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ORIGINAL ARTICLE

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Exploring the Antioxidant Potential and HPLC Profile of Fenugreek (*Trigonella foenum-graecum L.*) Seed Extracts

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ABSTRACT

Background: The growing concerns regarding the adverse effects of synthetic antioxidants, coupled with documented detrimental outcomes of adverse drug reactions, such as increased morbidity, mortality, and hospitalization, as highlighted by several studies, have emphasized the necessity of natural alternatives. Therefore, medicinal compounds derived from plants are increasingly advocated for the management of various diseases, due to their perceived minimal side effects and extensive health benefits.

Aims: The objective of the present study was to investigate the potential of *Trigonella foenum-graecum* seeds as a natural source of bioactive compounds by assessing their antioxidant activity employing various *in vitro* assays. Additionally, this study aimed to determine the total phenolic, falconoid, and tannin contents in both hydroethanolic (50% v/v) and hydromethanolic (50% v/v) extracts. Furthermore, phytochemical profiling of the fenugreek seed extract was performed utilizing High-Performance Liquid Chromatography (HPLC).

Materials and Methods: Following the extraction and quantification of total polyphenols, flavonoids, and tannins from hydroethanolic and hydromethanolic extracts, antioxidant activity was assessed employing three *in vitro* assays: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, the ferric reducing antioxidant power (FRAP) assay, and the total antioxidant capacity (TAC) assay. Phenolic compounds were separated and identified using HPLC with Diode Array Detection (DAD). The obtained data were analyzed using SPSS software.

Results: Phytochemical analysis revealed that the hydroethanolic extract exhibited a higher total polyphenol content (50.5 mg GAE/g) compared to the hydromethanolic extract (47.33 mgGAE/g). However, the evaluation of the antioxidant activity demonstrated that the hydromethanolic extract displayed the highest reducing power, as evidenced by the DPPH (IC_{50}=314,53 mg/L), FRAP (21.49 mg AAE/g), and TAC (74.53 mg AAE/g) assays. Chromatographic characterization by HPLC identified six phytochemical compounds in the hydromethanolic extract of *T. foenum graecum* powder, which was obtained through maceration.

Conclusions: In conclusion, *Trigonella foenum graecum* seeds represent a promising natural resource possessing significant biological activity. Their potent antioxidant properties render them valuable candidates for incorporation in pharmaceutical and cosmetic formulations to promote health and reduce damage induced by oxidative stress.

Keywords: *Trigonella foenum-graecum*, Polyphenols, Antioxidant activity, Chromatography.

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1 INTRODUCTION

Traditional medicine remains a primary healthcare resource for a significant portion of the global population (Ladoh *et al.*, 2014). For centuries, medicinal plants have been utilized to treat human diseases across various regions (Chabane *et al.*, 2013). Out of the 300,000 plant species recorded worldwide, more than 200,000 exist in Africa and other regions. Among the potential users of these plants, traditional medicine and pharmacopeia are predominant, with 70% of populations in developing countries relying on them (Sofowora, 2010).

However, several studies have highlighted the detrimental consequences associated with adverse drug reactions (ADRs), including increased morbidity, mortality, hospitalization rates, and considerable medical costs. In the United States of America (USA), ADRs are reported as the fourth leading cause of death (Jonville-Béra *et al.*, 2016). A study by the French Health Products Safety Agency, "EIM: *Incidence et*

Risque" (ADR: Incidence and Risk), estimates that 3.6% of hospital admissions are attributable to ADRs.

Consequently, antioxidant therapy has garnered significant attention in the treatment of several diseases, leading current research to focus on identifying natural antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage caused by free radicals and ROS, thereby preventing disease onset. Their mechanism of action involves interfering with the oxidation process through reactions with free radicals, chelation, and catalytic metals, and acting as deoxygenating agents (Halliwell, 2015; Teleanu *et al., 2019*).

