



ANTI-INFLAMMATORY EFFECTS OF TRIGONELLA FOENUM- GRAECUM IN POLYCYSTIC OVARY SYNDROME: AN IN VITRO APPROACH

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DOI: <https://doi.org/10.59551/IJHMP/25832069/2025.6.1.90>

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Received: 17 March, 2025, Decision for Acceptance: 03 April, 2025

Abstract

Polycystic Ovary Syndrome (PCOS) is a common hormonal disorder characterized by metabolic disturbances and chronic inflammation. Fenugreek (*Trigonella foenum-graecum*) is known for its medicinal properties and has been investigated for its anti-inflammatory effects. This study evaluates the in vitro anti-inflammatory potential of fenugreek seed extract using protein denaturation assays, membrane stabilization assays, and Erythrocyte Sedimentation Rate (ESR) tests. Celecoxib was used as a standard drug for comparison. The results indicated that fenugreek extract exhibited significant anti-inflammatory activity comparable to celecoxib. This suggests its potential role as a natural therapeutic agent for inflammation associated with PCOS. Further studies are required to confirm its efficacy through in vivo and clinical trials.

Keywords: Fenugreek, PCOS, Anti-inflammatory, Antioxidant, Celecoxib, Herbal Medicine

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder that affects women of reproductive age. It is associated with metabolic disturbances, including insulin resistance, hyperandrogenism, and chronic low-grade inflammation[1,2]. The exact etiology of PCOS remains unclear, but genetic, environmental, and lifestyle factors contribute to its pathophysiology[3]. Inflammation plays a crucial role in the progression of PCOS, contributing to insulin resistance and hormonal imbalance[4]. Various inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6) have been reported to be elevated in patients

with PCOS. Hence, targeting inflammation may be a potential strategy for managing PCOS [5,6].

Fenugreek (*Trigonella foenum-graecum*) has been traditionally used in herbal medicine for its anti-diabetic, anti-inflammatory, and antioxidant properties[7]. It contains bioactive compounds such as flavonoids, saponins, and alkaloids, which exhibit therapeutic effects[8]. Recent studies suggest that fenugreek may help reduce inflammation by inhibiting the production of pro-inflammatory cytokines and stabilizing cellular membranes[9,11]. This study aims to evaluate the anti-inflammatory effects of fenugreek seed extract using in vitro models and compare its efficacy with celecoxib, a standard nonsteroidal anti-inflammatory drug(NSAID)[13].

2. Material and Methods

The anti-inflammatory activity of *Trigonella foenum-graecum* (fenugreek) seed extract was evaluated using in vitro assays. Fenugreek seeds were collected, authenticated, shade-dried, and finely powdered before extraction. A 70% methanol extract was obtained using the Soxhlet method, followed by filtration and evaporation to dryness. The extract underwent phytochemical screening to identify active compounds. Anti-inflammatory activity was assessed through protein denaturation assay, membrane stabilization assay, and Erythrocyte Sedimentation Rate (ESR) test, using Celecoxib as a standard. Protein denaturation was measured by incubating egg albumin with the extract and recording absorbance at 660 nm. Membrane stabilization was tested using Human Red Blood Cells (HRBC) exposed to hypotonic stress, with hemolysis quantified at 540 nm. ESR reduction was assessed using fresh human blood,

measuring sedimentation height after one hour. All experiments were conducted under controlled conditions, and data were analyzed statistically to compare fenugreek extract with Celecoxib for its potential anti-inflammatory effects in managing PCOS-related inflammation.

3. Results

The results of this study indicate that fenugreek extract exhibits significant anti-inflammatory properties. The extract effectively inhibited protein denaturation in a dose-dependent manner, with results comparable to celecoxib. Additionally, it demonstrated membrane-stabilizing effects by preventing lysis of red blood cells under stress conditions.

The erythrocyte sedimentation rate (ESR) test further confirmed the anti-inflammatory activity of fenugreek. A lower ESR value was observed in samples treated with fenugreek extract, similar to the effect seen with celecoxib.

