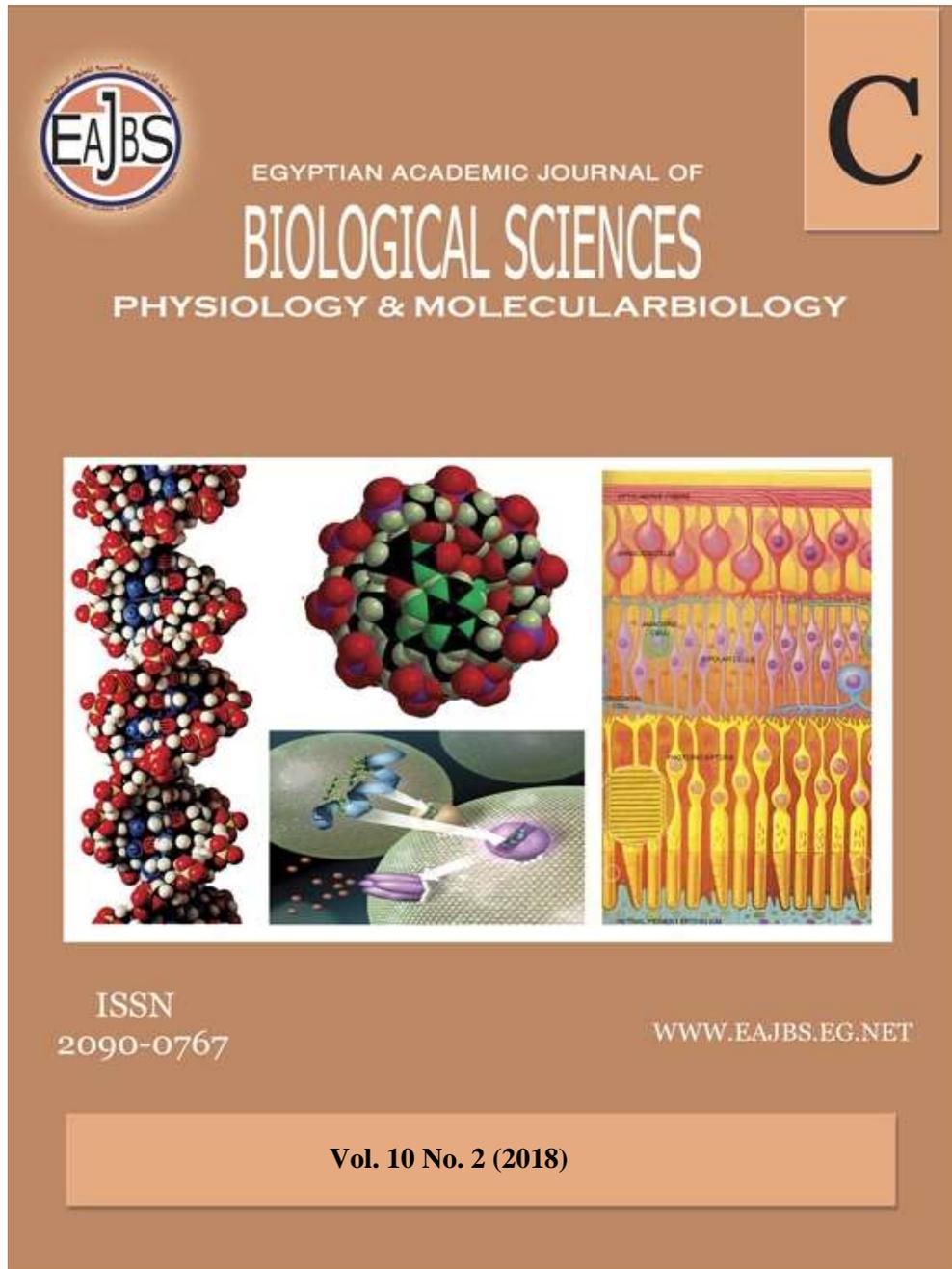


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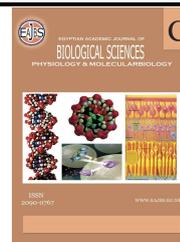
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## Screening the Antioxidant Activity of Aqueous and Ethanol Extracts from Fenugreek (*Trigonella foenumgraecum*) Seeds *in Vitro*

