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EVALUATION OF ANTI-ULCER ACTIVITY OF THE SEED EXTRACT OF TRIGONELLA FOENUM GRAECUM

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Abstract

In India, *Trigonella foenum-graecum,* an evergreen tree, is commonly used by traditional practitioners for gastric ulcer healing. In order to determine whether *Trigonella foenum-graecum* seed aqueous extract (AE) has antiulcer properties, this investigation was conducted in a lab. "Using an in-vitro technique called the H+/K+- ATPase inhibitory activity method, the anti-ulcer actions of different dosages of the aqueous extract (100 mg, 500 mg, 1000 mg, and 1500 mg) were evaluated. When compared to the gold standard of Aluminum hydroxide and Magnesium hydroxide (500 mg), the extract showed a drastic reduction in acid neutralizing capacity (ANC)." The antiulcer properties of Trigonella foenum-graecum seed powder have been extensively studied, highlighting the efficacy of its active constituents. Stress, long-term use of anti-inflammatory medicines, and other unknown variables have all been linked to on various native plants and their derivatives to treat peptic ulcers. The onset of ulcers in people. The fundamental process is a mismatch between aggressive elements and the body's inherent defensive systems for keeping the mucosal lining intact. Traditional healers have long relied. The main objective of this study is to estimate the in-vitro antiulcer activity of *Trigonella foenum-graecum* seed aqueous extract (AE).

Keywords: Antiulcer, Acid Neutralizing Capacity, Trigonella foenum-graecum, H+/K+-ATPase inhibitory.

INTRODUCTION

Peptic ulcers are a disease of the gastrointestinal system caused by an imbalance between aggressive forces (acid, pepsin, and Helicobacter pylori) and defensive factors (bicarbonate secretion, prostaglandins, gastric mucus, and inherent resistance of mucosal cells) [1]. Peptic ulcers arise when harmful stimuli outweigh protective mechanisms [2]. Infection with Helicobacter pylori, acid-pepsin hypersecretion, NSAIDs, tobacco use, psychological stress, rapid gastric emptying, and the syndrome of Zollinger-Ellison," characterized by excessive and uncontrollable acid production, can all disrupt this balance and lead to ulcer formation [3-5]. Side effects are common while using synthetic medications for ulcer treatment, including antacids, anticholinergics, antacids, cytoprotectants, demulcents, prostaglandin analogues, and proton pump inhibitors. Therefore, herbal treatments are seen as alternatives [6] that are superior for treating peptic ulcers. Nausea, stomach discomfort, constipation, and diarrhea have been linked to proton pump inhibitors (omeprazole, lansoprazole), whereas gynecomastia and decreased libido have been linked to H2 receptor antagonists (cimetidine). Research into medical plants is widespread because of the promise for novel treatments with fewer or no bad effects, which is a response to the problems associated with synthetic pharmaceuticals. Because of their low toxicity, cheap cost, and minimal risk of side effects, herbal drugs are often regarded as a secure option for ulcer therapy [7-8]. Herbal medicine, also called botanical medicine or herbalism, utilizes plants for healing purposes, treating ailments, and enhancing well-being. Herbalists, naturopaths, Ayurvedic doctors, homeopaths, and practitioners of traditional medicine are all involved. Natural plant chemicals are thought to have beneficial effects on health. [11].

Plant-based medicine operates on the idea that some plant compounds have therapeutic effects. Seeds, leaves, stems, bark, roots, flowers, and extracts have long been used in herbal medicine. These remedies were administered in various forms: raw, as teas or tinctures, topically, in liquid preparations, or as pills and capsules. Initially, plants were consumed raw or brewed into soups and teas. Over time, they were dried and crushed for different applications. Their usage was often based on superstitions or visual cues, but as scientific understanding advanced, herbal remedies became more refined. Today, herbs and plants serve as the foundation for many modern medicinal drugs. [12]. The purpose of this research was to determine whether *Trigonella foenum-graecum* seed extracts contain any of the components that have been shown to inhibit lipid peroxidation, reduce superoxide dismutase, H+K+ATPase, and boost catalase activity, all of which protect against oxidative damage to the gastric mucosa. The stomach parietal cells secrete H+ through the dimeric enzyme H+K+ ATPase. The stem component acts specifically to inhibit H+K+ ATPase.

