Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J5-211

Antioxidant, phytotoxic and cytotoxic activity of methanolic extract of *Trigonella foenum-graecum*

Amin Ullah[1], Said Hassan[1*], Muhammad Imran Khan[2], Muhammad Rizwan[1], Zafar Ullah[2], Mukaram Shah[3]

[1]*Center of Biotechnology and Microbiology, University of Peshawar, Peshawar KP, Pakistan*

[2]*Department of Biotechnology, University of Science and Technology, Bannu, Khyber Pakhtunkhawa, Pakistan*

[3]*Agriculture Research Institute, Tarnab Farm Peshawar, Khyber Pakhtunkhawa, Pakistan*

ARTICLE INFO

Article history:
Received 21 Oct 2015
Received in revised form 21 Dec 2015, 2nd revised form 5 Jan 2016
Accepted 18 Mar 2016
Available online 19 May 2016

Keywords:
1, 1-Diphenyl-2-picrylhydrazyl
Cytotoxic
Antioxidant
Trigonella foenum-graecum
Free radicals

ABSTRACT

Objective: To analyze the methanol extract of *Trigonella foenum-graecum* (*T. foenum-graecum*) for antioxidant, phytotoxic and cytotoxic activity.

Methods: The powder of *T. foenum-graecum* was extracted in diluted methanol with the help of random shaking method. All extracts of the plant were measured for cytotoxic activity (beside brine shrimp and antioxidant activity *vs.* 1, 1-diphenyl-2-picrylhydrazyl free radical).

Results: Various concentrations of methanolic extract of *T. foenum-graecum* were observed as 36.16% to 54.12% with rising concentrations of 50 to 1 000 μg/mL. Significantly phytotoxic activity (100 and 1 000 μg/mL) reduced the growth of roots (radicals) and shoots (hypocotyls) of rice when compared to control after 3 and 7 days' treatment. At a concentration of 10 μg/mL, the survival rate of cytotoxic activity of brine shrimp was maximum and at a concentration of 250 μg/mL, the death rate of brine shrimp was maximum.

Conclusions: *T. foenum-graecum* has potential activity against free radical mediated sickness and thus it is possible to treat cancer.

1. Introduction

In the living system, free radicals are created as components of the body's general metabolic growth. In the mitochondrial respiratory chain, the free radical chain reactions are frequently created, liver assorted purpose oxidases, atmospheric pollutants, throughout xanthine oxidase activity and from transitional metal catalysts, xenobiotics and drugs. Under different situations such as lactation, fever, exercise, infection and fasting, the mobilization of adding substance of fat store can improve radical activity and and reduce harm[1,2]. Number of human disorders appeared currently can cause by free radicals or oxidative injury. Peroxidation of lipids can be initiated by oxygen-free radical, which in turn accelerates the glycation of protein, while change in the arrangement, function of collagen cellar and additional membranes due to inactivation of enzymesand plays a vital role in long-term diabetes[3,4]. Antioxidants are the substances which prevent our body from the hazards by the effect of free radicals. Antioxidants have been detected in a number of agricultural and food products as well as cereals, fruits, vegetables and oil seeds[5-7]. Antioxidants can scavenge free radicals by balancing their electrons. Hydroxyl radical (OH), superoxide radicals (O2-), and peroxyl radicals (ROO), have been linked with carcinogenesis and heart disease[8]. These are more and more being encouraged because they proceed straight on oxidative processes and may be a technique to stop diseases and health troubles linked to aging. Therefore, separation of antioxidant biomolecules is a steady search for antioxidative natural resources.

Plants have been used as a source of medicine all over the world for more than 5 000 years old and are still carried on to occupy significant location and current system of medicines[9]. The traditional system of medicines using plants as a source has acquired superior impetus in the last two decades, as extreme use of synthetic drugs and antibiotics has been established to cause a number of side effects sometimes and is proved to be lethal.

