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Pharmacological Effects Of Trigonella Foenum Grecum L. Seeds On Cardiovascular And Antioxidant Stress Related Disease

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Abstract: In this present study we mainly focus to find out the antioxidant activity of Trigonella foenum grecumL seeds. In order to provide valuable documentation that supports and strengthens the antioxidant efficacy, we attempt to compare the clinical significance of the bioactive compounds already identified by GC-MS from these seeds. DPPH, SOD and CAT assays were used as standard methods to determine antioxidant activity. The concentration of the sample required to scavenge 50% of free radical, was calculated by plotting a graph between % inhibition vs concentration. The IC_{50} value of DPPH radical activity for aqueous and methanolic extract was found to be 3.8741 and 4.8372.The value is almost in the range of control ascorbic acid 3.1391.The % inhibition and specific activity ofCAT for aqueous extract was found to be 78.38% and 35.227µmolesof H_2O_2/min/ml.The% inhibition and specific activity ofCAT for methanolic extract was found to be 83.42% and 27.0153µmoles of H_2O_2/min/ml. The % inhibition and specific activity of SOD for aqueous extract was found to be 54.21% and 1.0843Units/mg. The % inhibition and specific activity of SOD for methanolic extract was found to be 60.24% and1.2848Units/mg. This result suggests that the methanolic extract from the seeds of Trigonella foenum grecum L had a more powerful antioxidant activity than the aqueous extract. The result of this in-vitro study confirmed that this species of Trigonella foenum grecum L authenticated to uses as raw drug, and definitely acts as best remedy to treat cardiovascular disease. In the future, in-vivo experiments may be performed to determine the antioxidant potential of these seeds of this species in the living system.

KEY WORDS: Trigonella foenum grecum, Flavonoids, Tannin, Apigenin, DPPH, SOD, CAT.IC_{50} value.

1. INTRODUCTION

Over the past century changes in human behaviour and lifestyle have led to a dramatic increase in the incidence of heart disease and high blood pressure. These illnesses are correlated with a higher concentration of cholesterol in serum, especially with cholesterol in low-density lipoprotein (LDL) fraction [2]. According to the world health organization,

cardiovascular disease (CVD) causes more than 17 million deaths in the world each year. CVD has a variety of protocols associated with various risk factors for its development, including hypercholesterolemia, hypertension, smoking habit, diabetes, poor diet, obesity or over weight, physical inactivity amongst others and stress related disease [3, 4, 5]. Recently, research data has raised a passionate debate as to whether oxidative stress is a primary or secondary cause of many cardiovascular diseases [5]. Further *in-vivo* and *ex-vivo* studies have provided precious evidence supporting the role of oxidative stress in a number of CVDs such as atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy and congestive heart failure [3, 4, 5, 6]. Antioxidants are biologically active substances that are used to protect our health from cell damage by eliminating free radicals.The indicated radicals can derive from chemicals by both synthetic and natural ones that immigrate the body from food, drinks and adulterated air. They pare the metabolism, which ultimately better the cells **[7].** Therefore, the convenient source of antioxidants in everyday life helps us to repel various diseases. Sources of antioxidant seeds, fruits, vegetables and green leafy vegetables mainly increase antioxidant potency.In the Indian system of medicine *Trigonella foenum grecum* is an important medicinal plant, its seeds and leaves have been used in various ailments and as a health tonic. It belongs to the Leguminosae family and is called fenugreek in English and Vendayam in Tamil, is a popular spice agent for aging, labour pains, boosting the immune system, improving mental function and adding vitality to the body and is also used for nervous disorders, dyspepsia, inflammation, tumours,cholesterolemic, hyperglycemic and ulcer [8].We are eagerly engaged in this research work from the above literature and are trying to provide additional information on the therapeutic efficacy of *Trigonella foenum grecum* L for free radical related diseases therapy for human benefit.

