IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 2, Issue 4, Apr 2014, 61-66

PHARMACOLOGICAL EVALUATION FROM LEAVES EXTRACTS OF *TRIGONELLA FOENUM-GRAECUM*

SADIQ AZAM[1], SALMAN SHEHZADA[2], FARAH SHIREEN[3], SAJJAD FIDA[4], HAMAYUN KHATTAK[5] & ZIAULLAH[6]

[1,3]Center of Biotechnology and Microbiology, University of Peshawar, KPK, Pakistan

[2,6]Aga Khan University and Hospital, Karachi, Pakistan

[4]Institute of Biotechnology and Genetic Engineering, IBGE, KPK, Pakistan

[5]Department of Biotechnology and Genetic Engineering, KUST, KPK, Pakistan

ABSTRACT

Medicinal herbal extracts are in vogue for over a century to cure human complications. Crude methanolic leaves extracts of competent herb *Trigonella foenum-graecum* were assessed for antibacterial, antifungal, free radical scavenging, hemagglutination and phytotoxic bioassay. Antibacterial bioassay was conducted via well diffusion protocol. And from the resulted zone of inhibition it was evinced that crude leaves extract possessed good activity against *E.coli* (47%), *P. mirabilis* (52%), *B. cereus* (60%) and *P. aeruginosa* (78%). Less antifungal aptitude against *T. harizanum* (30%) was validated. Rest of bacterial and fungal species was resistant. Less to moderate free radical scavenging capacity was demonstrated. Phytotoxic potentials at highest concentration was estimated i.e. 1000µg/ml (38%), manifesting moderate activity. No hemagglutination capacity was evidenced.

KEYWORDS: *Trigonella foenum-graecum,* Antibacterial, Antifungal, Free Radical Scavenging, Phytotoxic, Hemagglutination

INTRODUCTION

Approximately, 85% of herbal folk medicine commodity is being withdrawed from plant extracts which aid to treat ailments of about 80% occupants of the commenced countries (Chopra *et al.*, 1986). *Trigonella foenum-graecum* commonly known as Fenugreek (English), Helba (Arabic), Methi (Urdu, Hindi), Alholva, Feno-greco (Spanish) belongs to Family *Fabaceae*. The yellow flowered herb is cultivated in aquatic regions. The herb is widely distributed in Pakistan, India, Nepal, China, Bangladesh, France, and Spain. Fenugreek seeds are used as pulses, vegetable, spice and in preparation of pickles by the Asians.

The seeds possess anti-diabetic potentials (Allen *et al.*, 1981). The seeds are consumed by lactating women to increase milk supply for neonates (Boulenger *et al.*, 1966). Indians use the herb to prevent complications such as ageing, immuno-incompetance, labor pain, dyspepsia, cholesteroemia, ulcer, inflammation, cancer and other nervous disorders (Clifford A, 2001). Reports indicate that the pharmacological activities of *Trigonella foenum graecum* involve anti-diabetic, antipyretic, antifertility, antifungal, analgesic, ant-inflammatory, & immunomodulatory activities (Kamath *et al.*, 1959; Parthasarathy *et al.*, 2008). The research study was framed employing crude methanolic leaves extract of *Trigonella foenum-graecum* for antibacterial, antifungal, antioxidant, phytotoxic and hemagglutination bioassays.

MATERIALS AND METHODS

Plant Material

T. foenum-graecum (leaves) where collected from Charssada, KPK, Pakistan on January 2012 and were identified by Prof. Dr. Abdur-Rasheed, University of Peshawar.

Extraction

Leaves of *T. foenum-graecum* were hewed and then shade dried. Then plant material was powdered finely and the net weight of the powdered plant material was (1.2 kg). Further the plant material was macerated in commercial grade methanol for 14 days at 28^{o}C with occasional shaking. Then the methanol soluble materials were filtered off using Whattman filter paper No.1. Filtrate were intermingled and decoctured under vacuum at 40^{o}C using rotary evaporator. Finally blackish green crude methanolic leaves extract was obtained having 140g.

Antibacterial Bioassay

Antibacterial activity was preceded following reported well diffusion method (Ahmad *et al.*, 2013) against *Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Bacillus cereus,* and *Proteus mirabilis*.

