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Free radical scavenging activity of seeds of *Trigonella foenum-graecum* Linn.

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Abstract

The free radical scavenging potential of the seed of *Trigonella foenum-graecum* was studied by using different antioxidant models of screening. The ethanolic extract at 500 µg/ml showed maximum scavenging of the radical cation, 2,2-azinobis-(3- ethylbenzothiazoline-6-sulphonate) (ABTS) observed upto 97.56%; It also scavenged stable radical 1,1-diphenyl,2-picryl hydrazyl (DPPH) (88.12 %) and nitric oxide radical (72.86 %) at the same concentration.

Key words: *Trigonella foenum-graecum,* Antioxidant, Free radicals

1. Introduction

Free radicals have been implicated in causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity etc[1]. Together with other derivatives of oxygen they are, inevitable byproducts of biological redox reactions[2]. Reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydroxyl radical (.OH) and nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation [3], and thus have been shown to augment collagen synthesis and fibrosis. The increased production .of toxic oxygen derivatives lead to stress conditions.

Trigonella foenum-graecum (TFG) (Family-Leguminosae), a culinary vegetable, has been used in the Indian systems of medicine. Seeds are used in colic flatulence, diarrhoea, dyspepsia with loss of appetite, dropsy, enlargement of liver and spleen, rickets, gout, carminative, anthelmintic and possess antidysentric property. They are also used in piles, rheumatism and as a galactogogue, restorative. Diosgenin is one of the chemical constituent and is being used as a precursor for steroidal drugs [4-6].

The usage of the seeds has been reported as antidiabetic and hypocholesterolaemic in both animal and man [7]. In the present study it was aimed to evaluate free radical scavenging activity of ethanolic extract *Trigonella foenum-graecum* seeds.

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2. Materials and methods

The seeds of *Trigonella foenum-graecum* were purchased from local market and identified by Dr. S Vyas, Deptt.of Botany, Holkar science college, Indore. These were dried in sun light and crushed to moderately coarse powder and stored in air tight container. The powder was extracted with 90 % ethanol using a soxhlet apparatus. The vacuum dried extract (yield-22.5%) was used for free radical scavenging studies. Rutin used as standard for these studies.

Preliminary phytochemical tests were performed to detect the presence of flavonoids and free phenolic compounds. The qualitative chemical tests performed were, lead acetate test, ferric chloride test, shinoda test, ammonia fuming test, gibbs test, chalcones test, boric acid test, zirconium oxychloride test. All these tests confirmed the presence of flavonoids and free phenolic compounds [8].

2.1 ABTS radical cation scavenging activity [9]

ABTS radical cation ($ABTS.^{+}$) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate and the mixture were allowed to stand in dark at room temperature for 12-16 h before use. For the study different concentrations (2 - 500 µg/ml) of the ethanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1 ml. Absorbance was read at 532 nm using a spectrophotometer (Shimadzu 7100). The percentage inhibition was calculated by using the formula given below [10].

$$\text{Inhibition (\%)} = \frac{\text{(Control - Test)}}{\text{Control}} \times 100$$

2.2 DPPH radical scavenging activity

DPPH scavenging activity was measured by spectrophotometric method [11]. To an ethanolic solution of DPPH (200 µM) 0.05 ml of test compounds dissolved in ethanol were added at different concentrations (2 - 500 µg/ml). An equal amount of ethanol was added to the control. After 20 min the decrease in the absorbance of test mixtures (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition was calculated.

2.3 Nitric oxide radical scavenging activity [12-13]

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described previously [14,15]. Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations (2 - 500 µg/ml) of the ethanol extract dissolved in phosphate buffer (0.025 M, pH : 7.4), at 25°C for 5 h. Control experiments without the test sample but with equivalent amount of buffer, were conducted in an identical manner.

After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2%O-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 535 nm.

2.4 Statistical analysis

Linear regression analysis was used to calculate the IC_{50} values.

3. Results

Several concentrations, ranging from 2 - 500 µg/ml of the ethanolic extract of TFG were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extract in a concentration dependent manner upto the given concentration in all the models (Table 1). The maximum percentage inhibition in all the models *viz,*

Table 1.
Effect of ethanolic extract of TFG on different antioxidant models (Values are mean of 3 replicates)

Conc. (µg/ml)	Inhibition (%) ABTS	DPPH	Nitric oxide
500	97.56±4.26	88.12±2.82	72.86±4.2
250	88.41±3.98	79.86±3.8	67.20±3.68
125	86.46±1.62	77.62±3.42	54.98±2.82
63	73.65±2.68	35.70±1.12	51.44±2.33
32	48.20±4.7	18.22±2.1	43.76±2.7
16	33.80±1.1	2.32±0.52	41.46±1.64
8	26.12±2.3	1.72±0.1	40.54±1.28
4	15.90±0.8	1.15±0.09	38.41±1.06
2	8.42±0.4	0.56±0.02	35.49±0.9
IC_{50}(µg/ml)			
Ethanolic extract	40±2.1	110±3.6	60±2.54
IC_{50} (µg/ml)			
Rutin*	28±2.78	129±4.86	67±3.88

*Rutin used as standard

ABTS, DPPH & nitric oxide, were found to be 97.56, 88.12 and 72.86 respectively at 500 µg/ml concentration. The IC_{50} values of DPPH and nitric acid model are lower than the IC_{50} value of standard rutin, while IC_{50} value in ABTS model was slightly higher than that of standard rutin (Table 1).

On a comparative basis the extract showed better activity in quenching ABTS with an IC_{50} value of 40 µg/ml and DPPH radicals with an IC_{50} value of 110 µg/ml (Table 1). However, the extract showed significant response in quenching nitric oxide radicals with an IC_{50} value - 60 µg/ml.

4. Discussion

Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease [16].

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a characteristic long wavelength absorption spectrum [17]. The results obtained imply the activity of the extract either by inhibiting or scavenging the $ABTS^+$ radicals since both inhibition and scavenging properties of antioxidants towards $ABTS^+$ radicals have been reported earlier [18].

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichometrically depending on the number of electrons taken up [19].

From the present results it may be postulated that TFG reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles [17].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases [20-21]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by the ethanolic extract of TFG. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide [22] thereby inhibiting the generation of nitrite.

Seeds of TFG are rich in flavonoids like apigenin, luteolin, orientin, quercetin, vitexin and isovitexin [23]. Flavonoids are natural products, which have been shown to possess various