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PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *TRIGONELLA FOENUM-GRACUM* (LEAVES)

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ABSTRACT

Free radicals can cause some major complicated diseases like Diabetes mellitus, Coronary heart disease, Cancer. The present study was designed to evaluate the antioxidant properties of methanol extract of *Trigonella foenum-gracum.* Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The IC50 of the methanolic extract was 12.06µg/ml and that of ascorbic acid was 6.14 µg/ml. Phytochemical screening test indicates the presence of Saponins, Glycosides, Flavonoids and Alkaloids. The study reveals that the *Trigonella foenum-gracum* consumption would exert several beneficial effects by virtue of their significant antioxidant activity.

KEYWORDS: Phytochemical screening, Free radicals, *Trigonella foenum-gracum.*

INTRODUCTION

Oxidative stress can be defined as an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to cure the resulting damage. In humans, oxidative stress is thought to be responsible for development of cancer.[1] Parkinson's disease, Alzheimer's disease,[2][3] atherosclerosis, heart failure,[4] myocardial infarction,[5][6] fragile X syndrome.[7] Sickle Cell Disease,[8] lichen planus,[9] vitiligo,[10] autism,[11] infection,[12] and chronic fatigue syndrome.[13] Antioxidants terminate the chain reactions of oxidation by removing free radical intermediates, and inhibit other oxidation reactions. Antioxidants inhibit oxidation by being oxidized itselves, so they are often reducing agents such as thiols, ascorbic acid (vitamin C), or polyphenols.[14] Antioxidants are widely used in dietary supplements as well as it have been proved that they are effective for the prevention of diseases such as cancer, coronary heart disease and altitude sickness.[15] Modern research suggest Antioxidants as possible treatments for neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis,[16][17] beside this as a way to prevent noise-induced hearing loss.[18] On the other hand Antioxidants also have many other uses, such as in industries as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.[19] Fenugreek (*Trigonella foenum-graecum*) is an annual plant of the Fabaceae family, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop, and its seeds are a commonly used as an ingredient in dishes from the Indian subcontinent.[20] Although India is Major fenugreek-producing country but some other countries also produce fenugreek like Afghanistan, Pakistan, India, Iran, Nepal, Bangladesh, Argentina, Egypt, France, Spain, Turkey and Morocco. Fenugreek is used as a herb (dried or fresh leaves), spice (seeds) and vegetable (fresh leaves, sprouts and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell. Cuboid-shaped, yellow- to amber-colored fenugreek seeds are frequently used both whole and powdered in the preparation of pickles, vegetable dishes, daals, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavor.[21] In Turkish cuisine, fenugreek is used for making a paste known as çemen. In Persian cuisine, fenugreek leaves are the key ingredient and one of several greens incorporated into ghormeh sabzi and eshkeneh, often said to be the Iranian national dishes. In Egyptian add fenugreek seeds and maize to their pita bread to produce aish merahrah, a staple of their diet.[22]

MATERIALS AND METHOD

Plant material

The plant *Trigonella foenum-gracum* was collected from local market, Dhaka, Bangladesh.

Preparation of the crude extract

Cold extraction (Methanol extraction)

The collected plant parts (leaves) were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 180 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained.

Screening for the antioxidant activity

Antioxidant activity of the extract was determined on the basis of their scavenging potential of the stable DPPH free radical in quantitative assay.

Antioxidant tests[26-28]

Stock solution of the plant extract was prepared in methanol (10mg/ml) from which a serial dilution was carried out. At first 6 volumetric flasks are taken to make 6 different types of concentration 1, 5, 10, 50, 100 and 500 µg/ml. Test tubes and volumetric flasks are rapped with foil paper. In 6 volumetric flasks serial dilution of extract is done and marked them respectively.

Phytochemical Screening[23-25]

Phytochemical studied of methanolic extract of plant marerial extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book.

2ml of sample from each concentration and 2 ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. Then solution is kept in dark place for 30 minutes with raping each test tube with foil paper. In another test tube 2ml 0.004% DPPH & 2ml methanol is taken to prepare blank solution. Then absorbance is taken by UV Spectroscopy. The percent of inhibition is calculated by using following formula

$$\%\text{inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

RESULT AND DISCUSSION

Phytochemical Screening

Results of the phytochemical screening of the Methanolic Extract of *T. foenum-gracum.*

Table: 1. Results of Phytochemical Screening.

