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Antibacterial and Anti-oxidant Activities of Fenugreek (Trigonella foenum graecum L.) Leaves

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ABSTRACT

In the present study the polyphenolic content, antibacterial activity and antioxidant activity of Trigonella foenum graecum leaves in chloroform, hexane, methanol, ethanol and water extracts were carried out. Total phenolic content was estimated by Folin Ciocalteau method while the flavonoid content was determined using aluminum chloride method. Estimation of total phenolic and flavonoid contents revealed that, the ethanol extract had the highest phenolic and flavonoid contents of 4.9 and 0.47 mg g⁻¹, respectively. The antibacterial activity of various extracts was screened by disc diffusion method and ethanol extract was found to be more potent. The Minimum Inhibitory Concentration (MIC) of ethanol extract determined by broth dilution method showed a MIC value of 1 mg mL⁻¹ for Staphylococcus aureus and Pseudomonas aeruginosa. The antioxidant activities of the leaves were evaluated by various in vitro assays like ferrous reducing antioxidant assay, total reducing power, 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) scavenging activity, lipid peroxidation inhibitory activity and superoxide scavenging activity. Among the solvent extracts tested for antioxidant activities, ethanol extract had the highest activity compared to other solvent extracts. It had a total antioxidant activity of 47 µM Fe (II) g⁻¹ and an Inhibitory Concentration (IC₅₀) value of 0.3 and 0.7 mg mL⁻¹ for lipid peroxidation inhibitory activity and DPPH scavenging activity, respectively. These results suggested that ethanol extract of fenugreek leaf is not only an important source for antibacterial components but also a potential source of phenolic antioxidants.

Key words: Trigonella foenum graecum, solvent extracts, clinical isolates, antibacterial activity, antioxidant activity

INTRODUCTION

Nature has been a source of medicinal plants for thousands of years since the beginning of man. Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self medication by the general public for their biological effects. Beyond this pharmaceutical approach to plants, there is an increasing tendency to utilize herbal products to supplement the diet mainly with the intention of improving the quality of life (Maffei, 2003).

According to WHO, more than 80% of the world's population relies on plant based herbal medicines for their primary health care needs (Shanmugasundaram, 2005). The use of traditional

medicine is widespread in India (Jeyachandran and Mahesh, 2007). Indigenous plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties. In recent years, there is an increase in drug resistant strains of human pathogenic bacteria all over the world. One way to prevent antibiotic resistance of pathogen species is to use new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Systematic screening of plants may result in the discovery of novel effective compounds which would tackle the problem of drug resistance.

The overproduction of reactive oxygen species like hydroxyl radical, superoxide anion radical, hydrogen peroxide radical can contribute to oxidative stress (Braca et al., 2002). Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including diabetes, hypertension, coronary heart disease, cancer etc (Modi et al., 2010). Most of the reactive oxygen species are scavenged by endogenous defense systems. But these systems may not be completely efficient requiring them to depend on exogenous anti-oxidants from natural sources. Presently, there has been an amplified interest worldwide to identify antioxidant compounds which are pharmacologically effective or have low or no side effects for use in preventive medicine and the food industry (Sati et al., 2010). Several isolated plant constituents as well as crude extracts of vegetables like Ash gourd, Snake gourd, Ipomea aquatica (Prasad et al., 2004), mango ginger (Policegoudra et al., 2007) have been recognized to possess beneficial effects against free radicals in biological systems as anti-oxidants. Generally antioxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicine (Daniel, 2006).

Trigonella feonum graecum L. commonly known as Fenugreek belongs to the family Leguminosae which is an annual, herbaceou and aromatic plant. The seeds of this plant are used as a spice and the leaves are edible and used as a vegetable in many parts of India. Fenugreek seed is reported to have anti-diabetic (Karim et al., 2011), anti-fertility, anticancer, anti-microbial, anti-parasitic, lactation stimulant and hypocholesterolaemic effects (Al-Habori and Raman, 2002). In Ayurveda, both fenugreek seeds and leaves are used to prepare extracts or powder for medicinal use (Basch et al., 2003).

