VOLUME 9 ISSUE 1 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 1, 530-537 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Pharmacological Effects of the Seed Extract of *Trigonella foenum-graecum* L. on Cardiovascular and Anxiety Diseases

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Received: 22nd January, 2023; **Accepted:** 11th March, 2023; **Published online:** 28th March, 2023

https://doi.org/10.33745/ijzi.2023.v09i01.058

Abstract: The main objective of this study was to determine the antioxidant activity of *Trigonella foenum-grecum* L. seeds. The significance of the bioactive compounds found in these seeds was evaluated by GC-MS. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), Superoxide dismutase (SOD), and Catalase (CAT) tests are common ways to assess antioxidant activity. The concentration of the sample required to scavenge 50% of free radicals was calculated using a graph of per cent inhibition versus concentration. The IC_{50} values for 2,2-diphenyl 1-picryl-hydrazyl (DPPH) radical activity in aqueous and methanolic extracts were found to be 2.8741 and 3.8372, respectively. The figure is nearly identical to the control value for ascorbic acid, 2.5391. Catalase (CAT) specific activity and per cent inhibition for aqueous extract were found to be 34.227 moles of hydrogen peroxide H_2O_2/min/ml and 77.38 per cent, respectively. Catalase (CAT) specific activity and per cent inhibition for methanolic extract were evaluated to be 26.0153 moles of H_2O_2/min/ml and 82.42 per cent, respectively. The percentage of inhibition and the specific activity of SOD in aqueous extract were found to be 53.21% and 1.5843 units/mg, respectively. Superoxidedismutase (SOD) specific activity and per cent inhibition for methanolic extract were 1.3848 units/mg and 61.24 per cent, respectively. This showed that the antioxidant activity of the *Trigonella foenum-grecum* L. seed methanolic extract was higher than that of the aqueous extract. The results of this *in vitro* study showed that this species of *Trigonella foenum-grecum* L. is the greatest remedy for cardiovascular illness and suitable for use as a raw pharmaceutical (CVD). Further research regarding the antioxidant capacity of the seeds from this species in a living system may be performed *in vivo*.

Keywords: *Trigonella foenum-grecum*, Methi, Apigenin, Antioxidant, Flavonoids, DPPH, Pharmacological

Citation: Tripathi Arpan Kumar, Muztaba Mohammad, Baghel Pragati, Tajane Pravin Sampat, Gupta Sandeep and Chitrapu Prashanti: Pharmacological effects of the seed extract of *Trigonella foenum-graecum* L. on cardiovascular and anxiety diseases. Intern. J. Zool. Invest. 9(1): 530-537, 2023.

https://doi.org/10.33745/ijzi.2023.v09i01.058

Introduction

Due to changes in lifestyle and human behaviour during the past century, heart disease and high blood pressure have grown much more prevalent. A higher blood cholesterol level, especially the LDL fraction, is associated with several illnesses (Mattioli *et al.*, 2020). Almost 17 million individuals die from cardiovascular disease (CVD) worldwide each year. A growing amount of evidence has shown that oxidative stress contributes to a number of CVDs, including atherosclerosis, ischemia, hypertension, cardiac hypertrophy, and CHF (Xiang *et al.*, 2021). In the long run, antioxidants help the cells since they are physiologically active molecules that slow down metabolism. *Trigonella foenum-grecum* is known as fenugreek and methi dana in hindi and is a member of the Leguminosae family. It is a well-known spice remedy for ageing, labour pains, immune system support, and mental function enhancement. The main objective of this study was to determine the antioxidant activity of *Trigonella foenum-grecum* L. seeds. The significance of the bioactive compounds found in these seeds was evaluated by GC-MS.

Materials and Methods

Reagents:

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

Phytochemical screening:

Standard procedures were employed to identify the preliminary phytochemical elements in prepared extracts of *Trigonella foenum-grecum* L.

Carbohydrate Detection (Molish's examination): The presence of carbs was shown by the formation of a violet ring (Sathiya, 2017).

Amino acid testing (Ninhydrin Test): The presence of amino acid was shown by the formation of blue colour.

Test for triterpenoids: 2 ml chloroform was added to the 0.5 ml 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.

