Therapeutic Prospects and Comparative Bioactive Evaluation of Fenugreek Seed Extracts

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Ankit Kumar[1] and Vibha Rani[2]*

[1] PhD Scholar, Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62, Noida-201309, Uttar Pradesh

[2]Professor, Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62, Noida-201309, Uttar Pradesh.

vibha.rani@jiit.ac.in

Abstract

A medicinal plant with substantial therapeutic potential, fenugreek (*Trigonella foenum-graecum L.)* is rich in bioactive phytoconstituents. The phytochemical makeup and antioxidant properties of aqueous and methanolic fenugreek seed extracts were compared in this study. Phenolics, flavonoids, terpenoids, alkaloids, tannins, and saponins were found in both extracts, with the methanolic fraction showing greater intensity, according to preliminary phytochemical screening. The methanolic extract has higher levels of phenolic, flavonoid, and terpenoid content, according to quantitative analyses. Antioxidant analysis showed that the methanolic extract had better reducing potential and lipid peroxidation inhibition than the aqueous extract. Additionally, the methanolic extract showed increased capacity to scavenge free radicals from DPPH, ABTS, hydrogen peroxide, nitric oxide, superoxide, and hydroxyl radicals. The richer phytochemical composition of the methanolic extract may be responsible for its increased bioactivity. Overall, the results demonstrate the therapeutic value of fenugreek seed extracts, especially the methanolic extract, for possible phytopharmaceutical and nutraceutical uses in conditions linked to oxidative stress.

Keywords: Methanolic extract, Aqueous extract, Phytochemicals, Antioxidant activity, Free radical scavenging, Phenolic compounds, Flavonoids, Terpenoids, Oxidative stress, Therapeutic potential.

Introductions

Due to its many therapeutic benefits, fenugreek (*Trigonella foenum-graecum L.*) is a well-known medicinal and nutraceutical herb that is frequently utilized in traditional medical systems. Phenolics, flavonoids, alkaloids, terpenoids, saponins, and dietary fibers are among the many bioactive components found in fenugreek seeds that support antioxidant, anti-inflammatory, antidiabetic, cardioprotective, and antibacterial properties [1]. Because of these therapeutic qualities, fenugreek has drawn a lot of scientific interest in investigating its phytochemical makeup and biological potential. Geographical origin, climate, soil composition, harvesting season, and post-harvest processing techniques have all been found to have a significant impact on the phytoconstituent profile of medicinal plants. The biosynthesis and accumulation of secondary metabolites can be changed by variations in environmental and agroclimatic conditions, which might impact the medicinal effectiveness of plant extracts. Even though fenugreek has been the subject of many investigations, a thorough comparison of extracts made with various extraction techniques is still necessary to comprehend their therapeutic value and bifunctional characteristics [2]. The yield and variety of phytochemicals extracted from plant sources are largely dependent on the extraction technique. Because of their varying polarity and extraction efficacy toward various classes of phytoconstituents, aqueous and methanolic extraction systems were chosen for the current investigation. Aqueous extraction is very useful for separating hydrophilic substances like proteins, polysaccharides, glycosides, and certain phenolic components. It also imitates conventional herbal preparation techniques. On the other hand, methanolic extraction is frequently used to effectively recover bioactive molecules that are moderately polar to polar, such as flavonoids, terpenoids, tannins, and phenolics, which are frequently linked to increased antioxidant and medicinal properties. Therefore, a comparative analysis of different extraction systems offers important information

about the solvent-dependent recovery of bioactive compounds and the biological potential that goes along with them [3]. The current study was created to examine the bioactive profile and therapeutic potential of fenugreek seed extracts by a comparative analysis of aqueous and methanolic extracts, given the increasing interest in plant-derived medicines and natural antioxidants. The goal of the research is to improve knowledge of solvent-mediated phytochemical diversity and its potential effects on the creation of medicinal formulations based on fenugreek [4].

Material and methods:

Preparation of Fenugreek Seed Extracts

Fenugreek seeds were obtained from a nearby Noida local market and verified by morphological traits. The seeds were shade-dried at room temperature, thoroughly cleaned with distilled water to remove dust and other contaminants, and then mechanically milled into a fine powder. Until it was needed again, the powdered substance was kept in airtight containers [5]. The powdered seed material was combined with distilled water in a 1:10 (w/v) ratio for aqueous extraction, and the mixture was continuously stirred while being incubated at 60–70°C for four to six hours. After cooling, the extract was filtered using Whatman No. 1 filter paper. To extract methanol, the seed powder was macerated with 80% methanol while being continuously stirred for 48 hours at room temperature. The resulting extract was filtered and concentrated under reduced pressure [6], [7]. (Figure 1)