Many researchers have shown the antibacterial and antioxidant activities of fenugreek seeds. However, a detailed study on the antibacterial and antioxidant activities of the fenugreek leaves has not been carried out. So, the aim of the present study was to investigate the antibacterial and antioxidant activities of fenugreek leaves in various solvent systems and to determine the solvent that best extracts the compounds responsible for these activities.

MATERIALS AND METHODS

Plant material: Fresh and healthy leaves of *T. foenum graecum* were obtained from local market of Mysore (Karnataka state, India) in the year 2008. The sample specimen was identified based on the taxonomical characteristics and deposited in the herbarium of department of Applied Botany with a voucher number of KU/SG/JS/167. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were subsequently dried in a hot air oven at 40°C for 48 h, powered and used for extraction.

Test microorganisms: The test organisms used were clinical isolates causing Urinary Tract Infections (UTI) namely, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacter aerogens* were obtained from Department of Microbiology, JSS Medical College, Mysore. The bacterial cultures were maintained on nutrient agar medium.

Preparation of aqueous extract: Fifty grams of powdered leaves of *T. foenum graecum* was macerated with 100 mL sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in a brown bottle at 4°C until further use.

Preparation of solvent extract: Fifty grams each of the powered material was extracted initially with 300 mL of chloroform, hexane, methanol and ethanol separately for 24 h at 23±2°C. The extract was filtered with sterile whatman filter paper into a clean conical flask. Second extraction was carried out with same amount of solvent for another 24 h at 23±2°C and filtered. The extracts were later pooled and transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvents. The evaporated extract so obtained was weighed and preserved at 4°C in airtight bottle until further use. Ten milligram each of dried solvent extract was dissolved in 1 mL of respective solvent and used for the antibacterial assay.

Total phenolic content: Total soluble phenolic content was estimated by Folin-Ciocalteau method based on the procedure of Malik and Singh (1980).

Total flavonoid content: The total soluble flavonoid content was estimated by aluminium chloride colorimetric method (Woisky and Salatino, 1998).

Antibacterial screening: The antibacterial activity was carried out by disc diffusion method (Bauer *et al.*, 1966).

Determination of Minimum Inhibitory Concentration (MIC): The MIC was carried out by broth dilution method (Brantner and Grein, 1994).

Anti-oxidant activity assays

Ferrous reducing antioxidant power assay (Total antioxidant activity assay): The FRAP assay was carried out according to the method of Benzie and Strain (1996) with slight modifications. The FRAP solution contained 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL 2, 4, 6-tripyridyl-s-tri-azine (10 mM solution in 40 mM HCl) and 2.5 mL Ferric Chloride (20 mM). The temperature of the solution was raised to 37°C before use 0.15 mL of plant extracts were allowed to react with 2.85 mL of the FRAP solution for 30 min in the dark condition. Readings of coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in μM Fe (II) g⁻¹ dry mass and compared with a synthetic antioxidant [Butylated Hydroxyanisole (BHA)].

Total reducing power: The determination of reducing power was performed as described by Yen and Duh (1993).

DPPH radical scavenging activity: The DPPH scavenging assay was a modification of the procedure of Moon and Terao (1998). Briefly, 0.1 mL of test sample at different concentration (0.1-0.9 mg mL⁻¹) was mixed with 0.9 mL of Tris-HCl buffer (pH 7.4) and 1 mL of DPPH (500 μM in ethanol). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm in a spectrophotometer and compared with a synthetic

antioxidant [Butylated Hydroxyanisole (BHA)]. Radical scavenging potential was expressed as IC_{50} values which represents the sample concentration at which 50% of the radicals are scavenged. The percentage of DPPH scavenging was calculated using the following formula:

$$Percent\ scavenging = \frac{A_{\text{control}} \text{-} (A_{\text{samaple}} \text{-} A_{\text{sampleblank}})}{A_{\text{control}}} \times 100$$

Lipid peroxidation inhibitory activity: The lipid peroxidation inhibitory activity of was determined according to the method of Duh and Yen (1997).

