Trigonella Foenum Grecum L. Seed (Fenugreek) Pharmacological Effects on Cardiovascular and Stress Associated Disease

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ABSTRACT

The major goal of this study is to determine the antioxidant activity of *Trigonella foenumgrecum* L. seeds. The importance of the bioactive chemicals discovered by GC-MS in these seeds. Standard methods for determining antioxidant activity included the 2,2-diphenyl1-picryl-hydrazyl (DPPH), Superoxide dismutase (SOD), and Catalase (CAT) tests. By producing a graph of percent inhibition versus concentration, the concentration of the sample necessary to scavenge 50% of free radicals was estimated2.8741 and 3.8372 were reported to be the IC_{50} values for 2,2-diphenyl 1-picryl-hydrazyl(DPPH) radical activity in aqueous and methanolic extracts, respectively. The number is almost identical to the ascorbic acid control value of 2.5391. For aqueous extract, the percent inhibition and specific activity of Catalase (CAT) were found to be 77.38 percent and 34.227 µmoles of Hydrogen peroxide~H_2O_2/min/ml, respectively. For methanolic extract, the percent inhibition and specific activity of Catalase (CAT) were found to be 82.42 percent and 26.0153µmoles of H_2O_2/min/ml, respectively. For aqueous extract, the percent inhibition and specific activity of SOD were found to be 53.21 percent and 1.5843 units/mg, respectively. For methanolic extract, the percent inhibition and specific activity of Superoxidedismutase (SOD) were found to be 61.24 percent and 1.3848 units/mg, respectively. This indicates that the methanolic extract from *Trigonella foenum grecum* L seeds showed greater antioxidant activity than the aqueous extract. The findings of this in-vitro study indicated that this species of *Trigonella foenum grecum* L is suitable for use as a raw medication and is the best treatment for cardiovascular disease (CVD). In-vivo investigations may be conducted in the future to determine the antioxidant capability of these species' seeds in a living system.

KEYWORDS: Apigenin,Antioxidant, Flavonoids, *Trigonellafoenumgrecum*, methi

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1. INTRODUCTION

Heart disease and high blood pressure have become much more common in the last century as a result of changes in human behaviour and lifestyle. These disorders are linked to a greater level of cholesterol in the blood, particularly in the LDL portion [1]. Cardiovascular disease (CVD) kills more than 17 million people worldwide each year. More in-vivo and ex-vivo research have added to the growing body of data that oxidative stress plays a role in a variety of CVDs, including atherosclerosis, ischemia, hypertension, cardiac hypertrophy, and CHF[2]. Antioxidants are physiologically active molecule, they reduce metabolism, which benefits the cells in the long run.It is a popular spice agent for ageing, labour pains, boosting the immune system, improving mental function. It belongs to the Leguminosae family and is called fenugreek in English andmethi dana in english.

Figure. Fenugreek

2. MATERIALS AND METHODS

Reagents for chemistry

Methanol, Na_2CO_3, Sodium bicarbonate ($NaHCO_3$), Ethylenediaminetetraaceticacid (EDTA), Epinephrine, HCL, Sodium di-hydrogen phosphate, Hydrogen peroxide, Dichromate acetic acid, Potassium dichromate, DPPH and other analytical grade chemicals were acquired from Sigma-Aldrich in India.

Phytochemical screening

Standard procedures were employed to identify the preliminary phytochemical elements in prepared extracts of *Trigonella foenumgrecum* L. Carbohydrate Detection: Molish's examination. The presence of carbs was shown by the formation of a violet ring [3]. Amino acid testing: Ninhydrin Test. The presence of amino acid is shown by the formation of blue colour. Proteins should be tested: Xanthoproteic Test. Test fortriterpenoids, do the following: 2ml chloroform was added to the 0.5ml extract and thoroughly mixed. The side test tube has a few drops of strong sulphuric acid. The presence of triterpenoids is shown by the formation of a red brown ring at the intersection of two liquids. To test for coumarins, 1 mL of extract was mixed thoroughly with 1 mL of 10% sodium hydroxide. Tannins can be detected using the following method: Test for ferric chloride. Flavonoids were determined by alkaline reagent. The presence of flavonoids is indicated by the production of a bright yellow colour that fades to colorlessness when dilute acid is added.