2. MATERIALS AND METHODS

Chemical reagents

1,1-Dipheny-2-Picryl-hydrazyl (DPPH), Methanol, Sodium carbonate, Sodium bicarbonate, EDTA, Epinephrine, Hydrochloric acid, Sodium di-hydrogen phosphate, Di-Sodium hydrogen phosphate, Hydrogen peroxide, Dichromate acetic acid, Potassium dichromate, etc. are the analytical grade purchased from Sigma-Aldrich, India.

Plant material

Fresh dried seeds of *Trigonella foenum grecum* L. were commercially purchased from Nilgiris market Tambaram, Kanchipuram district, Tamil Nadu. The samples were approved on the basis of organoleptic and macroscopic examination and certified by the department staff.

Preparation of methanolic and aqueous extract

Trigonella foenum grecum L. seeds 500gm coarse powder and divided into two equal parts. They were then soaked separately in methanol and distilled water for 72 hours. Extracts were filtered by passed through Whatman No.1filter paper. Then both the filtrate was extracted with soxhlet apparatus, filtered and concentrated to dry [9].The last traces of solvents were removed by transferring the extract into a china dish. Allow the china dish to warm up by using a sand bath at normal temperature, carefully in order to prevent charring and denaturation of compounds caused by overheating. The yield of methanolic extracts 5gm

and aqueous extract 10gm extracts were noted. Dried crude extracts were kept in sterile amber coloured storage vials in refrigerator until used for my further studies.

Phytochemical screening

The formulated extracts of *Trigonella foenum grecum* L. were used for identifying the preliminary phytochemical constituents as described by standard methods [10,11,12].Test for Carbohydrates: Molish's test: To 2ml of extract, add two drops of alcoholic solution of α-naphthol and mixed well. Few drops of concentrated sulphuric acid were added along the sides of test tube. A violet ring was formed, which indicates the presence of carbohydrates. Benedict's test: To 0.5ml of extract, 0.5ml of Benedict's reagent was added and mixed well and kept in boiling water bath for 2 minutes. A characteristic-coloured precipitate was formed which indicates the presence of sugar.Test for amino acids: Ninhydrin Test: To the 0.5ml of extract, 0.25% w/v ninhydrin reagent was added, mixed well and kept in boiling water bath for few minutes. Formation of blue colour indicates, the presence of amino acid [13].Test for proteins: Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins [14].Test for saponins: Foam Test: 0.5gm of extract was mixed with 2ml of water and shaken well. If foam produced persists for ten minutes it indicates the presence of saponins [15].Test for phenol: Ferric Chloride Test:0.5gm of extract was mixed with3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [16].Test for triterpenoids: To the 0.5ml of extract 2ml of chloroform was added and mixed well. Few drops of concentrated sulphuric acid were at the side test tube. Formation of red brown coloured ring at the junction of two liquids indicates presence of triterpenoids [17].Test for coumarins: To 1ml of extract, 1ml of 10% sodium hydroxide was added and mixed well. Yellow colour will be appeared which indicates the presence of coumarins. Test for quinines: To 1ml of extract, 1ml of concentrated sulphuric acid was added slowly. Red colour will be formed which indicates the presence of quinines. Test for phlobatannin: To 1ml of extract, 1% hydrochloric acid and kept in boiling water bath. Formation of red coloured precipitate indicated the presence of pholobatannin. Test for anthraquinones: To 1ml of extract few drops of 10% ammonia solution was added and the appearance pink coloured precipitate indicated the presence of anthraquinones.Test for alkaloids (Mayer's test): To a few ml of extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids [18]. Wagner's test: A few drops of Wagner's reagent are added to a few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive [19].Test for glycosides (Legal's Test): Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Pink to blood red colour was formed, which indicates the presence of cardiac glycosides. Bail's test: Take a sample extract add sodium picrate reagent. If yellow to orange colour appeared it indicates the presence of glycoside.Test for sterols: To 1ml of extract, 1ml of chloroform was added, followed by a few drops of acetic anhydride and then 2 drops of concentrated sulfuric acid were added along the side of the test tube. Green colour solution appeared which indicates the presence of sterols.Test for Tannins: Ferric chloride test: To of plant extract, 1% ferric chloride was added and mixed well. Dark blur or greenish black colour was formed which indicated the presence of tannins[16].Test for Flavonoids:0.5gm of extract was mixed with a few drops of sodium hydroxide solution (alkaline reagent). The formation of an intense yellow colour, which becomes colourless on addition of dilute acid, which indicates the presence of flavonoids.Test for flavonoids (Lead acetate test: 0.5gm of extract was mixed with a few drops of lead acetate solution. Yellow coloured precipitate formed which indicates

