A Comparative Study of *in vitro* Nitric oxide radical, Hydroxyl radical and ABTS free radical Scavenging Activities of Leaf and Seed Extracts of *Trigonella foenum-graecum*

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Abstract

Free radicals are outcome of various metabolic activities and their excess production leads to many diseases. Therefore, it is necessary to neutralize excess free radicals. Diabetes is an oxidative stress related disorder and is emerging as a pandemic. The immediate need is to identify novel food based bioactive agents or drugs for curing or preventing diabetes, with comparatively fewer side effects. *Trigonella foenum-graecum* (Fenu greek) is one of the most promising vegetable providing treasures of secondary metabolites known to have health benefits against various diseases. In the present study free radical scavenging activities of ethyl acetate extracts of *Trigonella foenum-graecum* leaf (TGL) and *Trigonella foenum-graecum* seed (TGS) was studied, and it was found that Nitric oxide scavenging activity of all the extracts was found to be moderate with TGL 52% and TGS 54% when compared to ascorbic acid standard with 77% scavenging effect. The hydroxyl radical scavenging activity TGL and TGS were found to be 61% and 56%, the standard quercetin which showed a maximum of 64%. ABTS radical scavenging activity of TGL and TGS and standard ascorbic acid were found to be 73 %, 52 % and 68 %. Nitric oxide radical, Hydroxyl radical and ABTS radical Scavenging radical models for antioxidant studies showed that TGS had higher free radical scavenging activities when compared to TGL extracts. The antioxidant activity exhibited by *Trigonella foenum-graecum* shows promising scope in treatment and prevention of complications in diabetes.

Keywords: *Trigonella foenum-graecum*, Free radical scavengers, Antioxidants, Diabetes.

Introduction

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycaemic control [1]. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in the regimen for treatment of this devastating disease. Management of diabetes without any side effects is still a challenge for medical system [2].

Oxidative stress (formation of free radicals) is generated due to hyperglycaemic status through both enzymatic and non-enzymatic processes. These free radicals would damage cellular

proteins as well as mitochondrial DNA [3]. Overproduction of free radicals or reactive oxygen species (ROS) contributes to oxidative stress, that is associated with chronic degenerative diseases, including cancer, coronary artery diseases, hypertension and diabetes [4] and [5]. Free radicals are formed disproportionately during diabetes due to glucose oxidation and the subsequent oxidative degradation of glycated proteins [6].

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. A plant-based diet protects against chronic oxidative stress-related diseases. Dietary plants contain variable chemical families and amounts of antioxidants. It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants [7].

Antioxidants are abundantly present in leaf vegetables, fruits and natural food sources. Phytoconstituents with antioxidant potential include some cinnamic acids, coumarin derivatives, diterpenes, flavonoids, monoterpenes, phenylpropanoids, tannins and triterpenes. Natural antioxidants are present in all parts of higher plants like wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds. The occurrence of such oxidative mechanisms in plants clarifies why a plenty of antioxidant compounds have been recognized in plant tissue. Plants mostly those with elevated levels of powerful antioxidant compounds have an essential role in the cure and treatment of illness concerning oxidative stress including Diabetes Mellitus [8].

*Trigonella foenum graecum (*Fenugreek) is an annual leguminous bean and belongs to Fabaceae family. The seeds and green leaves of *Trigonella foenum graecum* used as food possess many medicinal applications. India is the largest producer of fenugreek in the world. Total fenugreek production in India was 113 thousand metric tonnes in the year 2012- 2013.In India; it is extensively used as ayurvedic medicine and in China as traditional medicine [9]. Fenugreek is consumed in various parts of the world in different forms and has been regarded as a treatment for many ailments known to man [10].

Research reports indicate that fenugreek possesses immunomodulatory, anti-carcinogenic, anthelmintic, anti-nociceptive, antioxidant, anti-microbial, anti-ulcer, gastro- and hepatoprotective, anti-obesity, anti-hyperglycemic, antidiabetic and hypocholesterolemic effects [11]. Leguminous plants are a rich source of proteins and peptides that are involved in plant defense, including proteinaceous amylase inhibitors [12].

The herb has an enormous potential to prevent or cure diabetes more than other plant species especially due to the presence of unique chemical constituents including quercetin, diosgenin, trignolline, galactomanin and unusual amino acid 4- hydroxy isoleucine. However, due to lack of enough scientific or clinical studies the use of fenugreek as hypoglycaemic official drug remains to be explored [13].

Materials and Methods

Plant Collection, Identification and Preparation of Extract

Trigonella foenum-graecum leaves and seeds were collected from local market identified and authenticated by botanist from Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The plant parts were dried, finely powdered and stored in air tight containers at room temperature for further use. Five grams of *Trigonella foenum-graecum* leaves (TGL) and *Trigonella foenum-graecum* seeds (TGS) were macerated with 50 ml ethyl acetate for 48 hours filtered and collected the solvent. The solvent was evaporated in water bath shaker to get dry extract and used for further analysis.