Fenugreek (*Trigonella foenum-graecum*), an annual species belonging to the legume family, is one of the oldest medicinal and culinary plant globally. Native to India and North Africa, it is widely cultivated across the Mediterranean basin (Sharma, 2022), with its seeds constituting a staple in daily diets (Faisal *et al.,* 2024). Historically, fenugreek seeds have been extensively used in Ayurvedic, Traditional Chinese, and Arabic medicine, primarily recognized for their anti-diabetic and cholesterol-lowering properties (Kumar, 2019). While previous studies have explored the antioxidant properties of crude fenugreek seed extracts (Liu *et al.*, 2012; Kenny *et al.*, 2013), comprehensive information regarding the antioxidant power of fenugreek seed extracts with solvents of intermediate polarity remains limited (Norziah *et al.*, 2015).

In this context, the present study aimed to evaluate the bioactive content of whole fenugreek seeds. Two primary objectives were pursued: first, to extract phenolic compounds using various solvents (water, ethanol, methanol) and different extraction methods; and second, to conduct a comprehensive phytochemical analysis, including the determination of total polyphenols, total flavonoids, and tannins, followed by the evaluation of their antioxidant activity and the identification of phenolic compounds using HPLC-DAD (High-Performance Liquid Chromatography with Diode Array Detection).

2 Material and Methods

2.1 Chemical Products

All chemicals utilized for metabolite determination, antioxidant activity, and chromatographic analysis were of analytical grade and procured from Sigma Chemical Co (Sigma-Aldrich, St. Louis, MO, USA).

2.2 Plant Material

Fenugreek (*Trigonella foenum-graecum*) seeds utilized in this study were sourced from a local herbalist imported from India. Prior to extraction, the whole fenugreek seeds were washed with distilled water and subsequently dried in an oven at 40°C for 24 hours. Subsequently, the dried seeds were then ground into a fine powder, which served as the raw material for extract preparation.

2.3 Preparation of Extracts

Extracts from fenugreek seed powder were prepared using classical extraction methods – infusion, maceration, and decoction – with various solvents. All extraction procedures were conducted in triplicate to ensure reliability and consistency of results.

2.3.1 Infusion

A10 g sample of fenugreek powder was added to a flask containing 100 mL of boiling solvent. The mixture was allowed to stand for 30 minutes until it reached room temperature. Subsequently, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under vacuum at 45°C. The resulting extract was then oven-dried at 45°C for 24 hours. The dried extracts were weighed and stored in a hermetically sealed sterile bottle for further analysis.

2.3.2 Maceration

A 10 g sample of fenugreek powder was placed into a flask containing 100 mL of solvent. The mixture was then subjected to magnetic stirring for 48 hours at room temperature. This process was repeated once with fresh solvent for a second extraction. After completion, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated. The resulting extract was stored in a hermetically sealed sterile bottle under the same conditions as the infusion extract.

2.3.3 Decoction

A 10 g sample of fenugreek powder was added to a flask containing 100 mL of solvent. The mixture was then boiled for 30 minutes. After cooling, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated, and the resulting extract was stored in a hermetically sealed sterile bottle under the same conditions as the infusion extract.

2.4 Determination of Secondary Metabolites

Total polyphenols were determined using the colorimetric Folin-Ciocalteu reagent, following the method outlined by Hameed *et al.* (2019). The concentration of total polyphenols was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Total flavonoid content (TFC) was determined utilizing the colorimetric method described by Nam *et al.* (2015), with quercetin as a standard. Results were expressed in milligrams

of quercetin equivalent per gram of extract (mg QE/g extract).

Condensed tannins were determined using the vanillin acid method described by Ba *et al.* (2010). Results were expressed in milligrams of catechin equivalent per gram of extract (mg CE/g extract).

Hydrolysable tannin content was determined following the method outlined by Catelani et *al.* (2016). Results were expressed in milligrams of tannic acid equivalent per gram of extract (mg TAE/g extract).

2.5 Assessment of Antioxidant Activity

2.5.1 DPPH Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a discoloration test that evaluate the antioxidant's ability to react directly with the radical (Gulcin, 2023). The method employed follows that described by Keshavarzi *et al.*, (2020). Results were presented as a percentage of inhibition, calculated based on the decrease in the mixture's color intensity using the following equation:

$$IP = \frac{(\text{Control OD} - \text{Extract OD})}{(\text{Control OD})} \times 100$$

Where:

- *IP: Inhibition Percentage (or IC_{50})*
- *Control OD: absorbance of the control*
- *Extract OD: absorbance of the extract*

The inhibition percentage data were used to determine the half maximal inhibitory concentration (IC_{50}), defined as the concentration of the extract required to scavenge 50% of the DPPH radicals. A lower IC_{50} value indicates higher antioxidant efficacy.

