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Original Articles

# Anti-inflammatory, Antidiabetic Potential, Antioxidant (Hydrogen peroxide scavenging and Peroxynitrite scavenging activity), and Phytochememical Constitute of *Trigonella foenum-graecum*

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**Abstract:**

**Background:** Diabetes, as it is known globally, is a chronic and severe metabolic disorder that directly affects the metabolism of carbohydrates, fats, and proteins. This clinical condition is also known as diabetes mellitus. Practically significant rates of morbidity and mortality are associated with it. High blood sugar, in addition to biochemical changes in glucose and fat metabolism, are among the distinct chemical characteristics of this condition. The development of oxidative stress, systemic inflammation, beta-cell destruction, insulin resistance, and type 2 diabetes are associated with both hyperglycemia and hyperlipidemia, respectively. We must also mention that these factors play a decisive role in the development of these conditions. One of the most important therapeutic agents that can actually be used in managing this disease is using natural medical herbal treatments.

**Objective:** Parts isolated from *Trigonella foenum-graecum* extract are studied in vitro for their bioactive components and possible anti-inflammatory, antioxidant, and antidiabetic properties.

**Methods:** The antioxidant activity was determined by conducting an experiment that utilized both peroxynitrite free radical scavenging and hydrogen peroxide scavenging. Also, *Trigonella foenum-graecum* was tested for its inhibitory effects on α-glucosidase and α-amylase using a substrate-based enzyme inhibition methodology. Researchers found that SGOT, SGPT, and ALP activities in rat serum had changed. A p-value of less than 0.05 was deemed to be statistically significant.

**Results:** In this study, the $IC_{50}$ value of the antioxidant activity (scavenging hydrogen peroxide) of the crude plant extract used in this study and its molecules was recorded from 65.23 ± 2.71 µg/ml to 29.11 ± 1.31 µg/ml. Likewise, the $IC_{50}$ value for α-amylase inhibition ranged from 119.14 ± 0.38 µg/mL to 43.05 ± 0.09 µg/mL. Similarly, the $IC_{50}$ value of crude methanol extract of *Trigonella foenum-graecum* against α-glucosidase was 65.55 ± 0.14µg/mL. On the other anti-inflammatory side, a significant decrease ($P < 0.01$) was recorded in the elevated SGPT and ALP activities in the serum of mice treated with *Trigonella foenum-graecum* extract (0.5 ml/kg).

**Conclusion:** Enzymes like α-glucosidase and α-amylase can be inhibited by the powerful antioxidant *Trigonella foenum-graecum*. More research is required to determine which compounds block, though.

**Keywords:** *Trigonella foenum-graecum*, α-Amylase, α-Glucosidase, Antioxidant, Anti-inflammatory.

## Introduction:

Fennel, also known as *Trigonella foenum-graecum*, is an aromatic annual plant that is cultivated in India [1]. This species is a distinct member of the Papilionaceae family and is commonly known as fennel. What distinguishes this plant is its characteristics, as the height of the plant can reach between 35 and 65 centimetres. Anatomical examinations have shown that the shape of the seeds of this type of plant is distinct and rhombic, and in fact their length ranges from three to five millimeters, and anatomically they are 2 millimeters thick. The color of the seeds varies between yellow and brown, and they have a distinctive and strong spicy smell. Recent studies have proven that *Trigonella foenum-graecum* has the ability of this plant to reduce blood sugar levels [2]. At the same time, clear and additional evidence of a practical hypolipidemic effect can be found in various extracts of seeds of this species. Other studies focused on the effect on microorganisms. It has been practically proven that seed extracts of this type are more effective against bacterial species, such as *salmonellae*, *Escherichia coli*, and *Staphylococcus aureus* [3]. It must be mentioned that the seed extract also contains another anti-worm agent. Furthermore, the entire plant has vital analgesic and antioxidant properties. Moreover, the herb possesses anti-inflammatory properties. Trigonelline, neuroin, quercetin, rutin, fenugrin B, and fenugreekine are some of the characteristic and well-known chemical elements of *Trigonella foenum-graecum* that have already been described in the past [4].

From a practical and laboratory standpoint, the biological oxidation process clearly leads in our bodies to the formation of important and well-known free radicals, which can be known by another name, which is free reactive oxygen species. Here it is very important to know that an excessive amount of active free radicals, including what are practically known as hydroxyl radical, superoxide anion radical, and hydrogen peroxide, can actually be very harmful and harmful to the body's cells and contribute significantly to the well-known biological oxidative stress. This distinct biological oxidative damage to DNA and proteins is associated with chronic diseases such as cancer, coronary artery disease [5, 6] and other distinct and well-known conditions. Hence we know that compounds that have a distinct ability to eliminate free radicals also have a great and clear ability to reduce the symptoms of various diseases. It is the job of the body's natural defensive mechanisms, such as peroxide-glutathione, superoxide dismutase, and catalase, to neutralize ROS [7]. Research in biological systems has shown that antioxidants can effectively neutralize free radicals [8]. The antioxidant capabilities of natural substances, including many raw extracts and unadulterated natural compounds, are well-known. The goal of this research is to determine whether there are any anti-inflammatory, antioxidant, or antidiabetic benefits in the *Trigonella foenum-graecum* extract and to identify its bioactive components.

## Materials and Methods:

### Methods for Collecting Plants and Making Extracts:

After the plant samples were collected, they were cleaned and left to dry at room temperature for about fifteen to twenty days. After that, they underwent any necessary processes to reduce their size. The prepared extract was made using the manufactured powder. The 100 grammes of plant material was extracted using 400 milliliters of petroleum ether for around 48 hours in a Soxhlet device [9]. The marc was defatted and then dried at 50 degrees Celsius in a hot air oven. Then, it was subjected to further extraction in a Soxhlet device with 400 mL of 95% ethanol until no more residue was visible upon evaporation. To make the watery extract, the mixture was macerated with 3% methanol and water for seven days at low heat, stirring frequently. Solvents were removed from the extracts using a rotating vacuum evaporator.