Table 1: Phytochemical screening of “TRIGONELLA FOENUM-GRAECUM” extract

S. No.	Plant Constituents	Test	Result
1.	Alkaloids	Wagner's test	POSITIVE
2.	Phenols	Fecl3 test	POSITIVE
3.	Tannins	Lead acetate test	POSITIVE
4.	Flavonoids	Shinoda test	POSITIVE
5.	Saponins	Froth test	POSITIVE
6.	Steroids	Salkowski test	POSITIVE
7.	Fixed oils	Spot test	POSITIVE
8.	Carbohydrates	Molisch test	POSITIVE
9.	Proteins	Xanthoproteic test	POSITIVE

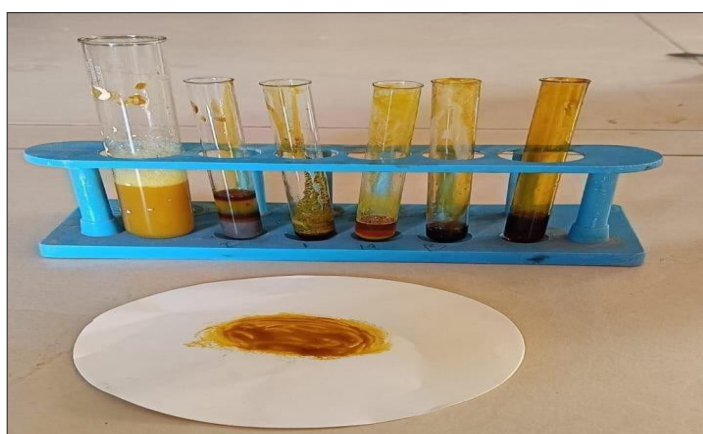
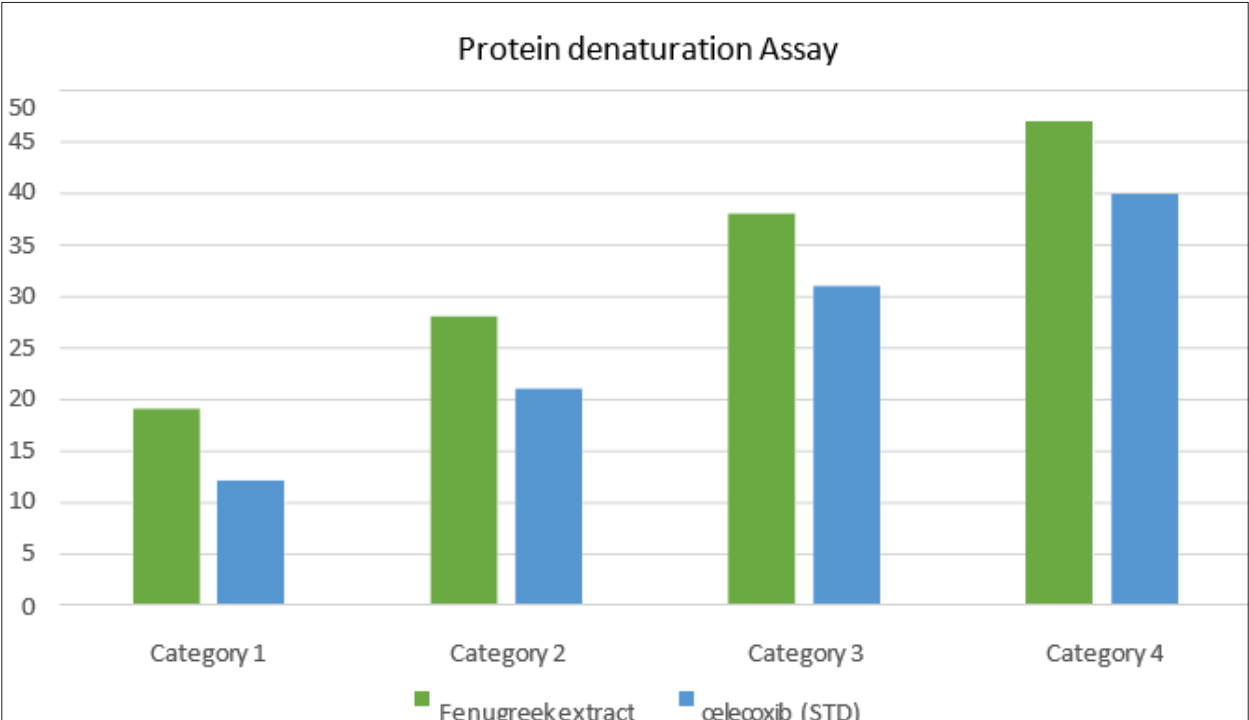