2.5.2 Ferric Reducing-Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay, based on the method outlined by Sano (2023), measures the capacity of antioxidants to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), resulting in a formation of blue colored complex. An increase in absorbance correlates with an increase in antioxidant activity. Results were expressed as the effective concentration (EC_{50}), defined as the sample concentration producing an absorbance of 0.5.

2.5.3 Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) assay followed the protocol described by Kasangana *et al.*, (2015). This assay is based on the reduction of molybdenum (VI) to molybdenum (V) by the plant extract. This reduction, conducted under acidic pH, leads to the formation of a green phosphomolybdenum (V) complex (Renugadevi *et al.*, 2018). Results were reported in milligrams of ascorbic acid equivalents (AAE) per gram of dry weight (mg AAE/g DW).

2.6 High-Performance Liquid Chromatography (HPLC) Analysis

Phenolic compounds were separated and determined using high-performance liquid chromatography (HPLC) with iodine detection (DAD). The Shimadzu Prominence-I LC-2030C 3D LC HP liquid chromatograph, equipped with an LC-2030 autosampler and an LC-2020/2040 Photodiode-Array 160 detection detector from Kyoto, Japan, was utilized for this purpose. A C18 column (150 mm × 4.6), 5 µm particle size; Ascentis C18 CLHP) was employed for detection at a wavelength of 280 nm. The mobile phase consisted of a gradient mixture of methanol and water (H_2O), with varying ratios of methanol from 5 to 100%.

2.7 Statistical Analysis

All data were analyzed using SPSS software (Version 30.0, IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted, and significant differences between mean values were determined using Duncan's multiple range test. Results were expressed as the mean ± standard deviation from samples analyzed in triplicate. Correlation analysis was conducted by determining the linear correlation coefficient (R), also known as Pearson's correlation coefficient. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Extraction Efficiency

Table 1 illustrates the extraction yields achieved using the three methods employed (N=11), indicating that maceration yielded the highest average yield 11.47 % compared to 10.12 % for decoction and 8.013% for infusion. Extraction yields varied significantly depending on the solvent ($p < 0.05$).

Notably, the water/ethanol mixture (50:50, v/v) proved to be the most effective solvent across all three extraction techniques, particularly in maceration (Db: 16.68%), followed by decoction (Dc: 13.55%), and then infusion (Da: 10.87%). Statistical analysis revealed significant differences between infusion and maceration ($p = 47\times10^{-5}$), maceration and decoction ($p = 305 \times 10^{-4}$), and infusion and decoction ($p = 559 \times 10^{-5}$) when utilizing the same solvent.

Table1. Extraction yield of fenugreek seed powder (*T. foenum graecum*)

Solvent	Extraction yield (%)		
	Infusion (a)	Maceration (b)	Decoction (c)
Water(A)	2.53 ± 0.33	1.81 ± 0.13	4.34 ± 0.46
Ethanol(B)	/	13. 35 ± 0.45	/
Methanol(C)	/	11.92 ± 0.05	/
Hydro–ethanolic (D)	10.87 ± 0.09	16.68 ± 0.22	13.55 ± 0.24
Hydro–methanolic (E)	10.64 ± 0.25	13.38 ± 0.292	12.49 ± 0.36
Average	8.013	11.47	10.12

Note: "/": not completed; Values are expressed as: Average ± Standard Deviation

3.2 Determination of Secondary Metabolites

The secondary metabolite contents of *Trigonella foenum graecum* extracts are presented in Table 2.