Nutrient broth (Sigma-Aldrich, Germany) was prepared in autoclaved test tubes and was incubated at 37^{o}C for 24 hours for sterility testing. Broth media was then inoculated with the test organisms. Nutrient agar medium was prepared in autoclaved petri-plates on same day of inoculation and incubated 37^{o}C for same sterility testing for 24 hours. Then the test cultures from the broth were relocated to sterile nutrient agar plates and homogeneously spread. Wells were made with the help of sterilized 6mm borer in the nutrient agar plates. Stock solution of crude methanolic leaves extracts were made at 3 mg/ml concentration in sterile DMSO (<1%). 100µl aliquots of stock solution were assigned into the wells. DMSO (<1%) was used as negative control and Amoxicillin drug was used as positive control. Plates were left undisturbed for better diffusion during incubation. Finally, after 24 hours, zone of inhibition by bacterial susceptibility was noted. Percent inhibition was computed using given formula;

$$\textbf{\textit{Percent growth Inhibition}} = \frac{\textbf{\textit{Zone of Inhibition of Sample (mm)}}}{\textbf{\textit{Zone of Inhibiton of Standard (mm)}}} \times \mathbf{100}$$

Antifungal Bioassay

Crude methanolic leaves extract confiscated for possible antifungal bioassay against *Alternaria alternata, Fusarium oxysporum, Trichoderma harizanum, Aspergillus niger, Aspergillus flavus, Aspergillus parasitica* and *Penecillium* as test organisms following reported agar tube dilution method (Ahmad *et al.*, 2013).

Stock solution of crude methanolic leaves extracts were prepared in DMSO (<1%). Then Sabouraud Dextrose Agar (SDA, Sigma-Aldrich, Germany) medium was prepared as per discussed procedure. Then to 4 ml SDA medium, 66.6 µl aliquots of stock solutions were transferred to autoclaved test tubes and were allowed to cool in slanting position to form slants at room temperature. Appendage media were then cultured with fungal strains. Sterile DMSO (<1%) was used as negative control and Miconazole drug was used as positive control. All the test tubes were incubated for 7 days at 28^{o}C. After incubation period, linear fungal mycelia growth was observed at 7th day of incubation. Percent growth inhibition was computed using following given formula;

$$Percent\ growth\ Inhibition = 100 - \frac{Linear\ growth\ in\ test\ sample(mm)}{Linear\ growth\ in\ control\ (mm)} \times 100$$

Free Radical Scavenging Bioassay

Free radical scavenging bioassay of crude methanolic leaves extract of *Trigonella foenum-graecum* was investigated following reported DPPH (1, 1-diphenyl-2- 8 picrylhydrazyl) radical scavenging method (Blois, 1958). The reaction composite holds different strengths of stock solution (20-500ppm) and 100 µM DPPH in methanol. Absorbance at 517 nm wavelength was observed after half an hour at room temperature. Radical scavenging strength was heeded and percent absorbance was computed by radical reduction formula given as follows. BHT (Butylated hydroxytoluene) was used as control.

$$Percent\ Absorbance = \frac{Control\ Absorbance - Extract\ Absorbance}{Control\ Absorbance} \times 100$$

Hemagglutination Bioassay

Crude methanolic leaves extract of *Trigonella foenum-graecum* were investigated for attainable hemagglutination bioassay as per reported procedure (Ahmad *et al.*, 2013).

1 mg/ml stock solution was prepared at the concentration of 1:2, 1:4, 1:8 and 1:16 in DMSO. Fresh blood samples from healthy volunteers were collected on the same day of the experiment and were centrifuged. From the centrifuged pellet RBC's were collected and 2% RBC's suspension was prepared using phosphate buffer. From every dilution, 1ml test sample was obtained in a clean test tube and to it 1 ml of the RBC's suspension was added. Incubate the test tubes at 37^{o}C for 30 minutes using incubator. Finally the test tubes were analyzed for agglutination reaction in form of smooth button formation, indicating positive results.

Phytotoxic Bioassay

Trigonella foenum-graecum methanolic leaves extracts were analyzed for phytotoxic activity against *Lemna minor* as per reported procedure (Ahmad *et al.*, 2013). Crude methanolic leaves extract were dissolved in methanol prepared at concentration of 20 mg/ml. Then 10, 100 and 1000µg/ml aliquots of stock solution were subjected into tidy flasks. Then allowed to rest at room temperature to evaporate the methanol content. Further 20 ml E-media were added to all flasks. Sixteen healthy *Lemna minor* plants were selected and designated to each flask separately. All the flasks were then incubated at room temperature for 7 days. Results were examined after 7 day of incubation. Paraquat was used as standard growth inhibitor. Percent growth regulation was then computed using following formula;

$$Percent\ growth\ regulation = \frac{Experimental\ Sample}{Standard} \times 100$$

RESULTS AND DISCUSSIONS

Antibacterial Bioassay

Crude methanolic extracts of *Trigonella foenum-graecum* leaves were checked for antibacterial bioassay against some pathogenic bacterial cultures. From the recorded zone of inhibition produced by bacterial culture the percent growth inhibition was computed, and it was analyzed that crude methanolic plant extract possessed good antibacterial potentials against *P.mirabilis* (52%), *E.coli* (47%), *B. cereus* (60%) and *P.aeruginosa* (78%). Previously, methanolic, ethyl acetate and acetone extract of spice *Trigonella foenum-graecum* seeds were investigated for antibacterial potentials against some

pathogenic gram negative bacteria and it was testified that the crude plant extract possessed highest antibacterial activity (Dash *et al.*, 2011; Tejaswini *et al.*, 2012).