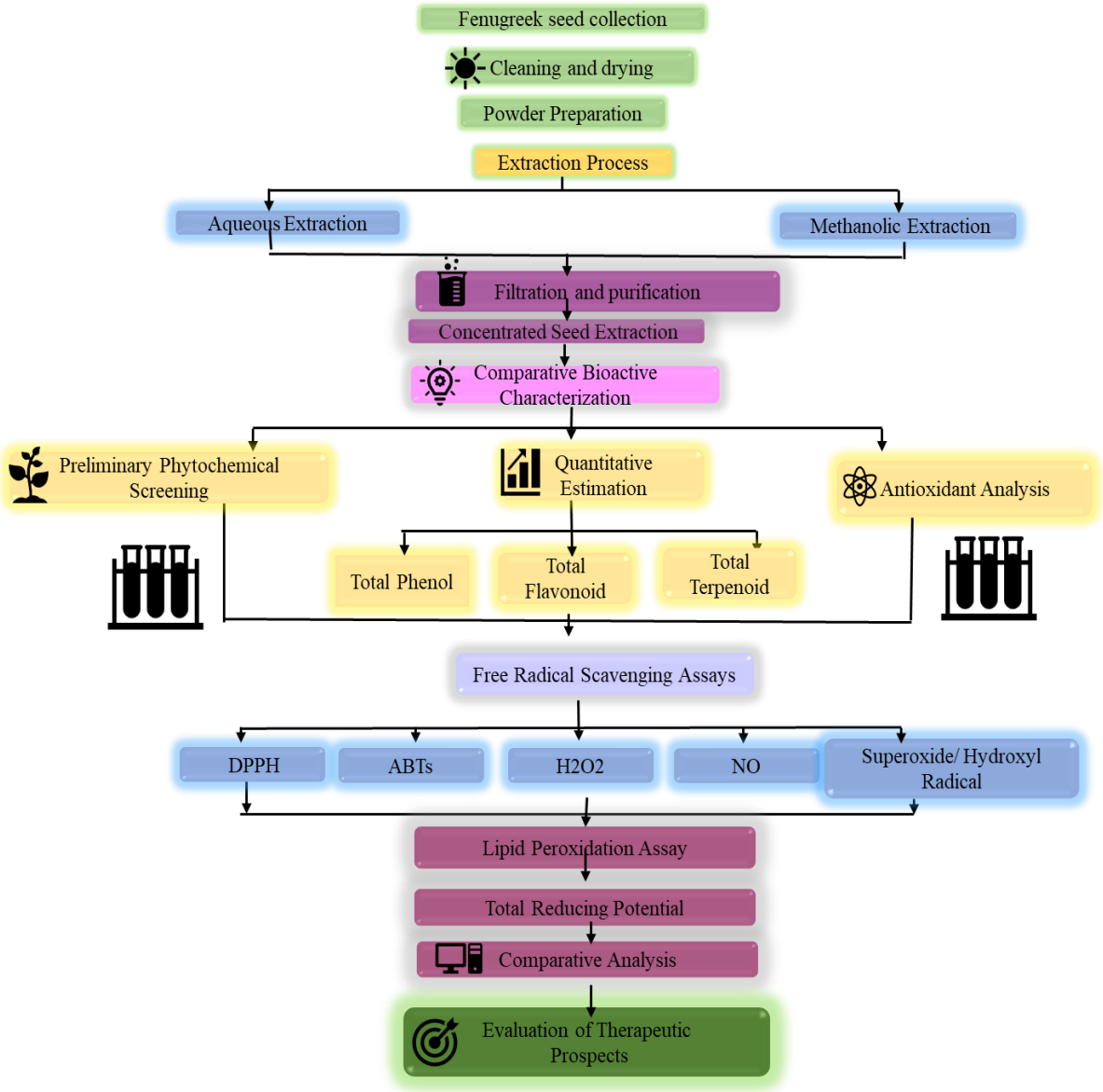


Figure 1. Experimental workflow for the preparation, phytochemical characterization, quantitative estimation, and antioxidant evaluation of aqueous and methanolic extracts of *Trigonella foenum-graecum* seeds.

Purification of Extracts

In order to exclude undesirable particulate matter and insoluble contaminants, the crude extracts were first purified. The concentrated extracts were filtered through 0.22 µm membrane filters after being centrifuged for 15 minutes at 10,000 rpm. Before biochemical studies, the pure extracts were lyophilized and reconstituted in suitable solvents.

Preliminary Phytochemical Screening

Standard biochemical techniques were used to qualitatively screen aqueous and methanolic extracts for important phytoconstituents such as alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins, glycosides, carbohydrates, proteins, and steroids. The presence of the corresponding phytochemicals was thought to be indicated by the formation of distinctive color changes or precipitates.

Determination of Total Phenolic Content
The Folin-Ciocalteu technique was used to estimate the total phenolic content. In short, the extract was combined with Folin-Ciocalteu reagent and then sodium carbonate solution, and it was left in the dark for half an hour. A UV-visible spectrophotometer was used to measure absorbance at 765 nm. The total phenolic content was reported as mg gallic acid equivalent (GAE)/g extract, with gallic acid serving as the standard [8].