Test for coumarins: 1 ml of extract was mixed thoroughly with 1 ml of 10% sodium hydroxide. *Flavonoids*: 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 foenum-grecum* L. methanolic and aqueous extracts was performed utilising DPPH, SOD, and CAT. The stock solution was made by dissolving 10 mg of each dry crude methanolic and aqueous extract from *Trigonella foenum-grecum* L seeds in 1 ml of suitable solution, filtering it with a muslin cloth, and storing it for further antioxidant evolution (Tiwari and Tiwari, 2021).

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 (Nengroo and Rauf, 2019). 1 ml of the DPPH solution (0.1 mM DPPH solution freshly prepared) was added to 3 ml 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: 200 mg/20 µl, 400 mg/40 µl, 600 mg/60 µl, 800 mg/80 µl, 1000 mg/100 µl, and 1200 mg/120 µl. Using methanol, the sample volume of all test tubes was made up to 1 ml. All the test tubes received 1 ml of DPPH. As a control, 1 ml of methanol and 1 ml 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 min, 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 517 nm. The proportion of free radical inhibition (DPPH) was computed as follows:

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

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 480 nm in a spectrophotometer reveals superoxide dismutase's capacity to inhibit. The method was used to measure SOD activity. 1.5 ml 0.1 M Carbonate-bicarbonate buffers (pH 10.3), 0.1 ml 30 mM EDTA, and 5 mg/0.5 ml methanolic and aqueous extracts from the stock solution were included in the 3 ml reaction mixture (suitable aliquot of enzyme preparation). In the test tubes labelled T1 and T2, the capacity was increased to 2.94 ml using double distilled water. The reaction was started in the test tubes by adding 0.06 ml of 15 mM 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 0.1 M Carbonate-bicarbonate buffers, which were placed in a separate test tube labelled B and run concurrently with the test (Hadwan, 2018). The reaction was observed by taking measurements of the change in optical density at 480 nm every 60 seconds for 3 min. One unit of enzyme activity was defined as the amount of extract required to prevent auto-oxidation of epinephrine by 50%. The per cent inhibition of the free radical (SOD) was computed using the equation:

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

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 620 nm (Hadwan, 2018; Yahia *et al.*, 2020; Poddar *et al.*, 2020; Farag *et al.*, 2020). After adding dichromate to the acetic acid mixture, the reaction was stopped at a specific time. The activity of the enzyme catalase was assessed (Yaldiz and Camlica, 2021). In the test tubes labelled T1 and T2, the reaction mixture contained 1 ml of 0.01 M phosphate buffer (pH 7.0), 1 mg/0.1 ml of methanolic and aqueous extract from the stock solution, and 0.5 ml of 2 M H_2O_2. The reaction in the test tubes was stopped by adding 2 ml of dichromate acetic acid reagent at 30 and 60 seconds (5 per cent potassium dichromate and glacial acetic acid in the ratio of 1:3). All of the materials were placed in a test tube labelled C as the control. The blank, which consisted of 1 ml of 0.01 M phosphate buffer and 0.5 ml of 2 M H_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 480 nm for 1 min at 30 and 60 second intervals. The enzyme activity was measured in µ moles of H_2O_2 degraded per min per milligram of extract. The (CAT) free radical inhibition % was computed as follows (Nandi *et al.*, 2019; Yaldiz and Camlica, 2021):

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

Results and Discussion

Phytochemical qualitative analysis:

Plants' therapeutic value is derived from the

Table 1: Preliminary phytochemical screening of *Trigonella foenum-grecum* L. methanolic and aqueous extracts

Phytochemical	Test	Methanol extract	Aqueous extract
Carbohydrates	Molisch's and 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 and Wagner's test	+ ve	+ ve
Glycosides	Legals and Baljets test	+ ve	- ve
Sterols	-	+ ve	+ ve
Tannins	Ferric chloride test	+ ve	+ ve
Flavonoids	Alkaline and Lead acetate test	+ ve	+ ve
Gum and mucilages	-	+ ve	+ ve

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

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 and mucilage. Flavonoids are an important phytochemical ingredient with a wide range of biological actions including anti-inflammatory, antioxidant, and antibacterial properties.

DPPH assay:

The per cent inhibition of (DPPH) radical scavenging activity for methanolic and aqueous extracts from *Trigonella foenum-grecum* L. seeds is shown in Table 2 and Figure 1. The per cent of radical scavenging activity increased dramatically as the concentration of the aqueous and methanolic extracts increased. Aqueous extract has a much higher per cent 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 this study. The IC_{50} values for aqueous and methanolic extracts from *Trigonella foenum-grecum* 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.