Chemical Groups	Methanolic Extract of *T. foenum-gracum*
Saponin	+
Glycoside	+
Flavonoids	+
Tannin	-
Alkaloids	+

Note: (**+**) = Indicates the presenc and (**−**) = Indicates the absence of the tested group.

Result of Anti-oxidants test

DPPH scavenging assay

Table-2: % inhibition of ascorbic acid and (*T. foenum-gracum).*

Conc. (µg/ml)	Absorbance (nm)			% of Inhibition	
	Blank	Ascorbic Acid	(*T. foenum-gracum)*	Ascorbic Acid	(*T. foenum-gracum)*
1	0.712	0.612	0.680	14.50	4.50
5		0.438	0.587	38.50	17.50
10		0.163	0.211	77.10	70.36
50		0.075	0.118	89.46	83.42
100		0.073	0.105	89.74	85.25
500		0.062	0.011	91.29	98.45

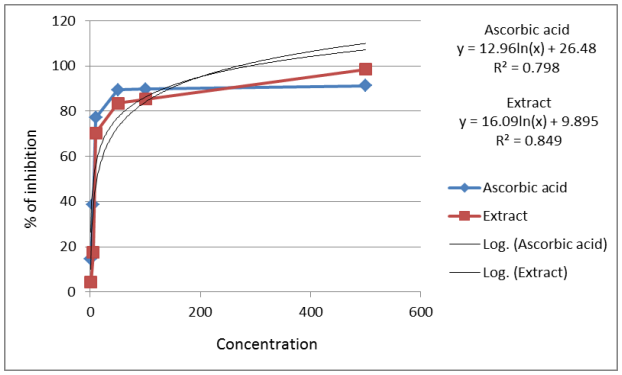

Figure-1: Anti-oxidant activity of ascorbic acid and *T. foenum-gracum*.

Table-3: IC_{50} values of the extracts of Ascorbic Acid and *T. foenum-gracum*.

Test Samples	Regression line	R^2	IC_{50} µg/ml
Ascorbic Acid	y = 12.96ln(x) + 26.48	R² = 0.798	6.14
T. foenum-gracum	y = 16.09ln(x) + 9.895	R² = 0.849	12.06

DISCUSSION

The antioxidant activity of the methanolic extract *T. foenum-gracum* was evaluated using DPPH free radical scavenging activity method. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts.[29-30] Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine.[31-32] The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties.[33] The methanolic extract of *T. foenum-gracum* leaf has significant anti oxidant activity. The IC_{50} of the *T. foenum-gracum* is 12.06µg/ml, whereas IC_{50} of Ascorbic Acid is 6.14 µg/ml.

CONCLUSION

The present study revealed that extracts of the *T. foenum-gracum* can be used as a source of antioxidant. At last we can say that further study is needed to do in-vivo antioxidant activity and find out the causative metabolites of *T. foenum-gracum* and possible mechanism.