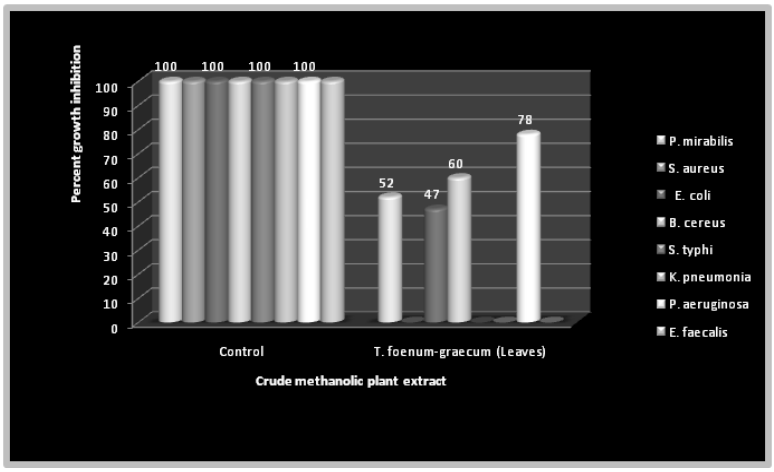

Figure 1: Antibacterial Bioassay from Crude Methanolic Leaves Extract of *T. foenum-graecum*

Antifungal Bioassay

Crude methanolic leaves extract of *Trigonella foenum-graecum* was examined for possible antifungal bioassay. From the percent growth inhibition computed by the linear mycelial growth, it was proved that the plant extract possessed less antifungal potentials against *T.harizanum* (30%). Rest of the fungal cultures was resistant to the plant extract. Research study conducted earlier on different fractions *T. foenum-graecum* roots, seeds, leaves and stem. From the results it was manifested that seeds extracts possessed excellent activity to cease pathogenic fungal mycelial growth (Haouala *et al.*, 2008).

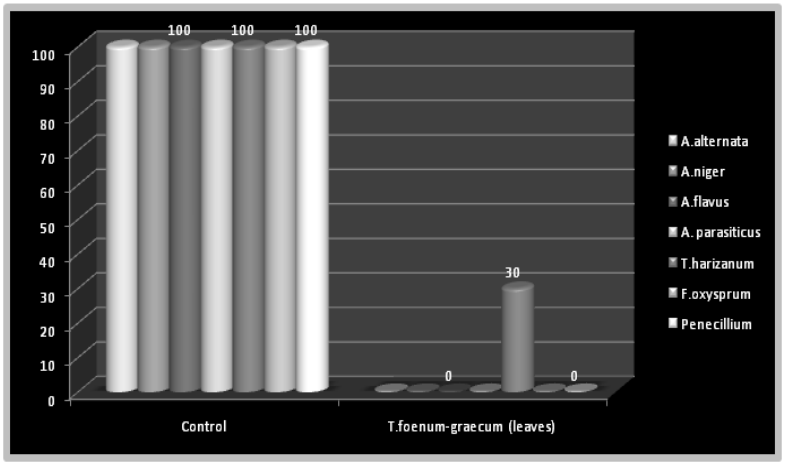

Figure 2: Antifungal Bioassay from Crude Methanolic Leaves Extracts *T. goenum-graecum*

Free Radical Scavenging Bioassay

Free radical scavenging activity of plant extract were inspected and from the percent absorbance recorded it was ratified that the crude methanolic leaves extract of *T. foenum-graecum* possessed less to moderate free radical scavenging activity at variable dilutions. From the research study conducted earlier, the seeds extract of the plant possessed highest free radical scavenging activity (Tejaswini *et al.*, 2012).

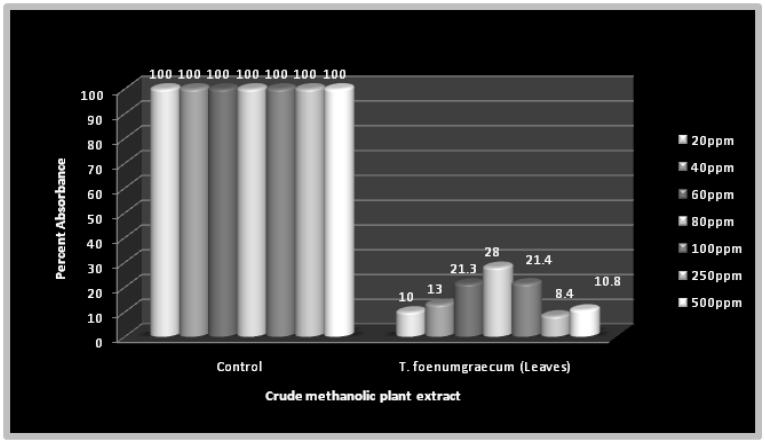

Figure 3: Free Radical Scavenging Bioassay from Crude Methanolic Leaves Extracts *T. foenum-graecum*

Hemagglutination Bioassay

Crude methanolic leaves extract of *T. foenum-graecum* were examined for hemagglutination bioassay against human erythrocytes of all blood groups (ABO) at inconstant dilution. The results disclosed that crude methanolic extract *T. foenum-graecum* lack smooth button formation manifesting as negative hemagglutination bioassay.