Figure 1: ***Trigonella foenum-graecum***

MATERIALS AND METHODS:

They brought back Trigonella Foenum-Graecum seeds from Dehradun, Uttarakhand. Dr. Sandeep Kumar of the Department of Botany and Microbiology at Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand, India, has verified its identity.

Preparation and Evaluation of Novel Extract

The plant was sanitized by rinsing it three times in running water and then spraying it with 70% alcohol. Drying the plant in the shade at normal temperature allows for frequent inspections for fungal infestation. Pestle and motor are used to ground the dried plant into a fine powder. The Soxhlet extraction technique is used to remove the crude medication from the fine powder using an aqueous solvent.

Chemicals used: The chemicals used were of an analytical standard [5]. These included aluminum hydroxide, sodium hydroxide, hydrochloric acid, sodium CMC, Tween80, sodium benzoate, orange oil, and magnesium hydroxide.

Extraction by Soxhlet Apparatus: This is an old and rudimentary technique of drug extraction. The specifics of how plant components are removed depend on their makeup. The Soxhlet apparatus is often used in conjunction with the aqueous solvent to get the crude extract. The apparatus consists of a condenser, a main jar to retain the substance, and a solvent, and a round-bottomed flask. The 100 g of plant material powder is transferred to the main Soxhlet container. Under low pressure, with the controlled heating mantle adjusted to bring the solvent to a boil between 60 and 80 degrees Celsius, A round-bottomed flask is used for the condensation process of the extract.

Through the main jar, solvent vapor is taken into the condenser, where it is cooled by a steady stream of water [6]. Dripping back over the packed material in the primary jar, the condensing solvent finally accumulates in a second jar. As the color of the solvent changes as a chemical dissolve in it, you can observe that the substance is being collected and extracted simultaneously in the main jar. The extraction of plant material has therefore been completed; typically, this takes between 7-8 hours. After the solvent was removed, a brown extract was obtained, which was then placed in the fridge for further use in research.

Test for Alkaloids:

- **Dragendorff's test**: Dragendorff's reagent (a potassium bismuth iodide solution) should be added to the 1 ml of extract. The presence of alkaloids may be seen as an orange-red precipitate.

Test for Saponins:

- To a graduated cylinder containing 20 ml of distilled water, add 5 milliliters of both the alcoholic and aqueous extracts and mix for 15 minutes. In the absence of saponins, no foam will form.

Test for Glycosides:

- **Legal's test:** To an alkaline solution of sodium nitroprusside, the extract is dissolved in pyridine. The absence of a pink to scarlet hue betrays the lack of glycosides.

Test for Carbohydrates:

- **Fehling's test:** A brick red precipitate appears in the presence of sugars when 1 ml of the extract is combined with 2 ml of Fehling's solutions A and B.

Test for Tannins:

- To conduct the test, combine a small sample of the solution to be tested with a basic lead acetate solution. If white precipitates form, tannins are present.

Test for Flavonoids:

- When sodium hydroxide is applied to the extract, a yellow color develops, revealing the presence of flavones.**Test for Phenol:**
- **Bromine Water:** To a bottle of distilled water, add 5 milliliters of bromine and shake well. The transparent liquid may be decanted.