Table 2. Secondary metabolites content of fenugreek extracts (*T. foenum graecum*)

Extract	TPC[a] mg GAE/g ext	TFC[b] mg QE/g ext	Condensed tanins[c] mg CE/g ext	Hydrolysable Tanins[d] mg TAE/g ext
hydro ethanolic	50.5 ± 0.41	68.27 ± 0.74	16.35 ± 0.06	0.93 ± 0.17
hydro methanoli	47.33 ± 0.47	88.57 ± 0.42	13.62 ± 0.52	4.05 ± 0.59

Note: a. Total polyphenols expressed as gallic acid equivalent (mg GAE/g extract); b. Flavonoids expressed as quercetin equivalents (mg QE/g extract); c. Condensed tannins expressed as catechin equivalents (mg CE/g extract); d. Hydrolysable tannins expressed as tannic acid equivalents (mg TAE/g extract). Values are presented as averages ± standard deviation.

3.2.1 Determination of Total Polyphenols

As presented in Table 2, the hydroethanolic extract of fenugreek seed powder exhibited a notably higher level of total phenols (50.5 ± 0.41 mg GAE/g) compared to the hydromethanolic extract (47.33 ± 0.47 mg GAE/). The statistically significant difference (p= 215×10^{-5}) suggests the superior efficacy of the hydroethanolic mixture as a solvent for extracting polyphenols from ground fenugreek seeds.

3.2.2 Determination of Flavonoid

Regarding the flavonoid content of fenugreek seed powder extracts, the hydromethanolic extract exhibited the highest content (88.57 ± 0.42 mg QE/g) compared to the hydroethanolic extract (68.27 ± 0.74 mg QE/g). The mean values for the two fractions are significantly different (p = 9.854 × 10^{-6}).

3.2.3 Determination of Tannin

Quantitative analysis of condensed and hydrolysable tannins in the two fenugreek seed extracts revealed that the hydroethanolic extract contained a higher content of condensed tannins (16.35 mg CE/g) compared to the hydromethanolic extract (13.62 mg CE/g). This difference was statistically significant (p = 5.218×10^{-5}). Conversely, no statistically significant difference was observed in the hydrolysable tannin content between the two fractions (p = 0.1074), with values of 0.9309 mg TAE/g in the hydroethanolic extract and 4.054 mg TAE/g in the hydromethanolic extract.

3.3 Assessment of Antioxidant Activity

3.2.4 DPPH Scavenging Assay

The DPPH radical scavenging assay, utilizing the DPPH radical as a substrate, is widely employed to evaluate the antioxidant capacity of plant extracts, where a higher level of inhibition indicates potent antioxidant activity. IC_{50}, the concentration eliminating 50% of the DPPH radical, has been calculated for each extract and the standard substance (ascorbic acid) (Table 3).

Table 3. The DPPH radical inhibition rate

Extract	mg/L	%Inhibition	IC_{50} (mg/L)
hydro Ethanolic	500	77.88 ±0.07	338.13 ±3.3
hydro Methanolic	500	77.97 ±1.75	314.53 ±2.57

Note: The values expressed are average± standard deviation; Abbreviations: IC, inhibiting concentration.

Tests conducted to determine the DPPH radical scavenging activity of the two fenugreek powder extracts at varying concentrations revealed a dose-dependent increase in scavenging activity. The hydromethanolic extract exhibited the highest antioxidant activity, achieving an inhibition rate of 77.97%, compared to the hydroethanolic extract, which showed 77.88% inhibition at the same concentration of 500 mg/L.

3.2.5 Ferric Reducing-Antioxidant Power Test (FRAP)

The FRAP analysis, a rapid and reliable method for assessing antioxidant activity based on reducing capacity, provided valuable insights into the antioxidant potential of the fenugreek extracts. The hydromethanolic extract exhibited superior reducing power, while the hydroethanolic extract showed a comparatively lower reducing power at the same concentration. Specifically, the hydromethanolic extract recorded a reading of 0.081, whereas the hydroethanolic extract measured 0.066 at a concentration of 200mg/ L. Both these values were lower than that of the standard substance, ascorbic acid (1.43).

Table 4 presents the effective concentration (EC_{50}) of the extracts, corresponding to an absorbance of 0.5, as obtained from linear regression curves. The ferric-reducing capacity (EC_{50}) of the hydromethanolic and hydroethanolic extracts was 1.32g/L and 1.42 g/L, respectively, with statistically significant differences (p = 3044×10^{-7}). Both extracts exhibited lower reducing power than the standard, ascorbic acid, which had an EC_{50} value of 0.042 g/ L.