Superoxide Dismutase (*SOD*) *assay*:

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 per cent inhibition of SOD's radical scavenging activity for methanolic and aqueous extracts from *Trigonella foenum- grecum* L. seeds is shown in the Tables 3a, 3b and Figures 2a, 2b. The per cent 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. SOD enzyme specific activity for methanolic and

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 517 nm with respect time period**					
		200 µg/ 20 µl	**400 µg/ 40 µl**	**600 µg/ 60 µl**	**800 µg/ 80 µl**	**1000 µg/ 100 µl**	**1200 µg/ 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

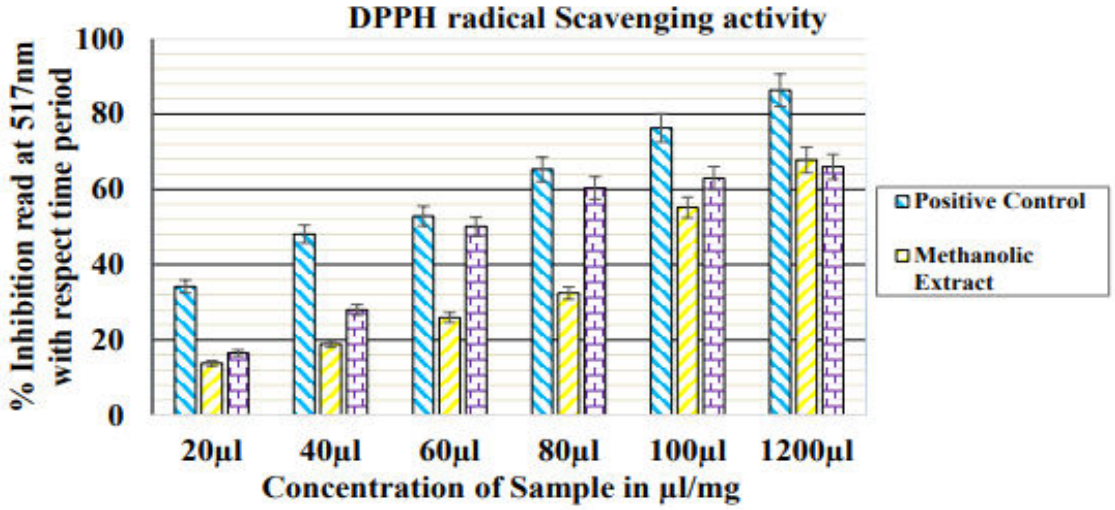


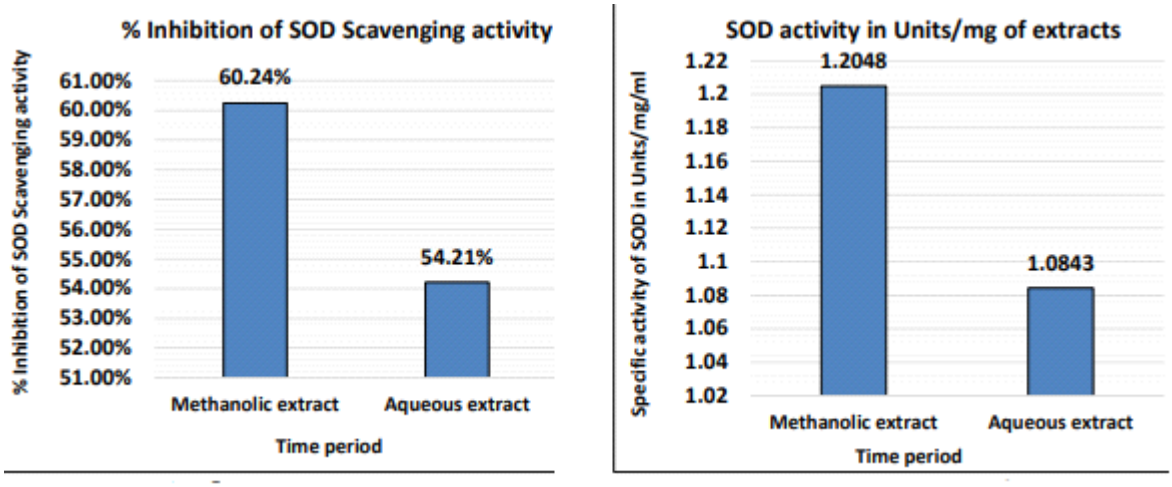
Fig. 1: DPPH radical scavenging activity (RSA %) of methanolic extract and aqueous extract of *Trigonella foenum-grecum* L.