Effect on antioxidant parameters

In-vitro evaluation of antioxidant effectiveness of *Trigonella foenumgrecum* L. alcoholic and

aqueous extracts utilising DPPH, SOD, and CAT. The stock solution was made by dissolving 10mg of each dry crude methanolic and aqueous extract from *Trigonella foenumgrecum* L seeds in 1ml of suitable solution, filtering it with a muslin cloth, and storing it for further antioxidant evolution [4].

DPPH Assay

The stable radical DPPH is used as a reagent in this spectrophotometric experiment. The Shimada scavenging activity method was employed to determine DPPH scavenging activity [5]. A 0.1mM DPPH solution was freshly produced, and 1ml of the DPPH solution was added to 3ml of the extracts. Six different amounts of methanolic extracts and aqueous extracts were added to the series of test tubes labelled T1 to T6 from the produced stock solution: 200g/20µl, 400mg/40µl, 600mg/60µl, 800mg/80µl, 1000mg/100µl, and1200mg/120µl.Using methanol, the sample volume of all test tubes was increased to 1ml. All of the test tubes received 1ml of DPPH. As a control, 1ml of methanol and 1ml of DPPH reagent were used and labelled as C. A positive control of ascorbic acid (1.0 mg/ml) was employed. For 25 to 30 minutes, the test tubes were kept in the dark. The purple-colored DPPH is a persistent free radical that can be reduced to the yellow-colored 2,2-diphenyl-1-picrylhydrazine by interacting with an antioxidant. Using a digital spectrophotometer, the optical density was measured and recorded as a decrease in absorbance at 517nm. The proportion of free radical inhibition (DPPH) was computed as follows: Inhibition as a percentage

$$(\text{DPPH}) = \frac{\text{Activity of control} - \text{Activity of extract}}{\text{Activity of the control}} \times 100$$

Superoxide dismutase (SOD) activity

The superoxide dismutase's O_2^- substrate is produced indirectly during the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. Observing the increase in absorbance at 480nm in a spectrophotometer reveals superoxide dismutase's capacity to inhibit. The method of was used to measure SOD activity. 1.5ml 0.1MCarbonate-bicarbonate buffers (pH 10.3), 0.1ml 30mM EDTA, and 5mg/0.5ml methanolic and aqueous extract from the stock solution were included in the 3.0ml reaction mixture (suitable aliquot of enzyme preparation).In the test tubes labelled T1 and T2, the capacity was increased to 2.94ml using double distilled water. The reaction was begun in the test tubes by adding 0.06ml of 15mM epinephrine. All of the materials, save the enzyme preparations, were placed in a test tube labelled C as the control. The blank was made up of 1.5 mL of double distilled water and 1.5 mL of 0.1MCarbonate-bicarbonate buffers, which were placed in a separate test tube labelled B and run concurrently with the test [6]. The reaction was observed by taking measurements of the change in optical density at 480nm every 60 seconds for 3 minutes. One unit of enzyme activity was defined as the amount of extract required to prevent auto-oxidation of epinephrine by 50%. The % inhibition of the free radical (SOD) was computed using the equation below,

$$\text{The \% inhibition} = \frac{\text{Enzyme activity of the control} - \text{Enzyme activity of the extract}}{\text{Enzyme activity of the control}} \times 100$$

Catalase (CAT) activity

When dichromate in acetic acid is heated in the presence of hydrogen peroxide, it is reduced to chromic acetate, with the creation of per-chloric acid as an unstable intermediate. Colorimetrically, the chromic acetate generated was measured at 620nm [7-10]. After adding dichromate to the acetic acid mixture, the reaction was stopped at a specific time. Sinha's approach was used to assess the activity of the enzyme catalase [11].In the test tubes labelled T1 and T2, the reaction mixture contained 1ml of 0.01Mphosphate buffer (pH 7.0), 1mg/0.1ml of methanolic and aqueous extract from the stock solution, and 0.5ml of $2MH_2O_2$. The reaction in the test tubes was stopped by

adding 2ml of dichromate acetic acid reagent at 30 and 60 seconds (5 percent potassium dichromate and glacial acetic acid mixed in the ratio of 1:3). All of the materials, save the enzyme preparations, were placed in a test tube labelled C as the control. The blank, which consisted of 1ml of 0.01M phosphate buffer and 0.5ml of $2MH_2O_2$ in a separate test tube labelled B, was performed concurrently with the test. The response was seen by taking measurements of the change in optical density at 480nm for 1 minute at 30 and 60 second intervals. The enzyme activity was measured in µ moles of H_2O_2 degraded per minute per milligram of extract. The (CAT) free radical inhibition % was computed as follows [11, 12]:

