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Antibacterial, Antioxidant and Antimicrobial Index Activities of Crude Alkaloids from the Seed of *Trigonella foenum-graecum*

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Abstract This study aimed to evaluate the anti-proliferative effects of crude alkaloids extracted from *Trigonella foenum-graecum* on some pathogenic bacterial cells. The effects of crude alkaloid extract from *plant* seed to antioxidant, the research explored the ability of crude alkaloid to induce arrest at the metaphase stage in human lymphatic cells, comparing its efficacy to the established drug Colchicine. The antimicrobial activity of crude alkaloid extract from the plant *Trigonella foenum-graecum* was tested against *Streptococcus. mutans S. vestiularis, Klebsilla Protues and E. coli by* using the diffusion technique. The higher inhibition zone was observed in *Streptococc mutans, S.vestiularis* were (18.5 mm, 17.4 mm), respectively, followed by Klebsiella (12.7), *Proteus* (16.1) and *E. coli* (13.2 mm). The antioxidant activity was tested using the stabilised free radical compound DPPH. The results showed that horsetail fern is effective in eliminating free radicals at the concentrations used The inhibition rate depends on *Trigonella foenum-graecum* the concentrations used, as it increases with increasing concentration The crude alkaloid extract has an inhibitory effect after interacting with DPPH. It started at a concentration of 15.1 µg/mL, the inhibition rate reached 73.47%. This percentage increases to 85.30, 88.10, 88.50, 89.17, 90.30 and 94.50% respectively. A significant difference was observed between the concentration of 15.1 µg/mL and ascorbic acid, while the differences were not significant in the rest of the concentrations.

Key Words *Trigonella foenum-graecum*, Antioxidant Activity, Alkaloids

INTRODUCTION

Trigonella foenum-graecum L. (fenugreek), an annual herbaceous legume belonging to the Fabaceae family, is recognised as one of the oldest multipurpose medicinal herbs. While primarily cultivated as a spice crop globally, its successful introduction for forage production has also been documented in certain regions. The precise origin of *T. foenum-graecum* L. remains a subject of ongoing debate, with recent scholarship suggesting either the Mediterranean region or Southeast Asia as potential native habitats [1]. Currently, fenugreek cultivation occurs across parts of Europe, Africa, West and South Asia, North and South America and Australia. India holds the distinction of being the world's largest producer, primarily utilising the crop for culinary and medicinal applications. Globally, fenugreek seeds are employed as a spice for seasoning, enhancing texture, [2] contributing to the sensory quality of food through the provision of characteristic flavour, as well as their incorporation into soups and pancakes. Furthermore, the green leaves and tender stems are utilised as leafy vegetables due to their nutritional composition, encompassing proteins, choline, minerals and vitamins [3].

An examination of historical texts concerning medicinal herbs indicates a long-standing tradition of fenugreek use in Indian Ayurveda and Traditional Chinese Medicine. Historically documented medicinal applications include wound healing, breast enlargement, lactation promotion, treatment of arthritis, dropsy, cardiac disease, splenomegaly and hepatomegaly, renal ailments, gastric stimulation and as an appetite stimulant [4]. Contemporary research, however, has extensively reviewed the medicinal and nutraceutical properties of fenugreek [5]. These attributed medicinal properties include... antidiabetic, cholesterol-lowering, anti-hyperthyroid, anticancer, antioxidant, antimicrobial, antihelminthic, anti-sterility, anti-androgenic, anti-allergic, anti-inflammatory and antipyretic activities. These pleiotropic effects have been attributed to its diverse phytochemical profile, including galactomannans, diosgenin, tigogenin, neotigogenin, triterpenoids, trigonelline, choline,

4-hydroxyisoleucine, flavonoids and various phenolic compounds [6]. Given the susceptibility of these bioactive constituents to variations in cultivation practices and environmental conditions, research efforts have focused on developing biochemically stable fenugreek lines capable of consistently yielding high-quality seed for industrial applications [7]. Despite the recognized importance of *T. foenum-graecum*, the study of its associated microbial communities, or phytosphere microbiology, has received comparatively limited attention within both fundamental and applied research domains [8]. Consequently, the intricate relationships between the plant and its phytosphere microbiome remain largely unexplored. The application of both conventional culture-dependent methodologies and culture-independent molecular techniques offers a promising avenue for elucidating microbial diversity and facilitating the discovery of novel functional metabolites produced by these communities [9]. This communication aims to review the existing literature regarding the association of microbiota with both above- and below-ground components of the fenugreek plant, examine host-microbe interactions and finally, highlight the potential of fenugreek and its associated microbiome for the development of plant growth-promoting substances, enhanced stress tolerance in plants, [10].