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 480 nm with respect to time period (0 min, 1 min, 2 min and 3 min)	
Time period	0 min	1 min	2 min	3 min
Control	0.035±0 .001	0.062± 0.001	0.074±0 .001	0.085± 0.001
Methanolic extract *Trigonella foenum-grecum*	0.046±0 .001	0.052± 0.001	0.057±0 .002	0.067± 0.002
Aqueous extract of *Trigonella foenum-grecum*	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%



(a) (b)

Fig. 2: Superoxide Dismutase (SOD) radical scavenging activity of methanolic extract and aqueous extract from the seeds of *Trigonella foenum-grecum*.

aqueous extracts from *Trigonella foenum-grecum* L. seeds was determined to be 1.3848 Units/mg and 1.5843 Units/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 per cent inhibition of SOD enzyme activity for methanolic extract was found to be significantly higher than that of aqueous extract from *Trigonella foenum-grecum* L. seeds, based on this finding.

Catalase (*CAT*):

Catalase is an antioxidant enzyme found in cells that uses dismutation to eliminate hydrogen peroxide. The per cent inhibition of radical scavenging activity of CAT for methanolic and aqueous extracts from *Trigonella foenum-grecum* L. seeds is shown in Tables 4a, 4b and Figures 3a, 3b. The per cent 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 26.0153 µmoles and 34.227 µmoles of H_2O_2/min/ml for methanolic and aqueous extract from *Trigonella foenum-grecum* L. seeds, respectively. Catalase transforms H_2O_2 to water and molecular oxygen, preventing the Fenton reaction from producing the extremely harmful hydroxyl radical (Gebicka and Krych-Madej, 2019).

Conclusion

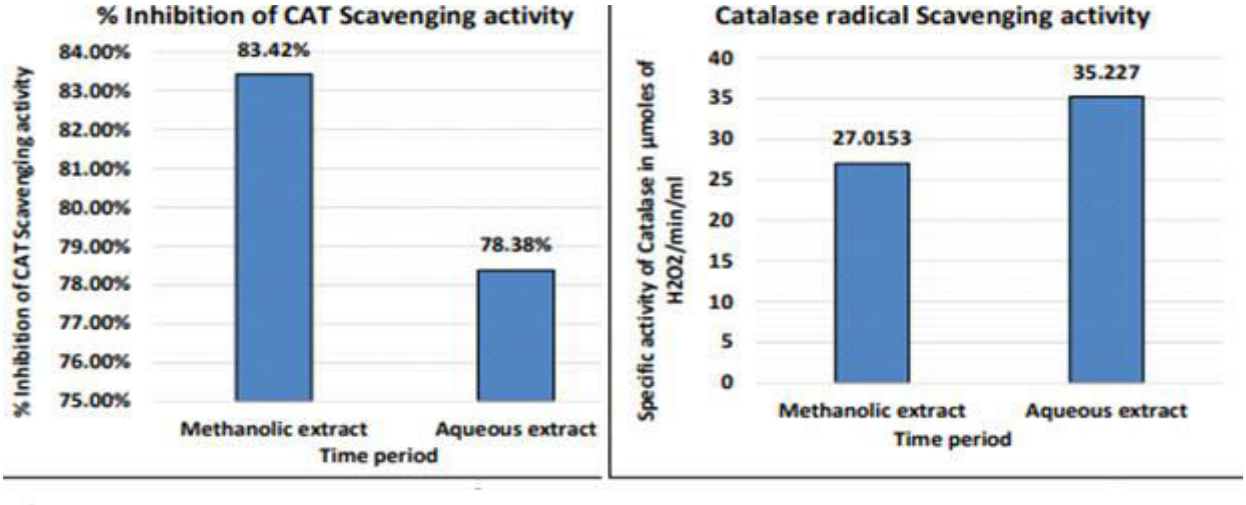
In conclusion, *Trigonella foenum-grecum* L. seeds are a natural source of dietary antioxidants that are essential in disease prevention, health care, and longevity enhancement. Consuming the seeds

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

Sample	% Inhibition of CAT Scavenging activity read at 480 nm 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 H_2O_2/min/ml	% Inhibition of CAT Scavenging activity
Control	163.9730	-
Methanolic extract	26.0153	84.42%
Aqueous extract	34.2270	77.38%



(a) (b)

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

of *Trigonella foenum- grecum* 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 foenum- grecum* 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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