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Research Article

Phytochemical, Characterization and In vitro Aldose Reductase Inhibitory Activity of Seed Extracts of *Trigonella foenum-graecum*

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ABSTRACT

This study investigates the phytochemical composition and aldose reductase (AR) inhibitory potential of ethanolic seed extracts from *Trigonella foenum graecum* (fenugreek). Qualitative and quantitative analyses confirmed the presence of alkaloids, flavonoids, saponins, tannins, and polyphenols, with saponins (87.56% total content) and flavonoids (68.25%) as dominant constituents. GC-MS analysis identified major bioactive compounds, including methyl α-D-glucopyranoside (74.54% peak area) and 3-O-methyl-D-glucose (16.11%). Aldose reductase inhibitory activity was assessed in vitro. From the results ethanol extract showed the highest potency. (IC50 = 101.35 ± 12.75 µg/mL), compare to ethyl acetate and n-hexane extracts. Fractionation via column chromatography yielded six fractions (A–F), with Fraction B showing potent AR inhibition (IC50 = 84.58 ± 19.61 µg/mL), compare to quercetin (81.67±18.16 µg/mL). FTIR of Fraction B revealed functional groups (O–H, C=O, N–O) associated with bioactive compounds. These results highlight fenugreek's potential as a natural source for managing diabetic complications via AR inhibition. These findings support the ethnomedicinal use of *T. foenum-graecum* and warrant further studies for therapeutic development.

Keywords: Aldose reductase; Characterization; Ethanolic; Inhibition; *In vitro*; Phytochemical

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, often associated with oxidative stress and complications such as cataract formation, neuropathy, and nephropathy (Ponnulakshmi *et al*., 2019). One target enzyme implicated in diabetic complications is aldose reductase, which catalyzes the conversion of glucose to sorbitol in the polyol pathway (Suryanarayana *et al*., 2005). Inhibiting aldose reductase has emerged as a promising therapeutic strategy to mitigate these complications. While synthetic AR inhibitors like epalrestat are available, their long-term use may lead to undesirable side effects. This has prompted increased interest in plant-based inhibitors that are sustainable and effective (Rahul *et al*., 2022). Numerous phytochemicals, especially flavonoids, phenolic acids and alkaloids, have shown aldose reductase inhibitory properties in both in vitro and in vivo studies (Priya *et al*., Priya). Given the global burden of diabetes and the increasing demand for natural therapeutic agents. Trigonella foenum-graecum L. (commonly known as fenugreek) is an annual herb belonging to the family Fabaceae, widely cultivated in Nigeria and Asia. It is traditionally valued as both a culinary spice and a medicinal plant, with its seeds recognized for their diverse pharmacological properties, particularly antidiabetic, antioxidant, anti-inflammatory and hypocholesterolemic effects (Ahmad *et al*., 2021). The seeds are rich in various bioactive constituents, including alkaloids, flavonoids, saponins, tannins, and phenolic compounds, which contribute to its therapeutic potential (Kaur & Kaur, 2020). Given these properties, T. foenum-graecum has attracted

considerable scientific interest as a natural source of phytochemicals with potential therapeutic applications, particularly in the management of diabetes and its associated complications (Kaur & Kaur, 2020). This study aims to evaluate the aldose reductase inhibitory activity of ethanolic extracts and chromatographic fractions of *Trigonella foenum-graecum* seeds. Furthermore, the study employs phytochemical screening, GC-MS, and FTIR analysis to characterize the active compounds and functional groups responsible for the observed bioactivity.

MATERIAL AND METHODS

Chemicals and Reagents

NADPH, DL-glyceraldehyde, dimethyl sulfoxide (DMSO), 3,5-dinitrosalicylic acid, dibasic sodium phosphate, and quercetin were procured from Sigma Aldrich (Germany). Monobasic sodium phosphate, sodium hydroxide, sodium bicarbonate, sodium chloride, and the solvents used for extraction were obtained from Haddis International, Samaru Zaria, Nigeria.

Plant Material

Fresh Fenugreek seeds were collected in March 2025 from Tudun wada, Kaduna South Local Government Area, Kaduna State. The plant was authenticated at the Herbarium Unit, Department of Biological Sciences, Kaduna State University. They were pulverized into fine powder using a mortar and pestle. The powdered material was stored in an airtight plastic container for subsequent experimental use.