Scientists are interested in investigating medicinal plants which are usually used by public and derived from folklore or anecdotal information[10,11]. About 80% of the globe population believe on conventional medicines for their main health care which was estimated by the World Health Organization[12]. Herbal medicines have frequently been used in the shape of fruits and vegetables, and

*Corresponding author: Said Hassan, Center of Biotechnology and Microbiology, University of Peshawar, Peshawar KP, Pakistan.
Tel: +92-345-9151715
E-mail: saidhassan15@yahoo.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

the drugs or their extracts can cure the diseases and protect health[13].

Currently, medicinal plants are a possible source of drugs or molecular models for new drugs. In present, vinblastine, irinotecan, topotecan, vincristine, taxanes *etc*. provided a lot of effective anticancer agents by various species[14]. Beside this, stomach aches, chills, lung and rheumatic diseases can be cured by the mixture of honey[15]. The perfume is manufactured from the extracted oil of the root and its smoking is used to cure flu and headaches[16]. Several wild food plants are also used for medicinal purposes[17-19]. There is new attention on strong wild food plants as they have specific pharmacological effects[14,20-22]. Moreover, they can help to decrease risk from a variety of chronic and inflammatory conditions, certain types of cancers, diabetes, menopausal symptoms and age associated diseases and are also useful to households afflicted by HIV/AIDS among others[12,16,23].

2. Materials and methods

2.1. Collection of plant

Trigonella foenum-graecum (*T. foenum-graecum*) was collected from Khyber Pakhtonkhwa Pakistan during summer, in the month of June, 2014 and was identified by Assistant Prof. Riaz Hussain, Department of Botany GDC Serai Naurang. The parts of the plant were washed and dried at room temperature and were grinded mechanically for 1 mm mesh size.

2.2. Preparation of plant extract

About 200 g powder of *T. foenum-graecum* was extracted for 600 mL methanol with the help of random shaking method. After 7 to 10 days, the extract was filtered with the help of qualitative Whatman filter paper No. 1.

2.3. Antioxidant assay

2.3.1. 1, 2-Dyphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH assay was conducted according to the method of Gyamfi *et al*. The experiment was conducted in duplicate test tubes with concentrations of 50, 100, 150, 200, 250, 500 and 1 000 μg/mL. Ascorbic acid concentration was used as reference. All the test tubes were labeled individually as extract, ascorbic acid and DPPH and shaken well and kept in incubator in dark at 25 ˚C for about 30 min. By means of spectrophotometer, the absorbance was taken and calculated as 517 nm. The results were expressed as percentage inhibition.

Percentage inhibition = [(A1 – A 2)/A1] × 100

where A1 = the absorbance of DPPH (control), A2 = the absorbance in the presence of sample.

2.4. Cytotoxic assay (brine shrimp assay)

Stock solution of 15 mg/mL was prepared from methanolic extract. Additional dilution was made from this stock solution (10, 50, 100, 150 and 200 μg/mL). About 2.8 g of sea salt was used in 100 mL distilled water to prepare saline solution. In the dark, the egg compartment tray covered by aluminum foil was placed and completely aerated. Larvae hatching occurred after incubation of 24 h under light at room temperature. Assay was done according to the procedure of Solis *et al*. and Potduang *et al*. to determine the inhibitory activity. About 50 μL of different concentrations of crude methanolic extracts (1 000, 500, 250, 200, 100 and 50 μg/mL) was taken and were repeated in triplicate. After incubation at room temperature for 24 h, the living brine shrimp was counted with the help of a hand magnifying lens and potassium dichromate was used as positive control.

2.4. Phytotoxic assay (Petri plate study)

Phytotoxic study was conducted in Petri plates with filter paper using rice seeds. The Petri plates were used in duplicates, sprayed with the solution of 100 μg/mL and 1 000 μg/mL and labeled. Water solution was used for treating of control sample. The plates were kept in growth room for 3 days after this process. Hypocotyls/shoot and radical/root inhibition was measured with the help of ruler after 3 days, with respect to the control and was averaged. Then, the plates were kept for 7 days and after 7 days, the length of hypocotyls/shoot and radical/root was measured and the mean value was taken.