the presence of flavonoids.Test for gum and mucilage: The extract (100mg) is dissolved in 10ml of distilled water and to this 2ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilages [20].

Effect on antioxidant parameters

Determination of antioxidant efficacy from alcoholic and aqueous extract of *Trigonella foenum grecum* L. using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Superoxide Dismutase (SOD), and Catalase (CAT) by *in-vitro* model. The stock solution was prepared by dissolving 10mg of each dry crude methanolic and aqueous extracts from *Trigonella foenum grecum* L seeds in 1ml of appropriate solution, filtered by a using muslin cloth and ready to proceed with further antioxidant evolution.

DPPH Assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a reagent [21].For the determination of DPPH scavenging activity method of Shimada [22],was used 0.1mM solution of DPPH was freshly prepared and 1ml of the DPPH solution was added to 3ml of the extracts. From the prepared stock solution six different concentrations of the methanolic extracts and aqueous extracts200µg/20µl,400mg/40µl, 600mg/60µl, 800mg/80µl,1000mg/100µl and 1200mg/1200µl were added to the series of test tubes labelled as T_1 to T_6. Sample volume of all test tubes were made up to 1ml by using methanol.1ml of DPPH was added to all the test tubes. 1ml of methanol and 1ml of DPPH reagent was taken as control and labelled as C. Ascorbic acid (1.0mg/ml) was used as a positive control.The test tubes were kept in the dark condition for 25 to 30 minutes. The purple-colored DPPH is a stable free radical, reduced to 2,2-diphenyl-1-picrylhydrazine (Yellow colored) by reacting with an antioxidant. The optical density was measured by thedecrease in absorbance at 517nm using digital spectrophotometer and recorded. The percentage inhibition of free radical (DPPH) was calculated as under:The % Inhibition

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

Superoxide dismutase (SOD) activity

The O_2^- substrate for the superoxide dismutase is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O_2^- builds in the solutions, the formation of adrenochrome accelerates because O_2^- reacts with epinephrine to form adrenochrome.Superoxide dismutase reacts with O_2 formed during epinephrine oxidation and therefore slows down the rate of formation of adrenochrome. The ability of superoxide dismutase to inhibit is found by observing the increase in absorbance at 480nm in spectrophotometer.SOD activity was assayed by the method of [23].3.0ml of reaction mixture contained 1.5ml 0.1MCarbonate-bicarbonate buffers (pH 10.3), 0.1ml 30mM EDTA, 5mg/0.5ml of methanolic and aqueous extract from the stock solution (suitable aliquot of enzyme preparation). The volume was made up to 2.94ml using double distilled water in the test tube labelled as T_1 and T_2.The reaction in the test tubes were started by addition of 0.06ml of 15mM epinephrine. The control consists of all the ingredients, except enzyme preparations was taken in a test tube labelled as C.The blank consists of 1.5ml of double distilled water and 1.5ml of 0.1MCarbonate-bicarbonate buffers was taken in another test tube labelled as B, were run simultaneously along with the test.The reaction was observed by

measuring the change in optical density at 480nm for 3 minutes at every 60sec interval. One unit of enzyme activity was defined which caused 50% inhibition of auto-oxidation of epinephrine by 1ml of extracts. The percentage inhibition of (SOD) free radicalwas calculated as under the following equation: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