### Examination of the *Trigonella foenum-graecum* methanol fraction using gas chromatography-mass spectrometry

The methanol fraction of *Trigonella foenum-graecum* was analyzed by (GC-MS) utilizing an Agilent Technologies 5890 Series gas chromatograph in conjunction with an Agilent 5973 Mass Selective Detector. The Agilent

Chemstation program was utilized to conduct the analysis. Agilent Technologies, a company based in Santa Clara, California, with its headquarters at Stevens Creek Boulevard, manufactured the eHP-5MS capillary column that was utilized. The column measured 30 meters in length, had an internal diameter of 0.25 mm, and a film thickness of 0.25 ɛm. As the carrier gas, ultrapure helium with a linear velocity of 27.5 cm/s and a flow rate of 0.57 mL/min was utilized. The temperature setting for the injector was 250 degrees Celsius. The oven was programmed to increase by four minutes between each temperature increment, from an initial setting of 50.0 degrees Celsius to a maximum of 250 degrees Celsius.

The rate of increase was 15 degrees Celsius per minute. The splitless mode was used to administer one microliter of injections at a twenty-to-one split ratio. [10] The mass spectrometer successfully carried out its tasks when the electron ionization mode was set to 70 eV and the electron multiplier voltage was set to 1859 V. Scan range of 50–700 amu, solvent delay of four minutes, ion source temperature of 230 degrees Celsius, and quadruple temperature of 150 degrees Celsius were among the other MS operating parameters. The unknown components of the investigated sample were compared to mass spectral data, fragmentation patterns, and retention durations found in the more than 75,000 chemical records held by the Wiley Libraries. The components might then be identified. Following this, we verified the components' identities by checking their structures, molecular weights, and percentages.

### *Trigonella foenum-graecum*'s Anti-inflammatory Functions

### Animals

The trials were conducted after the necessary permissions were obtained from Baghdad. The experiments were conducted using albino rats that weighed 200 to 250 grams. The animals were sourced from the animal breeding house. the eleventh The animals were housed in a controlled environment with a 12-hour day-night cycle, a temperature of 27 degrees Celsius, and unlimited access to food and water.

### Dosage and Drug Administration

Grounded nut oil (used as a vehicle control) and phenylbutazone (PBZ) were the positive controls in all trials involving *Trigonella foenum-graecum* methanol fraction. Group 1 consisted of groundnut oil at a concentration of 0.5 mL/kg, Groups 2, 3, and 4 of fractions of *Trigonella foenum-graecum* at concentrations of 0.20 mL/kg, 0.50 mL/kg, and 0.75 mL/kg, respectively. Group 5 contained phenylbutazone at a concentration of 100 mg/kg.

### Formaldehyde-Induced Paw Edema

Both the initial 0 and 48-hour post-formaldehyde measurement of paw volume were taken. The following is the formula for calculating anti-inflammatory activity:

**% Inhibition of inflammation = 1−(VT/VC) ×100**

Where VT represents the variation in paw volume for the treatment group and VC denotes the corresponding variation in paw volume for the control group.

### Blood Sampling and Serum Analysis

Animals were decapitated while under light ether anesthesia to obtain 4 mL of blood for serum analysis from the coronary artery. The tests for ALP, SGOT, and SGPT were performed on the serum samples using established kits.

### In *vitro* Antidiabetic assay

### α-amylase inhibitory assay

Slight adjustments were made to the conventional method in order to ascertain the inhibitory activity of the extract and fractions against α-amylase. [11] Eleventh Fifty microliters of phosphate buffer (100 mM, pH = 6.8), ten microliters of α-amylase (2 U/ml), and twenty microliters of different concentrations of extract and fractions (0.5 mg/ml) were incubated at 37°C for twenty minutes. Following this, the reaction mixture was poured into a 96-well plate. Subsequently, 20 microliters of 1% soluble starch were introduced to prepare the substrate. After an additional 30 minutes of

incubation at 37°C, 100 l of DNS color reagent was introduced and the mixture was brought to a simmer for 10 minutes. At 540 nanometers, the absorbance of the final mélange was determined utilizing a Multiplate Reader (Multiska Thermo Scientific, version 1.00.40). Carboxyse was employed in concentrations varying from 0.1 to 0.5 mg/ml as a standard. Every experiment was conducted in triplicate, and a control material, which did not contain any fractions or extracts, was also prepared in parallel. The findings, obtained through the employed methodology, were represented by the percentage inhibition.

**The following formula was used to obtain the inhibition percentage:**

**% Inhibition = ($Abs_{control}$ − $Abs_{extract}$) /$Abs_{control}$ × 100**

The graphic representation of the concentrations of fractions that allowed for a 50% inhibition of enzyme activity ($IC_{50}$) was used.

**Inhibitory Assay for α-Glucosidase**

The α-glucosidase inhibitory activity of the extract and fractions was assessed utilizing the standard methodology, with only minimal modifications. [12] A reaction mixture comprising 50 µl of phosphate buffer (100 mM, pH = 6.8), 10 µl of alpha-glucosidase (1 U/ml), and 20 µl of extract and fractions at various concentrations (0.5 mg/ml) was pre-incubated at 37°C for 15 minutes on a 96-well plate. Following this, 20 µl of P-NPG (5 mM) was added as a substrate and incubated for an additional 20 minutes at 37°C. The procedure was terminated by adding 50 µl of $Na_2CO_3$ (0.1 M). The Multiplate Reader measured the absorbance of the p-nitrophenol that was emitted at 405 nm. For the purpose of control, acarbose was added in concentrations between 0.1 and 0.5 mg/ml. In addition to performing each experiment in triplicate, a control group was established in which no test compound was administered.

The tripslicate tests were carried out and the α-glucosidase inhibitory activity was reported as % inhibition using the following expression:

**% Inhibition = ($Abs_{control}$ − $Abs_{extract}$) /$Abs_{control}$ × 100**

A control represents the absorbance of the control, while A extract represents the absorbance of the fraction. The graphic representation of the concentrations of fractions that allowed for a 50% inhibition of enzyme activity ($IC_{50}$) was used.

**Antioxidant test in *vitro***

**Hydrogen peroxide scavenging**

This activity was calculated using a slightly modified version of a previously described approach. For 30 minutes at room temperature, a mixture of 50 mM $H_2O_2$ and samples at concentrations ranging from 0 to 2 mg/ml was incubated in a 1:1 v/v ratio [12]. After that, the reaction mixture was given a good stir and left to incubate for half an hour at room temperature. The ferric-xylenol orange complex was found to have an absorbance of 560 nm. Sodium pyruvate served as the reference chemical, and each test was repeated six times.