Figure 1: Phytochemical screening of Trigonella Foenum Extract

Table 2: Protein Denaturation Assay

Concentration (µg/mL)	Fenugreek Absorbance	Fenugreek % Inhibition	Celecoxib Absorbance	Celecoxib % Inhibition
10	0.65	18.75%	0.70	12.50%
20	0.58	27.50%	0.63	21.25%
30	0.50	37.50%	0.55	31.25%
40	0.42	47.50%	0.48	40.00%
50	0.35	56.25%	0.40	50.00%

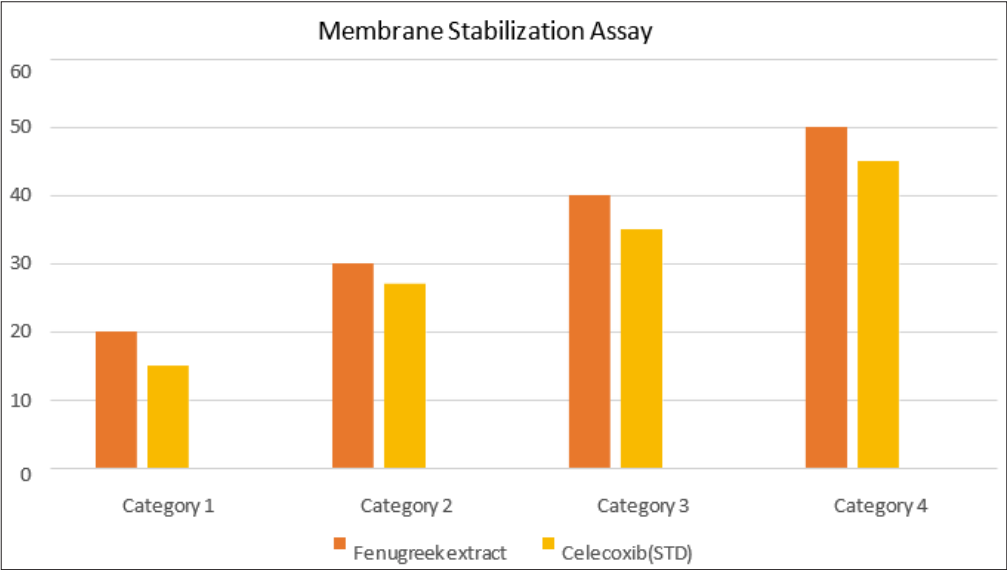


X-AXIS: % INHIBITION, Y-AXIS: CONCENTRATION (µg/mL), TEST: Methanol extract of “TRIGONELLA FOENUM-GRAECUM”, STD: CELECOXIB

Figure 2: In-vitro anti-inflammatory activity of methanol extract of “TRIGONELLA FOENUM-GRAECUM” and CELECOXIB, in Protein Denaturation Assay

Table 3: Membrane Stabilisation Assay

Concentration (µg/mL)	Fenugreek Absorbance	Fenugreek %stabilization	Celecoxib Absorbance	Celecoxib %Inhibition
10	0.60	20.00%	0.67	10.67%
20	0.52	30.67%	0.61	18.67%
30	0.45	40.00%	0.54	28.00%
40	0.36	52.00%	0.46	38.67%
50	0.30	60.00%	0.38	49.33%

