0 mg) and serum protein (10.0 mg), were mixed together into a 67 mM solution of sodium phosphate and maintained at pH 7.2. The reaction mixture was then incubated at 37°C for 48 hours. Absorbance was measured at 540 nm by using the spectrophotometer. Samples without D-glucose and metformin were used as controls [21]. The % inhibition was calculated as follows:

$$\% \text{Inhibitory Activity} = 100 \times \left[ \frac{\text{Abs c} / \text{Abs ex} - \text{Abs c}}{\text{Abs c}} \right] \quad (2)$$

**2.6.2. Alpha-Amylase Inhibitory Activity.** Alpha-amylase inhibition activity of extracts was measured by using the colorimetric method. In this method, 500  $\mu$ L of alpha-amylase enzyme (porcine pancreatic) was prepared into

0.02 M buffer maintained at pH 6.9 and the test samples (500  $\mu$ L) were incubated at 25°C for 10 minutes. Then, 1 mL of dinitrosalicylic acid (DNS) reagent was added to the reaction mixture and heated in a boiling water bath for 30 minutes. Buffer was used as negative control, and the acarbose drug positive was used as a positive control. Absorbance was measured at 540 nm by using the spectrophotometer [20]. The % inhibition was calculated as follows:

$$\% \text{ Alpha - Amylase inhibition: } (Ac - As/Ac) \times 100, \quad (3)$$

where Ac is absorbance of the control and As is absorbance of test samples.

$$\text{Acetylcholinesterase (AChE) inhibition: Absorbance (Control - Sample)/Absorbance control} \times 100. \quad (4)$$

## 2.7. Chemical Characterization

**2.7.1. HPLC Analysis.** HPLC was performed for the identification of different compounds in plant extracts. HPLC analysis was performed at Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan, under chromatographic conditions solvent A (acetic acid, water 6 : 94) and solvent B (acetonitrile). Shim-pack CLC ODS (C-18) column was used as a stationary phase with a 1 mL·min<sup>-1</sup> flow rate. Phenols and flavonoids were identified at 280 nm on the basis of comparison (retention times of peaks) of plant samples [23].

**2.7.2. FTIR Analysis.** Fourier transform infrared spectroscopy was performed for the structural identification of functional groups of different compounds in extracts. A thin film was prepared by mixing the powdered samples and potassium bromide and infrared spectrum were measured at 4 cm<sup>-1</sup> resolution and 25°C temperature. OPUS software was used to measure the IR spectra. The results were compared with standards for the detection of active groups [24].

**2.7.3. Statistical Analysis.** Data were subjected to analysis of variance (ANOVA), and a comparison between the means of two activities was carried out by using SPSS with the level of *p* significant at <0.05. Data were finally expressed as mean  $\pm$  SD or percentage.

## 3. Results and Discussion

### 3.1. Chemical Characterization

**3.1.1. HPLC Analysis.** Table 1 and Figure 1 show the different compounds in the extract of *Z. officinale* identified by the HPLC chromatogram. The HPLC analysis revealed the existence of phenolic acids and flavonoids, i.e., quercetin (18.17 ppm), gallic acid (2.71 ppm), vanillic acid (5.68 ppm), benzoic acid (15.93 ppm), chlorogenic acid (17.22 ppm), p-coumaric acid (1.16 ppm), ferulic acid (3.13 ppm), and cinnamic acid (0.37 ppm). Table 2 and Figure 2 show the

**2.6.3. Acetylcholinesterase Inhibitory Activity.** Acetylcholinesterase inhibitory activity of plant extracts was measured by mixing Ellman's reagent, phosphate buffer, and acetylcholinesterase. The reaction mixture was incubated at 25°C for 10 minutes. Then, a substrate (acetylcholine iodide) was added to the reaction mixture and absorbance was measured at 412 nm [22]. Extraction solvent was used as a negative control, whereas physostigmine was used as a positive control. Percentage acetylcholinesterase (AChE) inhibition was calculated by following the formula:

different compounds in the extract of *T. foenum-graecum* identified by the HPLC chromatogram. Quercetin was determined at the peak of 3.073, having a concentration of 4.12 ppm. While gallic acid, caffeic acid, vanillic acid, syringic acid, and M. cumeric acid were determined at peaks 4.487, 12.340, 12.973, 15.507, and 19.733, respectively, having concentrations of 0.43 ppm, 0.57 ppm, 0.61 ppm, 0.15 ppm, and 0.14 ppm.