Determination of Total Flavonoid Content
The aluminum chloride colorimetric assay was used to calculate the total flavonoid concentration. After combining the extract with distilled water, potassium acetate, and aluminum chloride, it was incubated for half an hour at room temperature. At 415 nm, absorbance was measured. Flavonoid concentration was measured in mg quercetin equivalent (QE)/g extract, with quercetin serving as the reference standard [9].

Determination of Total Terpenoid Content
The spectrophotometric approach was used to estimate the total terpenoid content. To get a color intensity that matched the terpenoid concentration, the extract was combined with concentrated sulfuric acid and chloroform. Results were represented as mg linalool equivalent/g extract, and absorbance was measured at 538 nm[10] .

DPPH Radical Scavenging Assay

The DPPH free radical scavenging experiment was used to assess the extracts' antioxidant capacity. After mixing various extract concentrations with DPPH solution, the mixture was left in the dark for 30 minutes. Radical scavenging activity was computed as a percentage inhibition compared to the control, and absorbance was measured at 517 nm [11] .

ABTS Radical Scavenging Assay
By reacting with potassium persulfate to produce ABTS radicals, the ABTS radical scavenging activity was measured. After combining the extract samples with the radical solution, they were incubated for ten minutes. At 734 nm, absorbance was measured, and the scavenging activity percentage was calculated [12].

Lipid Peroxidation Inhibition Assay
The thiobarbituric acid reactive substances (TBARS) assay was used to measure the extracts' inhibitory effect on lipid peroxidation. The thiobarbituric acid reagent was added after the reaction mixtures, including the lipid substrate, extract, and pro-oxidant system, were incubated. Absorbance was measured at 532 nm following heating and cooling. In comparison to the control, the degree of lipid peroxidation inhibition was computed.

Total Reducing Power Assay
The potassium ferricyanide reduction assay was used to measure the extracts' reducing potential. Extracts were incubated at 50°C after being combined with potassium ferricyanide and phosphate buffer. After incubation, the mixture was centrifuged, and trichloroacetic acid was added. After adding ferric chloride to the supernatant, the absorbance at 700 nm was measured. Higher reducing power was shown by increased absorption [13].

Hydrogen Peroxide (H_2O_2) Scavenging Assay

By combining extract samples with a hydrogen peroxide solution made in a phosphate buffer, the hydrogen peroxide scavenging activity was assessed. Absorbance at 230 nm was measured following incubation in comparison to a blank solution. The drop in absorbance was used to calculate the percentage of scavenging activity.

Nitric Oxide Scavenging Assay
Sodium nitroprusside was used as a nitric oxide donor to measure nitric oxide scavenging activity. Griess reagent was added after reaction solutions containing extract and sodium nitroprusside were incubated in light. At 546 nm, absorbance was measured, and the % suppression of nitric oxide scavenging activity was computed [14].

Superoxide Radical Scavenging Assay
The riboflavin–NBT photoreduction method was used to measure the superoxide radical scavenging activity. Light was applied to reaction mixtures comprising riboflavin, nitro blue tetrazolium (NBT), methionine, and extract samples. At 560 nm, absorbance was measured, and the percentage of superoxide radical inhibition was computed [15].

Hydroxyl Radical Scavenging Assay
The Fenton reaction method was used to test the activity of hydroxyl radical scavenging. $FeSO_4$, hydrogen peroxide, salicylic acid, and extracts were all included in the reaction mixture, which was incubated at room temperature. The efficacy of hydroxyl radical scavenging was assessed by measuring absorbance at 510 nm.

Statistical Analysis
Every experiment was carried out three times, and the results were reported as mean ± standard deviation. Proper software was used for statistical analysis, and one-way ANOVA and proper post hoc analysis were used to assess the significance between groups. Statistical significance was defined as a p-value of less than 0.05 [16].

Results and Discussion

Comparative Phytochemical Profiling
Both aqueous and methanolic fenugreek seed extracts included phenolics, flavonoids, terpenoids, tannins, alkaloids, saponins, and glycosides, according to preliminary phytochemical screening, is showing in Table 1. Stronger phytochemical intensity in the methanolic extract was found in comparative studies, indicating higher extraction efficiency for bioactive metabolites. Methanol's polarity, which promotes the solubilization of somewhat polar phytoconstituents, may be the cause of this improved recovery.
Quantitative measurement also showed that the methanolic extract had much larger concentrations of terpenoids, flavonoids, and total phenols than the aqueous extract. Improved extraction of secondary metabolites rich in antioxidants in methanol is indicated by the increased phytochemical abundance. The higher bioactivity seen in the methanolic extract is significantly correlated with the increased content of phenolic and flavonoid components, which are important contributors to antioxidative defence mechanisms.

Table 1. Preliminary phytochemical screening indicates the presence and relative abundance of bioactive constituents in aqueous and methanolic extracts of fenugreek seeds based on qualitative biochemical observations.

