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Evaluation of Antioxidant Activity of *Trigonella foenum graecum* Seeds Extract

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Abstract

In the current work, we completed a comprehensive assessment of the relative antioxidant activity in extracts from a few different medicinal plant species.The extracts' effectiveness at scavenging 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radicals was assessed spectrophotometrically. The highest degree of radical scavenging was 52.54%, while the IC_{50} value was 93.54.*Trigonella foenum graecum* extract demonstrates how the greater concentration of phytoconstituents chemicals leads to more powerful radical scavenging results.

Keywords: *Trigonella foenum graecum,* Antioxidant, DPPH, Phytochemical analysis

INTRODUCTION

Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer and AIDS. Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify in gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routs for producing free radicals in food, drugs and still living systems. Catalase and hydroperoxides enzymes change hydrogen peroxide and hydroperoxides to nonradical forms and purpose as natural antioxidants in human body. Owing to depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential. At present available synthetic antioxidants similar to butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity[1,2]. Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical induced tissue injury. Polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action[3]. A number of confirmations suggest that the biological actions of these compounds are related to their antioxidant activity[4]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases[5]. In particular, despite extensive use of wild plants as medicines in Iran, the prose contains few reports of antioxidant activity and chemical composition of these plants. In current study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plant species, which are being used traditionally: The seeds *Trigonella foenum graecum.* In the longer term, plant species (or their active constituents) recognized as having high levels of antioxidant activity *in vitro* may be of value in the design of additional studies to unravel novel treatment strategy for disorders connected with free radicals induced tissue damage. Besides well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements[6]. Also many other plant species have been investigated in the search for novel antioxidants[7,8] but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds[9].

MATERIALS AND METHODS

Selection, collection and processing of plant

Seeds of fenugreek were purchased from local retail market. The seeds were cleaned before drying it in oven at 50 °C for 24 h. Then, the dried seeds were ground using a mill with ultra-centrifugal equipped with ring sieve owning trapezoid holes sized 0.5 mm. The moisture content of the seed was (5.51 ± 0.14% d.w basis). The powdered seeds were kept in dark airtight container before extraction.

Reagents and chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol , ascorbic acid, potassium phosphate buffer-ph6.6 (dihydrogen phosphate , sodium hydroxide), potassium ferricyanide , trichloroacetic acid , ferric chloride .

Extraction

All the seeds are initially blended into coarse then into powder form. The powdered plant seeds were packed inside the Soxhlet extractor and were successively extracted with petroleum ether, and methanol for 8-10 hours and 40-60°C temperature of the heating mantle were adjusted. The extracts so collected were distilled on a water bath at atmospheric pressure and the last traces of solvent were removed using vacuum. Extracts were collected in air tight container[10].

Qualitative phytochemical analysis of plant extract

Following standard methods by Rani et al, 2021[11] the *Trigonella foenum graecum* extract obtained was subjected to the preliminary phytochemical analysis. The extract was screened to spot the presence or absence of many active constituents like carbohydrates, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, fats or fixed oils, protein, amino acid and tannins.

Activity (In-vitro anti-oxidant activity)

DPPH radical scavenging activity

a) Preparation of DPPH reagent

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

b) Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of extracts of *Trigonella foenum graecum* standard was prepared. 1 mg of extracts/standard was taken with methanol to make 1mg/ml stock solution. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

C) Preparation of control

For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm[12].Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100µg/ml.

Preparation of extracts of

Stock solutions of extracts of *Trigonella foenum graecum* was prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extracts of *Trigonella foenum graecum* (20 to 100µg/ml) in 1.0 ml of deionized water was mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard[13].

RESULTS

Table 1: Percentage yield of *Trigonella foenum graecum* extract

S. No	Solvent	Color of extract	Weight of Plant Material (gms)	Weight of extract (gms)	% Yield
1	Petroleum ether	Dark Green	180	4.32	2.4
2	Methanol	Green	164.71	10.68	6.484

Table 2: Qualitative phytochemical analysis

Phytoconstituents		Extracts	
		Pet. ether	Methanol
Alkaloids	Mayer	+++	+
	Dragendoff	+++	+
Flavonoids	Lead acetate	-	-
	Ferric chloride	+++	+++
Carbohydrates	Molish	+++	+++
	Bendict	-	+
Glycosides	Killer-lini	++	++++
Steroids	Salkowskis	-	+++
	Libermanburchard	+++	+++
Protein and amino acids	Ninhydrin	++++	+++
	Biuret	-	-
Phenolic compounds and tannins	Ferric chloride	-	-
	Gelatin	+++	+
	Lead acetate	++++	+
Terpenoids	Trim hill	-	-
	Libermann-burchard	+++	+++
Saponins	Foam test	+++	-
	Mercuric chloride	+++	++