**Peroxynitrite scavenging**

Following a one-second ice bath in which 5 milliliters of 0.7M H2O2 and 0.6M KNO2 were combined, an additional 5 milliliters of ice-cold water were introduced. The solution was acidic, consisting of 0.6M HCl. In addition, 1.2 M NaOH was added. The surplus $H_2O_2$ was extracted from the reaction mixture subsequent to its treatment with granular $MnO_2$ that had undergone prewashing with 1.2 M NaOH. The mixture was then allowed to rest at -20°C overnight. Spectrophotometric analysis was performed to ascertain the concentration at 302 nm (ε = 1670 M-1 cm-1) subsequent to the recovery of peroxynitrite solution from the surface of the frozen mixture. In order to evaluate the scavenging activity of peroxynitrite, an Evans Blue bleaching assay was employed. The investigation was conducted utilizing an altered iteration of a standard protocol. At 611 nm, the absorbance was measured following 30 minutes of incubation at 25°C. We compared the test and blank sample values to determine the ONOO - % scavenging. We ran each

test six times. The substance that served as the reference was gallic acid.

**Statistical analysis**

All of the statistical analyses were performed using the GraphPad Prism 5 Statistical Package, which is developed and owned by GraphPad Software in the United States. We analyzed the data using one-way analysis of variance (ANOVA) and then applied the Bonferroni test to the results. When calculating in vitro IC50 in triplicate, the results were shown as the mean value plus or minus the standard error of the mean. Phytochemical quantification was shown as the mean plus or minus the corresponding standard deviation, whereas free radical scavenging activities were shown as a percentage. A p-value below 0.05 was considered statistically significant.

**Results and Discussion:**

**Analysis of the methanol fraction from *Trigonella foenum-graecum* using gas chromatography-mass spectrometry**

These compounds were 5,7-Dihydroxyisoflavone, 3-(4-Hydroxyphenyl)-7-methoxy-4H-chromen-4-one, and 2'-hydroxyn-eobavaisoflavanone. 6.8-Diprenyl-kaempferol 3,5,7-Trihydroxy-2-(4-hydroxyphenyl)”-6,8-bis(3-methyl-2-butenyl)4,3-dihydro-4-(1-benzopyran-4-one), 1,1,2-benzopyran-4-one"-7- hydroxy-2- (4-hydroxy-phenyl)-, 3-(4-hydroxy-phenyl)"(-)-3-O-acetyl-spectaline, Isoorientin, Collettiside, Carpaine, Diosgenin glucoside, 3-hydroxypregn-5-en-20-yl hexopyranoside, and -7-methoxy-4H-chromen-4-one.

The results of the GC-MS study indicate that the fraction's phytoconstituents are associated with numerous biological activities, some of which are therapeutically significant. It is expected that the phytol actually present in this active substance is specifically and directly responsible for the antioxidant and anti-diabetic activities that were strongly and clearly stimulated in this work. It has been practically proven that 2-hexadesiloxane and stearic acid have good, distinct and obvious antioxidant potential. Here we must mention that phytol has chemical and physical antioxidant and blood sugar-lowering properties. This susceptibility, which has already been demonstrated in this research, can be explained by the presence of these laboratory-obtained biologically active components in *Trigonella foenum-graecum*.

**Effectiveness of fractions of Trigonella foenum-graecum as an inhibitor of α-amylase and α-glucosidase.**

The inhibitory activity of the methanol fraction that was screened and the ethyl acetate fraction that was produced from *Trigonella foenum-graecum* against α-amylase and α-glucosidase, respectively, are shown in Figures 1 and 2. The figures are displayed according to the order in which they were measured. Consequently, the data from the real enzyme inhibitor test showed that the inhibitory effectiveness of *Trigonella foenum-graecum* fractions on α-amylase and α-glucosidase was dependent on both the dosage and the fraction measured.

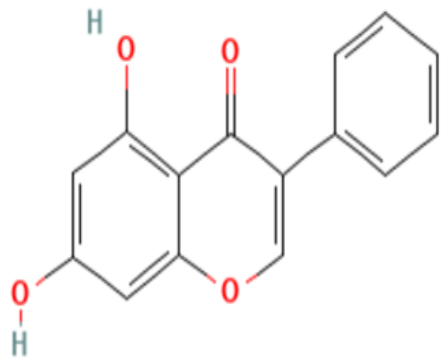
Based on the results obtained during this study, the maximum dose studied in this research showed significant and significant inhibition, and at the same time the low dose led to the lowest level of inhibition that was measured. Here, considering the type of extract used in the laboratory experiments (crude, ethyl acetate part, ethanol part, hexane part, water part and acarbose according to the standard) recorded (119.14 ± 0.38, 65.07 ± 0.14, 43.05 ± 0.09, 63.19 ± 0.11, 73.08 ± 0.18 and 23.10 ± 0.05) respectively, significant inhibitory power against alpha amylase. While (65.55 ± 0.14, 38.04 ± 0.09, 60.19 ± 0.12, 38.73 ± 0.09, 29.71 ± 0.11, and 13.12 ± 0.03) respectively, significant inhibitory power against α-glucosidase activity was recorded. The methanol and water fraction inhibited a substantially larger percentage of -amylase than the standard diabetic drug acarbose, as demonstrated by a significantly higher percentage ($P < 0.05$). Although methanol and the ethanol fraction showed a proportionally higher inhibition of α-glucosidase compared to acarbose, the disparity was not statistically significant ($P > 0.05$).

## Anti-Inflammatory and Antioxidant Properties of *Trigonella foenum-graecum* Extracts

In vitro antioxidant potential of the methanolic extract fractions derived from *Trigonella foenum-graecum* was revealed in Figures 3 and 4. And based on the type of extract used (crude, ethyl acetate part, ethanol part, hexane part, water part and quercetin as standard) (53.64 ± 2.49, 42.08 ± 2.21, 65.23 ± 2.71, 48.63 ± 2.30, 48.63 ± 2.30 and 17.09 ± 0.83) Respective hydrogen peroxide scavenging activity. While at the same time (724.22 ± 29.06, 685.12 ± 26.17, 731.82 ± 28.23, 657.79 ± 26.09, 715.90 ± 27.26 and 794.81 ± 30.01) were recorded, respectively.

