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Research Article

Phenolic Content and Antioxidant Activity of Fenugreek Seeds Extract

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum*) is a nutrient dense food rich in beneficial phytochemicals. In this study, three types of solvent extracts of fenugreek seeds were used to examine the effects of extraction solvent on total phenolics content (TPC), 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) were determined. Results showed that extraction solvent had significant effects on TPC and antioxidant activity of acetone extract. The highest content of TPC and antioxidant activity (FRAP and DPPH) were found in 50% acetone extracts. The TPC for fenugreek seeds from 25.90 to 15.45 mg GAE/100 g DW and antioxidant activity FRAP from 47.49 to 31.85 mg TE /100 g DW, DPPH were from 67.30 % to 43.61%). The largest amount of total phenol content which leads to more effective radical scavenging effect was shown by 50% acetone extract. Moreover, amount of phenolic compounds and antioxidant activities increased in acetone extract. Acetone 50% and methanol 50% solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced. It was concluded that extraction solvent play important roles on the phenolics compounds and their antioxidant activity of fenugreek seeds extract.

Key words: Fenugreek, Extraction Solvent, Antioxidant activity, Phenolics

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) belongs to the family Leguminosae. Fenugreek has been used traditionally to treat diabetes [1] coughs, congestion, bronchitis, fever, high blood pressure, headaches/ migraines, diarrhea, anemia, flatulence, irregular menstrual cycles, analgesic, inflammation and arthritis [2], to ease labor pains and menstruation pain, and as an appetite stimulant. Fenugreek leaves provide a good amount of various minerals and vitamins. They are especially rich in choline. Seeds are aromatic, bitter, carminative, galactogogue and antibacterial. It constitutes 50% unavailable carbohydrates (fiber) making its highest concentration among all the natural sources of fiber. The fiber portion consists of insoluble (30%) and soluble (20%) fraction which is mostly galactomannan [3]. Total lipids extracted from fenugreek seeds amounted to be 7.5% of the dry seeds [4] and consisted of 84.1% neutral lipids, 5.4% glycolipids and 10.5% phospholipids. Fenugreek contains approximately 4 to 8% saponins and about 1% alkaloids, which contributing to its bitterness. Fenugreek seed is widely used as a galactagogue (milk producing agent) by nursing mothers to increase inadequate breast milk supply [3]. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels [5]. Therefore, fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Indian and Chinese medicines [6]. It.s reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of different ulcers in digestive tract [7]. Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant [8].

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer [9].The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants [10]. Among the different factors (sample pre-treatment, solvent/sample ratio, solvent type, extraction time, and extraction temperature) affecting extraction efficiency, solvent type has been the most analyzed [11]. Frequently used solvents for antioxidant extraction include methanol, ethanol, and acetone either alone or in combination with an aqueous solution. Therefore, this study aimed to determine the effect of solvent for extracting antioxidant compounds from fenugreek seeds.

MATERIALS AND METHODS

Sample Collection and Preparation of Fenugreek Seeds Extract: The stem and fruits of fenugreek seeds were