3. Results

3.1. DPPH free radical scavenging assay

DPPH had strong potential to oxidize a range of compounds. So, it was extensively used for the judgment of *in vitro* antioxidant scavenging activities of medicinal plants. The percentage of scavenging activity of methanolic extract of *T. foenum-graecum* for free radicals of DPPH was shown in Figure 1. In this study, various concentrations of the methanolic extract of *T. foenum-graecum* were used. By the methanolic extract of *T. foenum-graecum* with increasing concentrations (50 μg/mL < 100 μg/mL < 150 μg/mL < 200 μg/mL < 250 μg/mL < 1 000 μg/mL), the important scavenging activity was observed. Ascorbic acid was used as a reference compound and the consequence was acquired by a variety of concentration of ascorbic acid (50–1 000 μg/mL).

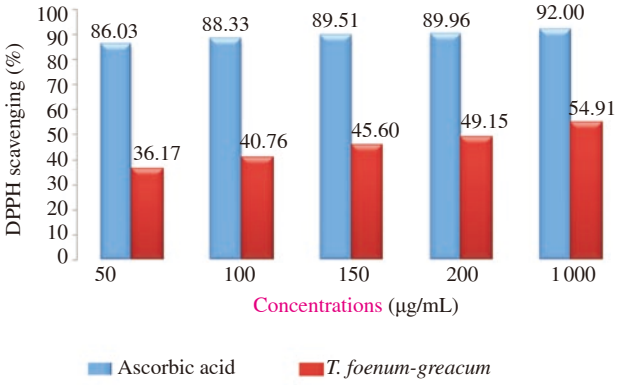

Figure 1. Percentage scavenging activity of methanolic extract of *T. foenum-graecum* for free radicals of DPPH.

3.2. Cytotoxic assay of brine shrimp

The cytotoxic assays were typically performed in pharmaceutical industry for screening/isolation of those natural bioactive compounds. These were used for the treatment of cancer by MTT, clonogenic and sulforhodamine B assays. In the cure of cancer, plants had a long history of use and herbal medicines had a fundamental function in the prevention and action of cancer[24,25]. The search for anticancer agents that could slow down cancer growth

was a significant aim for scientists.

At a low concentration of methanolic extract of *T. foenum-graecum,* the survival rate of brine shrimp was high and at a high concentration of methanolic extract of *T. foenum-graecum,* the death rate of brine shrimp was high, which indicated that the cytotoxic properties can be utilized for the treatment of tumors and cancer (Figure 2).

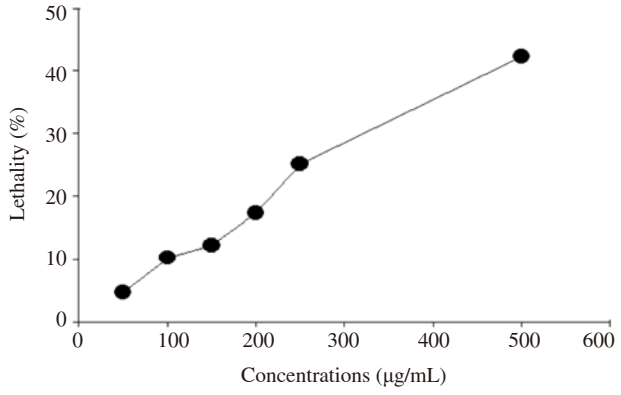

Figure 2. Cytotoxic effect of different concentrations of methanolic extract of *T. foenum-greacum* extract and ascorbic acid.

3.3. Phytotoxic activity (Petri plate study)

The samples of two different concentrations *i.e* 100 µg/mL and 1 000 µg/mL of the *T. foenum-greacum* were used for the phytotoxic activity. The consequence obtained from the methanolic crude extract of the sample demonstrated that these samples inhibited the growth of hypocotyls and radicals of rice as compared to the control after 3 and 7 days (Figures 3 and 4). Methanolic extract of *T. foenum-graecum* significantly inhibited roots growth as compared to non-treated water (control) group as shown in Figures 5 and 6.