Superoxide radical scavenging activity: The superoxide scavenging ability was assessed according to the method of Nishikimi et al. (1972) with slight modifications. The reaction mixture contained Nitroblue tetrazolium (0.1 mM) and Nicotinamide adenine dinucleotide (0.1 mM) with or without sample to be assayed in a total volume of 1 mL of Tris-HCl buffer (0.02 M, p^H 8.3). The reaction was started by adding Phenazine methosulphate (10 μ M) to the mixture and change in the absorbance was recorded at 560 nm every 30 sec for 2 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential was expressed as IC₅₀ value which represents the sample concentration at which 50% of the radicals are scavenged. The results were compared with that of quercetin. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\begin{aligned} \text{Percent scavenging} &= \frac{A_{\text{control}}\text{-}(A_{\text{samaple}}\text{-}A_{\text{sampleblank}})}{A_{\text{control}}} \times 100 \end{aligned}$$

Statistical analysis: The experimental results are expressed as Mean±Standard Deviation (SD) of triplicate measurements. The data was subjected to one way Analysis of Variance (ANOVA) and the significance of differences between the sample means was calculated by Turkeys test. Data was considered statistically significant at $p \le 0.001$. Statistical analysis was performed using Graph Pad statistical software.

RESULTS

Total phenolic and flavonoid content: The results obtained in the present study revealed that the level of polyphenols in the ethanol extract was 4.9 mg g⁻¹ which was higher when compared to other tested solvents (Table 1). Ethanol extract of the leaves had a flavonoid content of 0.47 mg g⁻¹. The flavonoid content of other extracts tested was lower than the ethanol extract. Aqueous extract had the least polyphenol and flavonoid content.

 ${\it Table 1: Polyphenol and flavonoid content of \it Trigonella \it foenum \it graecum \it leaves in \it different \it solvent \it extracts \it flavonoid \it flavonoid$

Solvents	Total phenols (mg g^{-1})	Flavonoid content (mg g ⁻¹)	
Ethanol	4.9 ± 0.25^{a}	0.47±0.01ª	
Methanol	4.2±0.15 ^a	0.32 ± 0.02^{b}	
Hexane	$2.6 \pm 0.27^{\mathrm{b}}$	$0.21 \pm 0.01^{\circ}$	
Chloroform	1.7 ± 0.26 bc	$0.16\pm0.02^{\circ}$	
Aqueous	$1.5 \pm 0.14^{ m d}$	0.18±0.01°	

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (p = 0.05)

Antibacterial activity of solvent extracts: The efficacy of various solvent extracts of *T. foenum graecum* leaf against pathogenic bacteria commonly associated with UTI infections showed varied level of inhibition (Table 2). Among various extracts, maximum *in vitro* inhibition of the tested bacteria *E. coli*, *E. aerogenes*, *Klebsiella* sp., *Proteus vulgaris*, *S. aureus* and *P. aeruginosa* was achieved in ethanol extract with zones of inhibition 12±0.8, 11±0.2, 10±0.3, 12±0.5, 14±0.5 and 13±0.0 mm, respectively. The least inhibition was observed with aqueous extract which showed least zones of inhibition.

Minimum inhibitory concentration: The ethanol extract was further subjected to broth dilution method to determine the MIC of T. foenum graecum leaf extract with concentrations ranging from 0.25-2 mg mL⁻¹ (Table 3). The maximum activity was observed against S.aureus followed by P.aeruginosa with a MIC value of 1 mg mL⁻¹. S. aureus and P. aeruginosa recorded an absorbance of 0.03 and 0.05 as against a control absorbance of 0.22 and 0.14, respectively at 600 nm. As with other bacteria, MIC values exceeded 2 mg mL⁻¹ as seen by the absorbance values.

Antioxidant activity assays

Ferrous reducing antioxidant assay: As shown in Table 4 the values obtained were significantly lower than that of the synthetic antioxidant (BHT) (63±0.35 μ M Fe (II) g⁻¹). Among the solvent extracts tested, ethanol extract had a reducing ability of 47±1.0 followed by methanol 31 μ M±1.0 Fe (II) g⁻¹. Aqueous extract had the least reducing ability.