Our results are agreed with the previous studies. A recent study reported that luteolin (15.80%), apigenin (14.41%), and glycosides (12.02%) were identified in the rhizome of *Z. officinale* [25]. Another study revealed that nonacylated compounds, apigenin (8.82%) and kaempferol (9.61%) derivatives and acylated apigenin (6.59%), glycosides (6.59%), and luteolin (5.60%), were screened in *Z. officinale*, respectively. These flavonoids were found to be the major compounds with 87.80% of the total identified compounds. The other compounds were present at lower relative percentages [26]. Another study revealed that quercetin and kaempferol isolated *T. foenum-graecum* leaves with concentration values of 12.6 mg/g and 11.2 mg/g, respectively [27].

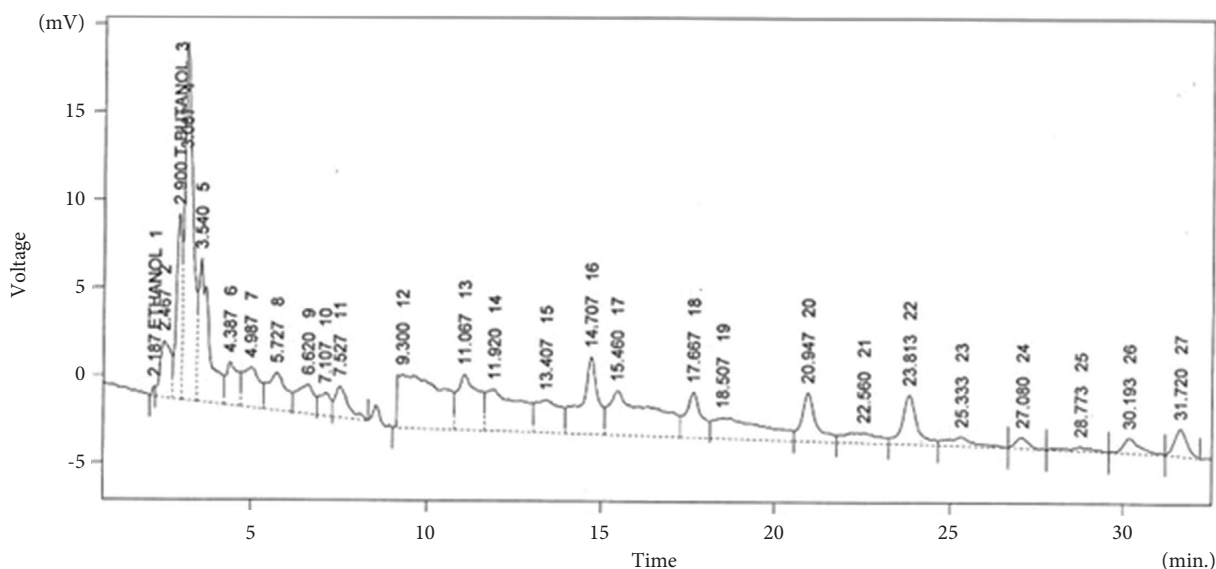
**3.1.2. FTIR Analysis.** Table 1 and Figure 3 show the different peaks of functional groups in the extract of *Z. officinale* identified by FTIR analysis. The broad peak of the O-H and N-H bond at 3276.3 cm<sup>-1</sup> indicates alcohols and secondary amines. Peaks at 928.1 cm<sup>-1</sup> as well as 991.5 cm<sup>-1</sup> and 2926.0 cm<sup>-1</sup> elucidate alkenes and alkanes, which projected out of plane bending due to =C-H and stretching of CH<sub>2</sub>, respectively. Table 2 and Figure 4 show the different peaks of functional groups in the extract of *T. foenum-graecum* identified by the FTIR analysis. Three functional groups were identified at medium peak, four at strong peak and one functional group at weak peak type in the range of 1000–3000. In medium peak type C-H, N-H, N-H, and O-H functional groups were identified at peaks 2900, 3400, 1530, and 1440, respectively, whereas in strong peak type functional groups -CH, N-O, S=O, and C=C were determined at

TABLE 1: Chemical characterization of *Zingiber officinale*.