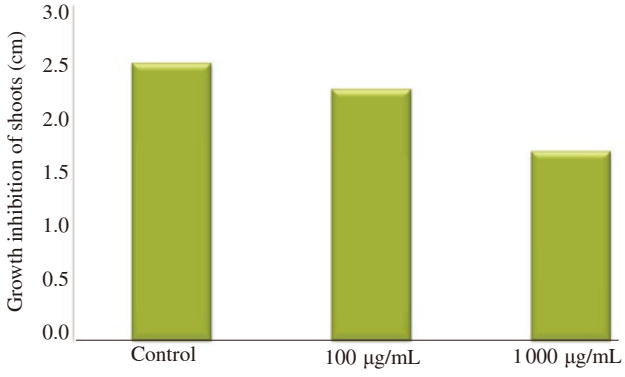

Figure 3. Growth of rice shoots in the presence and absence of different concentrations of *T. foenum-greacum* after 3 days of treatment.

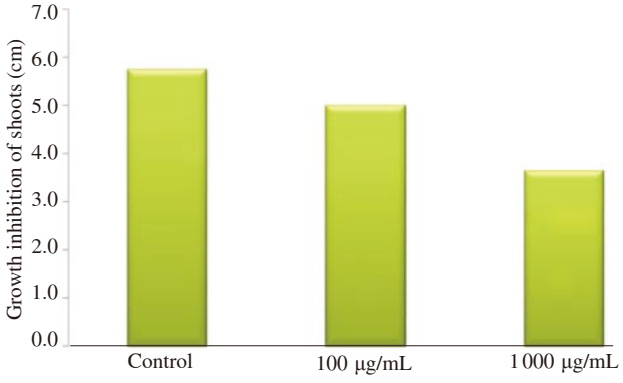

Figure 4. Growth of rice shoots in the presence and absence of different concentrations of *T. foenum-greacum* after 7 days of treatment.

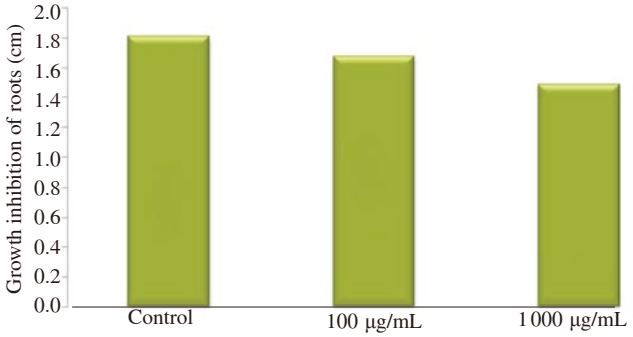

Figure 5. Growth of rice roots in the presence and absence of different concentrations of *T. foenum-greacum* after 3 days of treatment.

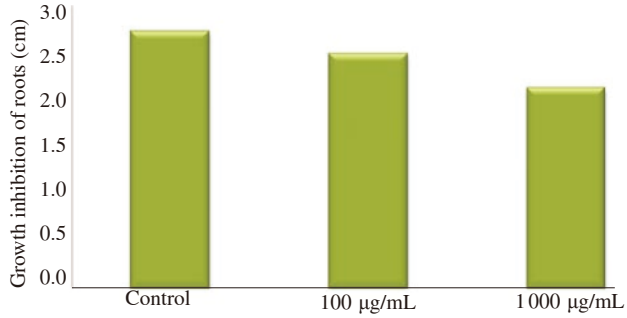

Figure 6. Growth of rice roots in the presence and absence of different concentrations of *T. foenum-greacum* after 7 days of treatment.