Test for Proteins:

Biuret test: To get a blue hue, combine 1 milliliter of the extract with 0.4 milliliters of a 40% sodium hydroxide solution and 0.2 milliliters of a 1% $CuSO_4$ solution. When a pinkish or purple-violet tint forms, proteins are present

“Detection	**Observation and Result**
Test for Alkaloids (Dragendorff’s test)	Dragendorff's reagent (a potassium bismuth iodide solution) should be added to the 1 ml of extract.The presence of alkaloids may be seen as an orange-red precipitate.
Test for Saponins	To a graduated cylinder containing 20 ml of distilled water, add 5 milliliters of both the alcoholic and aqueous extracts and mix for 15 minutes. In the absence of saponins, no foam will form.
Test for Glycosides (Legal’s test)	To an alkaline solution of sodium nitroprusside, the extract is dissolved in pyridine. The absence of a pink to scarlet hue betrays the lack of glycosides.
Test for Carbohydrates (Fehling’s test)	A brick red precipitate appears in the presence of sugars when 1 ml of the extract is combined with 2 ml of Fehlings solutions A and B.
Test for Tannins	To conduct the test, combine a small sample of the solution to be tested with a basic lead acetate solution. If white precipitates form, tannins are present.
Test for Phenol (Bromine Water)	To a bottle of distilled water, add 5 milliliters of bromine and shake well. The transparent liquid may be decanted.
Test for Proteins (Biuret test)	To get a blue hue, combine 1 milliliter of the extract with 0.4 milliliters of a 40% sodium hydroxide solution and 0.2 milliliters of a 1% CuSO4 solution. When a pinkish or purple-violet tint forms, proteins are present.
Test for Flavonoids	When sodium hydroxide is applied to the extract, a yellow color develops, revealing the presence of flavones.

Table No.-1 : Chemical Tests of Sample

Preparation of herbal suspension dosage form

The ingredients in a 100 ml solution of Trigonella foenum-graecum powder are shown in Table 2. By pulverizing the medication in solvents that are different added substances, such as Tween-80, sodium carboxymethyl cellulose (CMC), as an improving specialist, an enhancing specialist like orange oil, and a settling specialist like sodium benzoate, the medications are properly blended into fine particles of size 60 net during the period of practical application of the plan. After in vitro Evaluation of Fluid Concentrate from Trigonella foenum-graecum Seed for Antiulcer Activity [8], more research is needed to define antiulcer suspensions in vitro.

Table No. 2: Composition of aq. extract of seed of *Trigonella foenum-graecum* herbal suspension

S.No	Ingredients list	Quantities in suspension			
		F1	F2	F3	F4
1.	T.F. Extract	0.1gm	0.5gm	1gm	1.5gm
2.	Sodium CMC	0.6%	0.6%	0.6%	0.6%
3.	Tween80	0.1w/v	0.1w/v	0.1w/v	0.1w/v
4.	Sodium benzoate	1.5gm	1.5gm	1.5gm	1.5gm
5.	Orange oil	1ml	1ml	1ml	1ml
6.	Purified water q.s	100ml	100ml	100ml	100ml

In-vitro Evaluation of Antiulcer Activity

Acid Neutralizing Capacity (ANC):

Each suspension was produced fresh in a 250 ml beaker and heated to 37oC. The liquid in suspension was aerated. Constant rotation at 30 revolutions per minute of a magnetic stirrer was used to simulate the stomach. To determine the optimal pH for testing, 90ml of newly produced solution was combined with 3 drops of phenolphthalein and titrated with fake gastric juice. The amount of gastric juice replacement that was actually ingested was calculated to be V.

The total consumed H+ (mmol) which is also termed as ANC was measured as 0.063096 (mmol/ml) × V (ml).

The reference points are aluminum hydroxide (500 mg) and magnesium hydroxide (500 mg).

"H+/K+ - ATPase Inhibition Activity: To prepare the H+/K+ - ATPase enzyme, a fresh goat stomach was purchased from a local butcher, the gastric mucosa of the fundus was removed and the stomach was opened, and the inner layer of the stomach was scraped away for the parietal cell. The H+/K+- ATPase in the stomach was inhibited by homogenizing parietal cells in 16 mM Tris buffer at pH 7.4 with 10% Triton X-100 and centrifuging the mixture at 6000 rpm for 10 minutes. Bradford's method was used to determine protein concentration, with bovine serum albumin (BSA) serving as the reference."

