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Antioxidant and Antibacterial Activities of Trigonella foenum graecum Leaves

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ABSTRACT

Trigonella foenum graecum (Fenugreek) is one of the known traditional and most promising medicinal herbs belonging to the Leguminosae family. It is used as a vegetable, spice and a medicinal plant. Since antioxidant properties have been linked to health benefits of natural products, such property was studied in fenugreek leaves. The antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was studied. Also the study was aimed to investigate the in vitro antibacterial activity of Trigonella foenum graecum leaves extract against Staphylococcus aureus using agar disc diffusion method. The results indicated that the ethanolic extract Trigonella foenum graecum leaves possess antioxidant activity and thus can be a significant source of natural antioxidants. Also the hot (boiling) water extraction of fenugreek leaves contain active ingredients that have an inhibitory effect on the growth of Staphylococcus aureus.

KEYWORDS: *Fenugreek leaves, leguminosae, antioxidant activity, DPPH, antibacterial activity*

I. INTRODUCTION

Trigonella foenum graecum (Fenugreek) is one of the known traditional and most promising medicinal herbs belonging to the Leguminosae family. For more than 2500 years, this plant has been widely used due to its food and medicinal properties as a herbal remedy[1]. The seeds and leaves of this plant are extensively employed in medicinal purposes as an antimicrobial[2], antidiabetic[3-5], anticancer[6], anti-inflammatory[7] and antioxidant agent[8-10]. As a herbal medicine, fenugreek is used for its carminative, tonic, aphrodisiac and anticancer effects [6,11-12]. Fenugreek is reported to contain constituents like alkaloids, flavonoids, salicylate and nicotinic acid, and polyphenols[13-15]. These studies have also reported that the fenugreek leaves contain high amount of total phenolics and tocopherols.

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The excessive production of reactive oxygen species (ROS) like hydroxyl radical, superoxide anion radical, hydrogen peroxide radical can contribute to oxidative stress[16]. The oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases like diabetes, hypertension, coronary heart disease, cancer etc.[17]. Though most of the reactive oxygen species are scavenged by endogenous defence systems but these systems may not be completely efficient thus requiring the dependency on exogenous antioxidants from natural sources. Currently, there has been an amplified interest worldwide to identify natural antioxidant compounds which are pharmacologically effective or have low or no side effects, for use in preventive medicine and the food industry[18]. Several isolated phytoconstituents as well as crude extracts of vegetables like Ash gourd, Snake gourd, Ipomea aquatic[19], mango ginger[20] have been seen to possess beneficial effects against free radicals in biological systems as antioxidants.

Also the problem of drug resistance in microbes is increasing day by day[21,22] due to continuous and overuse of synthetic antibiotics which often produce tolerance in humans and create resistance in microbes for a particular antibiotic. In recent years, there has been a gradual revival of interest for the use of medicinal and aromatic plants to develop antimicrobial drugs because plant-derived drugs have been reported to be safe and without side effects[23]. Spices being an indispensable component of Indian cuisines are used since ancient times are considered as a rich source of bioactive antimicrobial compounds[24]. Most of the work regarding the antimicrobial activity of *T. foenum-graecum* has been done using seeds as a plant material but data on the use of leaves as a plant material is very limited. So, the aim of the present study was to evaluate the antimicrobial and antioxidant activities of fenugreek leaves in order to be used in some diseases.

II. MATERIALS AND METHODS

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals Co. ftAscorbic acid and all other chemicals and solvents used were of analytical grade.

Collection of plant part

Fresh leaves of *T. foenum graecum* were obtained from the local market of Ghaziabad. The leaves were washed thoroughly using distilled water and the surface water was removed by air drying under shade. Then the leaves were subsequently dried in a hot air oven at 40°C for 48 hours, powered and used for extraction.

Preparation of extract

Antioxidant activity: The extract of fenugreek leaves was prepared by soaking 100 g of dried powder in 900 ml of ethanol around for 48 hours. At the end of extraction, the plant extract was filtered using Whatman filter paper. The filtrate was then concentrated under reduced pressure in vacuum at 40 °C for 25 min using a rotary flash evaporator.

Antibacterial activity: Fenugreek leaves (powdered) were extracted by maceration in 200 ml of boiling water. The mixture was filtered with Whatman filter paper.

Antioxidant assay

DPPH radical scavenging assay

The free radical scavenging activity was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the method reported by Kumar *et al.*[24]. 0.1 mM solution of DPPH was prepared and 1 ml of the DPPH solution was added to 3 ml of the extracts. Five different concentrations of the ethanolic extracts were used. The mixture was shaken vigorously and incubated for 35 mins in the dark at room temperature. The absorbance was measured at 517 nm using UV-visible spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula:

(%) Inhibition = [(Absorbance of control–Absorbance of sample)/(Absorbance of control)] ×100.

Antibacterial assay

Antibacterial activity evaluation was performed on *Staphylococcus aureus* using agar disc diffusion method[25-26]. Gentamicin (10µg) was used as a positive control[27] and distilled water was used as a negative control. Samples were incubated at 37°C. The diameter of clear zone of inhibition surrounding each disc was measured in millimetres. The antibacterial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of two replicates.

III. RESULT AND DISCUSSION

In the present work, the ethanolic extract of *Trigonella foenum graecum* leaves was evaluated for its DPPH radical scavenging activity with respect to the standard ascorbic acid. The bleaching of DPPH indicates the free radical scavenging capacity of ethanolic leaves extract of *Trigonella foenum graecum*. The ethanolic leaf extract of *Trigonella foenum graecum* showed the highest antioxidant activity of 77.72% as compared to the standard ascorbic acid, 87.43% by DPPH radical scavenging method as shown in table 1.

Table 2 shows that the extraction of fenugreek leaves by hot (boiling) water has some inhibitory effect on the growth of *St. Aureus* bacteria in disc diffusion method with zone of inhibition of diameter of 18 mm.

Table 1: DPPH radical scavenging activity of ethanolic extract of *Trigonella foenum graecum* leaves

S.No	Concentration (µg/mL)	% Inhibition	
		Sample	Ascorbic Acid
1.	200	36.55	80.54
2.	400	54.84	83.86
3.	600	56.92	85.52

4.	800	75.63	86.56
5.	1000	77.72	87.43

Table 2: Effect of hot (boiling) aqueous extract of *Trigonella foenum graecum* leaves on *Staphylococcus aureus* by disc diffusion method.

Microorganism	**Zone of Inhibition (in mm)**		
	Hot (boiling) aqueous extract	**Control**	
		H_2O	**Gentamycin**
Staphylococcus aureus	18±2.0	-ve	22

-ve: Negative (no Zone of Inhibition is formed); Data of Mean ± SD.

IV. CONCLUSION

In vitro antioxidant activity of the ethanolic extract of leaves of *Trigonella foenum graecum* was performed. The results of DPPH assay shows that the extract has antioxidant activity when compared to the standard ascorbic acid indicating that this leaf extract can be a significant source of natural antioxidants, which might be helpful in preventing the diseases associated with oxidative stress.

Also biomolecules of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens. The results from our study showed that the hot (boiling) water extraction of fenugreek leaves has an inhibitory effect on the growth of *Staphylococcus aureus*. Therefore, it lays the foundation for further research to identify the active compounds responsible for the plant biological activity and its mechanism.

CONFLICT OF INTEREST

No conflict of interest lies between Authors.

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