This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of per-chloric acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 620nm. The catalase preparation is allowed to split hydrogen peroxide for a different period of time. The reaction stopped at a particular time by the addition of dichromate in acetic acid mixture and the remaining hydrogen peroxide is determined by measuring chromic acetate after heating the reaction mixture.The enzyme catalase activity was determined by the method of Sinha[24].Total volume of reaction mixture is 1.6ml which 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 $2MH_2O_2$ in the test tube labelled as T_1 and T_2. The reaction in the test tubes were stopped at 30sec and 60sec by the addition of 2ml of dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in the ratio of 1:3).The control consists of all the ingredients, except enzyme preparations was taken in a test tube labelled as C.The blank consists of 1ml of 0.01Mphosphate buffer and 0.5ml of $2MH_2O_2$ was taken in another test tube and labelled as B, were run simultaneously along with the test. The reaction was observed by measuring the change in optical density at 480nm for 1minuites at every 30sec and 60secinterval.The enzyme activity was expressed as µmoles H_2O_2 decomposed/min/mg of extract.The percentage inhibition of (CAT) free radical was calculated as under:The % inhibition = $\frac{\text{Enzyme activity of the control} - \text{Enzyme activity of the extract}}{\text{Enzyme activity of the control}} \times 100$

Statistical work

Each copy of the measurements was taken in three different standardinstructions and the mean values were recorded. The standard error of the mean (SEM) can be calculated using the following equation: $s_{\bar{x}} = \frac{S}{\sqrt{N}}$based on the SEM. The following are confidence intervals at different confidence levels.Depending on the field of study, 95% confidence level (or 5% statistical significance) is commonly used for data representation.

Sample Standard Deviation

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x}$$

Where: x_i is one sample value.

$\bar{x}$ is the sample mean

N is the sample size

Mean($\bar{x}$)= $\frac{\sum x}{n}$

3. RESULT AND DISCUSSION

Preliminary phytochemical qualitative analysis

Table 1: Preliminary phytochemical screening of methanolic and aqueous extract of Trigonella foenum grecum L

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)

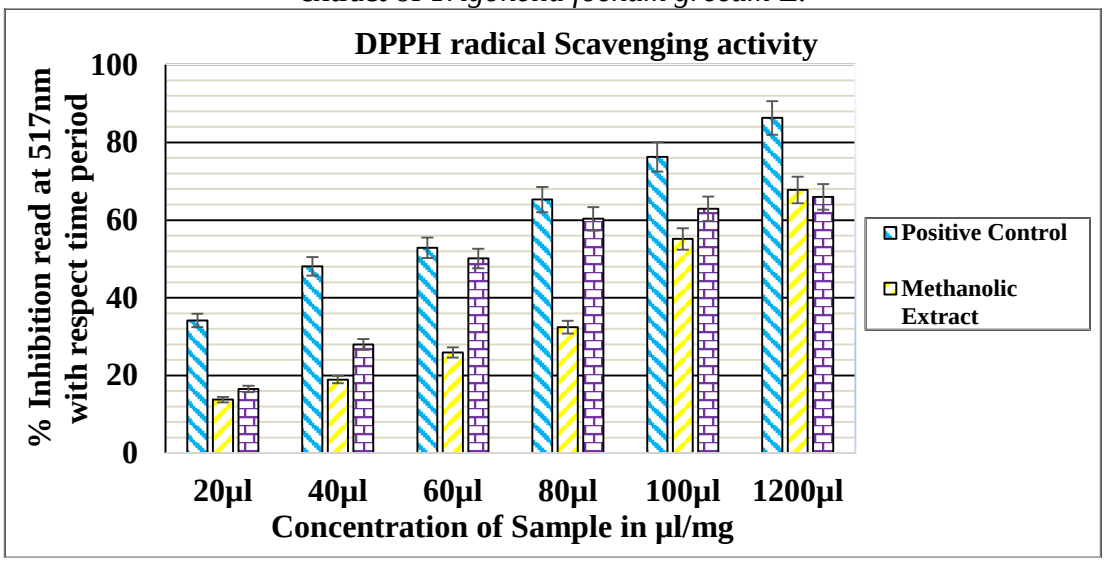
DPPH assay

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

Concentration of Sample	% Inhibition read at 517nm with respect time period						
		200 µg / 20µl	400 mg / 40µl	600 mg / 60µl	800 mg / 80µl	1000mg / 100µl	1200mg / 1200µl
Control	0.243±0.001	-	-	-	-	-	-
Ascorbic acid (-Ve control)	-	34.17±1.005	48.10±0.878	52.87±1.005	65.28±1.051	76.30±1.000	86.31±1.810
Meth	-	13.7	18.9	25.9	32.4	55.1	67.7