Total reducing power: The result indicates that the reducing ability of all the extracts increased with the concentration (Fig. 1a). Among all the extracts tested for their reducing abilities ethanol extract of *T. foenum graecum* showed better reducing power with an increase in the absorbance at

Table 2: Zone of inhibitory activity (in millimeter) of different solvent extracts of Trigonella foenum graecum leaves against clinical bacteria

Solvents	$E.\ coli$	E. aerogenes	Klebsiella sp.	$P.\ vulgaris$	S. aureus	P. aeruginosa
Ethanol	12±0.8 ^{ab}	11±0.2ª	10±0.3ª	12±0.5ª	14±0.5ª	13±0.0ª
Methanol	9 ± 0.4^{b}	8 ± 0.4^{b}	8±0.2 ^b	$10 \pm 0.4^{\rm bc}$	12 ± 0.7^{b}	10 ± 0.5^{b}
Hexane	5±0.7°	$5\pm0.4^{\circ}$	$4\pm0.4^{\circ}$	$5\pm0.5^{\circ}$	7 ± 0.2^{d}	5±0.3°
Chloroform	$3\pm0.4^{\circ}$	$2\pm0.7^{\circ}$	$1\pm0.2^{\rm d}$	2 ± 0.0^{d}	$4\pm0.7^{\rm e}$	3 ± 0.5^{d}
Aqueous	$2\pm0.2^{\rm cd}$	$1\pm0.4^{\circ}$	1 ± 0.5^{d}	$2\pm 0.7^{\rm d}$	3 ± 0.5^{ef}	2 ± 0.0^{de}
Streptomycin	15 ± 0.4^{a}	13 ± 0.5^{a}	11±0.1ª	12 ± 0.6^{a}	$11 \pm 0.4^{\circ}$	14 ± 0.4^{a}

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (p = 0.05)

Table 3: Minimum inhibitory concentration (MIC) of Trigonella foenum graecum leaf ethanol extract using broth dilution method

		Concentration (mg m L^{-1})			
D	G 1	0.05			
Bacteria	Control	0.25	0.5	<u> </u>	2
E. coli	0.27±0.0a	0.26 ± 0.0^{a}	0.17 ± 0.0^{b}	0.19 ± 0.0^{b}	0.25 ± 0.0^{a}
E. aerogenes	0.30 ± 0.0^{a}	0.30 ± 0.01^a	0.28 ± 0.0^{a}	0.27 ± 0.0^{a}	0.30 ± 0.0^{a}
Klebsiella sp.	0.25 ± 0.0^{a}	$0.23{\pm}0.01^{\rm ab}$	0.19 ± 0.0^{b}	0.19 ± 0.0^{b}	0.23 ± 0.0^{ab}
P. vulgaris	0.25 ± 0.0^{a}	0.23 ± 0.01^{ab}	0.23 ± 0.0^{ab}	$0.20{\pm}0.0^{b}$	0.22 ± 0.0^{ab}
S. aureus	0.22 ± 0.0^{ab}	$0.21{\pm}0.01^{\rm ab}$	0.17 ± 0.01^{b}	0.03±0.0°	0.09±0.0°
P. aeruginosa	0.14 ± 0.01^{b}	0.14 ± 0.01^{b}	0.06±0.01°	$0.05\pm0.0^{\circ}$	$0.07 \pm 0.01^{\circ}$

Values are absorbance at 600 nm. Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (p = 0.05)

Table 4: Total Antioxidant activity of Trigonella foenum graecum solvent extracts

Solvents	Total antioxidant activity (μsM Fe (II) g^{-1})
Ethanol	47.0±1.0ª
Methanol	31.0 ± 1.0^{b}
Hexane	22.2±0.85°
Chloroform	$11.2 \pm 0.47^{ m d}$
Aqueous	$6.5\pm0.5^{ ext{de}}$

Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (p = 0.05)