The % inhibition $=\frac{\text{Enzyme activity of the control}-\text{Enzyme activity of the extract}}{\text{Enzyme activity of the control}}\times 100$

3. RESULT

Phytochemical qualitative analysis

Table 1: Preliminary phytochemical screening of Trigonellafoenumgrecum L. methanolic and aqueous extracts.

Phytochemical	Test	Methanol extract	Aqueous extract
Carbohydrates	Molisch's& Benedicts test	+ ve	+ ve
Amino Acid	Ninhydrin test	+ ve	+ ve
Proteins	Xanthoproteic test	+ ve	+ ve
Saponin's	Foam test	+ ve	+ ve
Phenol	Ferric chloride test	+ ve	+ ve
Triterpenoid test	-	+ ve	+ ve
Coumarins test	-	+ ve	+ ve
Quinine's test	-	+ ve	+ ve
Phlobatannin test	-	+ ve	+ ve
Anthraquinones test	-	+ ve	+ ve
Alkaloids	Mayer's & Wagner's test	+ ve	+ ve
Glycosides	Legals&Baljets test	+ ve	- ve
Sterols	-	+ ve	+ ve
Tannins	Ferric chloride test	+ ve	+ ve
Flavonoids	Alkaline & Lead acetate test	+ ve	+ ve
Gum &mucilages	-	+ ve	+ ve

(+ve indicates presence of compounds) and (-ve indicates absence of compounds)

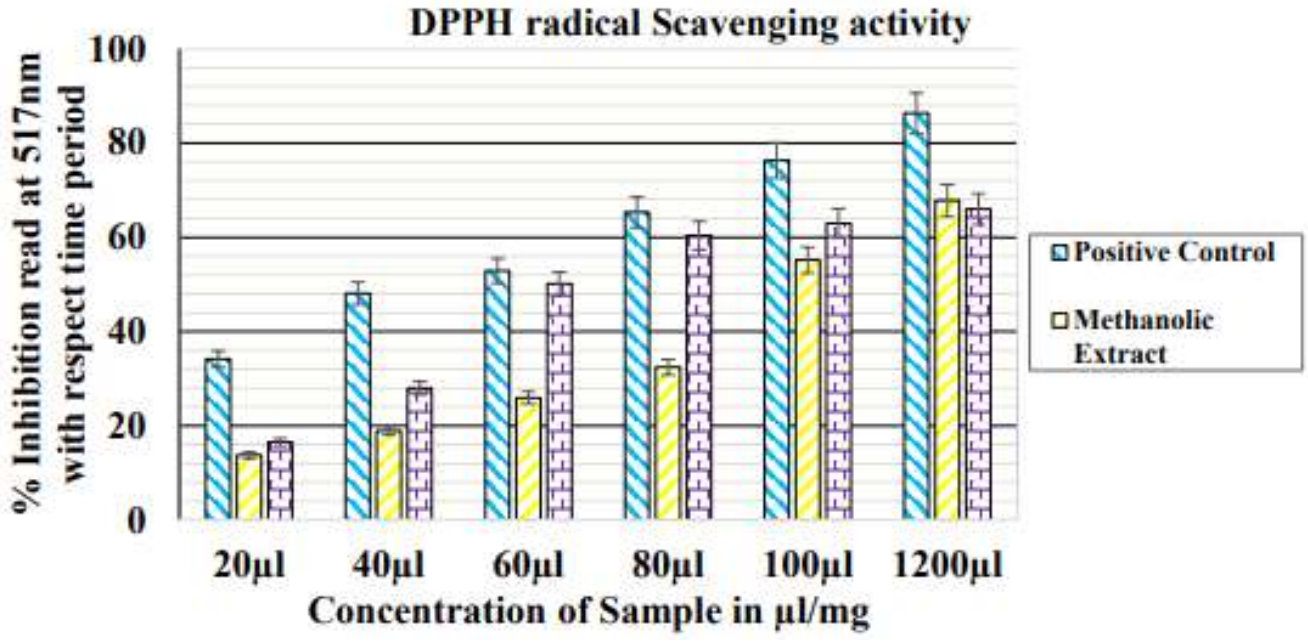
DPPH assay

Table 2: DPPH radical scavenging activity (RSA %) of methanolic extract and aqueous extract from the *Trigonella foenum grecum* L. seeds.