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### ABSTRACT

In this study was to prepare extracts aqueous and ethanol from Fenugreek *Trigonella foenumgraecum* Seeds powder, The aqueous extract and ethanol extract were used for analyzing the main phytochemical, nutrient and active groups composition of fenugreek seeds powder *Trigonella foenumgraecum*. The preliminary tests of active groups in extracts were carried out. It appeared to contain (alkaloids, flavonoids, phenolic compounds, tannins, terpenes and saponins). The extracts were different in their content of active groups quantitatively and qualitatively. And to evaluate *in vitro* antioxidant activity of aqueous and ethanol seeds extracts of *Trigonella foenumgraecum*, was used three different methods and all the three methods have proven the effectiveness of extracts aqueous and ethanol from Fenugreek *Trigonella foenumgraecum* Seeds compared to the reference standard antioxidant ascorbic acid, Showed the study is conducted to estimate the activity of the extracts prepared from Fenugreek *Trigonella foenumgraecum* Seeds as antioxidant *in vitro* by measuring the reducing power and their capacity of scavenging hydrogen peroxide as compared with the standard compound ascorbic acid. The results indicated that the extracts aqueous and ethanol Fenugreek *Trigonella foenumgraecum* Seeds exhibited the highest had a reducing power and a capacity of scavenging hydrogen peroxide, and demonstrated that extracts antioxidant capacity less than the standard for the reducing power and phosphomolybdate reduction methods. This is observed through increasing the intensity of absorbance with increasing the concentration.

### INTRODUCTION

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals (Benzie IFF.2003) free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Fang Y., *et al.*2002).

The most common reactive oxygen species (ROS) include super oxide anion ( $O_2^-$ ), hydroxyl radical (OH), hydrogen peroxide ( $H_2O_2$ ) peroxy radical, radicals ( $ROO^\cdot$ ). Nitrogen derived free radicals are nitric oxide ( $NO^\cdot$ ) and peroxy nitrite anion ( $ONOO^-$ ) (Nagendrappa, 2005). ROS have been implicated in over hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and cardiovascular malfunction (Nordberg, 2001; and Ray; Husain, 2002). In treatments of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and (ROS), any may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers

(Halliwell., Gutteridge, 1999; and Daniel; *et al.*1998). The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (Prabakaran, 2005). Poly phenol compounds such as flavonoids and phenolic groups widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-tumor (Irshad ; Chaudhuri, 2002; and Huang; *et al.* 2005) . They were also suggested to be a potential iron chelate. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties (Lee *et al.*; 2004). In Indian system of medicine *Trigonella foenumgraecum* is an important

medicinal plant and its leaves and a seed have been used in various ailments and as health tonic (Nadkarni, 1954). The seeds of fenugreek contain alkaloids, flavonoids, saponins, amino acids, tannins and some steroidal glycosides, proteins etc.

(Ayurvedic Pharmacopoeia, 1996), antioxidants or inhibitors of oxidation are the compounds which retard or prevent the oxidation in general and prolong the life oxidizable matter (Sharma; *et al.* 1990). Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalyzing metals and also by acting as reactive species scavenger. Polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activities of the individual compounds may depend on structural factors, such as number of phenolic, hydroxyl or methoxyl groups and other structural features (Shen and Zhang, 1994). Among the anti-oxidative compounds vitamin A, C, E, selenium, carotenoids, ascorbic acid show very strong intensity of anti-oxidative activities (Kalia, 2005). The objectives of the present study were to investigate the in vitro antioxidant activity of aqueous and alcohol extracts from Fenugreek *Trigonella foenumgraecum* seeds the H-donor activity, hydroxyl radical, hydrogen peroxide scavenging, total antioxidant activity by thiocyanate and phosphomolybdenum method, metal chelating.

#### **MATERIALS AND METHODS**

The purest chemical materials are used to analyzing the Evaluation of Antioxidant Activity of aqueous and ethanol extracts from Fenugreek *Trigonella foenumgraecum* seeds in vitro study.

#### **Gathering the Plants:**

The seeds fenugreek plant is gathered from local marked in Samarra. The seeds fenugreek are ground by a special grinder and conserved in the sealed container at the room temperature before used.