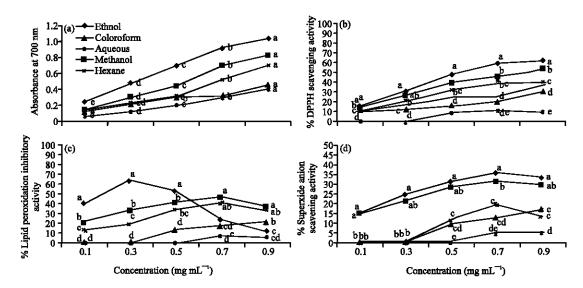


Fig. 1(a-d): Inhibitory activity of Trigonella foenum graecum leaf extract at different concentrations (mg mL⁻¹). A. Total reducing power B. DPPH scavenging activity (c). Lipid peroxidation inhibitory activity (d). Superoxide radical scavenging activity. Values are means of three independent replicates. Mean values with different superscripts are significantly different from each other as indicated by Tukey's HSD (p = 0.05)

700 nm from 0.24 ± 0.01 at 0.1 to 1.03 ± 0.02 at 0.9 mg mL⁻¹. Aqueous extract showed least reducing ability with an absorbance of 0.06 ± 0.02 at 0.1 and 0.40 ± 0.0 at 0.9 mg mL⁻¹.

DPPH radical scavenging activity: The DPPH radical scavenging activity of T. foenum graecum leaf extracts is shown in Fig. 1b. Among all the solvent extracts tested, ethanol extract had better scavenging activity (59.7±0.46%) followed by methanol (53.2±0.33%). When compared to the synthetic antioxidant (BHA) which had an IC_{50} value of 0.0053 mg mL⁻¹, the IC_{50} value of ethanol extract was quite high (0.7 mg mL⁻¹).

Lipid peroxidation inhibitory activity: The anti-oxidative action of T. foenum graecum leaf extracts in the liposome model is shown in Fig. 1c. Ethanol extract had an IC_{50} value of 0.3 mg mL⁻¹ which showed an inhibition of 62.3±0.35%. As with other solvent extracts, 50% inhibition of lipid peroxidation could not be achieved even at higher concentrations. BHA showed a very strong lipid peroxidation inhibitory activity with an IC_{50} value 0.012 mg mL⁻¹.

Superoxide scavenging activity: The decrease in absorbance at 562 nm with the plant extracts and the reference compound quercetin indicates their abilities to quench superoxide radicals in the reaction mixture. As shown in Fig. 1d the quenching ability generally was low with all the solvent extracts. Ethanol extract showed the highest inhibition of 33.3±0.4% at 0.9 mg mL⁻¹ and lowest inhibition of 14.9±0.23 at 0.1 mg mL⁻¹. Aqueous extract recorded the least inhibitions when compared to all other extracts (0% inhibition at 0.1 mg mL⁻¹ and 4.70±0.25% inhibition at 0.9 mg mL⁻¹. Quercetin was found more potent having an IC₅₀ value 0.155 mg mL⁻¹.

DISCUSSION

Polyphenol and Flavonoid contents: Phenols and flavonoids are very important plant constituents because of their antioxidant activity (Annegowda et al., 2010) and antimicrobial activity (Igbinosa et al., 2009). The antioxidant activity of phenolic compounds is mainly due to their redox properties which play an important role as free radical scavengers, reducing agents, quenchers of singlet oxygen and complexes of pro-oxidant metals (Butkhup and Samappito, 2011). The result obtained from the estimation of total polyphenol and flavonoid contents showed that alcohols are better solvents for the extraction of phenols and flavonoids. This is in agreement with the study by Tsao and Deng (2004) which showed that phenolic acids and flavonoids are generally better extracted using alcohols, water or a mixture of water and alcohols. Bukhari et al. (2008) had shown higher phenolic compounds in ethanol extract of fenugreek seed. Similar results were observed in the present study with fenugreek leaves.

Antibacterial activity of solvent extracts: It was clear from the present results that, ethanol leaf extract exhibited pronounced activity against all the tested six bacteria. The highest antibacterial activity as seen with ethanol extract may due to the presence of polyphenols because the phenol content as estimated in the present study was more in the ethanol extract than in any other solvent extracts. A study by Field and Lettinga (1992) has shown that antimicrobial properties exhibited by plants could be due to the presence of phenols and flavonoids. Broad spectrum activity of ethanol extract tends to show that the active ingredients of the leaves are better extracted with ethanol. Earlier studies have also shown the greater antibacterial activity of ethanol extracts than other solvent extracts (Aqil and Ahmad, 2003; Kannan et al., 2009). With least or no antibacterial activity as seen with other solvent extracts, may be due to loss of some active compounds during extraction process of the sample, lack of solubility of active constituents in the solvent (Sampathkumar et al., 2008).