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REFERENCE

1. Halliwell, Barry. "Oxidative stress and cancer: have we moved forward?" Biochem. J, 2007; 401(1): 1–11.
2. Valko, M., Leibfritz, D., Moncol, J., Cronin, MTD., Mazur, M., Telser, J. "Free radicals and antioxidants in normal physiological functions and human disease". International Journal of Biochemistry & Cell Biology, 2007; 39(1): 44–84.
3. Pohanka, M "Alzheimer´s disease and oxidative stress: a review". Current Medicinal Chemistry, 2013; 21(3): 356–364.
4. Singh, N., Dhalla, A.K., Seneviratne, C., Singal, P.K. "Oxidative stress and heart failure". Molecular and Cellular Biochemistry, 1995; 147(1): 77–81.
5. Ramond A, Godin-Ribuot D, Ribuot C, Totoson P, Koritchneva I, Cachot S, Levy P, Joyeux-Faure M. "Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia." Fundam Clin Pharmacol, 2011; 27(3): 252–261.
6. Dean OM, van den Buuse M, Berk M, Copolov DL, Mavros C, Bush AI. "N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and D-amphetamine-treated rats: relevance to schizophrenia and bipolar disorder". Neurosci Lett, 2011; 499(3): 149–53.
7. de Diego-Otero Y, Romero-Zerbo Y, el Bekay R, Decara J, Sanchez L, Rodriguez-de Fonseca F, del Arco-Herrera I. "Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency." Neuropsychopharmacology, 2009; 34(4): 1011–26.
8. Amer, J., Ghoti, H., Rachmilewitz, E., Koren, A., Levin, C. and Fibach, E. "Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants". British Journal of Haematology, 2006; 132(1): 108–113.
9. Aly, D. G.; Shahin, R. S. "Oxidative stress in lichen planus". Acta dermatovenerologica Alpina, Panonica, et Adriatica, 2010; 19(1): 3–11.
10. Arican, O.; Kurutas, EB. "Oxidative stress in the blood of patients with active localized vitiligo." Acta Dermatovenerol Alp Panonica Adriat, Mar 2008; 17(1): 12–6.
11. James, SJ.; Cutler, P.; Melnyk, S.; Jernigan, S.; Janak, L.; Gaylor, DW.; Neubrander, JA. "Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism." Am J Clin Nutr, 2004; 80(6): 1611–7.
12. Pohanka, M. "Role of oxidative stress in infectious diseases. A review." Folia Microbiologica, 2013; 584(6): 503–513.
13. Gwen Kennedy, Vance A. Spence, Margaret McLaren, Alexander Hill, Christine Underwood & Jill J. F. Belch. "Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms". Free radical biology & medicine, 2005; 39(5): 584–9.
14. Sies H. "Oxidative stress: oxidants and antioxidants". Experimental Physiology, 1997; 82(2): 291–5.
15. Baillie JK, Thompson AA, Irving JB, Bates MG, Sutherland AI, Macnee W, Maxwell SR, Webb DJ. "Oral antioxidant supplementation does not prevent

acute mountain sickness: double blind, randomized placebo-controlled trial". Qjm, 2009; 102(5): 341–8.
16. Di Matteo V, Esposito E. "Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis". Current Drug Targets. CNS and Neurological Disorders, 2003; 2(2): 95–107.
17. Rao AV, Balachandran B "Role of oxidative stress and antioxidants in neurodegenerative diseases". Nutritional Neuroscience, 2002; 5(5): 291–309.
18. Kopke RD, Jackson RL, Coleman JK, Liu J, Bielefeld EC, Balough BJ "NAC for noise: from the bench top to the clinic". Hearing Research, 2007; 226(1-2): 114–25.
19. Dabelstein W, Reglitzky A, Schütze A, Reders K "Automotive Fuels". Ullmann's Encyclopedia of Industrial Chemistry, 2007.
20. "Trigonella foenum-graecum information from NPGS/GRIN". Www.ars-grin.gov. Retrieved, 2008; 03-13.
21. "Fenugreek recipes". BBC Food.
22. "Aish Merahrah-Egyptian Fenugreek Corn Bread". The Taste of Aussie. Retrieved 29 June 2014.
23. Evans W.C.) Pharmacognosy: London, W.R. Saunders, 2002.
24. Mohammed Ali, Textbook of Pharmacognosy, Second Edition, 1998.
25. Abdul Ghani, Practical Phytochemistry. Firstedition, 2005.
26. CRC Handbook of Free Radicals and Antioxidants, 1989; 1: 209-221.
27. Antioxidant Vitamins Benefits Not Yet Proved (editorial) NEJM, 1994; 230(15): 1080 – 1081.
28. Antioxidants and Physical Performance, Critical Reviews in Food Science and Nutrition, 1995; 35(1&2): 131-141.
29. Koleva II, Van Beek TA, Linseen JPH, de Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal, 2002; 13: 8-17.
30. Suresh PK, Sucheta S, Sudarshana VD, Selvamani P, Latha S, Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol, 2008; 7: 1826-1828.
31. Beyer RE The role of ascorbate in antioxidant protection of biomembranes: interaction with vit-E and coenzyme. Q. J. Bioen. Biomemb, 1994; 24: 349-358.
32. Aqil F, Ahmed I, Mehmood Z Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol, 2006; 30: 177-183.
33. Rice-Evans CA, Miller NJ, Paganga G, Antioxidant properties of phenolic compounds. Trend. Plant Sci., 1997; 4: 152-159.