Sr.no	Compound name	HPLC	
		Peak area	Amount (ppm)
1	Quercetin	341.037	18.17
2	Gallic acid	75.336	2.71
3	Vanillic acid	91.680	5.68
4	Benzoic acid	150.354	15.93
5	Chlorogenic acid	220.818	17.22
6	p-Coumaric acid	81.639	1.16
7	Ferulic acid	42.235	3.13
8	Cinnamic acid	28.439	0.37

Sr.no	Wave number (cm <sup>-1</sup> )	FTIR	
		Functional group	Type of bond
1	3276.3	Secondary amide, alcohol	N-H stretching, O-H stretch
2	2926.0	Alkane	-CH <sub>2</sub> stretching
3	1636.3	Alkene, primary amines, water, ketone	C=C stretching, N-H bending, H-O-H, C=C-C=O
4	1148.0	Amine, alcohol	C-N, O-H stretching
5	1075.3	Amines, alcohol	C-N stretching, C-O stretching
6	991.5	Alkene	=C-H
7	928.1	Alkenes	=C-H
8	861.0	Ether	C-O stretching

FIGURE 1: Representative HPLC chromatogram of *Zingiber officinale*.

peaks 2820, 1520, 1190, and 990, respectively. Only one peak type was weak, identified at 1800 with functional group C-H. This data indicated the presence of polyphenols, alcohols, and carboxylic compounds. In a previous study, aldehyde and ketone functional groups were identified at 1705 cm<sup>-1</sup>.

According to the previous studies, *Z. officinale* exhibited wave numbers at 3443, 2970, 1475, 1439, 1386, and 1238 (cm<sup>-1</sup>) due to C-H/O-H stretch, C=C (aromatic), C-N (nitrile), C-O (acid and ester stretch), respectively [28]. A recent study revealed that *Z. officinale* exhibited N-H stretch of proteins, a symmetric stretch of C-O of COO groups, CH<sub>2</sub> bending of lipids and symmetric and asymmetric stretch of P=O of nucleic acids at 3290, 1236, 1400, and 1236 wave

numbers (cm<sup>-1</sup>), respectively [29]. Previous studies showed that the carbonyl group was found at 1790 cm<sup>-1</sup> in *T. foenum-graecum* leaves extract. Aromatic functional groups, including C-H, O-H, and N-H were identified at a spectral range of 3800 cm<sup>-1</sup>–2600 cm<sup>-1</sup> [30]. A recent study revealed that recognized peaks within similar spectral ranges that suggested the presence of methylene, carbonyl, and phenolic compounds similar to the current study [31].

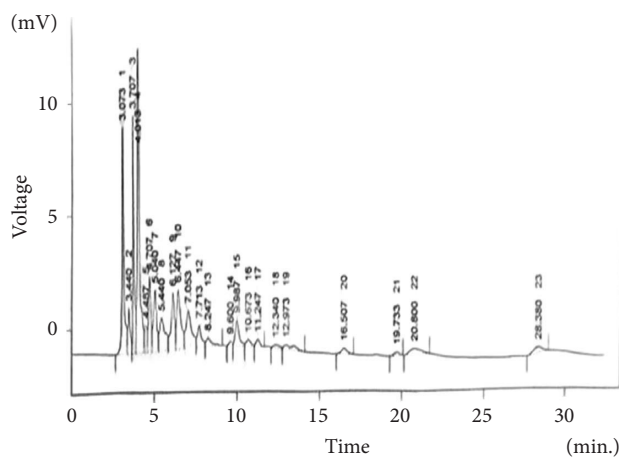
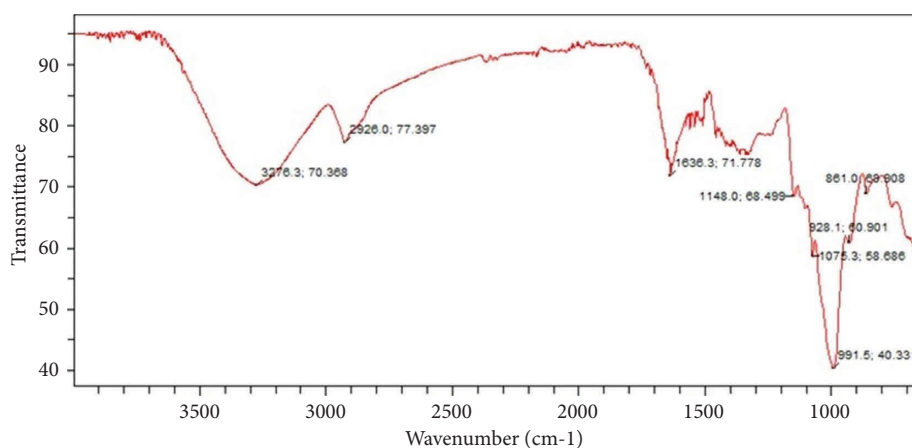
**3.1.3. Antioxidant Activity.** Table 3 shows that TPC in the extracts of *Z. officinal* rhizome lies in the range of 3.16 ± 0.77–6.15 ± 0.06 (GAE/100g), while Table 4 shows