METHODS

The extract is prepared according to [11] as follows: Take a quantity of 10g of dry plant ground and put it in a cylindrical container made of paper with pores called (Thimble) and put it in the place designated for it in the Soxhlet. Add 500 mL of methanol 70% to it and the extraction was carried out for 5 hours. Pour the methanolic extract into Petri dishes and then the extract was evaporated by using a rotor evaporator at room temperature and then filtrate. About 100 mL of chloroform was added for every 100 mL of the extract in the separating funnel, to adjust the pH a little weak base (ammonia) was added to get pH: 8 and left for 24 hours, two layers were obtained, the upper layer is the chloroform layer, which was disposed of and the lower layer is the aqueous layer containing the alkaloid, which was mixed with petroleum ether in equal quantities and then filtered using a 0.22mm filter unit.

The antioxidant activity of the crude alkaloid extract of horsetail fern was tested using [12] the stabilized free radical of the compound (DPPH 2,2 diphenyl-picryl hydrazyl). Measurements were made using a spectrophotometer with a wavelength of 517 nm and using ascorbic acid as a positive comparator. Equal volumes of 0.5 mL of DPPH solution and 0.5 ml of different concentrations of the extract were mixed. Crude alkaloid 15.1, 31.2, 62.5, 125, 250, 400 and 500 micrograms/ml using three replicates for each concentration and the samples were left at room temperature for 30 minutes.After that, the absorbance was measured at a wavelength of 517 nm, after which the inhibition percentage was measured according to the equation:

$$Scavening\ activity\ \% \frac{A517\,control - A517\,sample}{A517\,control} \times 100$$

A 517 control = It is a DPPH free radical absorbent with methanol only

A 517 sample = A free radical absorbance with different concentrations of crude alkaloid extract and ascorbic acid [13]

Study The Effect of extracted in Mitotic Index in Lymphocytes of Humans the effect of the extract at different concentrations on the mitotic index of lymphocytes was studied by using a short-term blood culture, based on the method of [14]. The provided text describes the experimental procedure for assessing the effect of an extract on lymphocyte division. Here's a breakdown of the steps involved.

Extract Preparation and Dilution

The extract was prepared at various concentrations (µg/mL). Each concentration was added to separate culture tubes containing complete RPMI-1640 medium. The final volume in each tube was adjusted to 5 mL. The experiment was conducted in triplicate for each concentration. Blood Sample and Lymphocyte Stimulation: 0.5 mL of blood was added to each culture tube.0.1 mL of Phytohemagglutinin (PHA), a lymphocyte mitogen, was added to stimulate lymphocyte proliferation. The contents of each tube were gently mixed. Incubation: The culture tubes were incubated at 37°C in a tilted position for 24 hours. The tubes were mixed every 12 hours during the incubation period. Control Group: A set of control tubes was included, containing only the complete RPMI-1640 medium, blood and PHA, without the addition of the extract. His experimental setup allows for the evaluation of the extract's effect on lymphocyte proliferation by comparing the lymphocyte response in the presence of different extract concentrations to the control group.

Statistical Analysis

The data of the current investigation is presented as means. Using the F test (ANOVA) and a significance level of $p \leq 0.05$ was applied.

RESULTS AND DISCUSSION

A weight of 0.4 g was obtained from 20 g of dry powder of *Trigonella foenum-graecum* L seed, The used to extraction crud alkaloid a greenish-brown color. The extract was dissolved in DMSO solution to prepare the concentrations used in the cytotoxicity tests: 15.1, 31.2, 62.5, 125, 250, 400 and 500 µg/mL. The aim of this study is anti-bacteria antioxidant and mitotic index activity. Plants in their various parts contain many compounds and small molecules that have anti-cancer activity, as some of these compounds and molecules are still undiscovered or have not been studied in detail [14,15]. There are also synergistic effects of the chemical compounds present in plant parts. These effects are responsible for their antioxidant and antibacterial properties.

Table 1: The Chemical Composition of *Trigonella foenum-graecum* L. Seed

	Results		Chemical compound
1	+	Detergent	Alkaloid
2	+	Dragendroff'	Flavonoid
3	+	Ferric chloride 1%	Glycosides
4	+	KOH	Tannins
		Lead acetate hydrate 1%	

Table 2: Effect of alkaloid extracted from *T. foenum-graecum* on Some Bacterial

	Inhibition zone (mm)
Type of Bacteria	Mean±SD
Streptococcus. mutans	18.5±1.0
S. vestibularis	17.4±1.1
Klebsiella. spp	12.7±1.1
Protues	16.1±1.2
E. coli	13.2±1.2

Significant Difference among more than two Independent Means at the Level ($p \leq 0.05$)

The results shown in Table 1 showed the presence of active compounds in the horsetail fern extract, such as alkaloids, flavonoids and glycosides. These compounds have toxic activity in other plants.