Evaluation of ATPase H+/K+ Inhibition:

The extracts were incubated at concentrations ranging from 20 to 100 g/ml in a reaction mixture consisting of 1 ml of 40 mM Tris-HCl buffer, pH 7.4, 2 mM MgC12, and 10 g membrane protein. After that, 2 mM ATP Tris salt was used to kick off the reaction, and the mixture was let to sit at 37°C for 20 minutes. To stop the reaction, 1 ml of cold trichloroacetic acid (10% v/v) was added. Different concentrations of the extract and omeprazole were tested for their effects on H+ -K+ ATPase activity. We used spectrophotometry to measure the quantity of inorganic phosphate that ATP hydrolyzed into at a wavelength of 400 nm. "Mean SEM (Standard Error of the Mean) % enzyme inhibition

was computed using the method [11] and results were compared to those obtained with the well-known anti-ulcer PPA inhibitor Omeprazole.

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100"

STATISTICAL ANALYSIS

Observed data from the biochemical and hematological analyses were expressed as mean ± SEM. One-way ANOVA was used to test the means. Values were considered statistically significant at $P < 0.05$. All results were represented as mean ± SEM (n = 6). Values with different superscripts were significantly different ($P < 0.05$).

RESULTS AND DISCUSSION:

Acid Neutralizing Capacity:

The extract's ability to neutralize Al(OH)3+Mg(OH)2(500mg) was investigated at four different concentrations (F1, F2, F3, and F4). Based on the calculated values, the acid-neutralizing capacity (ANC) of the extract at concentrations F1, F2, F3, and F4 was predicted to be 0.6760, 0.4343, 0.4332, and 0.4256, respectively, when compared to the 0.8109 ANC of the standard Al(OH)3+Mg(OH)2 (500 mg). A 1500 mg dose of the extract in F4 formulation was shown to have a smaller alkalinizing effect than the gold standard. The data is summarized in Table 3.

"Table No. 3: Effect of aqueous extract of seed of *Trigonella foenum-graecum* on acid neutralizing capacity

S.no.	Formulation / Concentration of extract(mg)	The consumed volume (V) of the artificial gastric juice	ANC was measured as 0.063096 (mmol/ml) × V (ml)."
1.	F1	10.15	0.6760
2.	F2	7.25	0.4343
3.	F3	6.22	0.4332
4.	F4	6.15	0.4256
5.	$Al(OH)_3$+$Mg(OH)_2$ 500mg	12.26	0.8109

H+/K+ - ATPase Inhibition Activity:

Different concentrations of aqueous extract (10µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50µg/ml respectively) were tested for their ability to block H+/K+ - ATPase activity and compared to the gold standard, Omeprazole. The extract exhibited strong dose-dependent efficacy. Extract at 50 µg/ml (F1) exhibited maximum percentage inhibition of 59.56 percent, whereas reference Omeprazole showed 66.98 percent. Tables 4, 5, 6, and 7 summarize the findings for generations F1, F2, F3, and F4.

Table No. 4: Effect of aqueous extract on In-vitro H+/K+ - ATPase Inhibition Activity for F1

S. No.	Concentration (µg/ml)	Percentage Inhibition (%)	
		Standard	Extract
1.	10	33.36	26.48
2.	20	46.03	28.27
3.	30	48.49	29.23
4.	40	54.18	44.23
5.	50	64.49	57.23

Table No. 5: Effect of aqueous extract on In-vitro H+/K+ - ATPase Inhibition Activity for F2

S. No.	Concentration (µg)	Percentage Inhibition (%)	
		Standard	Extract
1.	10	33.36	23.16
2.	20	46.03	26.12
3.	30	48.49	28.08
4.	40	54.18	43.09
5.	50	64.49	55.13