TABLE 2: Structural data analysis of *Trigonella foenum-graecum* leaves.

Retention time	Area (mV. s)	HPLC		Compound name
		Area (%)	Amount (ppm)	
3.07	77.54	12.2	4.12	Quercetin
4.49	12.09	1.9	0.43	Gallic acid
12.34	3.58	0.5	0.57	Caffeic acid
12.97	9.26	1.5	0.61	Vanillic acid
15.51	5.24	1.0	0.15	Syringic acid
19.73	3.75	0.5	0.14	m.Cumeric acid

Frequency range	Wave number	FTIR		
		Peak type	Functional group	Type of bonding
2840–3000	2900	Medium	-CH	Saturated -CH
2800–3000	2820	Strong	-CH	Saturated -CH
3400–3300	3400	Medium	N-H	N-H stretching
1550–1500	1520	Strong	N-O	N-O stretching
1440–1395	1440	Medium	O-H	O-H bending
1200–1185	1190	Strong	S=O	S=O stretching
995–985	990	Strong	C=C	C=C bending
1870–1540	1800	Weak	C-H	C-H bending

FIGURE 2: HPLC chromatogram of *Trigonella foenum-graecum* leaves.FIGURE 3: Representative FTIR spectrum of *Zingiber officinale*.

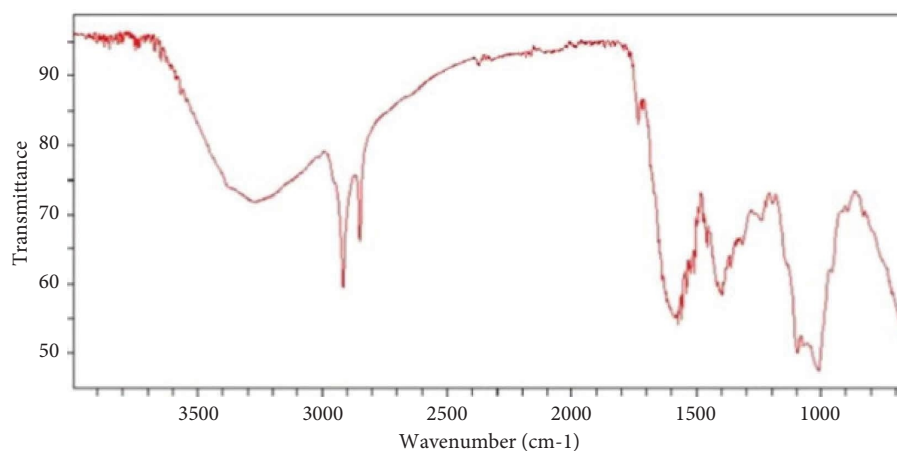


FIGURE 4: Representative FTIR spectrum of *Trigonella foenum-graecum* leaves.

TABLE 3: Different extracts and biological efficacies of *Zingiber officinale* rhizome.

Activities	Methanol	Ethanol	<i>n</i> -hexane	Aqueous	Control
TFC	10.04 ± 0.18	9.52 ± 0.10	7.09 ± 0.14	9.93 ± 0.09	—
TPC	5.56 ± 0.82	6.15 ± 0.06	3.75 ± 0.92	3.16 ± 0.77	—
DPPH (IC <sub>50</sub> )	21.11	88.82	38.14	50.23	90
Glycation	67	96	20	69	53
Alpha-amylase	28	54.97	6.01	15.27	13.58
AChE	40.8	17.3	46.88	0.37	59.51

Data expressed as mean ± SD or percentage. TPC: total phenolic contents expressed as g gallic acid equivalents/100 g dry weight; TFC: total flavonoid contents expressed as g catechin equivalents/100 g dry weight; DPPH: 2,2-diphenyl 1-picrylhydrazyl. Positive controls: BHT; butylated hydroxytoluene (antioxidant activity), metformin (antiglycation assay), glucobay ( $\alpha$ -amylase inhibitory assay), and physostigmine (AChE inhibitory assay).