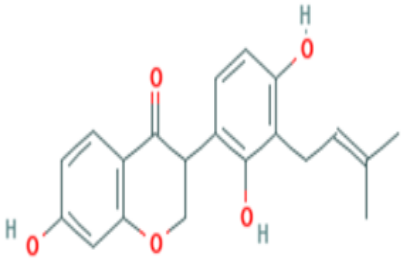
Figure 3 shows that compared to the standards, which were quercetin, the crude (methanolic extract) and hexane fraction had a significantly higher percentage of inhibitors against hydrogen peroxide scavenging activities ($P<0.05$). To see how well it scavenged peroxynitrite, we looked at the results in Figure 4. The figure shows that the ethyl acetate fraction and the hexane fraction inhibit a much higher percentage of peroxynitrite scavenging activity ($P<0.05$) compared to the standards.

Laboratory rats were subjected to in vitro experimental testing to determine the impact of *Trigonella foenum-graecum* oral administration of extract on the serum enzymes SGPT (serum glutamate-pyruvate transaminase), SGOT (serum glutamate-oxaloacetate transaminase), and ALP (alkaline phosphatase). recorded 78.38 ± 4.06, 295.48 ± 13.85. And 713.26 ± 22.64 respectively for *Trigonella foenum-graecum* extract while 97.41 ± 5.13, 232.57 ± 11.03 and 919.87 ± 31.70 were recorded respectively for using phenylbutazone and 122.16 ± 9.20, 312.77 ± 14.92 and 961.77 ± 33 were recorded. 10 to control. In other laboratory experiments with carotene and linoleic acid emulsion, it was laboratory revealed that freeze-dried fenugreek extract possesses physical and chemical antioxidant properties, with bioactivity similar to that of known ordinary commercial antioxidants [13, 14]. Hence, fenugreek extracts, which include methanol, ethanol, dichloromethane, acetone, hexane, and ethyl acetate, are considered powerful, distinctive and effective sources of antioxidants [15, 16]. There is other evidence that flavonoids already present in aqueous fenugreek extracts in particular have the potential to inhibit the production of free radicals [15, 16]. It has been laboratory and experimentally proven that fenugreek seeds can increase the levels of antioxidant activity and at the same time be effective in reducing the amount of lipid peroxidation in the livers of diabetic rats and rats that have been ingested with ethanol [17, 18]. *Trigonella foenum-graecum* seeds, when extracted with ethanol at a concentration of 500 µg/ml, actually clearly had the highest level of scavenging capacity for radical cations, at the same time indicated by the results of the study presented by the researchers [19]. Fenugreek seed extract is currently available in both aqueous and methanolic forms and possesses anti-radical and antioxidant capabilities and activities in vitro.