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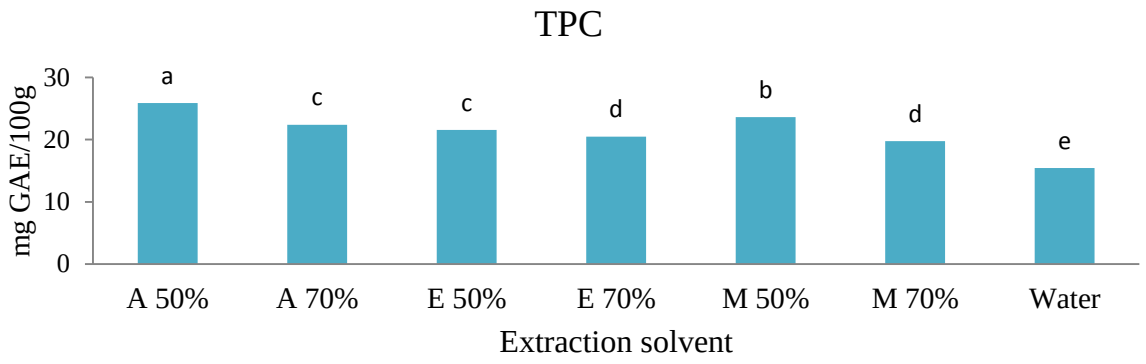
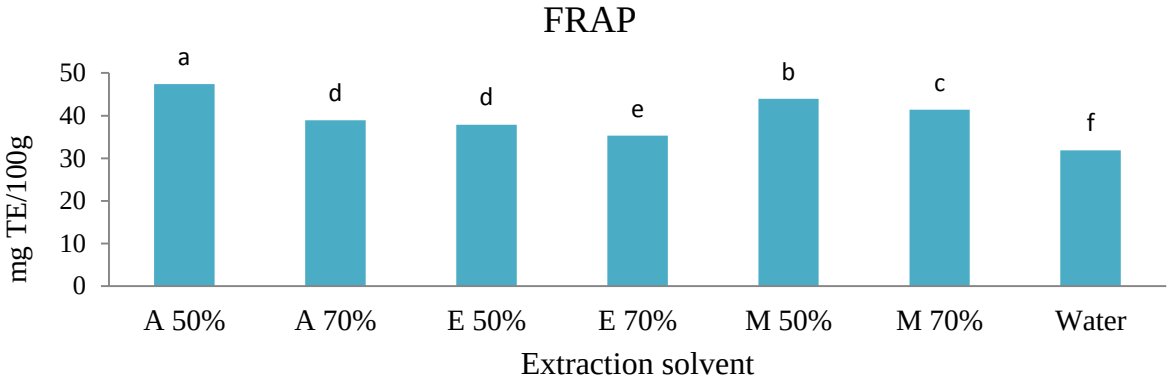
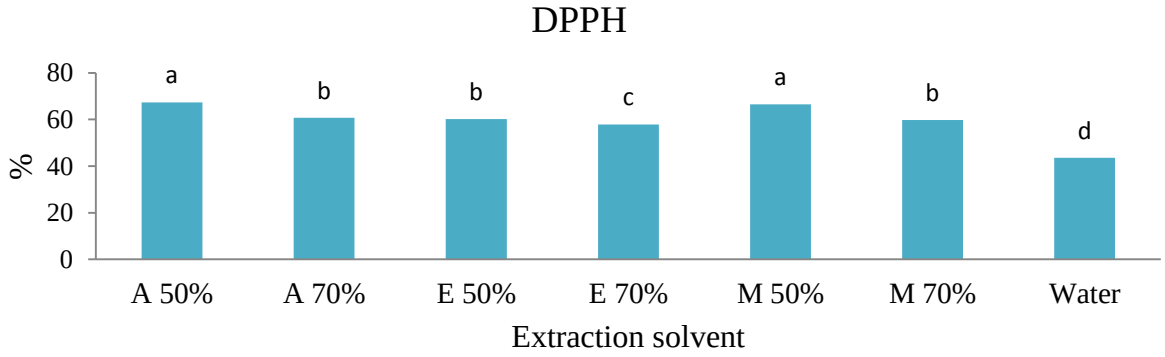


[a-e] *Mean with different letters within each column are significantly different ($p < 0.05$)*
Fig 1: Effect of extraction solvents on the total phenolic content of fenugreek seeds.



[a-f] *Mean with different letters within each column are significantly different ($P < 0.05$)*
Fig 2: Effect of extraction solvents on the FRAP content of fenugreek seeds



[a-d] *Mean with different letters within each column are significantly different ($P < 0.05$)*
Fig 3: Effect of extraction solvents on the DPPH activity of fenugreek seeds

obtained from the market in Thi-Qar city, Iraq. The stem and fruits of fenugreek seeds were cleaned and then oven dried at 50°C for 24 h. The dried sample was then pulverized using a mechanical grinder and passed through a 250 µm mesh and then stored at room temperature until use. In the extraction process, about 0.1 g of fenugreek seeds were weighed in universal bottles and 10 ml solvent was added. The different types of solvent used were absolute methanol, ethanol, acetone, water and their aqueous solutions at 50% and 70% concentrations, samples were then homogenized using homogenizer. All extracted samples were centrifuged by using table top centrifuge for 10 min. The supernatants were collected for further analysis.

Total Phenol Content (TPC): Antioxidant activity was determined using TPC based on the method of [11]. Approximately 0.4 mL distilled water and 0.5 mL diluted Folin–Ciocalteu reagent were added to 100 µL fenugreek seeds extracts. The samples extracts with Folin–Ciocalteu reagent) were set aside for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength using a spectrophotometer after 2 h. The calibration curve of gallic acid (GA) was used for the estimation of sample activity capacity. The result was

Table 1: Correlation coefficients of antioxidants activities of fenugreek seeds.

R^2	FRAP	DPPH
TPC	0.87	0.95

recorded in terms of mg of GA equivalents per 100 g of fresh sample (mg GA/100 g of FW).

Ferric Reducing Antioxidant Power (FRAP): [11] proposed the idea of determining antioxidant activity through FRAP . First, 300 mM acetate buffer FRAP reagent was prepared fresh as follows: pH 3.6 (3.1 g sodium acetate trihydrate plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ) in 40 mM HCl; and 20 mM FeCl3·6H2O in the ratio of 10:1:1 to provide the working reagent. In addition, approximately 1 mL FRAP reagent was added to 100 µL fenugreek extracts, and the absorbances were taken at 595 nm wavelength using a spectrophotometer after 30 min. The calibration curve of Trolox was established to approximate sample activity capacity. The result was recorded as mg of Trolox equivalents (TEs) per 100 g of fresh sample (mg TE/100 g of FW).