Fenugreek seeds possess a complex biochemical composition. Carbohydrates constitute a significant portion, ranging from 45 to 60%, with mucilaginous fibre, specifically galactomannans, [16] comprising 20 to 30% of the seed's weight. Proteins, rich in the essential amino acids tryptophan and lysine, are present at levels of 20 to 30%. Lipids, in the form of fixed oils, account for 5 to 10% of the seed's composition. A variety of alkaloids are present, including pyridine alkaloids such as choline [17], Flavonoids, Free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine and histidine, are also present, along with calcium and iron. Saponins and glycosides yielding steroidal compounds, including diosgenin and neotigogenin, are also found. Sterols, such as cholesterol and sitosterol, are present. Vitamins, including B, A, C and nicotinic acid [18].

The Effect of Alkaloid from *T. foenum-graecum* some Pathogenic Bacteria

The antimicrobial activity of crud alkaloids extract in 500 micrograms/mL concentrations was investigated against *Streptococcus. mutans S.vestiularis Klebsilla, Proteus,* by using the agar wells diffusion technique. The higher inhibition zone was observed in *Streptococci mutans* (18.5 mm), respectively, followed by *S. vestibularis* (17.4), *Klebsiella* (12.7) and *Proteus* (16.1), as shown in Table 2.

Trigonella foenum-graecum (fenugreek), a medicinal plant native to Northern Africa [19], has demonstrated antibacterial properties in numerous studies utilizing seed extracts. These effects are likely attributable to the presence of bioactive secondary metabolites, including alkaloids, steroids, tannins, phenolic compounds and flavonoids [20].

The Effect of Alkaloid to the Antioxidant Activity

The Effect of alkaloid to the antioxidant activity was tested using the stabilised free radical compound DPPH. The results of this study showed that horsetail fern is effective in eliminating free radicals at the concentrations used: 15.1, 31.2, 62.5, 125, 250, 400 and 500 micrograms/mL.

Table 3: Effect Crud Alkaloid Extracted from *T. foenum-graecum* on Antioxidant Activity

Standard deviation ± inhibition ratio	Con. µg/mL
2.2±73.47c	15.1
2.9±85.30b	31.2
2.5±88.10b	62.5
2.3±88.50b	125
2. 7±89.17b	250
2. 4±90.30a	400
2. 1±94.50a	500
1. 6±95.43a	Ascorbic acid

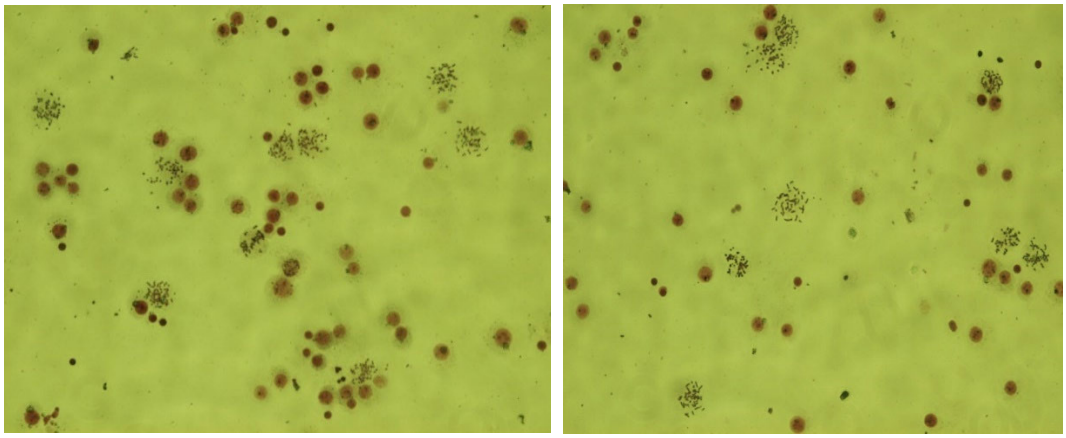
The Different Letters in the Column Indicate that there are Statistical Differences at the Level of ($0.05 \geq p$)

The inhibition rate depends on the concentrations used, as it increases with increasing concentration, as Table 3 shows that the alkaloid extract has an inhibitory effect after interacting with DPPH. It started at a concentration of 15.1 µg/mL and the inhibition rate reached 73.4%. This percentage increases to 85.3, 88.5, 88.8, 89.1, 91.3 and 94.5% for concentrations 31.2, 62.5, 125, 250, 400 and 500 µg/mL, respectively. A significant difference was observed between the concentration of 15.1 micrograms/mL and ascorbic acid, while the differences were not significant in the rest of the concentrations.