4. Discussion

4.1. Scavenging of free radicals

Oxidation is an important process in living organisms for the production of energy. During normal metabolism, oxygen consumption and free radicals of enzymatic reaction are produced. These free radicals are beneficial in minute amount in growth regulation as well as in signal transduction. So, the data of our present study of methanolic extract of *T. foenum-graecum* showed markedly scavenging of free radicals. The consequences have a similarity to that of Loo *et al.*, which stated that dichloromethane extract has potent antioxidant activity of the pyroligeneous acid of *Rhizophora apiculata*, which were actually suitable to the existence of high phenolic content[26]. The antioxidant activity of *T. foenum-graecum* extract could be due to the bioactive compounds *i.e* phenolic and polyphenolic compounds which considerably decrease free radicals because of oxidative stress. Similarly, the outcome is strongly supported by the results of Cook and Samman, in which the medicinal plants have antioxidants activities because of the phenolic compounds[27].

4.2. Cytotoxic property

Cytotoxic property of natural products is also linked to its anticancer potential[28,29]. During investigation 1 or 2, anticancer activities are joined and typically antitumor and cytotoxic activities go after each other[30]. In the current study of *T. foenum-graecum,* the standard concentrations of 10 µg, 50 µg, 100 µg, 150 µg, 200 µg and 250 µg projected by McLaughlin and Rogers were used for testing the cytotoxic impact of plant extracts. The results show that at the four strengths, the methanolic plant extracts were comparatively deadly. Comparable outcomes were achieved with negative control. But the 100% lethality is formed by positive control (potassium dichromate). This indicates that at a normal

concentration planned by McLaughlin and Rogers, the extract was moderately poisonous[31].

4.3. Phytotoxic potential

The methanolic extract of *T. foenum-graecum* revealed significantly phytotixc inhibition of shoots and roots growth of rice in comparison with control (non-treated plants). The comparable outcome were shown by Kordali *et al*. that some necessary oils and phenolic compounds, which are extracted from Turkish *Origanum acutidens* are said to be completely inhibitors for the growth of seedling/hypocotyls and roots compared to the standard[32]. Another study, Javaid showed results from the water extract of *Withania somnifera* and *Datura alba* and the number of bioactive compounds are isolated which extremely repressed the development of roots and shoots of *Rumex dentatus* L. and during allelopathic screening, it is a highly competitive weed in wheat[33].

In the present study, the phytochemical constituents of *T. foenum-graecum* extract showed that average cytotoxic and phytotoxic activity may be responsible for the potential activity. It is helpful against free radical mediated illness and also possible for treating cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Saha MR, Alam MA, Akter R, Jahangir R. *In vitro* free radical scavenging activity of *Ixora coccinea* L. *Bangladesh J Pharmacol* 2008; **3**: 90-6.

[2] Milella L, Bader A, De Tommasi N, Russo D, Braca A. Antioxidant and free radical-scavenging activity of constituents from two *Scorzonera* species. *Food Chem* 2014; **160**: 298-304.

[3] Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. *Am J Clin Nutr* 2003; **78**: 570S-8S.

[4] Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 2003; **73**: 167-79.

[5] Adom KK, Sorrells ME, Liu RH. Phytochemical profiles and antioxidant activity of wheat varieties. *J Agric Food Chem* 2003; **51**: 7825-34.

[6] Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharm Biomed Anal* 2006; **41**: 1523-42.

[7] Netzel M, Netzel G, Tian QG, Schwartz S, Konczak I. Native Australian fruits-a novel source of antioxidants for food. *Innov Food Sci Emerg Technol* 2007; **8**: 339-46.

[8] Steer P, Millgård J, Sarabi DM, Basu S, Vessby B, Kahan T, et al. Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. *Lipids* 2002; **37**: 231-6.

[9] Sher H, Hussain F. Ethnobotanical evaluation of some plant resources in Northern part of Pakistan. *Afr J Biotechnol* 2009; **8**(17): 4066-76.

[10] Namrata LK, Ghosh D, Dwivedi SC, Singh B. Wild edible plants of Uttarakhand Himalaya: a potential nutraceutical source. *Res J Med Plants* 2011; **5**(6): 670-84.

[11] Bernal J, Mendiola JA, Ibáñez E, Cifuentes A. Advanced analysis of nutraceuticals. *J Pharm Biomed Anal* 2011; **55**(4): 758-74.

[12] Kharb S, Singh V. Nutriceuticals in health and disease prevention. *Indian J Clin Biochem* 2004; **19**(1): 50-3.

