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Comparison of *in vitro* Antioxidant Activity of *Trigonella foenum-graecum* and *T. corniculata* Seeds

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Abstract: Successive methanolic and direct ethanolic extracts of *Trigonella foenum-graecum* and *T. corniculata* seeds were prepared and were investigated for their potential antioxidant activity against DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals. Seed extracts of *T. corniculata* showed better antioxidant activity than that of *T. foenum-graecum*. Ethanolic extract of *T. corniculata* was the most effective antioxidant among the extracts with 90.24% DPPH radical scavenging activity at 500 µg mL⁻¹. The antioxidant activity of the extracts increased with the increasing amount of the concentration. It was concluded that the seeds of *T. corniculata* had better antioxidant than *T. foenum-graecum*. Moreover the ethanolic extracts showed significantly better activity than the successive methanolic extracts.

Key words: Antioxidant activity, *Trigonella foenum-graecum* and *T. corniculata* seeds, DPPH

INTRODUCTION

Trigonella or Fenugreek or Greek Hayes is cultivated as a leafy vegetable, condiment and as medicinal plant. *Trigonella foenum-graecum* Linn. belongs to family, Fabaceae. There are two species of the genus Trigonella which are of economic importance viz., *T. foenum-graecum*, the common *methi* and *T. corniculata*, the *Kasuri methi*. These two differ in their growth habit and yield. The latter one is a slow growing type and remains in rosette condition during most of the vegetative growth period (Kumar *et al.*, 1997a, b; Warriar *et al.*, 1996).

A poultice of the leaves is applied for swellings and burns. Seeds are used for fever, vomiting, anorexia, cough, bronchitis and colonitis. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Brasch *et al.*, 2003). Fenugreek is reported to have anti-diabetic, anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic, effects (Al-Habori and Raman, 2002). In India, fenugreek is used as a lactation stimulant (Tiran, 2003). Fenugreek seed in powder or germinated form exhibits anti-diabetic properties (Broca *et al.*, 2004; Acharya *et al.*, 2006; Dixit *et al.*, 2005). Hair oils enriched with seeds of Trigonella, are used as hair growth promoter in traditional folk medicine practices in Uttarakhand. Many studies have reported the preparations of hair growth formulations of Trigonella (Purwal *et al.*, 2008; Semalty *et al.*, 2008).

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Seeds are bitter, mucilaginous, aromatic, carminative, tonic, diuretic, thermogenic, galactagogue, astringent, emollient, amorphodisiac, antirheumatic, CNS depressant and antiimplantation. Fenugreekine is hypoglycaemic, diuretic, hypotensive, cardiogenic, antiphlogistic (Mustafa *et al.*, 2005). It showed 80% inhibition of vaccinia virus.

Seeds contain saponin-diosgenin, its 25-epimer(yamogenin), tigogenin, gitogenin, yuccagenin, 25-2-spirosta-3-5-diene and its β -epimer. Seeds also contain a C₂₇-steroidal saponin-peptide ester-fenugreekine. Seeds, in addition, contain 4-hydroxyisoleucine and saponins-fenugrins A-E:two furostanol glycosides-trigonellose C and (25S)-22-O-methyl-52-firostan-3 β , 22, 26, triol-3-O- α -rhamnopyranosyl(1-2) C- β -D-glucopyranosyl (1-3)- β -Dglucopyranoxide-26-O- β -D-glucopyranoxide (1).

Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Andlauer and Furst, 1998). Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years (Jayapvakesha *et al.*, 2003). So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

As the antioxidant activity had been reported in the leaves of *Trigonella foenum graecum*, the same activity may be found in the seeds of *T. foenum graecum* and *T. corniculata* (Gupta and Prakash, 2009). Therefore, the present study deals with the antioxidant activity of seeds of two different species of *Trigonella* of Garhwal Himalayan region.

MATERIALS AND METHODS

The study was conducted as the part of doctoral research work and was conducted from July 2008 to March 2009 in the Department of Pharmaceutical Sciences and Department of Chemistry of H.N.B. Garhwal University Srinagar (Garhwal), India.

Chemicals

Butylated hydroxyanisole (BHA) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from E. Merck Mumbai and Himedia Mumbai respectively. All other chemicals and reagents used were of analytical grade.

Plant Material and Extract

The seeds of the *T. foenum-graecum* and *T. corniculata* were collected from the local farmers of District Pauri, Uttarakhand, India. The seeds of the plants were identified and authenticated from the Department of Botany, H.N.B. Garhwal University Srinagar (Garhwal) India. The seeds were dried under shade and made to a coarse powder.