#### **Preparation of the Raw Aqueous And Ethanol Extracts:**

The Fenugreek *Trigonella foenumgraecum* seeds powder is dried by using the air-drying oven at (40°C). Then alcohol are extracted by (95%) of ethyl alcohol and the aqueous extract was attended by using the distilled water boiled intercooler, when (50gm) of dried fenugreek powder used the stirring magnetic par for 48 hours and at room temperature, with ratio (1:10) of solvent to the solid material. The solution of extracts are filtered under the vacuum. The solution of extracts are entirely dried by air oven at temperature (35-40°C). And the extracts are conserved after its dryness in the tied sealed plastic containers in conditions free of moisture by refiguring until it is used. According to the method Ruch (Ruch and Worf, 2001).

#### **Preliminary Phytochemical Screening of Aqueous and Ethanol Extracts from Fenugreek *Trigonella foenumgraecum* Seeds:**

A chemical tests are carried out on the powdered of seeds fenugreek to their

aqueous extract and ethanol extract prepared are subjected to the tests to identify their chemical constituents alkaloids (Kokate *et al.* 2009; and Mahmoud, 2008), tannins and phenolic compounds (Kokate; *et al.* 2009), flavonoids (Rashant; *et al.* 2011; and Sofowara, 1993), terpenes (Gibbs, 1974), saponins (Mahmoud, 2008), by using standard procedures to preliminary phytochemical screening.

#### **Methods of Estimating the Activity of Antioxidant of Aqueous and Ethanol Extracts from Fenugreek *Trigonella foenumgraecum* Seeds *in Vitro*:**

The antioxidant activity of antioxidant of aqueous and alcohol Extracts from Fenugreek *Trigonella foenumgraecum* seeds *in vitro* This is observed through increasing the intensity of absorbance with increasing the concentration by the following methods:

#### **Reducing Power Assay:**

##### **Principles:**

The reducing power of aqueous and ethanol extracts was determined by the method of Oyaizu (Oyaizu, 1986). Substances, which have reduction potential, react with potassium ferricyanide-Fe<sup>+3</sup> to form potassium ferrocyanide-Fe<sup>+2</sup>, which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

#### **Antioxidant**

Potassium ferricyanide + Ferric chloride  $\longrightarrow$  Potassium ferrocyanide + ferrous chloride

#### **Preparation of Samples and Standard:**

Stock samples and standard 250µg/ml: the stock solution of aqueous and alcohol extracts from Fenugreek *Trigonella foenumgraecum* seeds, ascorbic acid were prepared by dissolving 25mg of powder extract aqueous in 75ml of deionized DW, mixed and used ultrasonic for 10 min at

35°C, then volume were completed to 100 ml by the same solvent, when the extract alcohol used ethanol solvent.

Different concentration of Extracts from Fenugreek *Trigonella foenumgraecum* seeds and ascorbic acid were prepared, as stated in Table (1) below:

Table 1: Preparation of extracts from Fenugreek *Trigonella foenumgraecum* seeds and Ascorbic Acid.

Tube No.	Stock volume (ml)	Distill water (ml)	Final conc. (mg/dl)
1-	1	9	25
2-	2	8	50
3-	4	6	100
4-	6	4	150
5-	8	2	200

**Procedure:**

One milliliter of sample or standard was added to 2.5ml of (0.2M, pH6.6) phosphate buffer, mixed by vortex and then 2.5ml of potassium ferricyanide 1% was added.

The tubes were incubated at 50°C for 20 min.

The tubes were cooled and 2.5 ml of 10% trichloroacetic acid was added to each tube mixture, and then centrifuged at 3000rpm for 10 min.

(2.5) ml of the upper layer of solution was taken and mixed with 2.5ml of deionized DW and 0.5ml of freshly prepared ferric chloride 0.1% solution. The absorbance was measured at 700 nm against blank reagent. The reducing power tests were run in triplicate. Increase in absorbance of the reaction mixture indicated the reducing power of the samples.

**Scavenging of Hydrogen Peroxide:**

The ability of the fractions to scavenge hydrogen peroxide was determined according to the method of Muhammad (Muhammad; *et al.* 1987).

**Preparation of Samples and Standard:**

Different concentrations of extracts from Fenugreek *Trigonella foenumgraecum* seeds and ascorbic acid were prepared in the same procedure mentioned in (2-4A-II).

**Procedure:**

Four milliliters of extracts from Fenugreek *Trigonella foenumgraecum* seeds and ascorbic acid (for each conc.) was mixed with 0.6ml of 40mM H<sub>2</sub>O<sub>2</sub> solution [prepared in 0.1M phosphate buffer, pH7.4].

Incubated for 10min, then absorbance of the solution was taken at 230nm.