Conc. Of sample		**%Inhibition read at 517nm with respect time**					

		period					
		200µg/20 µl	**400µg/40 µl**	**600µg/60 µl**	**800µg/80 µl**	**1000µg/100 µl**	**1200µg/200 µl**
control	**0.243±0.001**	-	-	-	-	-	-
Ascorbic acid(- ve control)	-	34.18 ±1.005	48.11±0.878	52.86±1.005	65.27±1.051	76.31±1.000	86.32±1.810
Methanolic extract	-	14.76±0.476	17.93±0.945	24.92±0.970	33.42±0.428	54.14±0.970	66.76±0.692
Aq. extract		15.55±0.506	28.95±1.045	51.13±1.001	61.35±1.106	63.91±0.985	64.94±1.003

Graph: 1 DPPH radical scavenging activity (RSA %) of methanolic extract and aqueous extract of *Trigonella foenum grecum* L.



Superoxide Dismutase (SOD) assay

Table 3a: Radical scavenging activity of methanolic and aqueous extracts from *Trigonella foenum grecum* L. seeds using Superoxide Dismutase (SOD).

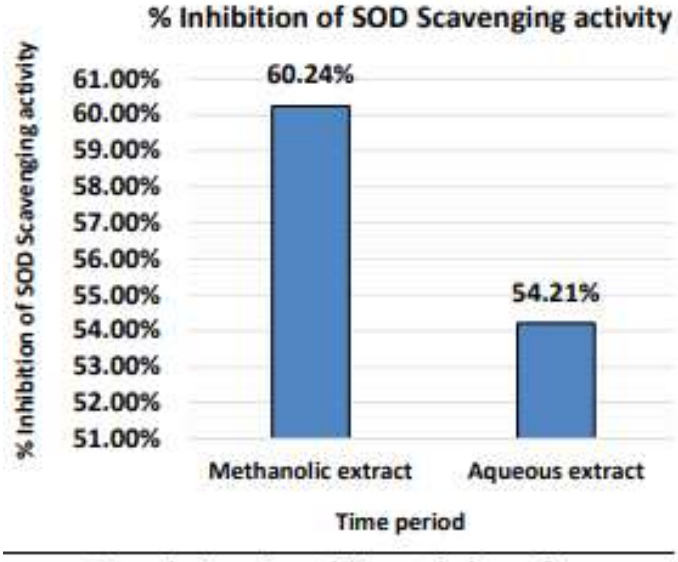
Sample			% Inhibition of SOD Scavenging	

			activity read at 480nmwith respect to time period (0min, 1min, 2min and 3min)	
Time period	0 mins	1 min	2 mins	3 mins
Control	0.035±0 .001	0.062± 0.001	0.074±0 .001	0.085± 0.001
Methanolic extract T-graecum L.	0.046±0 .001	0.052± 0.001	0.057±0 .002	0.067± 0.002
Aqueous extract of T- graecum	0.035±0 .001	0.044± 0.001	0.048±0 .001	0.058± 0.001

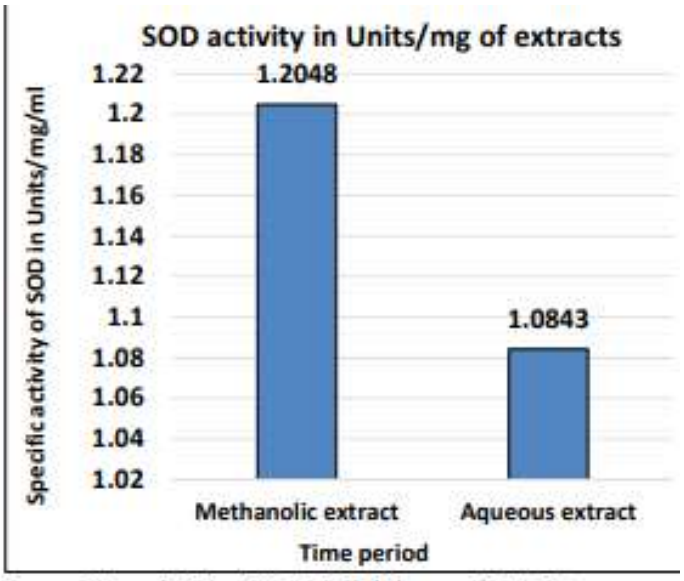
Table 3b: SOD activity for control Methanolic extract and Aqueous extract from the seeds of *Trigonella foenum grecum* L.