anolic extract		**6±0.476**	**3±0.945**	**2±0.970**	**2±0.428**	**4±0.970**	**6±0.692**
Aqueous extract	**-**	**16.55±0.506**	**27.95±1.045**	**50.13±1.001**	**60.35±1.106**	**62.91±0.985**	**65.94±1.003**

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



Superoxide Dismutase (SOD) assay

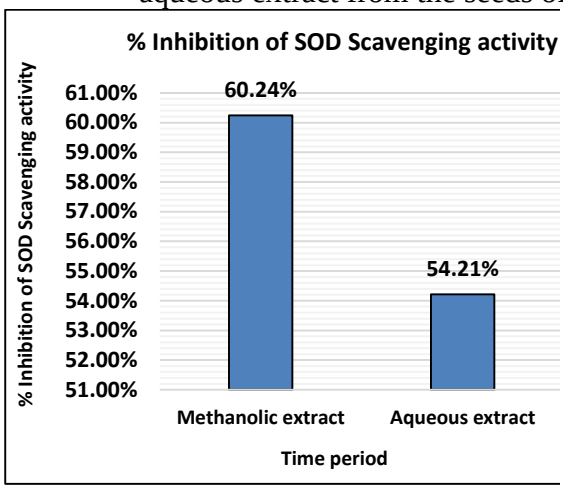
Table 3a: Superoxide Dismutase (SOD) radical scavenging activity of methanolic extract and aqueous extract from the seeds of Trigonella foenum grecum L.

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	**0.034±0.001**	**0.061±0.001**	**0.073±0.001**	**0.084±0.001**
Methanolic extract *T-graecum* L.	**0.045±0.001**	**0.051±0.001**	**0.058±0.002**	**0.065±0.002**
Aqueous extract of *T-graecum* L.	**0.034±0.001**	**0.043±0.001**	**0.049±0.001**	**0.057±0.001**

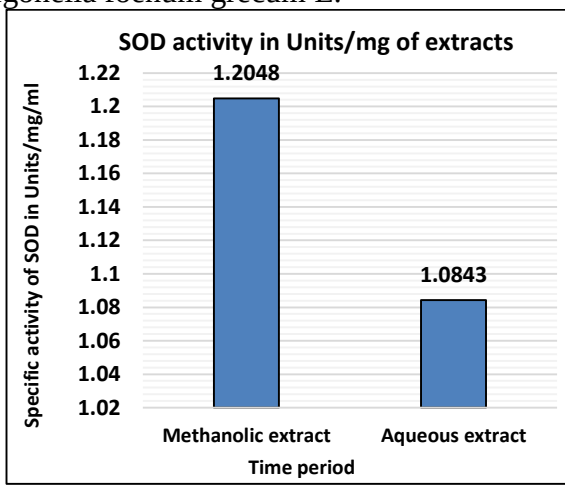
Table 3b: SOD activity for control Methanolic extract and Aqueous extract from the seeds ofTrigonella foenum grecum L.

Groups	**SOD activity in Units/mg**	**% Inhibition of SOD Scavenging activity**
Control	**-**	**-**
Methanolic extract	**1.2048**	**60.24%**
Aqueous extract	**1.0843**	**54.21%**

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



Graph 2a: Specific activity of Superoxide Dismutase



Graph 2b: % Inhibition of SOD Scavenging activity

Catalase (CAT)

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

Sample	**% Inhibition of CAT Scavenging activity read at 480nm with respect to time period.**		
Time period	**0 sec**	**30 sec**	**60 sec**
Control	**0.368±0.003**	**0.391±0.004**	**0.428±0.001**
Methanolic extract	**0.222±0.001**	**0.225±0.001**	**0.228±0.001**
Aqueous extract	**0.227±0.001**	**0.232±0.001**	**0.235±0.001**

Table 4b: Catalase (CAT) activity for control, Methanolic extract and Aqueous extract from the seeds ofTrigonella foenum grecum L.