Nitric oxide radical Scavenging Activity [14].

Three ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (200 - 1000µg) of TGL and TGS solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Ascorbic acid was used as a standard. Percentage radical scavenging activity of the sample was calculated as follows:

% NO radical scavenging activity = (control OD-sample OD / control OD) x 100

Hydroxyl radical Scavenging Activity [15].

Different concentrations of the TGL and TGS extracts (200 - 1000µg) were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%) and 1ml of DMSO. The reaction was initiated by adding 0.5 ml of ascorbic acid and incubated at 80-90°C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1ml of ice-cold TCA. Three millilitres of Nash reagent was added and left at room temperature for 15 min. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. Quercetin was used as the standard. The % hydroxyl radical scavenging activity was calculated as follows:

% Hydroxyl radical scavenging activity = (control OD-sample OD / control OD) × 100

ABTS free radical Scavenging Activity [16].

ABTS was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (1:89 v/v) and equilibrated at 30^0 C to give an absorbance at 734 nm of 0.700 ± 0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 µl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10 µl of sample TGL and TGS (200-1000 µg/ml), absorbance was measured

at 734 nm at exactly 30 min after the initial mixing. Samples were analyzed in triplicate. Ascorbic acid is used as the standard. Percentage radical scavenging activity of the sample was calculated as follows:

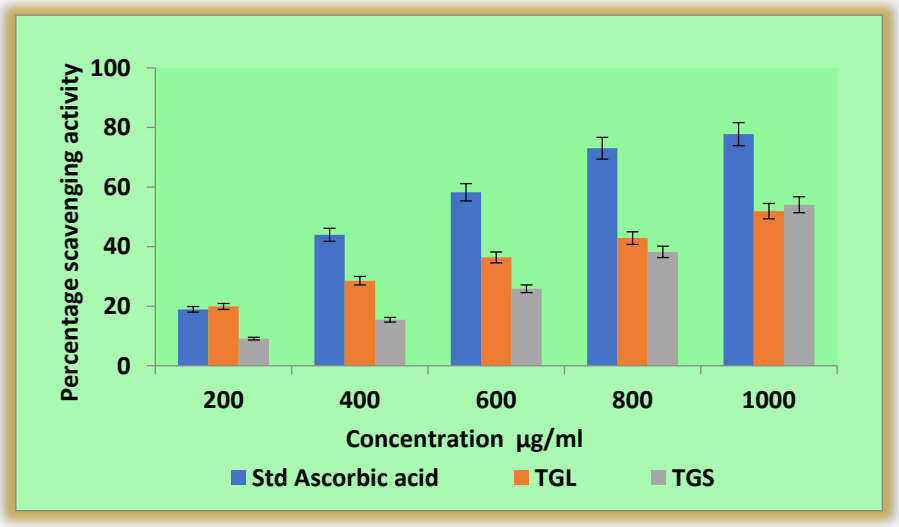
% ABTS radical scavenging activity = (control OD-sample OD / control OD) × 100

Results and Discussion

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity of *Trigonella foenum-graecum* leaf (TGL) and *Trigonella foenum-graecum* seed (TGS) extracts was found to be TGL 52% and TGS 54% when compared to ascorbic acid standard with 77% scavenging effect as shown in the Figure 1.

Figure 1: Nitric oxide radical scavenging activity of *Trigonella foenum-graecum*



Values are mean ±SEM of triplicates

TGL-*Trigonella foenum graecum* leaves TGS-*Trigonella foenum graecum* seeds

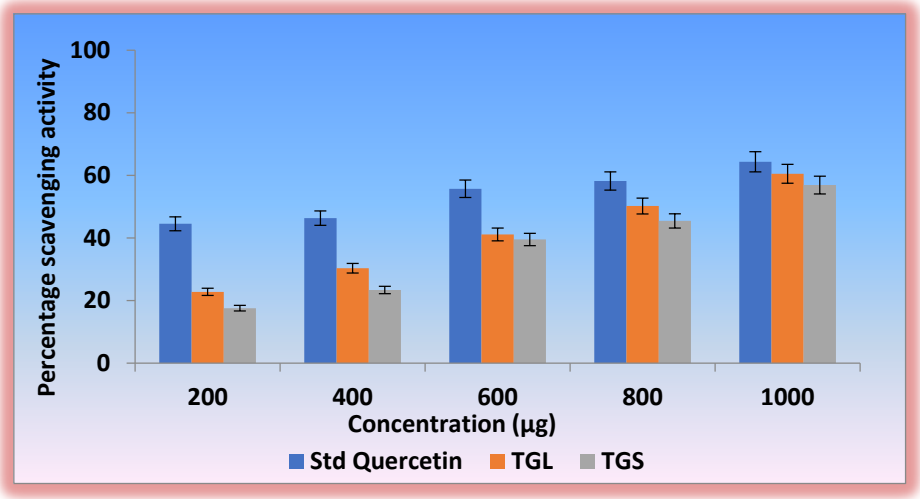
Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal [17]. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [18]. From the present study, we can find that the selected plant extracts exhibited moderate nitric oxide scavenging activity in a dose dependent

manner. The scavenging effect might be due to the antioxidant compounds from plant extracts which may compete with the O_2 to react with the NO and thus inhibits the generation of nitrite.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *Trigonella foenum graecum* is shown in Figure 2 The peroxyl radical scavenging activity was determined and the results were compared with standard quercetin. The hydroxyl radical scavenging activity of TGL and TGS were found to be 61% and 56%.