TABLE 4: Different extracts and biological efficacies of *Trigonella foenum-graecum* leaf.

Sample	ME	EE	NHE	AE	Control
<i>Antioxidant contents and activity</i>					
TFC	2.38 ± 0.26	1.88 ± 0.31	1.51 ± 0.58	2.93 ± 0.09	—
TPC	6.23 ± 0.76	6.18 ± 0.06	5.75 ± 0.86	5.74 ± 0.81	—
DPPH	32.56	45.78	38.14	50.27*	90
<i>Antidiabetic activity—percentage inhibition</i>					
Glycation	29*	23	16	20	53
Alpha-amylase	24.95*	17.93	11.30	9.43	48.20
<i>Antiamnesic activity</i>					
AChE inhibition	14.08	8.37	4.62	10.37	59.51

Data expressed as mean ± SD or percentage of triplicate measurements. \* Significant at  $p < 0.05$ . ME: methanol extract; EE: ethanol extract; NHE: *n*-hexane extract; AE: aqueous extract. TPC: total phenolic contents expressed as g gallic acid equivalents/100 g dry weight; TFC: total flavonoid contents expressed as g catechin equivalents/100 g dry weight; DPPH: 2,2-diphenyl 1-picrylhydrazyl free radical scavenging potential expressed as percentage. Positive controls: BHT; butylated hydroxytoluene (antioxidant activity), metformin (antiglycation assay), physostigmine (AChE inhibitory assay), glucobay (alpha-amylase inhibitory assay), and acarbose (alpha-glucosidase).

that different extract fractions of *T. foenum-graecum* leaves with TPC in the range 5.74 ± 0.81–6.23 ± 0.76 (GAE/100 g). Total flavonoid contents (TFC) varied from 3.52 ± 0.13–17.54 ± 0.58 (GAE/100 g) in the extract fractions of *Z. Officinale* rhizome. TFC (g CE/100 g) in different extract fractions of *T. foenum-graecum* leaves varied from 1.51 ± 0.58–2.93 ± 0.09 (GAE/100 g). Free radical scavenging activity was maximally (88.82%) shown by ethanol fraction while minimum (21.11%) by methanol fraction in *Z. officinale* rhizome extracts. Regarding antioxidant activity

in the *T. foenum-graecum* leaf extracts, DPPH reducing activity was observed in the case of aqueous extract (50.27%).

Oxidative stress is induced during lipid and carbohydrate metabolisms that lead to damage to the biological membranes and biomolecules [32]. The presence of phytochemicals such as polyphenols supports the use of medicinal plants in alternative and traditional medicines and has received continued attention since being responsible for supporting plant, animal, and human health [2, 33]. The phenolic compounds, including flavonoids, contribute to



human health through antioxidant activity, free radical scavenging, and antimicrobial properties [2].

Our findings are agreed with the previous studies. A recent study revealed that *T. foenum-graecum* had  $46.08 \pm 0.15$  mg GAE/g phenolic compounds,  $13.02 \pm 0.44$  mg/g flavonoids content, and  $45.41 \pm 2.1\%$  antioxidant activity (DPPH) [34]. Another study reported that methanolic extract of *Z. officinale* possessed  $13.5 \pm 2.26$  g GAE/100 g phenolic contents [35]. As reported earlier, *Z. officinale* extract possessed 2.80 mg CE/g flavonoid contents, which agreed with the current study. A recent study showed that methanolic extract *Z. officinal* exhibited 15% DPPH scavenging activity [36], and the aqueous extract showed 52.50% radical scavenging activity [37], which is in agreement with the findings of the present study.