Groups	SOD activity in Units/mg	% Inhibition of SOD Scavenging activity
Control	-	-
Methanolic extract	1.3848	61.24%
Aqueous extract	1.5843	53.21%

Graph 2: Superoxide Dismutase (SOD) radical scavenging activity of methanolic extract and aqueous extract from the seeds of *Trigonella foenumgrecum* .



Graph 2a: Specific activity of Superoxide Dismutase



Graph 2b: % Inhibition of SOD Scavenging activity

Catalase (CAT)

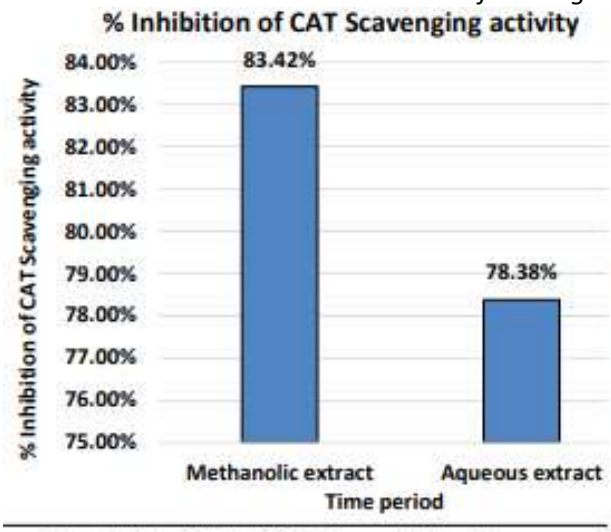
Table 4a: Catalase (CAT) radical scavenging activity of *Trigonella foenum grecum* L. methanolic and aqueous extracts.

Sample	% Inhibition of CAT Scavenging activity read at 480nm with respect to time period.		
Time period	0 sec	30 sec	60 sec
Control	0.369±0.003	0.390±0.004	0.429±0.001
Methanolic extract	0.223±0.001	0.224±0.001	0.229±0.001
Aqueous extract	0.228±0.001	0.231±0.001	0.236±0.001

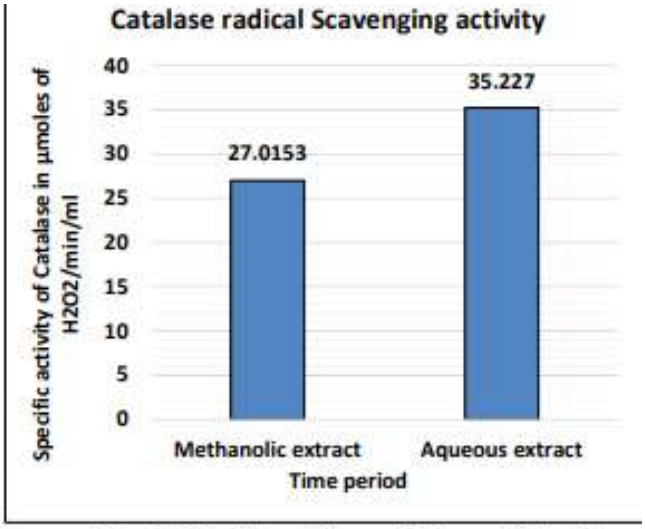
Table 4b: Catalase (CAT) activity in Trigonella foenum grecum L. seeds (control, methanolic extract, and aqueous extract).

Groups	CAT activity in µmoles of H2O2/min/ml	% Inhibition of CAT Scavenging activity
Control	163.9730	-
Methanolic extract	26.0153	84.42%
Aqueous extract	34.2270	77.38%

Graph 3: Catalase (CAT) radical-scavenging activity of methanolic and aqueous extracts from *Trigonella foenumgrecum* L. seeds.