Extraction and Fractionation

A total of 5 g of the powdered seeds sample was separately extracted for 48 hours in 500 ml of three solvents: n-hexane (HEX), ethyl acetate (EtOAc) and ethanol (EtOH). Each extract was filtered through Whatman filter paper and concentrated using a rotary evaporator. Since the ethanol extract showed the strongest inhibitory activity, 5 g of it was subjected to column chromatography using Merck silica gel 60 (0.040–0.063 mm). Elution was performed with solvent systems of increasing polarity, namely HEX:EtOAc and EtOAc:MeOH, with polarity increments of 10% at each step. A total of 68 fractions were collected. Thin-layer chromatography (TLC) analysis was used to group similar fractions, yielding six major pooled fractions. These fractions displayed notable inhibitory effects against Aldose reductase enzyme.

Determination of Aldose Reductase Activity

Preparation of Bovine Lens Homogenate:

Bovine lens aldose reductase was prepared following the method of Hayman and Kinoshita, (1965) with slight modifications. Fresh transparent, non-cataractous bovine lenses were carefully enucleated and pooled. The lenses were homogenized in 100 mM phosphate buffer saline (PBS, pH 6.2). The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C, and the supernatant containing aldose reductase was collected for subsequent assays.

Bioassay of Plant Extracts:

Plant extract test solutions were prepared by dissolving 2 mg of the extract in 50 mL of 5% DMSO and then diluting to 1 mL, yielding final test concentrations ranging from 25 to 200 µg/mL. Each 1 mL assay mixture contained equal units of enzyme, 0.3 mM NADPH, 10 mM DL-glyceraldehyde (substrate), and the plant extract. For the negative control, the mixture contained 0.3 mM NADPH, 10 mM substrate, the enzyme preparation, and 5% DMSO in place of the inhibitor. For the blank, double-distilled water and 100 mM PBS (pH 6.2) were used instead of the inhibitor to make up the final 1 mL volume.

Enzyme Activity Measurement:

Aldose reductase activity was monitored spectrophotometrically by measuring the decrease in NADPH absorbance at 340 nm following substrate addition. Measurements were taken every 30 seconds over a 2-minute period. All bioassays were conducted in triplicate, and the mean percentage inhibition was calculated.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The fraction labeled B, which demonstrated the strongest inhibitory activity, was analyzed using GC–MS (Shimadzu QP 2010 series, Tokyo, Japan) equipped with a VF-5 ms fused silica capillary column (30 m × 0.25 mm × 0.25 µm). Ultra-pure helium served as the carrier gas at a flow rate of 0.7 ml/min with a linear velocity of 37 cm/s. The injector was maintained at 250 °C. The oven temperature was initially set at 60 °C, increased at 10 °C/min to 280 °C and held for 3 minutes. A 2 µl sample was injected in splitless mode with a manual split ratio of 20:1. The mass spectrometer operated in electron ionization mode at 70 eV with an electron multiplier voltage of 1859 V. Additional parameters included an ion source temperature of 230 °C, quadrupole temperature of 150 °C, solvent delay of 4 minutes, and a scanning range of 50–700 amu. The resulting spectra were compared against the National Institute of Standards and Technology (NIST) library database for compound identification.

FTIR Analysis

B-fractions of the sample were subjected for elucidation of the possible functional group by FTIR spectra at room temperature (20^{-}C) using attenuated total reflectance (ATR) and an internal reflection element made of diamond using Spectrum Two instrument (Perkin Elmer

Inc., USA). The ATR crystal portion between the measurements was carefully cleansed using solvent and acetone before the dried extracts of the two fractions were analyzed directly on the diamond ATR crystal, without any preparation. The spectral region was 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were measured by Spectrum software (Perkin Elmer Inc., USA).

Data Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) with SPSS version 20.0. Post-hoc comparisons were carried out with Duncan post-hoc test. A p-value of less than 0.05 was considered statistically significant.

RESULT

Qualitative analysis in Table 1below revealed the presence of major bioactive groups: alkaloids, flavonoids, tannins, phenols, saponins, and cardiac glycosides, all of which are known to have antidiabetic and antioxidant properties. Quantitative analysis of Table 2 showed that, Saponins (87.56%) and flavonoids (68.25%) were the most abundant components. Tannins and polyphenols were present in smaller amounts.

Table 3 showed the major compounds identified in the ethanol extract include: Methyl-α-D-glucopyranoside (74.54%), 3-O-Methyl-D-glucose (16.11%). Other minor compounds: aziridine derivatives, squalene and bicyclo-heptanone. The dominant sugar derivatives and bioactive lipids detected are known for their protective and metabolic regulatory roles in diabetes management.