**Total Antioxidant Capacity by Phosphomolybdenum Method:**

The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate Mo(V) complex at acidic pH and at higher temperature with a maximum absorption at 695nm (Nabavi; *et al.* 2009).

**Reagents:**

(250µg/ml) of samples and standard were prepared.

Ammonium molybdate (4mM): prepared by dissolving 0.0989g in 10 ml DW, Mixed and complete the volume to 20ml with DW.

Sodium phosphate (28 mM): prepared by dissolving 0.0672g in 10 ml DW, Mixed and

complete the volume to 20ml with DW.

**Procedure:**

One milliliter of 600mM of sulfuric acid was mixed with the same volume of sample or standard.

The tubes were capped with silver foil and then incubated at 95°C for 90 min, cooled down to room temperature, and the absorbance of the green phosphomolybdenum complex was measured at 695 nm against a blank.

**RESULTS AND DISCUSSION****Preliminary Phytochemical Screening of Aqueous and Ethanol Extracts from Fenugreek *Trigonella foenumgraecum* Seeds**

The concentration of phytochemical compounds in the two solvent extracts the aqueous and ethanols were significantly different from each other. The aqueous extract was found to contain rich amounts of alkaloids, flavonoids, phenolic

compounds and tannins, terpenoids, phenolic compound and tannins, saponins. Ethanol extract of fenugreek seeds was also rich in flavonoids, terpenoids and small amount of alkaloids and saponins. As shown in Table (2).

Table 2 Phytochemical Screening for aqueous and ethanol Extracts of fenugreek seeds

Phytochemical constituents tests		Extracts prepared from fenugreek seeds	
		Aqueous	Ethanol
Alkaloids	Mayer	+++	++
	Dragendoff	+++	++
Flavonoids	Lead acetate	-	-
	Ferric chloride	+++Deposit	+++Deposit
phenolic compounds and tannins	Ferric chloride	-	-
	Gelatin	+++	+++
	Lead acetate	++++Deposit	+++
Terpenoids	Trim-Hill	-	-
	Liebermann-Burchard	+++	+++
Saponins	Foam test	+++	-
	Mercuric chloride	+++	+

++++ Very large quantity    +++ large amount    ++ small amount    - absent

**Estimation the Antioxidant Activity of Aqueous and Ethanol Extracts from Fenugreek *Trigonella foenumgraecum* Seeds in vitro:**

**The Reducing Power:**

The results indicated that the extract of aqueous and ethanol extracts from Fenugreek *Trigonella*

*foenumgraecum* seeds had a reducing power to the compared standard ascorbic acid (vitamin C), which shows the absorption corresponds to concentration. Recognized by increasing the intensity of the absorbance by raising the concentration as in the Figure (1).

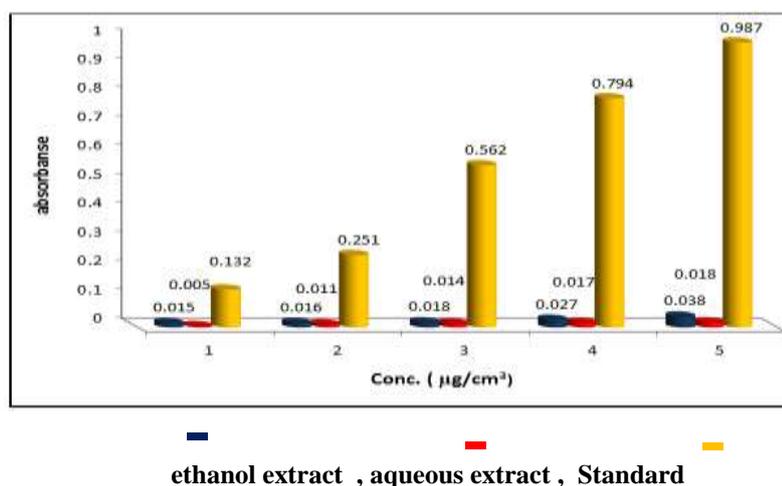


Fig. 1: the reducing power for the standard ascorbic acid and the aqueous and ethanol extracts from Fenugreek *Trigonella foenumgraecum* seeds

**Capacity of Scavenging Hydrogen Peroxide:**

The result of the present study is given that the extracts of aqueous and

ethanol from Fenugreek *Trigonella foenumgraecum* seeds had a capacity of scavenging Hydrogen Peroxide superior to the compared standard ascorbic acid

(vitamin C), which shows the absorption corresponds to concentration. Recognized through increasing the

intensity of the absorbance by raising the concentration as in the Fig. (2).