Note :-+++ Very large quantity, +++ large quantity, +small quantity, - absent

Table 3: DPPH activity of Ascorbic acid

Ascorbic acid		
Concentration (µg/ml)	**Absorbance (nm)**	**% Inhibition**
20	0.447	46.97
40	0.353	58.12
60	0.235	72.12
80	0.137	83.74
100	0.069	91.81
Control	0.843	
IC50 24.34		

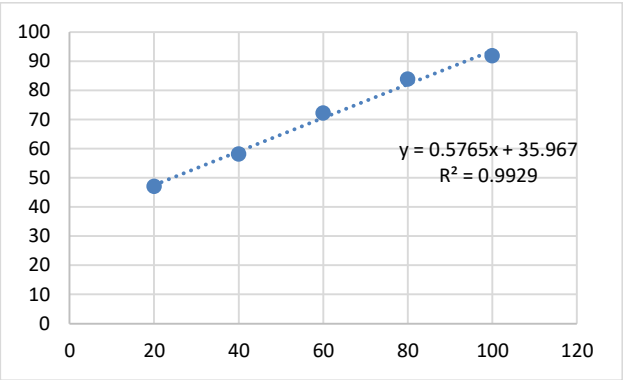

Figure 1: Graph represents the percentage inhibition *vs* concentration of ascorbic acid

Table 4: DPPH activity of *Trigonella foenum graecum* extract

Trigonella foenum graecum		
Concentration (µg/ml)	**Absorbance (nm)**	**% Inhibition**
20	0.49	16.9492
40	0.43	27.1186
60	0.38	35.5932
80	0.33	44.0678
100	0.28	52.5424
Control	0.59	
IC50 93.4568		

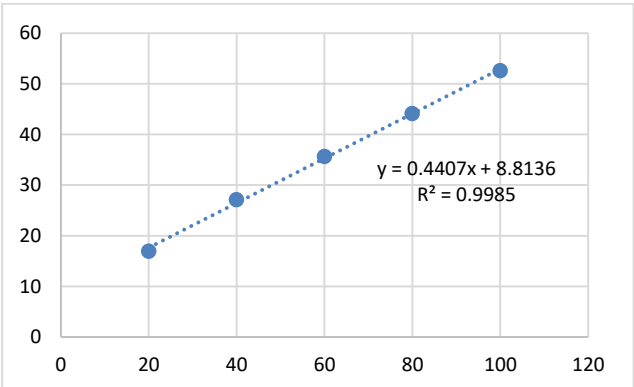

Figure 2: Graph represents the percentage inhibition *Vs* concentration of extract

Table 5: Reducing power activity of Ascorbic acid

Concentration (µg/ml)	Absorbance (nm)
20	0.14
40	0.244
60	0.367
80	0.43
100	0.505

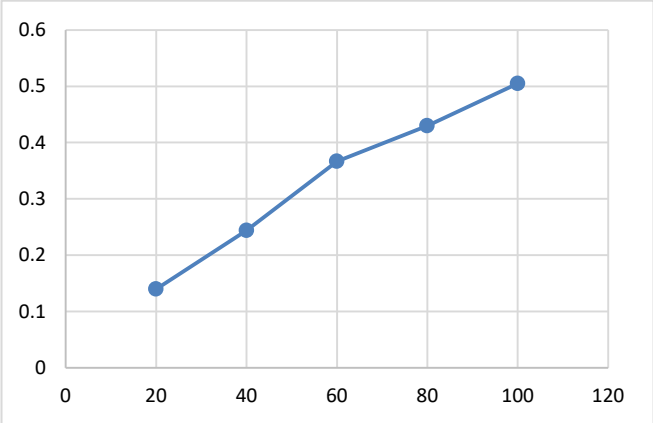

Figure 3: Graph represents the percentage inhibition *Vs* concentration of ascorbic acid