Phytochemicals	Aqueous Extract (AE) Observation	AE Inference	Methanolic Extract (ME) Observation	ME Inference
Alkaloids	Light yellow precipitate	+	Creamy precipitate	++
Flavonoids	Light yellow color	++	Intense yellow color	++
Phenolics	Greenish blue color	++	Deep blue color	+++
Tannins	Dark green color	+++	Dark green color	+++
Saponins	Froth (1 cm)	++	Froth (2 cm)	+++
Terpenoids	Reddish-brown interface	++	Dark reddish-brown interface	+++
Glycosides	Light pink color	+	Deep pink color	++
Steroids	Yellow color	+	Intense yellow color	++
Carbohydrates	Brick red precipitate	+	Deep brick red precipitate	++
Proteins	Light violet color	+	Deep violet color	++
Fixed oils & fats	Slight oily stain on filter paper	+	Intense permanent oily stain	++
Reducing sugars	Deep brick red precipitate	++	Light brick red precipitate	+
Coumarins	Pale yellow fluorescence	+	Bright yellow fluorescence	++
Quinones	Light pink coloration	+	Deep reddish pink coloration	++
Anthraquinones	No pink/red coloration	-	Pink to red coloration	+

The methanolic extract had a far larger phytochemical content than the aqueous extract, according to the quantitative estimate of bioactive components. In the aqueous extract, the total phenolic content was 62.35 ± 2.71 mg GAE/g extract, while in the methanolic extract, it was 108.67 ± 3.24 mg GAE/g extract. Similarly, in the aqueous and methanolic extracts, the total flavonoid concentration was determined to be 45.12 ± 2.10 mg QE/g extract and 92.48 ± 2.83 mg QE/g extract, respectively. The aqueous extract had a total terpenoid content of 22.18 ± 1.38 mg linalool equivalent/g extract, while the methanolic extract had 48.73 ± 1.92 mg linalool equivalent/g extract. The mean ± standard deviation (SD) of triplicate experiments (n = 3) is used to express all values (Figure 2).

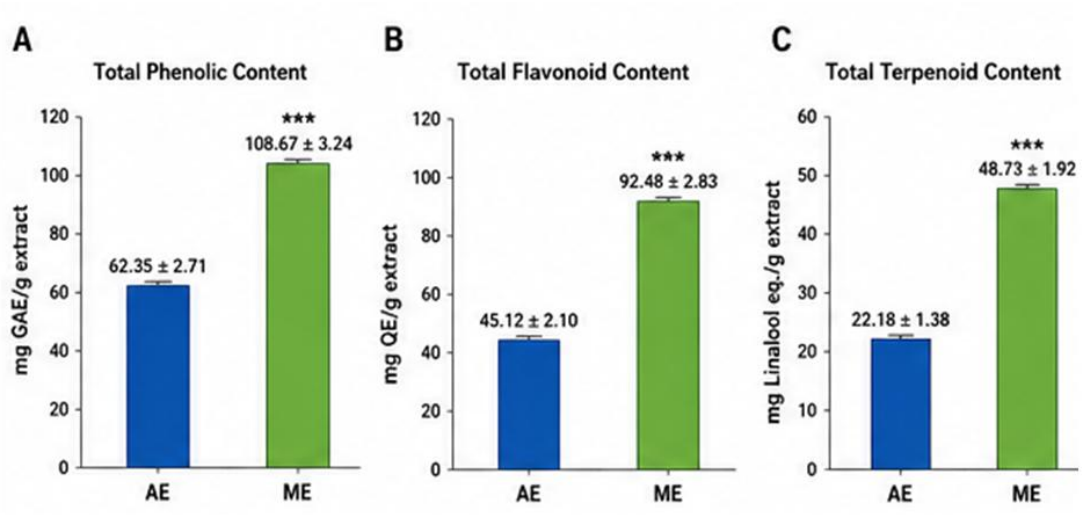
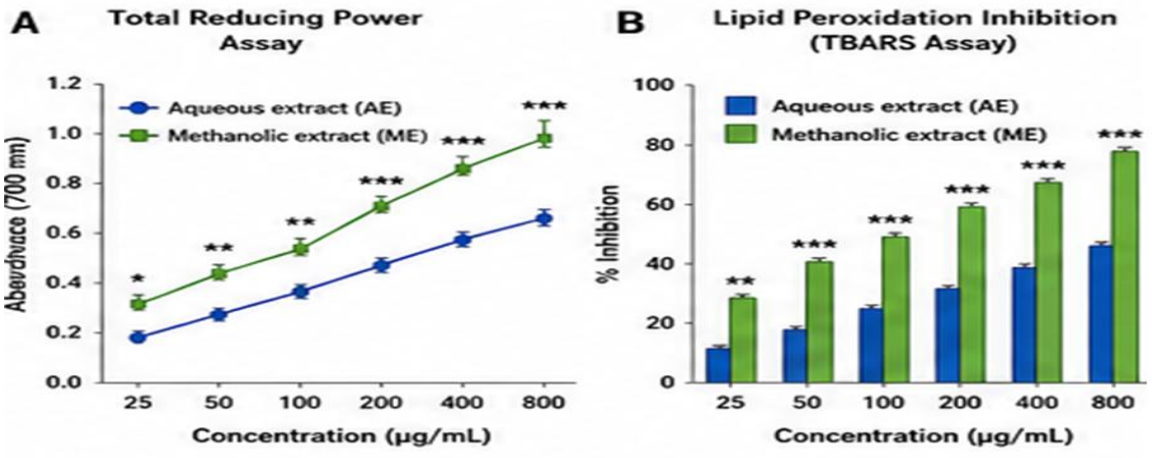