**5,7-Dihydroxyisoflavone**

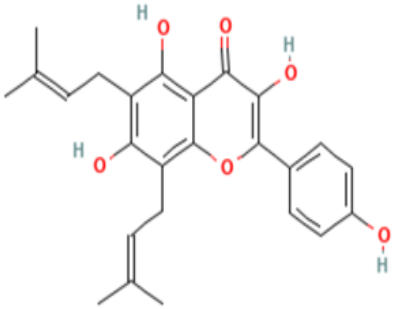
**Molecular Weight: 254.24 g/mol**



**2'-hydroxyneobavaisoflavanone**

**Molecular Formula: $C_{20}H_{20}O_5$**

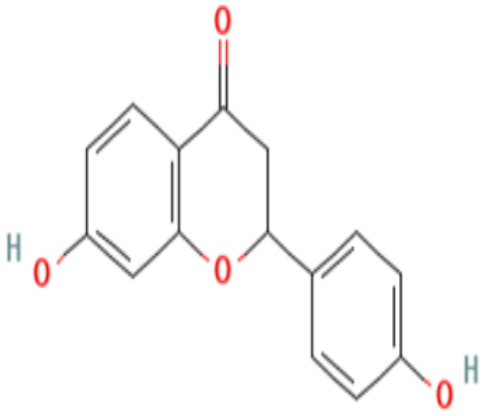
**Molecular Weight: 340.4 g/mol**



**6,8-Diprenylkaempferol**

**Molecular Formula: $C_{25}H_{26}O_6$**

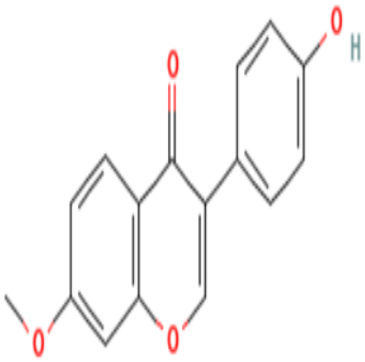
**Molecular Weight: 422.5 g/mol**



4H-1-Benzopyran-4-one, 2,3-dihydro-7-hydroxy-2-(4-hydroxyphenyl)-

Molecular Formula: $C_{15}H_{12}O_4$

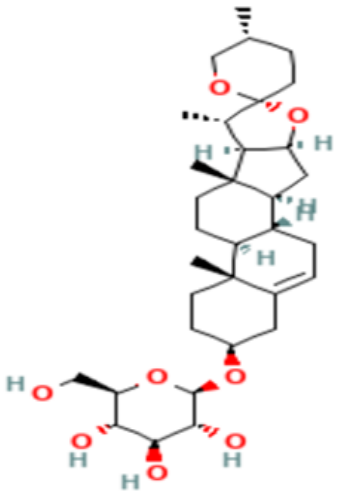
Molecular Weight: 256.25 g/mol



3-(4-Hydroxyphenyl)-7-methoxy-4H-chromen-4-one

Molecular Formula: $C_{16}H_{12}O_4$

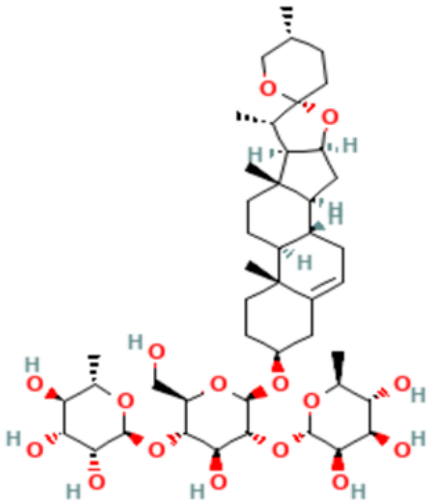
Molecular Weight: 268.26 g/mol



Diosgenin glucoside

Molecular Formula: $C_{33}H_{52}O_8$

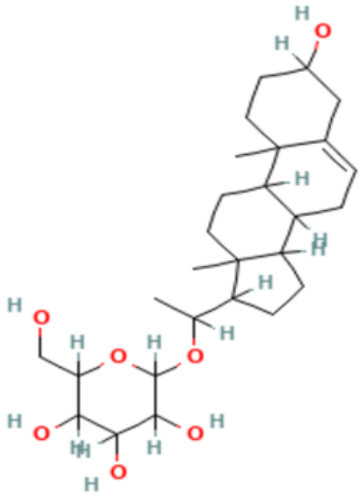
Molecular Weight: 576.8 g/mol



Collettiside III

Molecular Formula: $C_{45}H_{72}O_{16}$

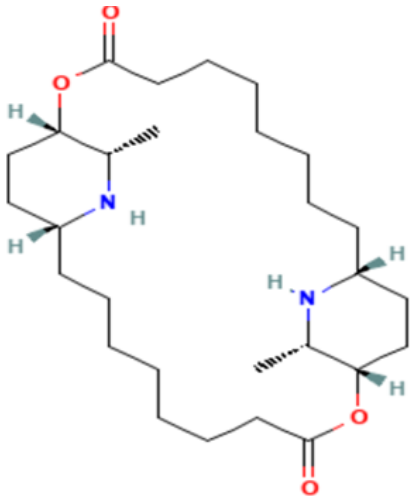
Molecular Weight: 869.0 g/mol



3-hydroxypregn-5-en-20-yl hexopyranoside

Molecular Formula: $C_{27}H_{44}O_7$

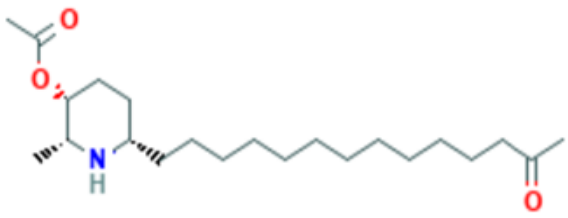
Molecular Weight: 480.6 g/mol



Carpaine

Molecular Formula: $C_{28}H_{50}N_2O_4$

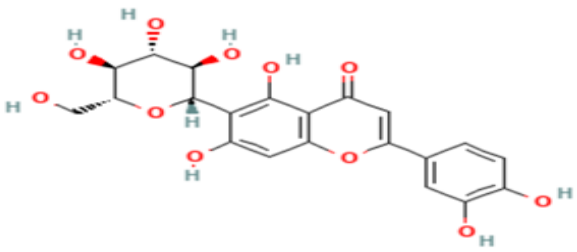
Molecular Weight: 478.7 g/mol



(-)-3-O-acetylspectaline

Molecular Formula: $C_{22}H_{41}NO_3$

Molecular Weight: 367.6 g/mol



Isoorientin

Molecular Formula: $C_{21}H_{20}O_{11}$

Molecular Weight: 448.4 g/mol

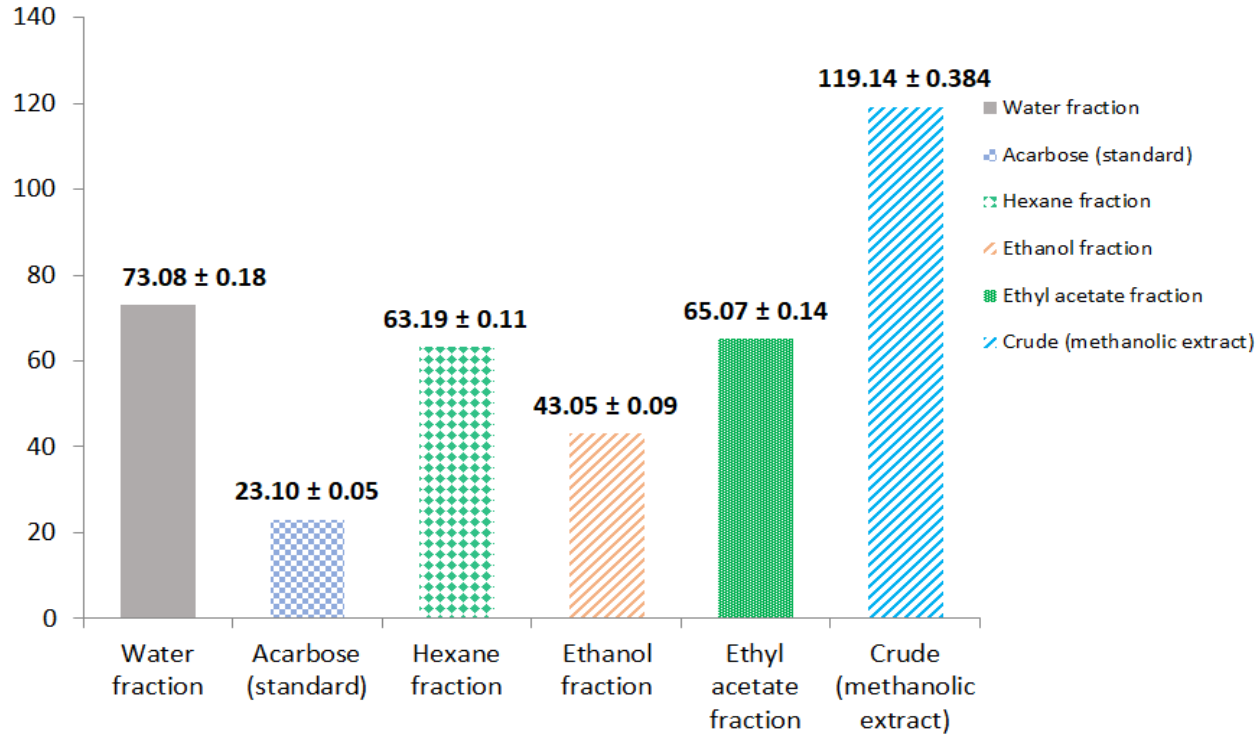



**Figure 1. Inhibitory potency of *Trigonella foenum-graecum* fractions against α-amylase compared with Acarbose - standard**