The Functional groups of Table 4 identified in Fraction B include: Hydroxyl (O–H) group: 3235 cm^{-1}, Alkane (C–H): 2852 cm^{-1}, Carbonyl (C=O) and nitro (N–O) groups. These functional groups are characteristic of flavonoids and polyphenols, supporting the compound class detected in the phytochemical and GC-MS analyses.

Table 1. Qualitative analysis of Ethanolic seed extract of *Trigonella foenum graecum*

Test	Inference
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenol	+
Saponins	+
Tannin	+

Keys; present (+), Absent (-)

Table 2. Quantitative Phytochemical analysis of Ethanolic seed extract of *Trigonella foenum graecum*

Constiteunts	Concentration	Absorbance	Total content	%Total Content
Alkaloid	0.385	0.049	75mg/g	16.24
Flavanoid	6.125	1.854	4.95mg/g	68.25
Polyphenol	0.315	2.589	1.98mg/AE/g	04.25
Saponin	2.124	0.912	36.95mg/g	87.56
Tannin	0.124	0.998	0.512mg/g	1.594

Table 3. Result Showing the Compounds Present in Ethanol Extract of Trigonella foenum Graecum from the GCMS Analysis

RT	Name of compounds	Compound Molecular Formula	Molecular Weight	Peak area (%)
10.09	á-D-Glucopyranoside, methyl	C7H14O6	194	74.54
10.77	3-O-Methyl-d-glucose	C7H14O6	194	16.11
13.74	Heptanoic acid, 2-ethyl-	C9H18O2	158	0.09
5.03	1-Azabicyclo [2.2.2] octane, 4-methyl-	C8H15N	125	0.42
3.25	Aziridine, 1,2,3-trimethyl-, trans-	C5H11N	85	2.41
3.87	2-Propen-1-amine, N-ethyl-	C5H11N	85	3.43
16.04	Bicyclo [3.1.1] heptan-3-one, 2,6,6-trimethyl-	C10H16O	152	0.14
25.13	Squalene	C30H50	410	0.83

Table 4. Functional Groups and Modes of Vibration in B-fraction of *Trigonella foenum graecum* seed extract fractions by FTIR Spectrum Analysis

S/N	Functional Group	Degree of Bending/Stretching	Prominent Peaks (cm^{-1})	Band Range (cm^{-1})
1	O-H	Strong and stretching	3235	3650-3200
2	C-H	Medium and stretching	2852	2960-2850
3	C=O	Medium and stretching	1893	2280-2200
4	N-O	Medium and bending	1850	1850-1650
5	-C-H	Stretching	940	940-819

The Aldose Reductase Inhibition. Among the crude extracts, ethanol extract had the strongest inhibition: IC50 = 101.35±12.75 µg/mL, second only to quercetin (81.67 18.16 µg/mL). In the fraction study (Figure 1 and Table 5), Fraction B showed high potency (IC50 = 84.58 ±19.61 µg/mL), close to quercetin 81.67 18.16 µg/mL and stronger than other fractions. Fraction B likely contains the most bioactive compounds, possibly flavonoids, which effectively inhibit aldose reductase—an enzyme implicated in diabetic complications.