Figure 2. Quantitative estimation of bioactive compounds in aqueous (AE) and methanolic (ME) extracts of *Trigonella foenum-graecum* seeds showing (A) total phenolic content, (B) total flavonoid content, and (C) total terpenoid content.

Both the aqueous and methanolic extracts of fenugreek seeds showed concentration-dependent increases in antioxidant activity, with the methanolic extract showing noticeably higher activity, according to the comparative antioxidant study. The absorbance of the methanolic extract ranged from 0.31 to 0.98 at equivalent concentrations in the total reducing power assay, while the absorbance of the aqueous extract increased from 0.18 at 25 µg/mL to 0.66 at 800 µg/mL. In a similar vein, the TBARS assay for lipid peroxidation inhibition showed that the methanolic extract had more inhibitory action than the aqueous extract. The methanolic extract showed significantly greater inhibition, ranging from 28.4% to 76.8% across doses of 25–800 µg/mL, whereas the aqueous extract showed inhibition values ranging from 11.2% to 46.2%. The observed antioxidant potential indicates the stronger free radical scavenging and reducing capacity of the methanolic extract, likely due to its higher phenolic, flavonoid, and terpenoid contents.



Values are mean ± SD (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 vs. AE

Figure 3. Comparative antioxidant potential of aqueous (AE) and methanolic (ME) extracts of *Trigonella foenum-graecum* seeds showing (A) total reducing power assay and (B) lipid peroxidation inhibition (TBARS assay).

Comparative Antioxidant Potential

Total reducing power and lipid peroxidation inhibition tests were used to assess the antioxidant capability of fenugreek seed extracts. Both extracts showed concentration-dependent antioxidant activity, although the methanolic extract was consistently far more effective. Increased absorbance values in the total reducing power assay demonstrated a significantly improved reducing potential in the methanolic extract in Figure 3A. The findings show that methanol-extracted phytoconstituents had increased reductive activity and a greater potential to donate electrons.

Similarly, compared to the aqueous extract, the methanolic extract showed noticeably greater prevention of lipid peroxidation in Figure 3B. Improved antioxidant stability and successful avoidance of oxidative membrane damage were demonstrated by decreased production of thiobarbituric acid reactive compounds (TBARS). The enhanced phenolic and flavonoid makeup of the methanolic extract may be responsible for its improved antioxidant efficacy.

Comparative Free Radical Scavenging Activities

DPPH, ABTS, hydrogen peroxide, and nitric oxide radical scavenging assays were used to assess the free radical scavenging capabilities of aqueous and methanolic fenugreek seed extracts. Both extracts showed concentration-dependent antioxidant activity; however, at all tested doses 25–800 µg/mL, the methanolic extract showed a far greater scavenging potential than the aqueous extract. The methanolic extract showed greater inhibition in the DPPH experiment, ranging from 18.2% to 78.4%, while the aqueous extract demonstrated radical scavenging activity ranging from 6.4% to 52.6%. ABTS radical scavenging activity also rose from 21.5% to 90.1% in the methanolic extract and from 7.3% to 56.3% in the aqueous extract. The aqueous extract's hydrogen peroxide scavenging activity ranged from 6.8% to 54.1%, whereas the methanolic extract's ranged from 20.2% to 87.6%. The methanolic extract showed more activity in the nitric oxide scavenging experiment, ranging from 22.4% to 85.2%, whereas the aqueous extract showed inhibitory values ranging from 8.1% to 51.8%. The higher quantity of phenolics, flavonoids, and terpenoids in the methanolic extract may be responsible for its increased antioxidant activity. These compounds are known to play a major role in reducing oxidative stress and neutralising free radicals.