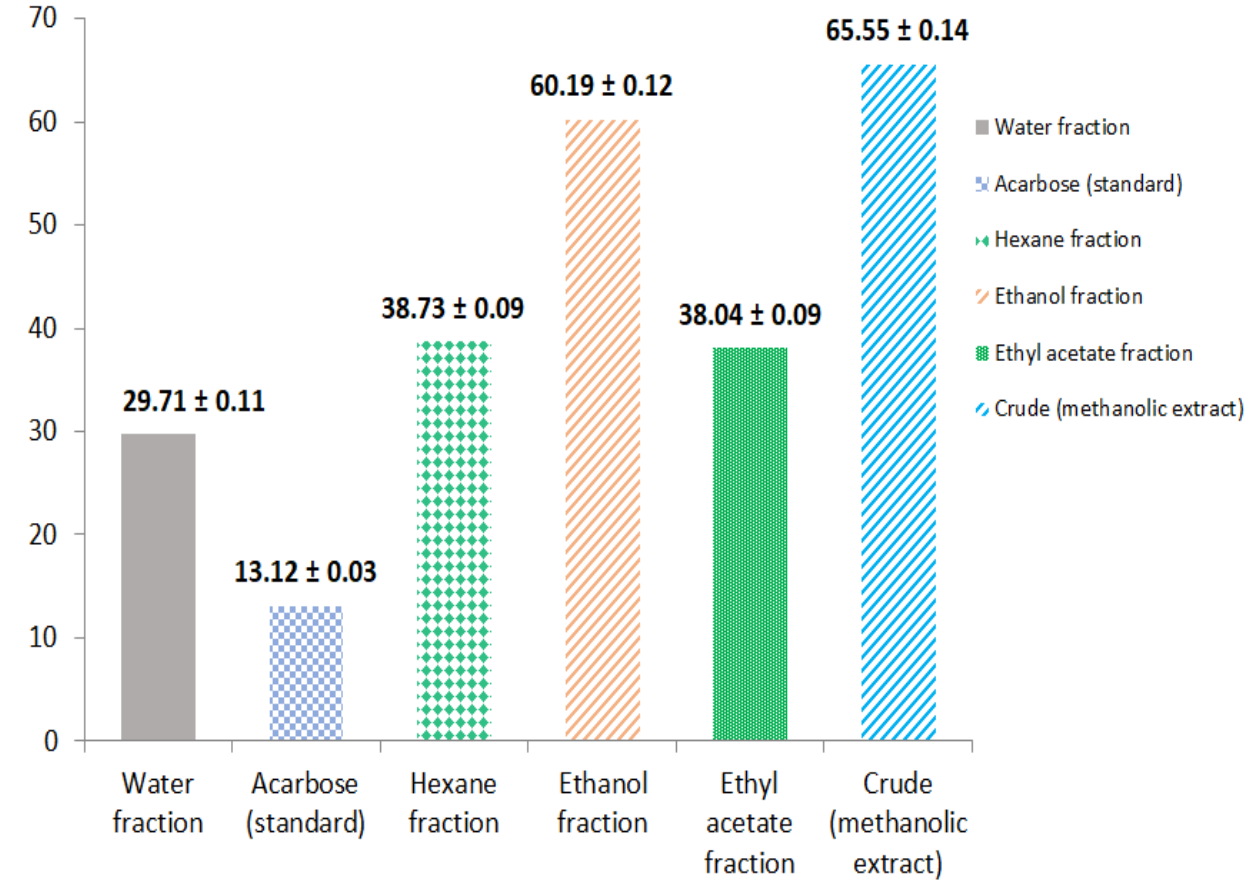



**Figure 2. Inhibitory potency of *Trigonella foenum-graecum* fractions against α-glucosidase compared with Acarbose - standard**

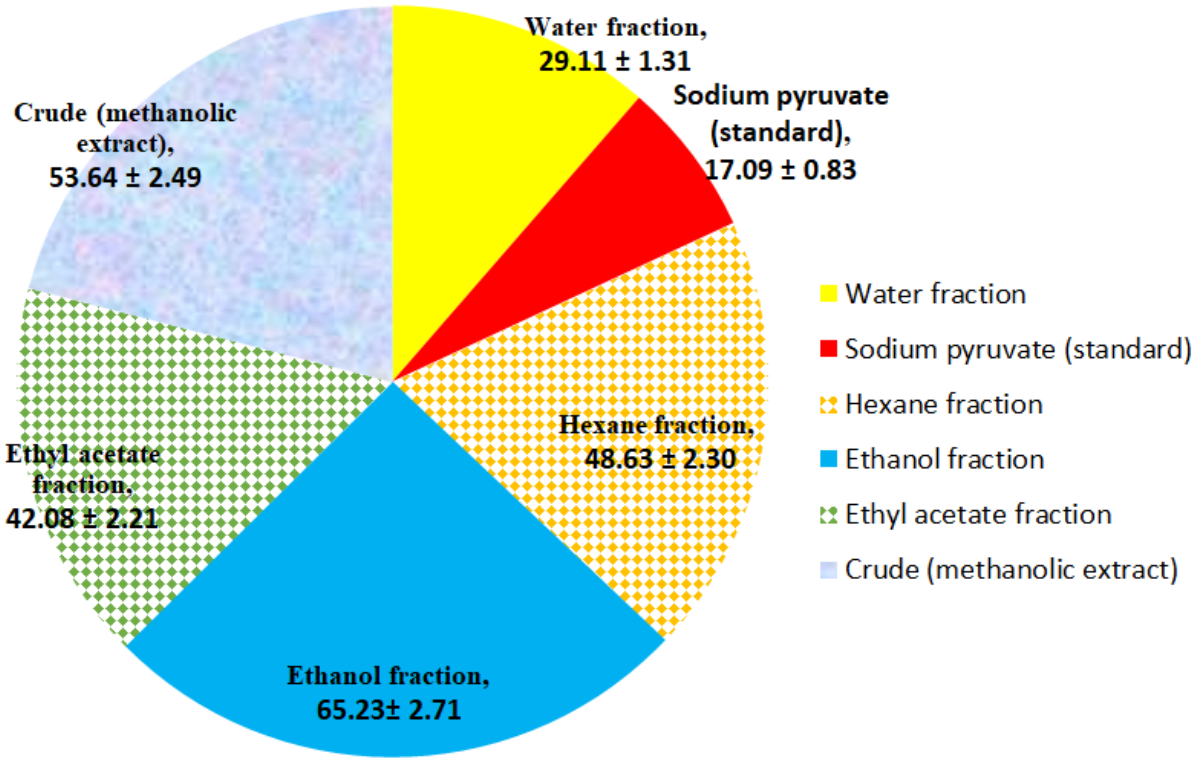



**Figure 3. Free radical scavenging potential of Hydrogen peroxide scavenging of *Trigonella (foenum-graecum* crude extract and fractions compared with Sodium pyruvate (Standard**