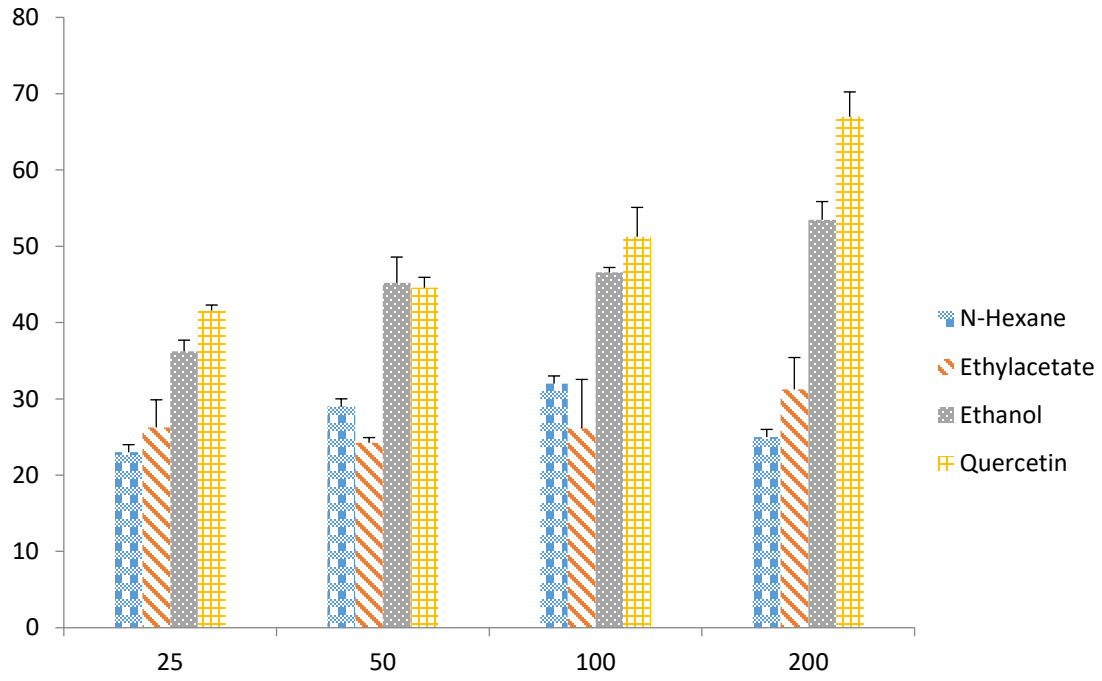

Figure 1. The Effect of Aldose reductase *In vitro* activity by the extracts of *Trigonella foenum graecum* seed extract
Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the bars for a given concentration for each extract are significantly different from each other ($p < 0.05$. Duncan post-hoc test, IBM, SPSS, version 20).

Table 5. IC50 Values for the Inhibition of Aldose reductase activity by the extracts of *Trigonella foenum graecum* seed extract

S/N	Extracts/Standard	Aldose Reductase IC50 (µg /mL)
1	n-Hexane	213.12±3.52[b]
2	Ethyl acetate	278.23±4.59[c]
3	Ethanol	101.35±12.75[a]
4	Quercetin	71.23±21.47[a]

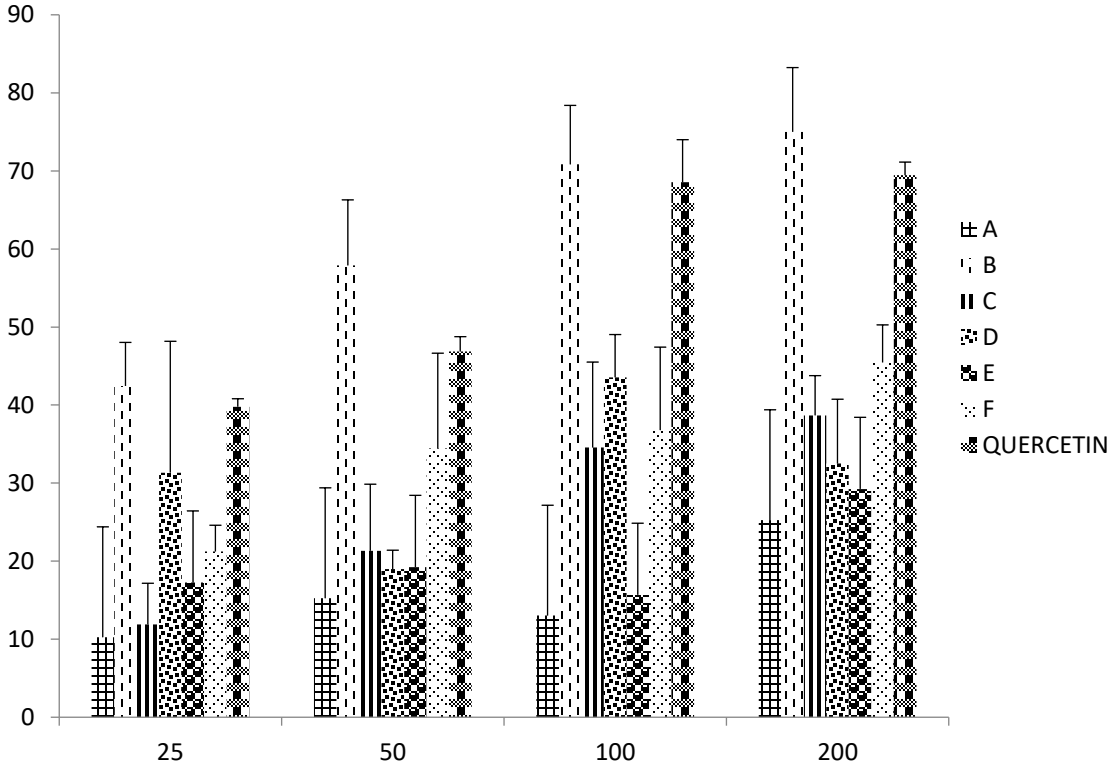

Figure 2. The Effect of Aldose reductase *In vitro* activity by the fractions of *Trigonella foenum graecum* ethanolic seed extract

Data are presented as mean ± SD of triplicates of analysis. Different letters presented above the bars for a given concentration for each extract are significantly different from each other ($p < 0.05$. Duncan post-hoc test, IBM, SPSS, version 20).