[13] Sahito SR, Memon MA, Kazi TG, Shar GQ. Evaluation of mineral contents in medicinal plant *Azadirachta indica* (neem). *J Chem Soc Pak* 2003; **25**(2): 139-43.

[14] Soetan KO. Pharmacological and other beneficial effects of anti-nutritional factors in plants-a review. *Afr J Biotechnol* 2008; **7**(25): 4713-21.

[15] Al Faïz C, Thami Alami I, Saïdi N. Domestication of some MAP species in biological diversity, cultural and economic value of medicinal, herbal and aromatic plants in Morocco; 2006- 2007 : 15-22.

[16] Ebadi M. *Pharmacodynamic basis of herbal medicine*. 2nd ed. Boca Raton: CRC Press; 2007.

[17] Adwan G, Abu-Shanab B, Adwan KM, Abu-Shanab F. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turk J Biol* 2006; **30**: 239-42.

[18] Jaca TP, Kambizi L. Antibacterial properties of some wild leafy vegetables of the Eastern Cape Province, South Africa. *J Med Plants Res* 2011; **5**(13): 2624-8.

[19] Cseke LJ, Kirakosyan A, Kaufman PB, Warber S, Duke JA, Brielmann HL. *Natural products from plants*. 2nd ed. Boca Raton: CRC Press; 2006.

[20] Pieroni A, Price LL. *Eating and healing traditional food as medicine*. Oxford: Haworth Press, Inc; 2006.

[21] Agea JG, Okia CA, Abohassan RAA, Kimondo JM, Obua J, Hall J, et al. Wild and semi-wild food plants of Bunyoro-Kitara Kingdom of Uganda: growth forms, collection niches, parts consumed, consumption patterns, main gatherers and consumers. *Environ Res J* 2011; **5**(2): 74-86.

[22] Guarrera PM, Savo V. Perceived health properties of wild and cultivated food plants in local and popular traditions of Italy: a review. *J Ethnopharmacol* 2013; **146**: 659-80.

[23] Kaschula SA. Wild foods and household food security responses to AIDS: evidence from South Africa. *Popul Environ* 2008; **29**: 162-85.

[24] Cragg GM, Newman DJ. Plants as a source of anticancer agents, in ethnopharmacology. In: Elisabetsky E, Etkin N, editors. *Encyclopedia of life support systems (EOLSS), developed under the Auspices of the UNESCO*. Oxford: Eolss Publishers; 2006.

[25] Sakarkar DM, Deshmukh VN. Ethnopharmacological review of traditional medicinal plants for anticancer activity. *Int J Pharm Tech Res* 2011; **3**(1): 298-308.

[26] Loo AY, Jain K, Darah I. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. *Food Chem* 2007; **104**: 300-7.

[27] Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *J Nutr Biochem* 1996; **7**(2): 66-6.

[28] Rumzhum NN, Rahman MM, Kazal MK. Antioxidant and cytotoxic potential of methanol extract of *Tabernaemontana divaricata* leaves. *Int Curr Pharm J* 2012; **1**(2): 27-31.

[29] Habib MR, Rana MS, Hasan MR, Imam MZ, Hasan SMR, Saha A. Antioxidant, cytotoxicity and antibacterial potential of different extract of *Geodorum densiflorum* (Lam.) Schltr. root. *J Herb Med Toxicol* 2011; **5**(1): 63-70.

[30] Hussain A, Zia M, Mirza B. Cytotoxic and antitumor potential of *Fagonia cretica* L. *Turk J Biol* 2007; **31**: 19-24.

[31] Agarwal A, D'Souza P, Johnson TS, Dethe SM, Chandrasekaran C. Use of *in vitro* bioassays for assessing botanicals. *Curr Opin Biotechnol* 2014; **25**: 39-44.

[32] Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. *Bioresour Technol* 2008; **99**: 8788-95.

[33] Javaid A. Role of effective microorganisms in sustainable agricultural productivity. Advances in sustainable agriculture. Springer 2009.