Groups	**CAT activity in µmoles of H_2O_2/min/ml**	**% Inhibition of CAT Scavenging activity**
Control	**162.9730**	**-**
Methanolic extract	**27.0153**	**83.42%**
Aqueous extract	**35.2270**	**78.38%**

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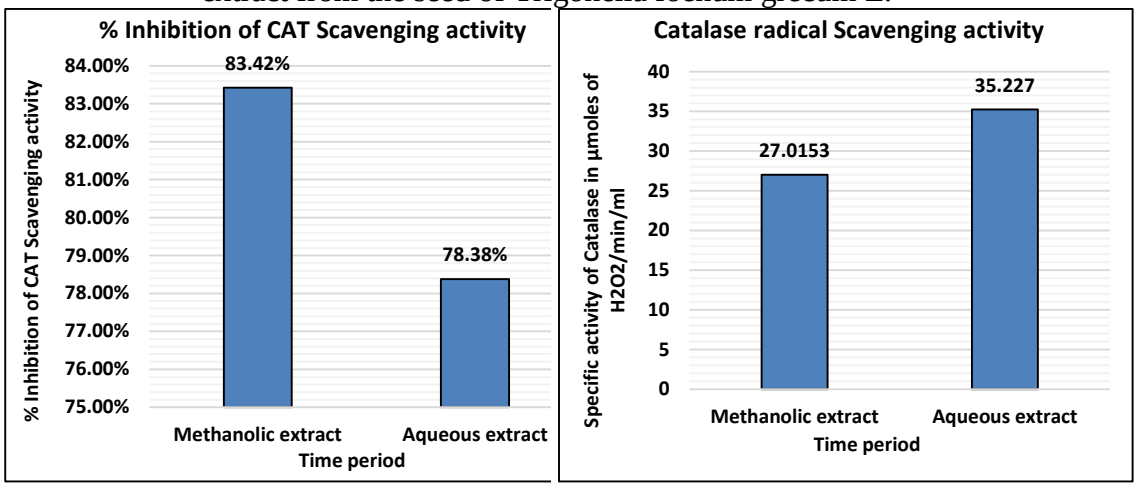
Graph 3: Catalase (CAT) radical scavenging activity of methanolic extract and aqueous extract from the seed of Trigonella foenum grecum L.



Graph 3a: % Inhibition of Catalase Scavenging activity

Graph 3b: Specific activity of Catalase

The medicinal value of plants lies in the phytochemicals substance that forms a definite physiological process in the human body. The most important bioactive compounds in plants are alkaloids, flavonoids, tannins and phenolic compounds [25].Table 1 shows that both methanolic and aqueous extract of *Trigonella foenum grecum* L. reveals 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 & mucilages. As previously reported, tannins, flavonoids and phenolic compounds are the main group of phytochemicals that act as primary antioxidants or free radical scavengers [26]. Among the phytochemical constituent, flavonoids are an important phytochemical constituent, exhibits a large number of biological activities like anti-inflammatory, antioxidant and antimicrobial properties [27]. In this current study, both methanolic and aqueous extracts from the seeds of *Trigonella foenum grecum* L. contain extensive secondary metabolites. Hence the result obtains from this preliminary screening of phytochemical emphasize that seeds of *Trigonella foenum grecum* L. is a naturally occurring pharmacological agent and may provide scientific support for the folklore claims on the use of plant materials for treatment of numerous diseases in traditional medicine.