Table No. 6: Effect of aqueous extract on In-vitro H+/K+ - ATPase Inhibition Activity for F3

S. No.	Concentration (µg)	Percentage Inhibition (%)	
		Standard	Extract
1.	10	33.36	21.07
2.	20	46.03	25.12
3.	30	48.49	27.10
4.	40	54.18	39.13
5.	50	64.49	52.26

Table No. 7: Effect of aqueous extract on In-vitro H+/K+ - ATPase Inhibition Activity for F4

S. No.	Concentration (µg)	Percentage Inhibition (%)	
		Standard	Extract
1.	10	33.36	19.11
2.	20	46.03	23.07
3.	30	48.49	25.10
4.	40	54.18	34.11
5.	50	64.49	49.03

CONCLUSION:

Back titration may be used to determine an antacid's acid-neutralizing capacity (ANC), which is the maximum amount of acid that it can neutralize. The ANC of 50 µg/ml of *Trigonella foenum-graecum* seed aqueous extract was much lower than the ANC of 0.6760 of the whole seed. Additionally, at a concentration of 50 µg/ml, the extract demonstrated a maximum percentage inhibition of 57.23% for the H+/ K+ - ATPase inhibitory activity, whereas the reference drug Omeprazole showed a value of 64.49%. Based on these findings, Trigonella foenum-graecum seed may serve as a useful antiulcer medication. It was investigated here whether or not aqueous extract from goat stomach might block H+ -K+ ATPase in vitro. To assess the phytochemicals' potential for cellular entry and to illustrate how they interact with the stomach ATPase, in vitro experiments are deemed important. Important enzyme system H+ -K+ ATPase is found in the apical secretory membrane of a half cell.

The results showed that both omeprazole and the extract were able to dose-dependently inhibit the enzyme, suggesting that the Trigonella foenum-graecum aqueous extract could strongly inhibit the acid-secreting enzyme H+ -K+ ATPase. It follows that Trigonella foenum-graecum seed protects against the inhibition of the stomach proton pump through the inactivation of H+ -K+ ATPase, and that this finding paves the way for the isolation and identification of the active chemicals responsible for this activity.

References

1. Bonlawar, J., Setia, A., Challa, R.R., Vallamkonda, B., Mehata, A.K., Vaishali, , Viswanadh, M.K., Muthu, M.S. (2024). Targeted Nanotheransotics: Integration of Preclinical MRI and CT in the Molecular Imaging and Therapy of Advanced Diseases. *Nanotheranostics*, 8(3), 401-426. https://doi.org/10.7150/ntno.95791.
2. Pasala, P. K., Rudrapal, M., Challa, R. R., Ahmad, S. F., Vallamkonda, B., & R., R. B. (2024). Anti-Parkinson potential of hesperetin nanoparticles: *in vivo* and *in silico* investigations. *Natural Product Research*, 1–10. https://doi.org/10.1080/14786419.2024.2344740
3. Suseela, M. N. L., Mehata, A. K., Vallamkonda, B., Gokul, P., Pradhan, A., Pandey, J., ... & Muthu, M. S. (2024). Comparative Evaluation of Liquid-Liquid Extraction and Nanosorbent Extraction for HPLC-PDA Analysis of Cabazitaxel from Rat Plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 116149. https://doi.org/10.1016/j.jpba.2024.116149
4. Chakravarthy, P.S.A., Popli, P., Challa, R.R. *et al.* Bile salts: unlocking the potential as bio-surfactant for enhanced drug absorption. *J Nanopart Res* 26, 76 (2024). https://doi.org/10.1007/s11051-024-05985-6
5. Setia, A., Vallamkonda, B., Challa, R.R., Mehata, A.K., Badgujar, P., Muthu, M.S. (2024). Herbal Theranostics: Controlled, Targeted Delivery and Imaging of Herbal Molecules. *Nanotheranostics*, 8(3), 344-379. https://doi.org/10.7150/ntno.94987.
6. Dhamija P, Mehata AK, Tamang R, Bonlawar J, Vaishali, Malik AK, Setia A, Kumar S, Challa RR, Koch B, Muthu MS. Redox-Sensitive Poly(lactic-*co*-glycolic acid) Nanoparticles of Palbociclib: Development, Ultrasound/Photoacoustic Imaging, and Smart Breast Cancer Therapy. Mol Pharm. 2024 May 5. doi: 10.1021/acs.molpharmaceut.3c01086. Epub ahead of print. PMID: 38706253.