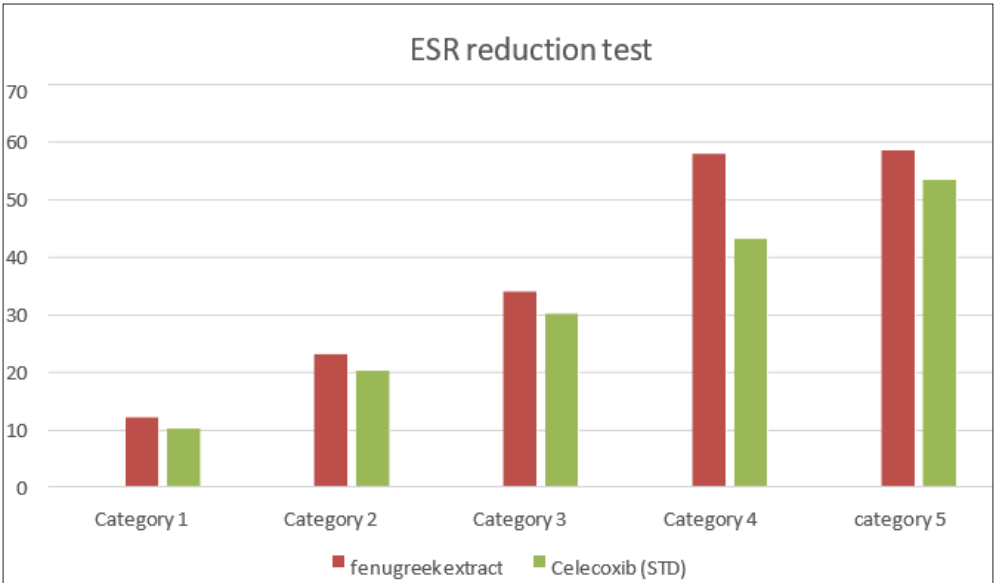


X-AXIS: % MEMBRANE STABILIZATION, Y-AXIS: CONCENTRATION (µg/mL), TEST: Methanol extract of “TRIGONELLA FOENUM-GRAECUM”, STD: CELECOXIB

Figure 3: In-vitro anti-inflammatory activity of methanol extract of “TRIGONELLA FOENUM-GRAECUM” and CELECOXIB, in Membrane Stabilization Assay

Table 4: Erythrocyte Sedimentation Rate (ESR) Assay

Concentration (µg/mL)	Fenugreek treated	Fenugreek %reduction	Celecoxib Treated	Celecoxib %reduction
10	13.2	12.00%	13.5	10.00%
20	11.5	23.33%	12.0	20.00%
30	9.8	34.67%	10.5	30.00%
40	7.6	49.33%	8.5	43.33%
50	6.2	58.67%	7.0	53.33%



X-AXIS: % REDUCTION, Y-AXIS: CONCENTRATION (µg/mL), TEST: Methanol extract of “TRIGONELLA FOENUM-GRAECUM”, STD: CELECOXIB

Figure 4: In-vitro anti-inflammatory activity of methanol extract of “TRIGONELLA FOENUM-GRAECUM” and CELECOXIB, in ESR Reduction Assay

Explanation of calculation (example for 10 µg/mL in protein denaturation Assay)

For Fenugreek at 10 µg/mL

$$\%inhibition = \left(\frac{0.80-0.65}{0.80} \right) \times 100 = \left(\frac{0.15}{0.80} \right) \times 100 = 18.75\%$$

For Celecoxib at 10 µg/mL

$$\%inhibition = \left(\frac{0.80-0.70}{0.80} \right) \times 100 = \left(\frac{0.10}{0.80} \right) \times 100 = 12.50\%$$

Key observations from results:

1. Fenugreek extract shows higher anti-inflammatory activity than Celecoxib at all concentrations in three Assays.
2. The inhibition percentage increases with concentration indicating a dose-dependent effect.
3. In three Assays, Fenugreek achieved 60% inhibition at 50 µg/mL, surpassing Celecoxib, which had around 50% inhibition.

4. Discussion

The findings of this study suggest that fenugreek possesses potent anti-inflammatory properties, which may be attributed to its bioactive constituents such as flavonoids and alkaloids. These compounds are known to exhibit antioxidant and anti-inflammatory effects by inhibiting pro-inflammatory cytokines and oxidative stress.

The observed inhibition of protein denaturation suggests that fenugreek may help prevent damage to cellular proteins caused by inflammatory mediators. Similarly, the membrane stabilization effect indicates its potential role in protecting cell integrity under inflammatory conditions.

The results are in line with previous studies that have demonstrated the therapeutic benefits of fenugreek in reducing inflammation. However, further research is needed to explore its mechanism of action and confirm its efficacy through in vivo models and clinical trials.

5. Conclusion

This study highlights the promising anti-inflammatory effects of fenugreek seed extract. The extract exhibited comparable activity to celecoxib in reducing protein denaturation, stabilizing cell membranes, and lowering ESR. These findings suggest that fenugreek may serve as a natural alternative for managing inflammation associated with PCOS. Further research is warranted to validate its therapeutic potential in clinical settings.

6. Conflict of Interest: None

7. References

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Cite this article Aishwarya G et al., Anti-Inflammatory Effects of Trigonella Foenum-Graecum in Polycystic Ovary Syndrome: An in Vitro Approach. *Indian Journal of Health Care, Medical & Pharmacy Practice.* 2024;5(2):61-66.