DPPH usually refers to a free radical, its practicality act as a scavenger is positioned based on the transmission of electrons from one molecule to another molecule. Its crystals are insoluble in water but when dissolved in methanol it appears as a violet tinted solution [28]. The DPPH serves as a simple and quick mode for evaluating oxidants by spectrophotometry.The rule in the background of the assay is the decrement of DPPH by virtue of the appearance of hydrogen devoting antioxidant [29]. The table 2 and graph 1 shows the % inhibition of (DPPH) radical scavenging activity for methanolic and aqueous extract from the seeds of *Trigonella foenum* grecum L. As the concentration of the aqueous and methanolic extract increases, the % of radical scavenging activity also significantly increased. The % of radical scavenging activity for aqueous extract was found to be significantly higher than that of methanolic extract and control. But more or less similar to that of standard ascorbic acid. The anti-oxidant activity could be associated with the phenolic

components present in the extract from the seeds of fenugreek [30], like alkaloids, flavonoids, saponins, amino acids, tannins and some steroidal glycosides, proteins etc, [31]. In general flavonoids are widely known for their antioxidant properties, various literature studies reported that apigenin one among the flavonoid's possesses excellent antioxidant properties[32]. In addition to that they have anti-hyperglycemic[33], anti-inflammatory [34], and (in myocardial ischemia) anti-apoptotic effects [34], Flavonoids are polyphenolic compounds that are found everywhere in plant foods. Flavonols and flavones are subclasses of flavonoids [35,36,37, 38]. Early report says that average daily intake of flavonols (quercetin, kaempferol, and myricetin) and flavones (apigenin and luteolin) rich diet [39],Intake of these five dietary flavonoids was associated with a reduced risk of ischemic heart disease and stroke in several cases [40,41,42]. Earlier through GC-MS analysis we isolated a compound called apigenin from the seeds of *Trigonella foenum grecum* L, which is usually dietary flavonoid that has many physiological functions such as anti-inflammatory, antioxidant and antibacterial activity. It has health-promoting properties such as lowering blood pressure [43]. In this current research work, the IC_{50} value for DPPH of both the extract was calculated. The IC_{50} value range between 3.8741 to 4.8372 for aqueous extract and methanolic extract from the seeds of *Trigonella foenum grecum* L. The IC_{50} value is almost in the range of control ascorbic acid 3.1391.The very good antioxidant activity of the extracts for DPPH may be probably due to the presence of phytochemical compounds with an available hydroxyl group.

Superoxide Dismutase (SOD) catalyses the dismutation of the superoxide radical (O_2) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2) and as such provides an important defence against the toxicity of the superoxide radical [44]. The table (3a, 3b) and graph (2a, 2b) show the % inhibition of radical scavenging activity of SOD for methanolic and aqueous extract from the seeds of *Trigonella foenum grecum* L. In this present study, it was found that the % inhibition of SOD enzyme activity for methanolic extract was found to be significantly higher (60.24%) than that of aqueous extract (54.21%) from the seeds of *Trigonella foenum grecum* L. The specific activity of SOD enzyme for methanolic and aqueous extract from the seeds of *Trigonella foenum grecum* L was found to be 1.2848Units/mgand 1.0843Units/mg. Earlier report says that superoxide dismutase is a biological antioxidant enzyme that directly scavenges free radicals or prevent their conversion to toxic foodstuffs[45, 46]. High levels of this radical, hydrogen peroxide, and the hydroxyl radical, reactive oxygen species (ROS) cause oxidative stress and high cell toxicity because they react with many organic molecules. The term "oxidative stress" is referred to an imbalance between high levels of ROS and low cellular antioxidant defences [47]. In physiological conditions, the superoxide dismutase, together with the non-enzymatic ROS scavengers such as vitamins tocopherol, retinol, and ascorbic acids, which maintain a steady state between oxidant and antioxidant systems [48]. Variation in redox homeostasis is determined by the imbalance between ROS production and scavenging capacity, which determines significant cellular damage such as membrane lipoperoxidation, nucleic acid, and neurotransmission and structural changes in proteins that contribute to cardiovascular diseases.Superoxide dismutase enzyme is an important cellular antioxidant enzyme, which converts the superoxide radical (O^{-}_2) into either hydrogen peroxide (H_2O_2) or ordinary molecular oxygen (O_2) [49]. From this result it was found that the % inhibition of SOD enzyme activity for methanolic extract was found to be significantly higher than that of aqueous extract from the seeds of *Trigonella foenum grecum* L.