**3.1.4. Antidiabetic Potential.** Table 3 shows the results of the antidiabetic potential of the rhizome extract of *Z. officinal*. Ethanol fractions of *Z. officinal* showed a 96% antiglycation effect. The ethanol fraction of *Z. officinale* showed maximum (54.97%) inhibition of  $\alpha$ -amylase. Table 4 shows the results of the antidiabetic potential of *T. foenum-graecum* leaf extract, showing inhibitions of alpha-amylase are 9.43 to 24.95%.

Secondary metabolites like polyphenols are helpful for controlling the glycemic index in diabetic patients [38]. Previous studies reported that an aqueous fraction of *Z. officinale* exhibited  $\alpha$ -amylase inhibition activity by 50% ( $3.14 \pm 0.05$  mg·ml<sup>-1</sup>) in a dose-dependent manner and also indicated that phytoconstituents of *Z. officinale* can actively inhibit amylase enzyme and glycation processes [39]. Another study revealed aqueous extract of *Z. officinale* showed  $\alpha$ -amylase 68% inhibition which also supports our findings [40].

Another study revealed the glycation potential of ethanolic and aqueous extracts of *T. foenum-graecum*. The hindrance of alpha-amylase and alpha-glucosidase activities reduces the production of monosaccharides and absorption through intestinal epithelial cells and controls hyperglycemia [41].

**3.1.5. Acetylcholinesterase Inhibitory Activity.** Table 3 shows the inhibitory effect of the extract of *Z. officinale* on acetylcholinesterase activity. Results revealed that the extract of *Z. officinale* showed a maximum of 46.88% inhibitory effect on acetylcholinesterase activity. Table 4 shows that all the test samples of *T. foenum-graecum* showed 4.62%–14.08% acetylcholinesterase inhibitions.

Alzheimer's disease, the most prevalent type of neurological disorder, has fewer treatment opportunities. The activity of acetylcholinesterase is linked with Alzheimer's disease, which can be blocked through the action of medicinal plants. Plant-based medications have the neuroprotective potential for controlling neurodegenerative disorders [42]. Previous studies showed that *Z. officinale* extract showed more than 80% acetylcholine esterase inhibition [43]. Similarly, another study reported  $2.422 \pm 0.133$  mg/mL AChE inhibition by *Z. officinale* extract [44]. Another study revealed that *T. foenum-graecum* seed has a potential AchE inhibitory

activity and is considered a promising therapeutic option to treat Alzheimer's disease [45]. A recent study showed the positive healing efficacies of *T. foenum-graecum* in neurodegenerative conditions. Several studies have reported antidepressant and anti-anxiety effects along with modulation of cognitive behaviour by *T. foenum-graecum* [46].

## 4. Conclusion

*T. foenum-graecum* and *Z. officinale* are consumed as traditional medicinal plants worldwide and are also used as phytomedicine for the treatment of infectious diseases and bacterial infections, having numerous medicinal properties. The comparative analysis of *T. foenum-graecum* and *Z. officinale* showed that *Z. officinale* showed higher therapeutic effects due to the presence of quercetin, gallic acid, caffeic acid, vanillic acid, syringic acid, cumeric acid, benzoic acid, chlorogenic acid, p.Coumaric acid, ferulic acid, and cinnamic acid. This research laid the foundation for the discovery of optimized, cost-effective *in vitro* bioassays of active constituents of different plants. *T. foenum-graecum* and *Z. officinale* could be used as nutraceutical adjuncts as they have immense prospects to be further explored in animal and human trials. Furthermore, these medicinal plants are potential sources of significant natural antioxidant and marked antimicrobial agents. Furthermore, isolation, purification, and investigations of the bioactive constituents of these plants are required to reveal more health benefits for the public and therapeutics uses in the medical field.

## Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Conception and design of the study were performed by Javaria Hafeez; data acquisition was performed by Muhammad Naem, Tayyab Ali, and Javaria Hafeez; data analysis and interpretation were performed by Haroon Ur Rashid, Muhammad Nadeem, Fatma Hussain, and Bushra Sultan; drafting manuscript was performed by Ibrahim Shirzad, Muhammad Naem, Tayyab Ali, and Javaria Hafeez; critical revision of manuscript was performed by Muhammad Naem, Tayyab Ali, Ibrahim Shirzad, and Fatma Hussain; and final approval was performed by Ibrahim Shirzad, Fatma Hussain, Muhammad Naem, Tayyab Ali, Bushra Sultan, and Javaria Hafeez.

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