Minimum inhibitory concentration: The MIC values for ethanol extract indicated that *S. aureus* was more susceptible than any other bacteria followed by *P. aeruginosa*. In spite of this permeability difference between gram positive and gram negative bacteria, the ethanol extract has a broader spectrum of inhibitory activity. This tends to show the involvement of more than one active principle of biological significance (Ming *et al.*, 2005). Earlier study by Bonjar (2004) has shown the broad spectrum activity of fenugreek seeds. Similar to the earlier findings in seeds, we have also shown the wide spectrum activity of fenugreek leaves. The possible mechanism for this broad spectrum activity of the extract may due to the ability of compounds present in the extract to complex with cell wall (Cowan, 1999).

Antioxidant activity assays

Ferrous reducing antioxidant assay: The antioxidant activities of the leaf extracts were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). It was found that, there was an increase in the antioxidant activity which was proportional with the polyphenol content as shown by the increased activity of ethanol extract. Antioxidant compounds such as polyphenols are more efficient reducing agents for ferric iron (Wong et al., 2006).

Total reducing power: The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Hazra *et al.*, 2008). The reducing power of the leaf extracts at different concentrations is associated with its anti-oxidant activity. All the extracts exhibited concentration dependent activity and showed a positive correlation with the polyphenol content. As reported by Gordon (1990) the presence of compounds with-OH groups in the extract may be responsible for reduction power.

DPPH radical scavenging activity: Anti-oxidants react with DPPH which is a stable free radical and convert it to α , α -dipehnyl- ρ -picryl hydrazine. The degree of discolouration indicates the radical scavenging potential of the anti-oxidant (Singh *et al.*, 2002). The ethanol extract recorded the highest phenol and flavonoid contents and also had the highest scavenging activity. There was a linear correlation between the antioxidant activity and total phenol and flavonoid contents of *T. foenum graecum* leaves. This suggests that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. The result was consistent with the findings of Tawaha *et al.* (2007) who reported positive correlation between total phenols and scavenging activity.

Lipid peroxidation inhibitory activity: Lipid peroxidation inhibitory activity mainly depends on the solubility, hydrophobicity of the compounds present in the respective extracts (Son and Lewis, 2002). The result in this study showed that ethanol extract had the highest inhibition of lipid peroxidation which may be due to the presence of high amounts of polyphenols. The inhibitions recorded were higher than the inhibitions recorded with DPPH assay. The results were contrary with the findings of Anokwuru et al. (2011) who showed greater inhibitions in DPPH assay than in lipid peroxidation inhibition assay. There was a gradual decrease in the inhibition of lipid peroxidation even with the increase in concentration of the extract which may be due to the degration or peroxidation of the source (Bendich et al., 1986).

Superoxide scavenging activity: Superoxide anion is harmful to cellular components (Korycka-Dahl and Richardson, 1978). The superoxide scavenging ability of the extracts may be due to the presence of flavonoids as reported by Robak and Gryglewski (1988). From the results, it appeared that the superoxide scavenging activity of the plant extract was generally low with all the extracts. Even though, the IC_{50} values could not be reached there was a positive correlation with flavonoid content and SOD scavenging activity of the extract.

CONCLUSIONS

In the present study, ethanolic extract of *T. foenum graecum* leaf gave the highest phenolic and flavonoid contents. Ethanol extract showed a broad spectrum of activity against all six bacteria of which *S. aureus* and *P. aeruginosa* were more susceptible. It was evident from the present study

that ethanolic extract showed potent lipid peroxidation inhibitory activity and DPPH scavenging activity. Superoxide scavenging ability was low with all the extracts. On the basis of the results obtained, it can be concluded that ethanol is the best solvent for extracting both antibacterial and antioxidant compounds from leaves. Further studies are needed for the characterization of individual phenolic compounds to elucidate the mechanism underlying bioactive principles and the existence of possible synergism, if any, among these compounds.

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Res. J. Med. Plant, 5 (6): 695-705, 2011

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