The 200 g seed powder was extracted in the soxhlet extraction apparatus with 700 mL of petroleum benzene (60-80°C) followed by 650 mL of chloroform and then by 500 mL methanol for 72 h by hot continuous successive extraction method. The solvents were evaporated under reduced pressure in a rotary vacuum evaporator (Perfit Model No. 5600 Buchi type) and dried in vacuum. The dried extract thus obtained was used directly for the assessment of antioxidant activity.

In the ethanol extraction, the same amount of coarse seed powder was extracted with 700 mL of ethanol in a soxhlet apparatus until extraction solvents become colorless.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the *T. foenum-graecum* and *T. corniculata* seed extracts was measured and compared with the activity of butylated hydroxy anisol (BHA) for radical-scavenging ability using the stable radical DPPH (Blois, 1958). The free-radical scavenging activities of extracts and BHA (used as a standard) were measured by decrease in the absorbance of methanol solution of DPPH.

The 0.1 mM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 3.5 mL of extract solution in water at different concentrations (50-500 µg mL⁻¹). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \left(\frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \right) \times 100$$

where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

RESULTS AND DISCUSSION

The DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). Methanolic DPPH (0.1 mM) solution gives a violet color which shows λ_{max} at 517 nm. When an antioxidant is mixed with this solution, the concentration of the stable free radical 2, 2-diphenyl-1-picryl-hydrazyl or DPPH is reduced which can be detected by the decrease in the optical absorbance of DPPH at 517 nm.

The ethanolic extracts showed better antioxidant activity for both the species of *Trigonella*. Table 1 shows the percent inhibition or DPPH radical scavenging activity of 500 µg mL⁻¹ of extracts. The free radical scavenging activity was found to be in the following order BHA > *T. corniculata* (EtOH) > *T. foenum-graecum* (EtOH) > *T. corniculata* (Successive MeOH Extract) > *T. foenum-graecum* (Successive MeOH Extract).

It was also evident that the seeds of *T. corniculata* showed better antioxidant activity in terms of free radical scavenging activity than that of *T. foenum-graecum*. Ethanolic extract of *T. corniculata* was the most effective antioxidant among the all extracts with 90.24% DPPH radical scavenging activity at 500 µg mL⁻¹. The antioxidant activity of the extracts increased with the increasing amount of the concentration.

Table 1: Antioxidant activities of *Trigonella* seeds

Compound	Concentration (µg mL ⁻¹)	DPPH radical scavenging activity (% inhibition±SEM)**
BHA	500	93.68±0.00060
Tf (Successive MeOH extract)	500	76.28±0.00060
Tf (EtOH)	500	84.65±0.00560
Tc (Successive MeOH extract)*	500	79.77±0.00005
Tc (EtOH)	500	90.24±0.00010

*Highest percentage inhibition was 86.67% at 200 µg mL⁻¹. Tf: *T. foenum-graecum*; Tc: *T. corniculata*; BHA: Butylated hydroxyanisol; **n = 3

In an ethanol toxicity rat study, an aqueous extract of fenugreek seeds prevented the rise in lipid peroxidation and enhanced antioxidant potential (Thirunavukkarasu *et al.*, 2003). These results are supported by *in vitro* evidence in diabetic human erythrocytes, that polyphenol acids from fenugreek seeds showed a concentration-dependent inhibition of lipid peroxidation (Kaviarasan *et al.*, 2004). In the present study, the presence of greater fraction of phenolic compounds in the direct ethanolic extracts in comparison of successive methanolic extracts, might be the reason of better antioxidant activity shown by the ethanolic extracts. In another study, it was proved that higher the amount of the phenolic compounds and reducing power, higher the percent DPPH scavenging activity (Bukhari *et al.*, 2008).

CONCLUSION

Reactive Oxygen Species (ROS) or free radicals are mediators of process of aging. They accelerates the ageing as well as cause tissue injury. The ROS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart diseases, stroke, atherosclerosis, diabetes and cancer (Tanizawa *et al.*, 1992; Hertog *et al.*, 1993). Most of the mammals have an inherent mechanism to prevent and neutralize the ROS or free radical induced damage. However, natural antioxidant mechanism can be inefficient and hence dietary intake of antioxidant compounds is necessary. The herbal antioxidants may be the safer alternatives than the carcinogenic synthetic antioxidants like BHA or BHT. It can be concluded that the seeds of *T. corniculata* have better antioxidant than *T. foenum-graecum*. Moreover, the ethanolic extracts show significantly better activity than the successive methanolic extracts.

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