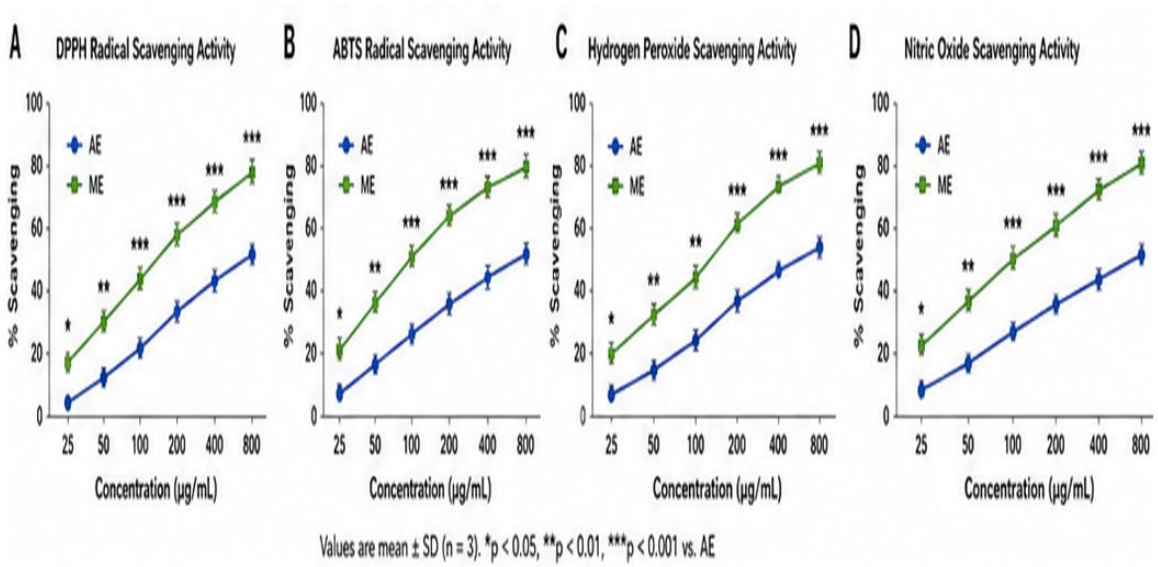


Figure 4. Comparative free radical scavenging activities of aqueous (AE) and methanolic (ME) extracts of *Trigonella foenum-graecum* seeds showing (A) DPPH radical scavenging activity, (B) ABTS radical scavenging activity, (C) hydrogen peroxide (H_2O_2) scavenging activity, and (D) nitric oxide (NO) scavenging activity.

The aqueous extract exhibited scavenging activity ranging from 8.7% to 54.1% in the superoxide radical scavenging experiment, while the methanolic extract showed significantly greater inhibition ranging from 23.4% to 82.3%. Similarly, the methanolic extract showed increased scavenging activity ranging from 22.6% to 82.6% in the hydroxyl radical scavenging assay, whereas the aqueous extract showed inhibitory values between 8.2% and 47.4%. The increased content of phenolic chemicals, flavonoids, and terpenoids in the methanolic extract may be responsible for its improved antioxidant activity. These substances are crucial for neutralising reactive oxygen species and shielding biological systems from oxidative damage.

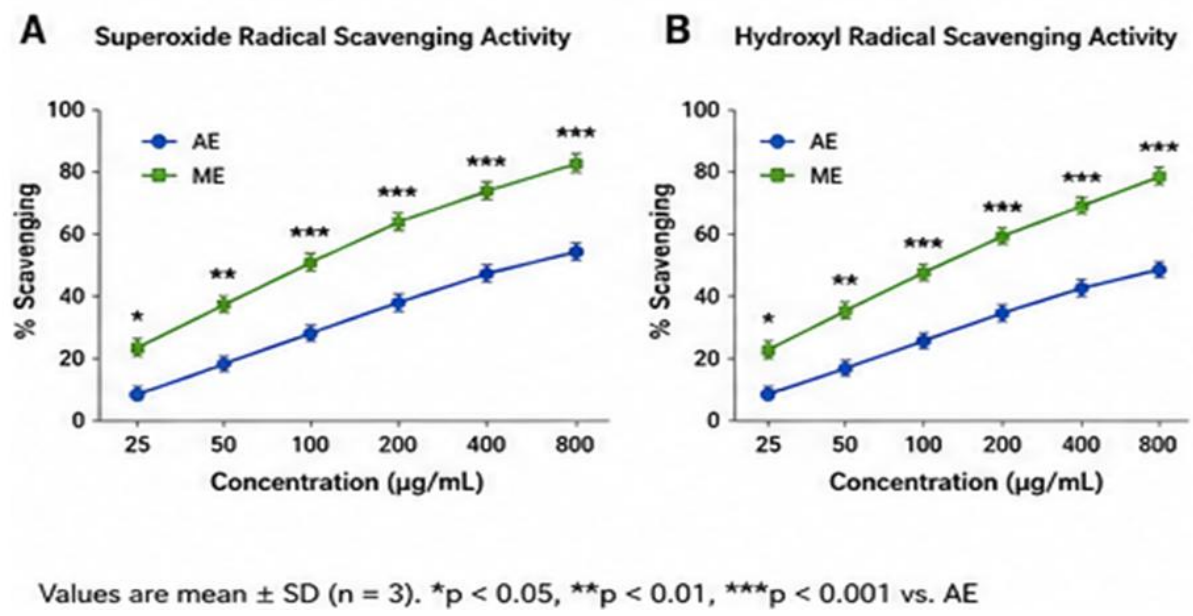


Figure 5. Comparative free radical scavenging activities of aqueous (AE) and methanolic (ME) extracts of *Trigonella foenum-graecum* seeds showing (A) superoxide radical scavenging activity and (B) hydroxyl radical scavenging activity.