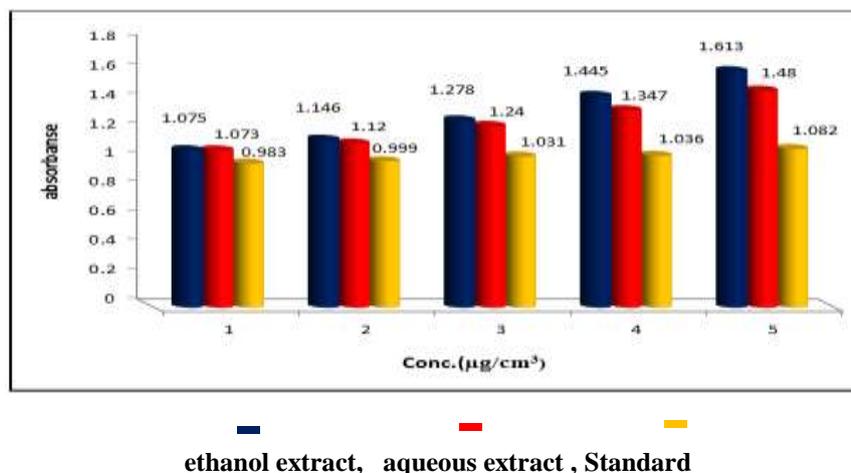


Fig. 2: Capacity of the standard ascorbic acid and the aqueous and ethanol extracts from Fenugreek *Trigonella foenumgraecum* seeds in scavenging hydrogen peroxide

### Total Antioxidant Capacity by Phosphomolybdenum:

Results indicated that the total antioxidant capacity for phosphomolybdate reduction of the extracts aqueous and ethanol from Fenugreek *Trigonella foenumgraecum* seeds to the compared standard ascorbic acid (vitamin C), which shows the absorption corresponds to concentration. Recognized by increasing the intensity of the absorbance by raising the concentration as in the Figure (3).

In the present study, three different assays were employed in order to determine and compare the antioxidant properties of aqueous and ethanol extracts from Fenugreek *Trigonella foenumgraecum* seeds as well as to elucidate their mode of action. The Scavenging of Hydrogen Peroxide assay has been widely used to determination of antioxidant activity of various antioxidant substances. In this method with the aqueous and ethanol extracts from Fenugreek *Trigonella foenumgraecum* seeds show figure(2) the results have the higher absorbance may be due to its strong H<sub>2</sub>O<sub>2</sub> scavenging activity as compared to ascorbic acid and its

showed antioxidant increases with the increase in amount of sample and standard concentrations. Either assays the reducing power and the total antioxidant capacity for phosphomolybdate reduction show Figure (1) and (3) the results decreased in activity to the reference antioxidant.

The constituents that are understood to be responsible were flavonoids and phenolic compounds which generally marks their presence in the polar solvent system due to their self-polar nature. Thus, due to the ability of fenugreek extracts to quench the radicals, it can be a useful candidate to alleviate the harmful effects of various diseases and thus can be used for treatment purposes. It has been documented in various studies that fenugreek bears potential of a powerful antioxidant in which the presence of flavonoids and polyphenols have been found to be responsible for the same (Rababah *et al.* 2004; and Ghaskadbi; *et al.* 2005). In addition, the antioxidant property of fenugreek has been studied in vivo and in vitro in ethanol-induced toxic rats which reduced lipid peroxidation and prevented enzyme leakage (Thiruna vukkarasu; *et*

*al.* 2003) Reported Bukhari *et al* that fenugreek seed extract with methanol, ethanol, and solvents other has a radical scavenging activity (Bukari; *et al.* 2008). As such reported (Naidu; *et al.* 2010) that the proximate composition of fenugreek seeds exhibited antioxidant activities by free radical scavenging activity (Naidu; *et al.* 2010). (Joglekar *et al.* 2012) observed has the highest Phenolic and flavonoids content in fenugreek antioxidant property was checked by reducing power, NBT assay and H<sub>2</sub>O<sub>2</sub> scavenging, thus show the highest reducing power, superoxide and free radical scavenging. Indicated fenugreek seeds contains Phenolic and flavonoid compounds which help to enhance its antioxidant capacity (Thiruna vukkarasu; *et al.* 2003).

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