Table 6. IC50 Values for the Inhibition of Aldose reductase activity by *Trigonella foenum graecum* seed extract fractions

S/N	Fractions/Standard	Aldose Reductase IC50 (µg /mL)
1	A	175.18±11.52[d]
2	B	84.58±19.61[a]
3	C	131.59±9.48[c]
4	D	112.85±4.41[c]
5	E	206.59±25.22[e]
6	F	114.64±20.12[bc]
7	Quercetin	81.67 ± 18.16 [ab]

DISCUSSION

The current study provides strong evidence that the ethanolic extract and chromatographic fractions of *Trigonella foenum-graecum* seeds possess significant aldose reductase inhibitory activity, which may contribute to their antidiabetic potential. The study also

confirms a rich phytochemical profile dominated by flavonoids, saponins, and phenolic compounds, which are well-known for their role in modulating oxidative stress and diabetic complications.

The quantitative phytochemical analysis indicated high levels of saponins (87.56%) and flavonoids (68.25%), with lower concentrations of tannins, alkaloids and polyphenols. These results are consistent with the findings of Ahmad *et al*., (2021) who reported a flavonoid content of 66.4% and significant saponin content in fenugreek extracts using ethanol as the solvent. Flavonoids and saponins are known to modulate glucose metabolism and improve insulin sensitivity (Mohammadi et al., 2020).

The GC-MS analysis identified methyl-α-D-glucopyranoside (74.54%) and 3-O-methyl-D-glucose (16.11%) as the dominant constituents (Lohvina *et al*., 2021). These compounds are sugar derivatives known for their osmoprotective and antioxidant properties, which can indirectly reduce diabetic oxidative stress. Comparable findings were reported by Kaur and Kaur, (2020) who also detected similar glucose analogs in the GC-MS analysis of fenugreek seed extracts and linked their presence to potential antidiabetic and anti-glycation activity. The ethanolic extract showed the highest AR inhibition with an IC50 of 101.35 ± 12.75 µg/mL, followed by n-hexane (213.12±3.52 µg/mL) and ethyl acetate (278.23±4.59 µg/mL). Among the fractions, Fraction B demonstrated significant potency (IC50 = 84.58 ± 19.61 µg/mL), nearly matching the standard quercetin (81.67 ± 18.16 µg/mL). This result aligns with Khatune *et al*., (2019), who reported that flavonoid-rich fractions of *T. foenum-graecum* exhibited potent aldose reductase inhibition, with IC50 values ranging from 70–110 µg/mL. Similarly, Rahul *et al*., (2022) showed that purified flavonoid compounds from fenugreek seeds reduced aldose reductase activity and sorbitol accumulation in lens epithelial cells.

FTIR spectroscopy of Fraction B confirmed the presence of hydroxyl (O–H), carbonyl (C=O), and C-H groups, which are typical of phenolics and flavonoids. The peak at 3235 cm^{-1} corresponds to O–H stretching, indicative of polyphenolic content. These functional groups have been associated with aldose reductase inhibitory potential in prior studies, particularly in flavonoid-rich plant extracts (Jiang *et al*., 2017). A comparable FTIR profile was reported by Sharma *et al*., (2023) in their analysis of *Ocimum sanctum* extract, linking these peaks to compounds responsible for AR inhibition. Quercetin, a well-established natural aldose reductase inhibitor, had the lowest IC50 (81.67± 18.16 µg/mL). The close inhibitory potential of Fraction B (84.58± 19.61 µg/mL) suggests that the bioactive content in fenugreek may be responsible for this potent activity. This supports previous findings that fenugreek is a functional food with AR inhibitory capacity, comparable to standard quercetin.

CONCLUSION

The ethanolic extract of *T. foenum graecum* seeds is rich in saponins, flavonoids, and glycosides. Fraction B, enriched with O–H/C=O/N–O functional groups, demonstrates potent AR inhibition (IC~50~ ≈ 85 µg/mL), comparable to quercetin. This validates fenugreek's traditional use in diabetes management and identifies Fraction B as a promising source of natural AR inhibitors. Future studies should isolate active compounds from Fraction B and validate efficacy in *in vivo* models.

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Conflict of Interest: No Conflict of Interest

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