Strong free radical scavenging action was shown by both aqueous and methanolic extracts against a variety of reactive oxygen and nitrogen species; however, the methanolic extract exhibited noticeably higher inhibition in all tests. In comparison to the water extract, the methanolic extract demonstrated higher DPPH and ABTS radical scavenging activity in Figure 4A and Figure 4B, suggesting improved hydrogen-donating capacity and increased electron-transfer potential. The improved antioxidant capability of methanol-extracted phytochemicals is reflected in increased scavenging effectiveness against these radicals. Greater neutralization ability in the methanolic extract was shown by the hydrogen peroxide scavenging assay in Figure 4C, indicating improved defence against oxidative stress-mediated cellular damage. Stronger anti-inflammatory and cytoprotective qualities were also demonstrated by the much higher nitric oxide scavenging activity in the methanolic extract compared to the water extract in Figure 4D. Additionally, the methanolic extract demonstrated significantly improved superoxide and hydroxyl radical scavenging in Figure 5A and Figure 5B. Improved inhibition indicates better protective efficacy against oxidative deterioration of lipids, proteins, and nucleic acids because these radicals are extremely reactive and can cause biomolecular damage.

Overall Comparative Evaluation and Therapeutic Implications

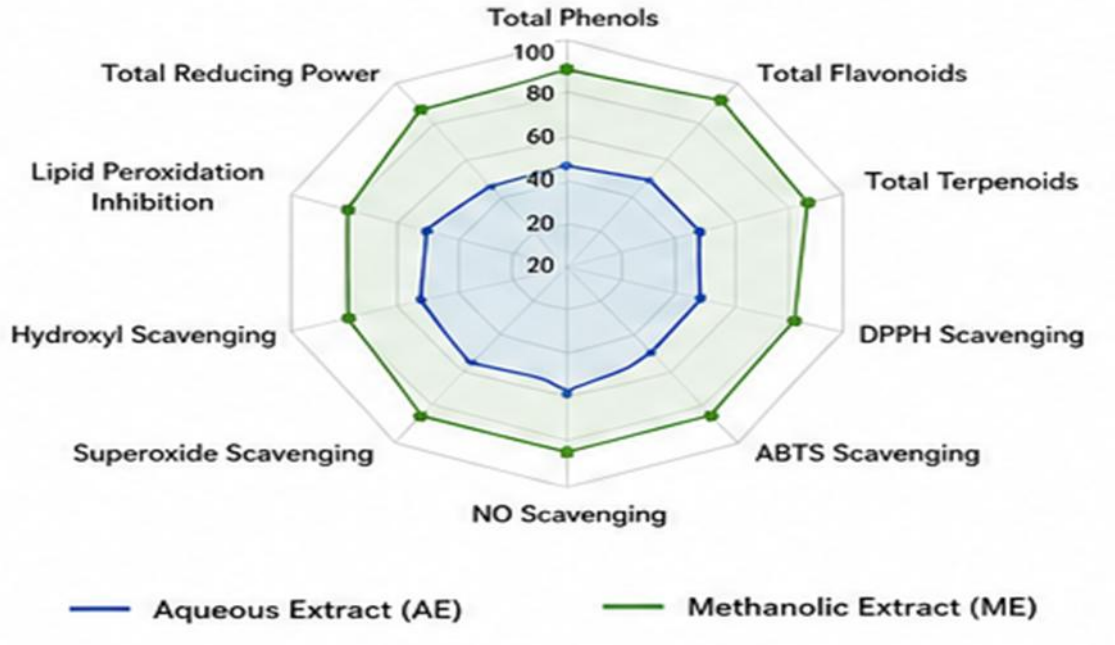


Figure 6. Overall comparative summary of the phytochemical composition and antioxidant activities of aqueous (AE) and methanolic (ME) extracts of *Trigonella foenum-graecum* seeds, presented as a radar plot highlighting the superior bioactive and antioxidant potential of the methanolic extract.

When compared to water extraction, the results showed that methanolic extraction produced bioactive-rich fractions with much higher phytochemical abundance and superior antioxidant activity in Figure 6. Stronger reducing power, suppression of lipid peroxidation, and higher free radical scavenging effectiveness were all facilitated by the methanolic extract's increased concentrations of phenolics, flavonoids, and terpenoids. These findings demonstrate the therapeutic value of methanolic fenugreek seed extract and bolster its probable use in the creation of nutraceuticals and phytopharmaceutical formulations rich in antioxidants that may have anti-inflammatory, cardioprotective, and cytoprotective properties.