Table 6: Reducing power activity of *Trigonella foenum graecum* extract

Concentration (µg/ml)	Absorbance (nm)
20	0.02
40	0.03
60	0.05
80	0.06
100	0.08

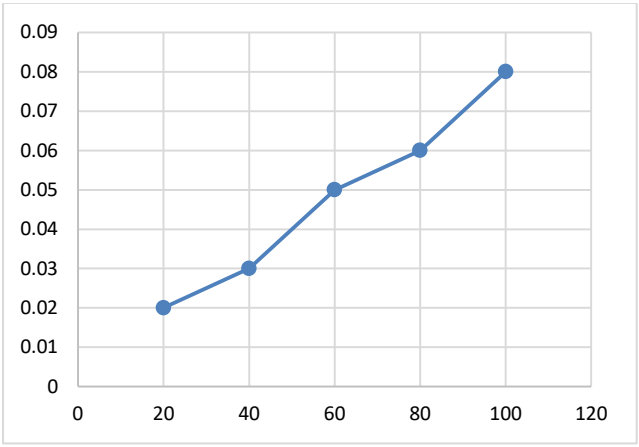

Figure 4: Graph represents the percentage inhibition *Vs* concentration of extract

CONCLUSION

The current study concluded that *a Trigonella foenum graecum* seed which was purchased from local market of Bhopal, Madhya Pradesh is rich source of phytochemicals. Results showed the presence of alkaloids, amino acids, carbohydrates, flavonoids, phloba tannins and tannins in the seeds of *Trigonella foenum graecum*. Results of DPPH assay showed that *Trigonella foenum graecum* (100µg/ml) has about 52.54% scavenging activity. Therefore, it is concluded from this preliminary study that *Trigonella foenum graecum* can be used for isolation of important compounds with medicinal and pharmacological importance.

REFERENCES

1. Barlow SM. Toxicological aspects of antioxidants used as food additives. In Food Antioxidants, Hudson BJF (ed.) Elsevier, London, 1990; pp 253-307. https://doi.org/10.1007/978-94-009-0753-9_7

2. Branen AL. Toxicology and biochemistry of butylatedhydroxyanisol and butylatedhydroxytoluene. J. American Oil Chemists Society, 1975; 5:59- 63. https://doi.org/10.1007/BF02901825

3. Frankel E. Nutritional benefits of flavonoids. International conference on food factors: Chemistry and Cancer Prevention, Hamamatsu, Japan. Abstracts, 1995; C6- 2.

4. Gryglewski RJ, Korbut R, Robak J. On the mechanism of antithrombotic action of flavonoids. Biochemical Pharmacol, 1987; 36:317- 321. https://doi.org/10.1016/0006-2952(87)90288-7

5. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 2002; 13:8-17. https://doi.org/10.1002/pca.611

6. Schuler P. Natural antioxidants exploited commercially, In Food Antioxidants, Hudson BJF (ed.). Elsevier, London, 1990; pp 99-170. https://doi.org/10.1007/978-94-009-0753-9_4

7. Mantle D, Eddeb F, Pickering AT. Comparison of relative antioxidant activities of British medicinal plant species in vitro, J. Ethnopharmacol. 2000; 72:47- 51. https://doi.org/10.1016/S0378-8741(00)00199-9

8. Merghem M, Dahamna S, In-Vitro Antioxidant Activity and Total Phenolic Content of *Ruta montana* L. Extracts, Journal of Drug Delivery and Therapeutics 2010; 10(2):69-75 https://doi.org/10.22270/jddt.v10i2.3919

9. Cook NC, Samman S. Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry, 1996; 7:66-76. https://doi.org/10.1016/0955-2863(95)00168-9

10. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of *Fumaria officinalis* leaf extract. Journal of Advanced Scientific Research. 2020; 11(03):176-82.

11. Rani D, Kharkwal H, Jha M, Rai N. Assessment of the total flavonoid, phenol, alkaloid content and sun protection factor *in Grewia abutilifolia* leaf extract. Journal of Pharmaceutical Research International.2021; 33(49A):42-51. https://doi.org/10.9734/jpri/2021/v33i49A33300

12. Jha M, Ganesh N, Sharma V In vitro evaluation of free radical scavenging activity of *Pistia stratiotes*. International Journal of Chemtech Research 2010; 2(1):180-184

13. Jha M, Sharma V, Nema N, Hussain T. Effect of the topical application of the extract of *Glycosmis pentaphylla* on excisional wound model in mice. Pharmacologyonline. 2009; 3:356-60.