Figure 2: Hydroxyl radical scavenging activity of *Trigonella foenum-graecum*



Values are mean ± SEM of triplicates

TGL- *Trigonella foenum graecum* leavesTGS- *Trigonella foenum graecum* seeds

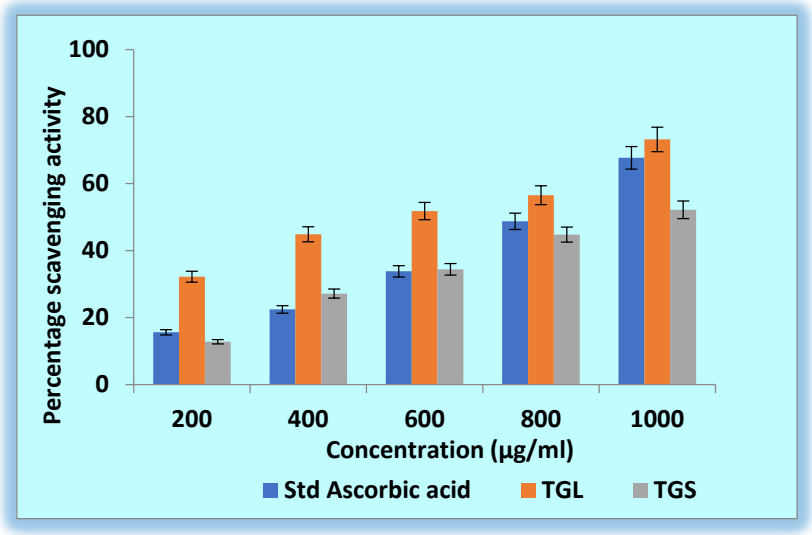
The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to many products. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore and determined at 532 nm. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. Thus, removing hydrogen peroxide as well as O_2^- is very important for protection of food systems [19] and [20] had reported that *Juglans regia L* could reduce oxidative deoxyribose damage in dose dependent manner [4] had reported that ethyl acetate extract of *Calycopteris floribunda* exhibited a maximum hydroxyl radical scavenging activity. The ethyl acetate

extract of *C. floribunda* was found to be more effective than petroleum ether and methanolic extract.

ABTS free radical scavenging activity

To determine the ABTS [2,2--azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid)] radical scavenging activity of *Trigonella foenum graecum* cationic ABTS radical decolorization was carried out. The results are shown in Fig 3.

Figure 3: ABTS radical scavenging activity of *Trigonella foenum-graecum*



Values are mean ±SEM of triplicates

TGL-*Trigonella foenum graecum* leaves TGS-*Trigonella foenum graecum* seeds

The ABTS radical scavenging activity of TGL and TGS and standard ascorbic acid were found to be 73 %, 52 % and 68 %. The scavenging activity was dose dependent in all the extracts. ABTS radical scavenging activity is often used for screening complex antioxidant mixtures such as plant extracts and involves a more drastic radical, chemically produced [21]. ABTS radical is relatively stable but readily reduced by antioxidants. The scavenging activity against cationic ABTS radical indicates the ability of fractions to act as electron donors or hydrogen donors in free radical reactions [22]. The present study results are supported by the findings of [23] who had reported that *Crataegus azarolus* leaf ethyl acetate extracts exhibited a strongest antioxidant activity in ABTS method and suggested that the effect of ethyl acetate extracts might be probably attributed to their high phenolic compounds and flavonoids.

Ethyl acetate and water extracts of *Trigonella foenum graecum* leaves demonstrated its hypoglycemic activity to be mediated through its dose dependent inhibitory activity on carbohydrate hydrolysing enzymes, α-amylase and α-glucosidase [24].

Summary and Conclusion

Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. The present study demonstrates that *Trigonella foenum graecum* leaves and *Trigonella foenum graecum* seeds showed a concentration-dependent inhibition of free radicals The various antioxidant mechanisms of *Trigonella foenum-graecum* may be attributed to their effectiveness as good scavengers of nitric oxide, hydroxyl radical and ABTS free radicals. This may be due to the active compounds present in ethyl acetate extracts. Hence, *Trigonella foenum graecum* leaves and *Trigonella foenum graecum* seeds might be useful in the control of hyperglycaemia without any side effects. Due to its potent antioxidant properties this plant-based diet might protect against chronic oxidative stress-related diseases and in prevention of complications in diabetes.

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