Table 4. Ferric reducing power (FRAP test)

Extract	mg/L	OD	EC_{50} (g/L)	mg AAE/g
hydro Ethanolic	200	0.066 ± 0.0004	1.42 ± 0.0012	19.58 ± 0.14
hydro Methanolic	200	0.081 ± 0.0008	1.32 ± 0.0004	21.49 ± 0.08

Note: Values are presented as Average ± Standard Deviation; OD, optical density; EC, effective concentration.

3.2.6 Total Antioxidant Capacity (TAC) test

The total antioxidant capacity (TAC) test of *T foenum graecum* extracts results are depicted in Table 5.

Table 5. Total Antioxidant Capacity

Extract	mg AAE/g
hydro ethanolic	73.07±3.86
hydro methanolic	74.53±1.57

Note: Values shown are means ± standard deviation; Abbreviations: AAE, ascorbic acid equivalent.

3.3 Chromatographic Analysis

Molecular separation of the hydromethanolic extract of fenugreek seeds by HPLC was conducted at a wave length of 280 nm. The results are depicted in chromatograms displaying the peaks and retention times for each molecule (Figure 1).

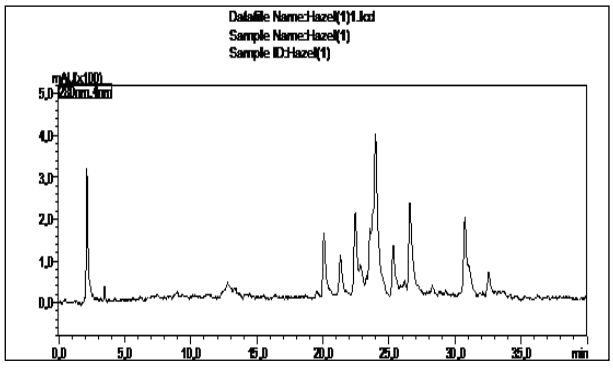

Figure 1. Chromatogram of fenugreek seed hydromethanolic extract at 280 nm

HPLC analysis of the hydromethanolic extract of *T. foenum graecum* powder, obtained through maceration, revealed the presence of 12 components. Identifying these molecules requires comparing their retention times (Rt) with pure standards under identical experimental conditions. The compounds identified in the hydromethanolic extract of fenugreek seeds are listed in Table 6.

Table 6. HPLC detected phytochemical molecules in hydromethanolic extracts of *fenugreek* seeds

Retention Time (minutes)	Molecules
2.126	3,4dihydroxybenzoic acid
3.459	Vanillic Acid
20.073	Luteolin
21.332	Epigallocatechin gallate
25.322	Apigenin
26.578	Rutin

4 Discussion

The observed discrepancies in extraction yields across the employed methods can be attributed to the inherent characteristics of each technique and the physicochemical properties of the solvents used, particularly their polarity (distilled water; 10.2; ethanol: 4.3; methanol 5.1.) The extended contact duration between the solvent and fenugreek molecules, characteristic of maceration (48 hours), likely played a significant role in achieving higher yields. Moreover, the ambient laboratory temperature of 23°C during maceration limits the evaporation of ethanol and methanol, unlike the higher temperatures utilized for decoction and infusion, which facilitate the evaporation of these organic solvents (methanol boiling point: 65°C, ethanol: 79°C).

In comparison to other studies, Bouhenni *et al.* (2021), reported an extraction yield of 17.66% via methanolic maceration, while Bukhari *et al.* (2008) reported higher yield of 25.32% and 25.89% using ethanolic and methanolic Soxhlet extraction, respectively. Decoction with distilled water resulted in the highest extraction yield in our study, likely due to the combined effects of elevated temperature and prolonged contact time, which facilitate the breakdown of fenugreek particles and the release of water-soluble molecules.