DPPH Radical Scavenging Activity: Based on the method of [11] the antioxidant activity was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system. The stock solution was obtained by dissolving 40 mg DPPH in 100 mL methanol, which was stored at -20 °C until further use. Approximately 350 mL stock solution was mixed with 350 mL methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using a spectrophotometer (Epoch, Biotek, USA). In the dark, approximately 100 µL *Trigonella foenum-graecum* seeds extracts with 1 mL prepared methanolic DPPH solution was stored overnight for scavenging reaction. The percentage of DPPH scavenging activity was determined based on the following equation: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$, where A is the absorbance.

Statistical Analysis: Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed byone-way ANOVA using SPSS ver.19. Significant differences ($P<0.05$) among the solvent extraction were analyzed by Duncan 'triplicates range test [12]

RESULTS AND DISCUSSION

Total Phenol Content (TPC): Figure 1 showed significant difference ($P<0.05$) in the total phenolic of fenugreek seeds. The total phenolic content in different solvent extracts (acetone, ethanol and methanol) of the fenugreek seeds is shown in Fig1. With increase in solvent polarity, TP content increased in extract. High amount of TPC (25.90 mg GAE/g DW) was obtained from acetone % extract. After acetonel 50%, methanol 50% had high content of phenolic content in extract. As found in this study, in a mixture with no aqueous content, the extraction efficiency was low and negative. It is clear that the addition of some amount of water enhance the extraction efficiency. One possible reason for the increased efficiency with the presence of some water might be due to the increase in bulge of plant material by water, which increased the contact surface area between the plant matrix and the solvent [13]. Our results is similar to that reported by [11], where acetone solvent was most effective in extracting phenolic components from ginger fruit. [14] reported that solvent with different polarity had significant effect on phenolics compound and antioxidant activity in higher content in more polar solvents.

Ferric Reducing Antioxidant Power (FRAP): For measurement of the reductive ability, the $Fe3^{+}$- $Fe2^{+}$ transformations in the presence of fenugreek seeds extracts was investigated. Figure 2 shows FRAP values for different solvent extraction (acetone, ethanol and methanol). Both acetone 50% and methanol 50% were the best solvent for finding extracts with higher antioxidant activity. The FRAP value obtained by acetone 50% 47.39 mg TE/100 DW was higher significantly ($P<0.05$) than the extract obtained by methanol 43.92 mg TE/100 DW. However, for FRAP values sample extracted by acetone 70% were not significantly ($P<0.05$) different from ethanol 50%. When comparing the results from this study with other study, values from different sources seriously differ. The FRAP mean value in this study showed that fenugreek seeds were higher than that of [15]. The better extraction power of aqueous solvent indicates that the mixing of a non-polar solvent with water may increase the polarity index of solvents, thereby consequently enhancing the extraction power of a certain solvent. Our findings are consistent with those of [11] who found that the increase in polarity of a solvent (up to 50% water) enhances the solubility of antioxidant compounds

DPPH Radical Scavenging Activity**:** Figure 3 shows free radical scavenging activity values for different solvent extraction of fenugreek seeds. The results in Figure 3 showed that antioxidant activity were sensitive to extraction solvents; generally acetone and methanol 50% gave the highest extraction recovery. DPPH values of fenugreek seeds extracted decrease with increase in the organic solvent concentration. Aqueous organic solvent were found to give the highest values. Acetone 50% was the best solvent for obtaining extracts with high antioxidant activates of fenugreek seeds followed significantly ($P<0.05$) with methanol 50%. The results are higher than those of (Musa et al. 2011), where the DPPH scavenging percentages for fenugreek seeds ranged from 67.30 to 43.61 %, despite the use of the same types of solvents (water and methanol). The difference in findings might possibly be attributed to the different extraction methods and solvents used [16]. The different results obtained from the previous studies may be attributed to different cultivars, growing conditions, maturity stage at harvest, or the storage conditions and time elapsed before the fruits were analyzed. Sample preparation method may also influence the results.

Correlation of Total Phenol Content and Antioxidant Activity: A correlation analysis among total phenolic content TPC and antioxidant activity (FRAP, DPP) was performed regardless of the extraction solvent used. A high correlation (Table 1) was found between TPC and antioxidant activity (FPAP and DPPH). Thus, we can reasonably conclude that in the extract, antioxidant activity

is related to the active component. Findings of researches of correlation analyses among TPC and antioxidant activities (FRAP and DPPH) are high [11]. There have been significant effects on the antioxidant activities of fenugreek seeds based on the solvent.

CONCLUSION

The results of this study showed that the type of solvent used had a significant effect ($P<0.05$) on the extraction of antioxidant compounds from fenugreek seeds .Total phenol content, ferric reducing antioxidant power and free radicals scavenging activity of fenugreek extracts decreased with increase in the organic solvent concentration. In fact, it can be concluded that the extracts obtained using higher polar solvents were more effective than less ones. The addition of 50% water to methanol, acetone or ethanol can enhance the extracting power and antioxidant activity estimation especially acetone and methanol. The total phenolic content showed a good correlation with antioxidant activity FRAP and DPPH.

ACKNOWLEDGEMENT

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