Antioxidants function by neutralising free radicals, thereby offering protection against infections and degenerative diseases. These compounds are broadly classified as either natural or synthetic. Synthetic antioxidants include butylated Hydroxyanisole (BHA) and gallic acid esters. While effective in inhibiting oxidation, these substances may also function as chelating agents, such as ethylenediaminetetraacetic acid [21], binding metals and thus reducing their pro-oxidant activity. However, concerns exist regarding potential adverse health effects associated with synthetic antioxidants, including mutagenesis and carcinogenesis [22]. Consequently, a significant shift towards utilizing naturally occurring antioxidants for the prevention of free radical-mediated diseases has emerged Plants have many extracts with antioxidant activity, as the study conducted by [16] showed that the plant extracts of many plants: *Curcuma longa L. rhizomes, Commiphora myrrha L. gums and Ginkgo biloba L.* leaves have antioxidant activity at a significant level of $p \leq 0.05$ compared to ascorbic acid [23]. Natural antioxidants mitigate the formation of free radicals and reactive oxygen species, or alternatively, inhibit their interaction with biological structures.

Effect of Crud Alkaloids from *T. foenum-graecum* in Mitotic Index

To test the efficacy of crud alkaloids from *T. foenum-graecum* in stopping lymphocyte division, the treatment led to an increase in the rate of lymphocyte division as the concentration increased, as shown in Table 4. The percentage of suspended cells stopped in the metaphase was 0.15, 1.51, 2.02, 2.92, 3.18,3.81,3.92 at concentrations 15.1, 32.2, 65,250,400 and 500 µg/mL, respectively, while the percentage of stopped cells in the control was 4.02. There was no significant difference between the two concentrations, 62.2 and 125 µg/mL and no significant difference between 250 and 400 µg/mL, respectively and the difference was significant between the percentages of suspended cells at other concentrations, as shown in Figure 1.



(A) (B)

Figure 1: Comparison between Lymphocytes that were Treated and Untreated with Crud Alkaloids extract at a Concentration of 500 µg/mL for 24 hours of Exposure at 37°C (x20) by using Crystal Violate Stain. (A) Representing Control Lymphocytes (B) Representing Treated Lymphocytes with Crud Alkaloids at a Concentration of 500 µg/mL

Table 4: Effect of crude alkaloids Extract in Mitotic Index in Human Lymphocytes in 24 Hours of Exposure at 37°C and Compared with Colchicine

Cell ratio in metaphase inhibition ratio ± Standard deviation	Con. µg/mL
4.02±0.01	Control(Colchicine)
0.15±0.14	15.1
1.51±0.11	31.2
2.02±0.07	62.5
2.92±0.14	125
3.18±0.24	250
3.81±0.24	400
3.92±0.14	500

The different letters in the same column indicate that there are statistical differences at the level of (0.05≥p)

The genotoxic potential of aqueous and alcoholic extracts derived from *Peganum harmala* L. seeds was investigated using an *Allium cepa* (onion) root assay. Roots were exposed Results demonstrated that *P. harmala* extracts significantly inhibited onion root growth rate across all tested concentrations and exposure periods when compared to the control group [24]. Despite significant advancements in pharmaceutical development, medicinal plants continue to be widely employed in the treatment and prophylaxis of a range of ailments, owing to their inherent medicinal and nutraceutical properties. *Trigonella foenum-graecum* L., commonly known as fenugreek, exemplifies such a plant. Belonging to the Fabaceae family, this self-pollinating annual herbaceous aromatic crop is also referred to as bird's foot, Greek hayseed, halba and methi [25] Fenugreek seeds and leaves are utilized as both a spice and a culinary ingredient across numerous countries. Its applications extend to functional and traditional foods, as well as nutraceutical and physiological contexts. Furthermore, the high fibre, protein and gum content of fenugreek has led to its recent adoption as a food stabilizer and emulsifying agent [26].

CONCLUSION

Crude alkaloids extracted from *Trigonella foenum-graecum* seeds exhibited significant antibacterial, antioxidant and anti-mitotic activities. The extract showed marked antibacterial effects, particularly against *Streptococcus mutans* and *S. vestibularis* and demonstrated strong, concentration-dependent free-radical scavenging activity comparable to ascorbic acid. In addition, a dose-dependent reduction in the mitotic index of human lymphocytes, accompanied by metaphase arrest, was observed. Collectively, these findings indicate that fenugreek seed alkaloids represent a promising source of multifunctional bioactive compounds with potential pharmaceutical applications, meriting further isolation and mechanistic investigation.

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