Conversely, extraction with distilled water resulted in the lowest yields (2.53%, 1.81% and 4.34%) across all three extraction methods (infusion, maceration, and decoction) when compared to other solvents. This phenomenon may be attributed to the high viscosity of fenugreek powder in water at a low solids-to-solvent ratio (10%), as reported by Norziah *et al.* (2015). An increased water content may

induce a saturation effect, thereby elevating the local density around bioactive molecules (Bulgarevich *et al.*, 2002). Our findings contrast with those of Benziane *et al.* (2019), who reported that maceration with tap water yielded three times higher extraction efficiencies than decoction of fenugreek seeds (45% and 15%) at a solid-to-solvent ratio of 25%. It is well-established that the recovery of bioactive compounds, such as phenolic compounds, from plant materials is influenced by various factors, including the nature of these compounds, the choice of extraction solvent, solids-to-solvent ratio, and the specific extraction conditions (Wijekoon *et al.*, 2011)

Regarding polyphenol contents, our findings align with several studies that have quantified polyphenols in fenugreek seed extracts using the Folin-Ciocalteu method with gallic acid as a standard. For instance, Norziah *et al.* (2015) reported values of 44.96 mg GAE/g for ethanolic and 43.15 mg GAE/g for methanolic extracts, while Bukhari *et al.* (2008) found values of 6.86 mg EAG/g and 5.75 mg GAE/g for ethanolic and methanolic extracts, respectively. Conversely, Saxena *et al.* (2011) reported 5.75 mg GAE/g and 14.23 mg GAE/g for the two solvents, respectively. Variations in the total phenolic content of fenugreek seed extracts can be attributed to factors such as solvent polarity, extraction method, and extraction duration (Wijekoon *et al.*, 2011; Kowalczyk *et al.*, 2013). It is important to acknowledge that while the Folin-Ciocalteu assay is used to estimate total phenolic compounds, it is not entirely specific to polyphenols, as other interfering compounds may react with the reagent, yielding seemingly high phenolic concentrations (Pérez *et al.*, 2023). Additionally, factors such as genetic variation, environmental conditions, seed age, and extraction processes can influence the secondary metabolite content of a plant species (Hazrati *et al.*, 2024).

Based on the results of flavonoid composition, it is hypothesized that compounds with higher molecular weights compared to that of polyphenols, are more effectively extracted using high-polarity solvents such as water and methanol. These findings are consistent with the results obtained by Aylanc *et al.* (2020), who reported 89.90 mg QE/g and 30.08 mg QE/g for *Trigonella spruneriana*. However, they differ from the results reported by Norziah *et al.* (2015) for methanolic and ethanolic extracts of *T. foenum graecum*, which were 9.48 mg CE /g and 14.2 mg CE /g, respectively. Bukhari *et al.* (2008) found 607 µg QE/g and 653 µg QE/g for both extracts. Other studies have reported lower values, such as 20.8 mg CE/g in the methanolic extract, as reported by Belguith-Hadriche *et al.* (2013), and 80.98 mg QE/100 g DM for the hydromethanolic extract, as reported by Bouhenni *et al.* (2021).

Regarding the quantitative determination of condensed tannins and hydrolysable tannins in the two fenugreek seed extracts, other studies have reported lower values. Bouhenni *et al.* (2021) found 2.2 mg CE/100g DM of condensed tannins and 1 mg TAE/100g DM of hydrolysable tannins in the hydromethanolic extract, while Abdouli *et al.* (2012) and Abdouli *et al.* (2014) found 3.3 mg /g DM and 2.59 mg/g DM of total tannins, and 0.027 mg/g DM of condensed tannins, respectively. The amount of tannin in the extract can be affected by the various solvents, and the polarity of ethanol also have an impact on the extract's yield and the tannin content of the plant seeds (Bosso *et al.*, 2024).

Consequently, the two extracts recorded IC_{50} values of 314.53 mg/L and 338.13 mg/ L, respectively, which support the suggestion that the hydromethanolic extracts contain more compounds capable of donating hydrogen to free radicals, thereby neutralizing the unpaired electrons responsible for radical reactivity. It is noteworthy that the standard substance (Ascorbic Acid) exhibited a considerably lower IC_{50} value of 2.21 mg/ L. The significant difference ($p = 1764 \times 10^{-6}$) in IC_{50} inhibition rates between the two fractions may be attributed to their respective flavonoid content (68.27 mg QE/g for hydroethanolic and 88.57 mg QE/g for hydromethanol extracts).