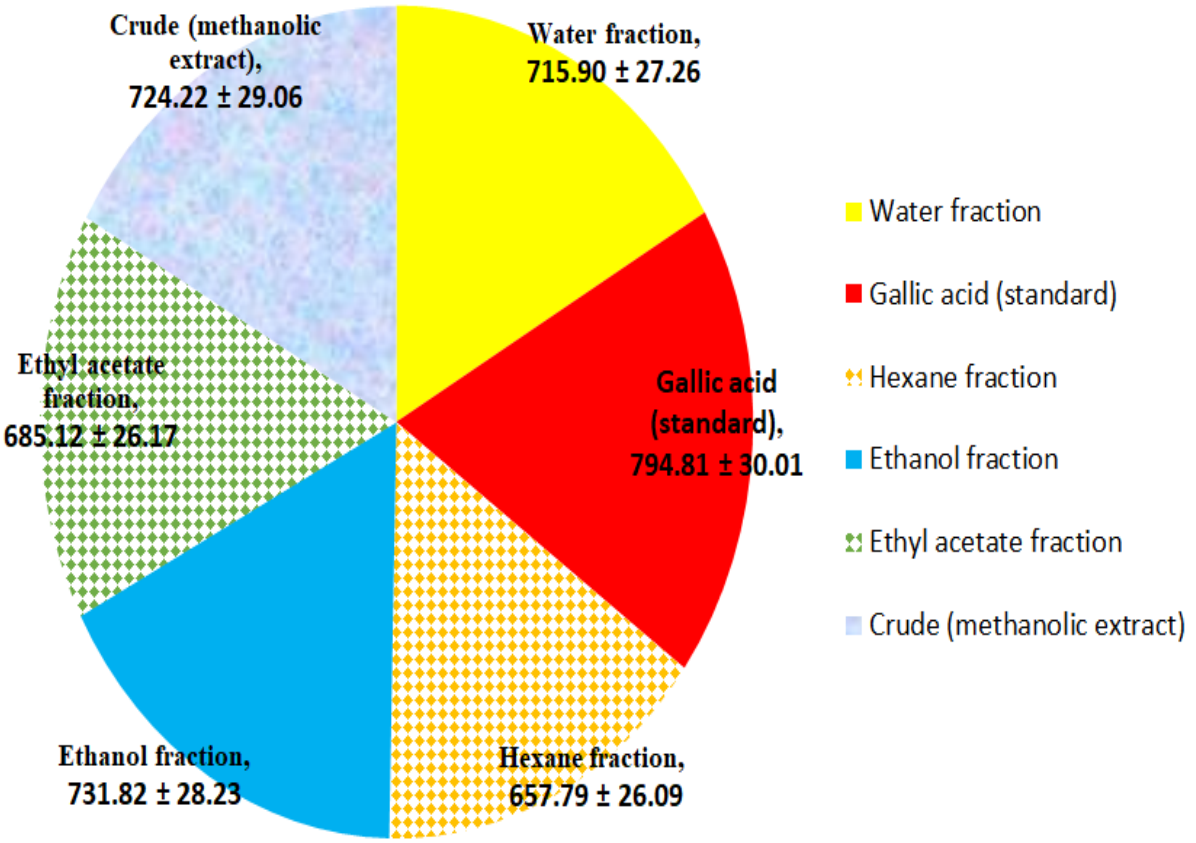



**Figure 4. Free radical scavenging potential of Peroxynitrite scavenging of *Trigonella foenum-graecum* crude extract and fractions compared with Gallic acid (Standard)**

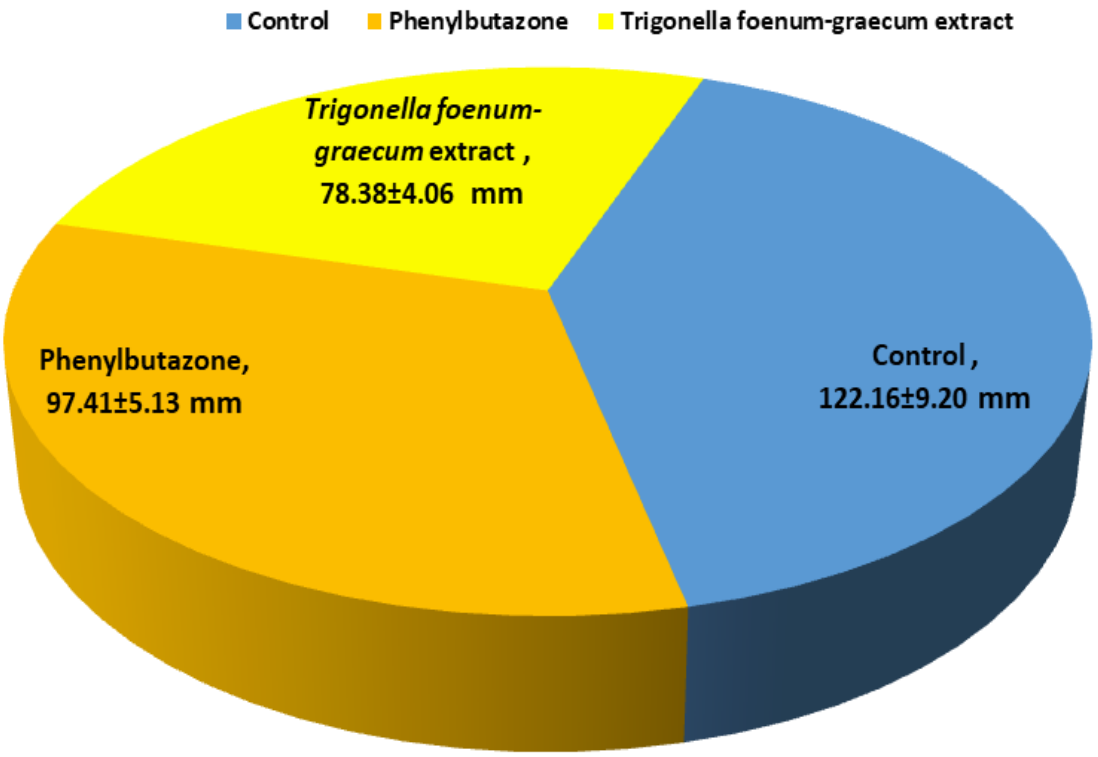



**Figure 5. Effect of oral administration of *Trigonella foenum-graecum* extract and Phenylbutazone on serum SGPT (Serum glutamate-pyruvate transaminase)enzyme**