Phytotoxic Bioassay

Phytotoxic bioassay of crude methanolic leaves extract of *T. foenum-graecum* against *Lemna minor* testified that even that highest dilution moderate phytotoxic activity was recorded. i.e. 1000µg/ml (38%).

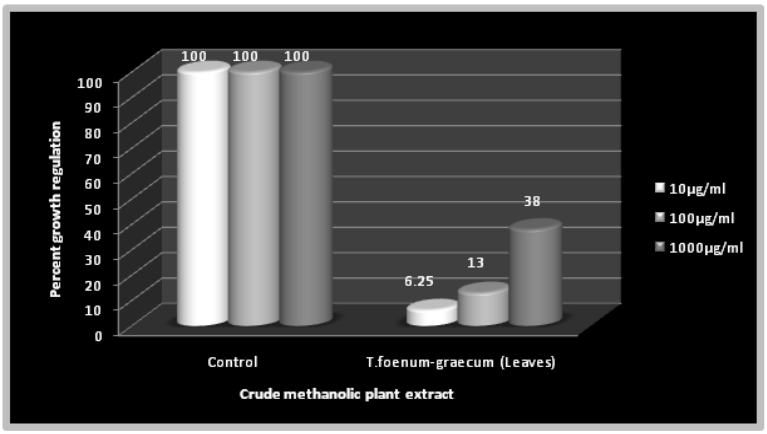

Figure 4: Phytotoxic Bioassay from Crude Methanolic Leaves Extracts *T. foenum-graecum*

CONCLUSIONS

From the research work conducted earlier on crude methanolic leaves extracts of *Trigonella foenum-graecum* for possible antibacterial, antifungal, free radical scavenging, hemagglutination and phytotoxic bioassay, we concluded that the methanolic leaves extracts own good antibacterial capability and can be used in many herbal formulations to ease many human and animal ailments. Moderate antifungal activity was recorded against *T. harizanum* only, remaining fungal species were unaffected. At inconsistent dilutions of crude plant extract, less to moderate free radical scavenging and phytotoxic activity was recorded. No hemagglutination activity indicated due to lack of phytoglutinins in the crude leaves extract.

REFERENCES

1. Allen O, Allen N (1981). The Leguminosae; A source book of characteristic uses and nodulation, Macmillan Publishers Ltd, University of Wisconsin Press., Madison, pp.511-515.
2. Ahmad K, Shireen F, Atif M, Mehreen and Bahar S (2013). Antibacterial, Antifungal and Phytotoxic properties of Caralluma tuberculata. Indo-American Journal of Pharmaceutical Research, 3:7:5465.
3. Boulenger, Albert G (1882). Catalogue of the Batrachia salientia S. ecaudatain the collection of the British Museum, 2nd Edition, Wesley London, pp.503.
4. Blois MS (1958). Antioxidant determination by the use of stable free radicals, Nature. 26:1199-2000.
5. Chopra RN et al (1986). Glossary of Indian medicinal plants, Council of Scientific & Industrial Research., New Delhi, pp.119.
6. Clifford A (2001). Mediterranean vegetables: a cook's ABC of vegetables and their preparation in Spain, France, Italy, Greece, Turkey, the Middle East, and north Africa with more than 200 authentic recipes for the home cook, Illustrated ed., Harvard Common Press., pp. 181–182.
7. Dash BK et al (2011). Antibacterial Activities of Methanol and Acetone Extracts of Fenugreek (Trigonella foenum) and Coriander (Coriandrum sativum), Life Sciences and Medicine Research., 2011:1-8.
8. Haouala R et al (2008). Aqueous and organic extracts of *Trigonella foenum-graecum* L. inhibit the mycelia growth of fungi, Journal of Environmental Sciences., 20:12:1453–1457.
9. Kamath SH, Sohonie K (1959). Nutritive value of proteins of green vegetables. Indian Jounal of Medicinal Research, 47:93.
10. Parthasarathy VA et al (2008). Organic spices. New India Publishing Agency., New Delhi, pp.694.
11. Tejaswini BA et al (2012). Invitro antioxidant and antimicrobial activities of crude extracts of *Trigonella foenum-graecum* seeds, Asian Journal of Chemistry., 24:11:5019-5022.