Catalase is an antioxidant enzyme ubiquitous in mammalian and non-mammalian cells that destroy hydrogen peroxide by dismutation. Eukaryotic catalases are heme enzymes found in high concentrations in the liver, kidney, and erythrocytes, while low concentrations in connective tissues and also concentrated in the peroxisome subcellular organelles. The estimation of catalase was performed by the standard method [24]. The table (4a, 4b) and graph (3a, 3b) show the % inhibition of radical scavenging activity of CAT for methanolic as well as aqueous extract from the seeds of *Trigonella foenum grecum* L. In this present study, it was found that the % inhibition of CAT enzyme activity for methanolic extract was found to be significantly higher (83.42%) than that of aqueous extract (78.38%) from the seeds of *Trigonella foenum grecum* L. The specific activity of CAT enzyme for methanolic and aqueous extract from the seeds of *Trigonella foenum grecum* L was found to be 27.0153µmoles and 35.227µmoles of H_2O_2/min/ml. Previously we had reported that the seed of *Trigonella foenum grecum* L contains bioactive compound such as Glucopyranoside, α-D-Glucopyranoside and 3,5-Decadiyne, 2,2-dimethyl (2,2-dimethyldeca-3,5-diyne) and etc., which exhibits strong antioxidant, anti-inflammatory, anti-tumour and anti-diabetic activity [43].As previously reported, fenugreek extract exhibits antioxidant property [50], which protects the functional organs and thereby increases body weight. Catalase is one of the biological antioxidant enzymes that either directly scavenge the free radicals or prevent their conversion to toxic products [45,46]. Catalase converts H_2O_2 to water and molecular oxygen, thus preventing the formation of extremely dangerous hydroxyl radical from H_2O_2 via the Fenton reaction [51].

Epidemiological studies have suggested positive associations between the consumption of phenolic-rich, foods or beverages and the prevention of ailments. The effects have been attributed to antioxidant components such as phenolics, flavonoids and phenylpropanoids among others [52]. The seeds of *Trigonella foenum grecum* L eaten in winters as to improve immunity and protects heart, brain and other vital organs of the body through its medicinal properties. The recent researches have proved it beneficial for atherosclerosis, constipation, diabetes, high cholesterol and hyper-triglyceridemia. [53]. In this current research work, methanolic and aqueous extract shows significant antioxidant activity for all the enzymes assay and enzyme activities are close to the range of control. This significance anti-oxidative potentiality of both the extracts may be attributed might be due to either an increase of the synthesis of antioxidant enzymes or to the occurrence of some phyto-constituents, phenols, flavonoids such as apigenin, tannins, glucopyranoside, α-D-glucopyranoside and 3,5-decadiyne, 2,2-dimethyl (2,2-dimethyldeca-3,5-diyne) and etc., found in the extract, which may behave as scavengers of free radicals can reduce or repair the damage cells caused by free radicals cell and thereby protect the functional organs.

4. CONCLUTION

Overall, in conclusion, the seeds of *Trigonella foenum grecum* L is a source of natural dietary antioxidants that are important in the prevention of disease, health care and promotion of longevity. From this *in-vitro* study, consuming the seeds of *Trigonella foenum grecum* L with regular diet confirms the availability of nutritional and medicinal phytochemicals in the system, that can effectively prevent the risk of developing ischemic heart disease and stroke. In addition to *Trigonella foenum grecum* L it imports flavour into food, its potential use as a value-added ingredient to stabilize food against lipid peroxidation reaction and it has health and other properties such as lowering blood pressure. In the future, a comprehensive and

systematic approach to identifying the active principle compounds for this antioxidant process is in progress.

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