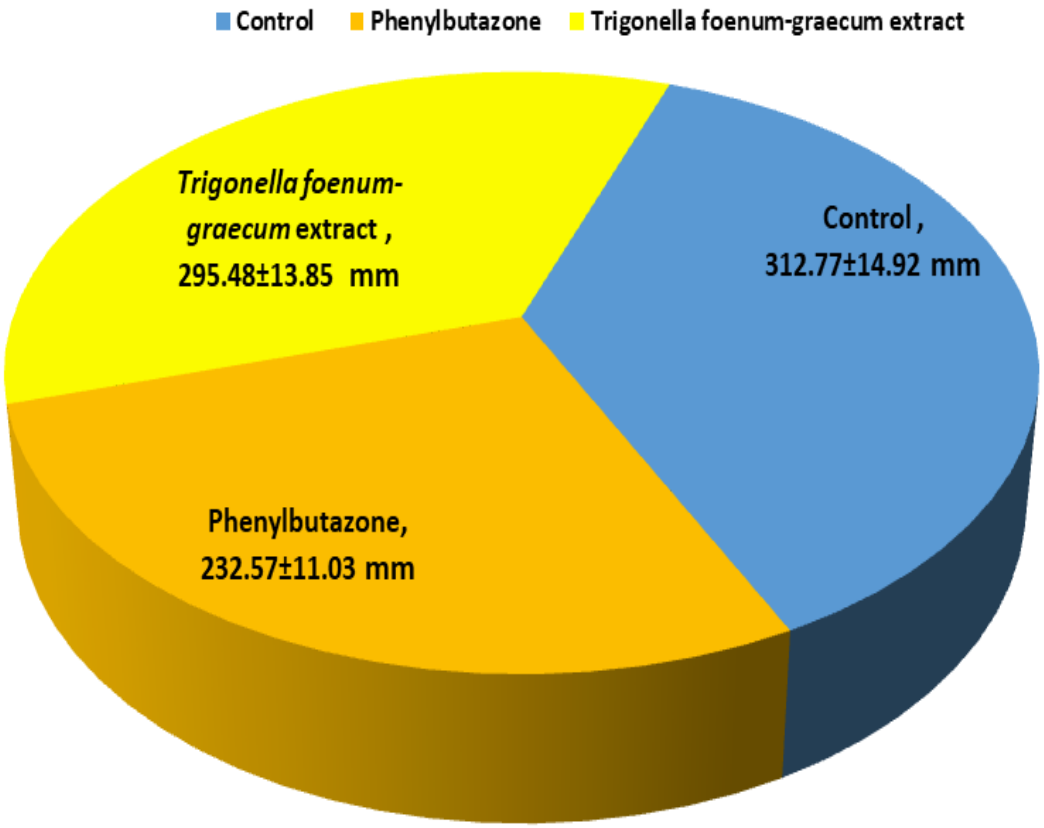



**Figure 6. Effect of oral administration of *Trigonella foenum-graecum* extract and Phenylbutazone on serum SGOT (Serum glutamate-oxaloacetate transaminase) enzyme**

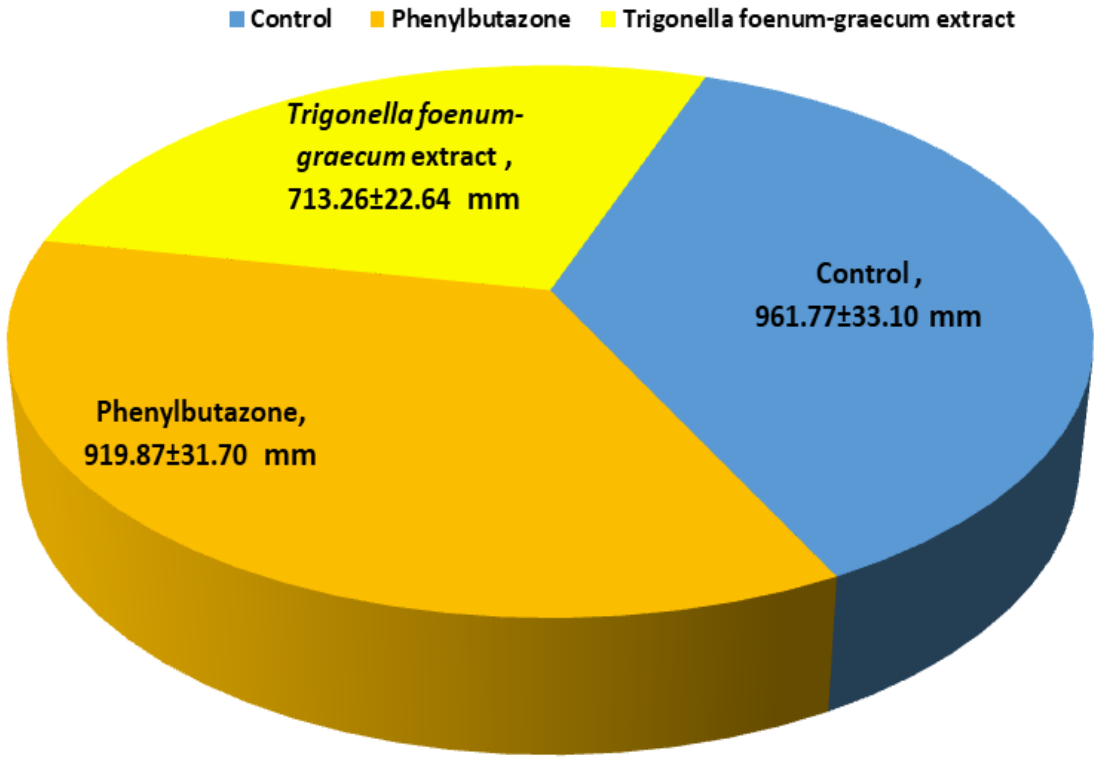



**Figure 7. Effect of oral administration of *Trigonella foenum-graecum* extract and Phenylbutazone on serum ALP (Alkaline phosphatase) enzyme**

**Conclusion:**

In this study, the methanolic extract and its fractions from *Trigonella foenum-graecum* were experimentally examined to determine its total natural chemical components, its antioxidant properties and activities, and the in vitro ability to inhibit the activities of both α-glucosidase and α-amylase enzymes. In addition to having significant antioxidant activity, the plant also has the ability to prevent inflammation by checking and measuring liver enzymes. Furthermore, significant and enhanced research should be conducted to diagnose, isolate and identify the bioactive molecules directly responsible for these effects.

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