Discussion

In comparison to aqueous extract, the current study showed that methanolic fenugreek seed extract has greater phytochemical richness and antioxidant capacity, indicating its increased therapeutic value. Improved reducing potential, prevention of lipid peroxidation, and increased free radical scavenging activities were all substantially linked with the much higher amounts of phenolics, flavonoids, and terpenoids in the methanolic extract. These results are consistent with many other studies that found that because methanolic solvents are polar and may dissolve semi-polar bioactive chemicals, they are more effective at removing antioxidant-rich phytoconstituents from medicinal plants.

It has long been known that fenugreek is a medicinal herb with potential therapeutic uses, especially in metabolic and cardiovascular conditions. Its antidiabetic qualities may be significantly influenced by the antioxidant-rich profile found in this study. One of the main pathogenic factors linked to insulin resistance, pancreatic β-cell malfunction, tissue damage caused by hyperglycaemia, and the consequences of diabetes is oxidative stress. The methanolic extract's increased capacity to scavenge free radicals indicates that it can lower oxidative stress and boost cellular redox equilibrium. Previous research has shown that phytoconstituents produced from fenugreek, including flavonoids, diosgenin, trigonelline, and polyphenols, are essential for boosting insulin sensitivity, increasing glucose metabolism, and reducing inflammatory pathways linked to the development of diabetes.

Fenugreek seed extracts may have cardioprotective qualities, as evidenced by the reported prevention of lipid peroxidation and increased reducing power. One of the main causes of endothelial dysfunction, atherosclerosis, myocardial damage, and cardiac remodelling is oxidative stress-mediated lipid oxidation. Fenugreek phytoconstituents may help shield cardiac tissues from oxidative stress by effectively scavenging reactive oxygen species and avoiding membrane lipid oxidation. Fenugreek has been shown in numerous experimental trials to have cardioprotective benefits via controlling lipid metabolism, lowering blood cholesterol, reducing inflammatory mediators, and enhancing antioxidant enzyme defence mechanisms. The current study's findings regarding the high antioxidant effectiveness of methanolic extract thus provide credence to its potential use in cardiovascular protection and the treatment of cardiac diseases associated with oxidative stress.

The study's findings regarding the scavenging of superoxide and nitric oxide radicals reinforce the anti-inflammatory and cytoprotective properties of fenugreek seed extracts. Overproduction of reactive oxygen and nitrogen species is linked to tissue deterioration, apoptosis, mitochondrial malfunction, and chronic inflammation. The methanolic extract's improved scavenging capacity suggests that it may play a part in preventing cellular damage and preserving physiological homeostasis. These results are consistent with previous findings

that fenugreek-derived antioxidants may enhance cellular resistance to oxidative assaults and modify inflammatory signalling pathways. The study also highlights how crucial extraction techniques are to phytochemical output and medicinal effectiveness. Methanolic extraction was found to be more successful in retrieving antioxidant-rich phytoconstituents, even though aqueous extraction resembles conventional herbal preparation methods. This emphasizes how important it is to use appropriate extraction methods to maximize bioactive recovery and create standardized plant-based formulations.

Beyond their antioxidant properties, fenugreek seed extracts have medicinal value. Fenugreek may be a useful source for nutraceuticals, functional foods, and phytopharmaceutical uses because it contains a variety of phytoconstituents. A solid foundation for investigating fenugreek in the treatment of diabetes, cardiovascular illnesses, inflammatory disorders, and other oxidative stress-mediated pathologies is provided by the potent antioxidant and free radical scavenging qualities found in this study. The current study is restricted to in vitro biochemical tests, despite encouraging results. To confirm the therapeutic effectiveness and molecular pathways linked to fenugreek bioactive, more research using cellular and in vivo experimental models is required. Future research should concentrate on toxicity profiling, bioavailability analysis, molecular interaction studies, isolation and characterization of specific phytoconstituents, and assessment of signalling pathways implicated in cardiometabolic and antioxidant protection. Further insights into phytochemical diversity and therapeutic optimization may be obtained by comparative research based on geographic origin, growth circumstances, and extraction technologies. Methanolic fenugreek seed extract is a powerful source of bioactive antioxidants with potential therapeutic uses, according to the study's overall findings. The methanolic extract's increased phytochemical richness and antioxidative effectiveness demonstrate its potential use in the creation of evidence-based natural remedies and formulations for preventive healthcare.

Conclusion

The results of this study showed that fenugreek seed extracts have strong bioactive and antioxidant potential, with the methanolic extract being more effective than the aqueous extract. The methanolic fraction's higher concentrations of phenolics, flavonoids, and terpenoids boosted free radical scavenging capabilities, increased reducing power, and inhibited lipid peroxidation. These results demonstrate the therapeutic value of fenugreek seed extracts, especially in conditions like diabetes and cardiovascular illnesses that are linked to oxidative stress. All things considered, methanolic fenugreek extract might be a good option for phytopharmaceutical and nutraceutical uses.

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Conflict of interest

The authors declare no conflict of interest.

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