7. Eranti, Bhargav and Mohammed, Nawaz and Singh, Udit Narayan and Peraman, Ramalingam and Challa, Ranadheer Reddy and Vallamkonda, Bhaskar and Ahmad, Sheikh F. and DSNBK, Prasanth and Pasala, Praveen Kumar and Rudrapal, Mithun, A Central Composite Design-Based Targeted Quercetin Nanoliposomal Formulation: Optimization and Cytotoxic Studies on MCF-7 Breast Cancer Cell Lines. Available at SSRN: https://ssrn.com/abstract=4840349 or http://dx.doi.org/10.2139/ssrn.4840349
8. Setia A, Challa RR, Vallamkonda B, Satti P, Mehata AK, Priya V, Kumar S, Muthu MS. Nanomedicine And Nanotheranostics: Special Focus on Imaging of Anticancer Drugs Induced Cardiac Toxicity. Nanotheranostics 2024; 8(4):473-496. doi:10.7150/ntno.96846. https://www.ntno.org/v08p0473.htm
9. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.
10. Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).
11. Bhandari S, Chauhan B, Gupta N, et al. Translational Implications of Neuronal Dopamine D3 Receptors for Preclinical Research and Cns Disorders. *African J Biol Sci (South Africa)*. 2024;6(8):128-140. doi:10.33472/AFJBS.6.8.2024.128-140
12. Tripathi A, Gupta N, Chauhan B, et al. Investigation of the structural and functional properties of starch-g-poly (acrylic acid) hydrogels reinforced with cellulose nanofibers for cu2+ ion adsorption. *African J Biol Sci (South Africa)*. 2024;6(8): 144-153, doi:10.33472/AFJBS.6.8.2024.141-153
13. Sharma R, Kar NR, Ahmad M, et al. Exploring the molecular dynamics of ethyl alcohol: Development of a comprehensive model for understanding its behavior in various environments. Community Pract. 2024;21(05):1812-1826. doi:10.5281/zenodo.11399708
14. Mandal S, Kar NR, Jain AV, Yadav P. Natural Products As Sources of Drug Discovery: Exploration, Optimisation, and Translation Into Clinical Practice. African J Biol Sci (South Africa). 2024;6(9):2486-2504. doi:10.33472/AFJBS.6.9.2024.2486-2504
15. Kumar S, Mandal S, Priya N, et al. Modeling the synthesis and kinetics of Ferrous Sulfate production: Towards Sustainable Manufacturing Processes. African J Biol Sci (South Africa). 2024;6(9):2444-2458. doi:10.33472/AFJBS.6.9.2024.
16. Revadigar RV, Keshamma E, Ahmad M, et al. Antioxidant Potential of Pyrazolines Synthesized Via Green Chemistry Methods. African J Biol Sci (South Africa). 2024;6(10):112-125. doi:10.33472/AFJBS.6.10.2024.112-125
17. Sahoo S, Gupta S, Chakraborty S, et al. Designing, Synthesizing, and Assessing the Biological Activity of Innovative Thiazolidinedione Derivatives With Dual Functionality. African J Biol Sci (South Africa). 2024;6(10):97-111. doi:10.33472/AFJBS.6.10.2024.97-111
18. Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.

19. Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768