These results are consistent with the findings of Bouhenni *et al.* (2021), who reported an IC_{50} of 343.75 µg/mL for the methanolic extract of *Trigonella foenum-graecum*, and Norziah *et al.* (2015), who recorded IC_{50} values of 345 µg/mL for the ethanolic extract and 361 µg/mL for the methanolic extract. Other studies have reported values ranging from 3.87 to 5.28 mg/mL (Aylanc *et al.*, 2020). Differences in antioxidant activity between extracts are primarily attributed to variations in solvent polarity and its influence on the extractability of antioxidant compounds (Zeng *et al.*, 2023).

Similar trends in reducing power were observed between the DPPH radical scavenging and FRAP tests for both extracts. The antioxidant activity of *T. foenum graecum* extracts appears to be proportional to the solvents' polarity, likely due to the differential solubility of antioxidant compounds with varying polarities. Correlation analysis revealed a strong relationship between TFC and FRAP, as observed in previous studies (Sathisha *et al.*, 2011; Smeriglio *et al.*, 2016).

The two extracts studied demonstrated an average reducing power of 19.58 mg AAE/g for the hydroethanolic extract and 21.49 mg AAE/g for the hydromethanolic extract. These results are higher compared to those obtained by Benziane *et al.* (2019) (56.37 mg AAE/100g), but lower than those recorded by Aylanc *et al.* (2020), who reported 78.95 mg TE/g for the hydromethanolic extract and 77.57 mg TE/g for the hydroethanolic extract.

In determining total antioxidant capacity, both hydromethanolic and hydroethanolic extracts of *T. foenum graecum,* exhibited 74.53 mg AAE/g and 73.07 mg AAE/g values, respectively, indicating a slight advantage for the hydro-methanolic extract. However, the difference between the two was not statistically significant (p = 0.6575). Additionally, the study showed a statistically significant correlation between flavonoid contents and the phosphomolybdenum assay. Other authors have reported TAC values of 162.51 mg AAE /g for hydroethanolic extract (Fatima *et al.*, 2022). While Aylanc *et al.*, (2020) reported values of 65.75 mg TE/g for the hydromethanolic extract and 47.00 mg TE/g for the hydroethanolic extract for *T. spruneriana*.

A statistically significant correlation was observed between phenolic compounds and antioxidant activity, with a strong correlation for tannin content and a perfect correlation for phenol and flavonoid content. It has been noted that an extract with the highest total phenolic content does not always exhibit the highest biological activity, likely due to the diversity of phenolic compounds and their varying concentrations.

HPLC chromatographic analysis of the hydromethanolic extract of *Trigonella foenum graecum* revealed the presence of several molecules, including protocatechuic acid (3,4-dihydroxybenzoic acid, vanillic acid, luteolin, epigallocatechin gallate, apigenin, and rutin. However, the remaining molecules appearing on the chromatograms could not be identified.

5 Conclusion

This study reports that various fenugreek seed extracts, obtained using three extraction techniques (maceration, infusion, and decoction) and different solvents, presented extraction yields ranging from 1.81% to 16.68%. These extraction rates were influenced by the specific parameters of each extraction technique, such as temperature and contact time, as well as the polarity of the solvents employed.

An analytical study of the phytochemical composition and antioxidant activity of hydroethanolic (50 :50) and hydromethanolic (50 :50) extracts of *Trigonella foenum graecum* seed powder revealed that this plant possesses a unique metabolism enabling it to produce a rich array of bioactive molecules, primarily phenols, flavonoids, and tannins. These compounds are widely recognized for their beneficial and protective effects against biological and abiotic stresses on the plant.

Based on our findings, the bioactive compounds in *T. foenum graecum* extracts exhibited strong antioxidant activity, as assessed using the DPPH radical scavenging, FRAP, and TAC assays. HPLC chromatographic analysis of the hydromethanolic extract of *Trigonella foenum graecum* further elucidated the presence of several key molecules, including protocatechuic acid (3,4-dihydroxybenzoic acid), vanillic acid, luteolin, epigallocatechin gallate, apigenin, and Rutin.

In conclusion, *Trigonella foenum graecum* seeds are promising candidates for pharmaceutical and cosmetic formulations, offering potential for promoting health and reducing damage caused by oxidative stress.

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