Graph 3a: % Inhibition of Catalase Scavenging activity



Graph 3b: Specific activity of Catalase

Plants' therapeutic value is derived from the phytochemicals material, which is involved in a specific physiological process in the human body .Table 1 shows that both the methanolic and aqueous extracts of *Trigonella foenum grecum* L. contain a significant amount of secondary metabolic products and phytochemical compounds such as carbohydrates, amino acids, proteins, flavonoids, saponins, phenols, triterpenoids, coumarins, quinines, phlobatannins, anthraquinones, alkaloids, sterols, tannins, flavonoids, gum & mucilage. Flavonoids are an important phytochemical ingredient with a wide range of biological actions including anti-inflammatory, antioxidant, and antibacterial

properties. The percent inhibition of (DPPH) radical scavenging activity for methanolic and aqueous extracts from Trigonella foenumgrecum L seeds is shown in table 2 and graph 1. The percent of radical scavenging activity increased dramatically as the concentration of the aqueous and methanolic extracts increased. Aqueous extract has a much higher percent of radical scavenging activity than methanolic extract and control. However, it is more or less the same as that of normal ascorbic acid. The phenolic components included in the extract from the seeds of fenugreek, such as alkaloids, flavonoids, saponins, amino acids, tannins, and certain steroidal glycosides, proteins, and others, may be responsible for the anti-oxidant activity. The IC_{50} value for DPPH of both extracts was calculated in our current study. The IC_{50} values for aqueous and methanolic extracts from *Trigonella foenumgrecum* L seeds range from 2.8741 to 3.8372. The IC_{50} value is almost identical to that of the control ascorbic acid, which is 2.5391.

SOD is a catalytic enzyme that catalyses the dismutation of the superoxide radical (O_2) into H_2O_2 and elemental oxygen, providing a crucial defence against the superoxide radical's toxicity. The percent inhibition of SOD's radical scavenging activity for methanolic and aqueous extracts from Trigonella foenum grecum L seeds is shown in the table (3a, 3b) and graph (2a, 2b). The percent inhibition of SOD enzyme activity for methanolic extract was found to be significantly higher (61.24%) than for aqueous extract (53.21%) from the seeds of *Trigonella foenum grecum* L. in this study.SOD enzyme specific activity for methanolic and aqueous extracts from *Trigonella foenum grecum* L seeds was determined to be 1.3848Units/mg and 1.5843Units/mg, respectively. SOD react with many organic molecules, excessive amounts of this radical, hydrogen peroxide, and the hydroxyl radical, reactive oxygen species (ROS), produce oxidative stress and significant cell toxicity. An imbalance between high amounts of ROS and low cellular antioxidant defences is referred to as "oxidative stress". The percent inhibition of SOD enzyme activity for methanolic extract was found to be significantly higher than that of aqueous extract from *Trigonella foenumgrecum* L. seeds, based on this finding.

Catalase is an antioxidant enzyme found in cells that uses dismutation to eliminate hydrogen peroxide. The percent inhibition of radical scavenging activity of CAT for methanolic and aqueous extracts from Trigonella foenumgrecum L seeds is shown in table (4a, 4b) and graph (3a, 3b). The percent inhibition of CAT enzyme activity for methanolic extract was found to be significantly higher (84.42%) than for aqueous extract (77.38%) from the seeds of Trigonella foenum grecum L. in this study. The specific activity of the CAT enzyme was reported to be 26.0153µmoles and 34.227µmoles of H2O2/min/ml for methanolic and aqueous extract from *Trigonella foenumgrecum* L seeds, respectively. Catalase transforms H_2O_2 to water and molecular oxygen, preventing the Fenton reaction from producing the extremely harmful hydroxyl radical[13].

4. CONCLUSION

In conclusion, *Trigonella foenumgrecum* L seeds are a natural source of dietary antioxidants that are essential in disease prevention, health care, and longevity enhancement. Consuming the seeds of Trigonella foenumgrecum L with a regular diet verifies the presence of nutritional and medicinal phytochemicals in the system, which can effectively prevent the development of ischemic heart disease and stroke, according to this in-vitro study. In addition to Trigonella foenumgrecum L, it imparts flavour to food, has the potential to be used as a value-added ingredient to stabilize food against lipid peroxidation, and has health benefits such as blood pressure reduction. A complete and systematic approach to discovering the active principle molecules for this antioxidant mechanism is being developed in the future.

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