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Original Research Article

Pharmacognostic Evaluation and Anti-Inflammatory Potential of Flavonoid Rich Extract of Trigonella Foenum-Graecum Seed Ameliorate

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Abstract: *Trigonella foenum graecum* has been traditionally claimed that seeds of *Trigonella foenum graecum* are useful in the management of anti-inflammatory and anti-oxidant activity. In the present study, anti-inflammatory and anti-oxidant activity of *Trigonella foenum graecum* hydroethanolic extract was evaluated. The physicochemical parameters were found to be within acceptable limits, while phytochemical analysis confirmed the presence of Steroids, Alkaloids, Flavonoids, Saponins and tannins. The prepared plant extract demonstrated significant antioxidant activity with an IC50 value of 18.64 µg/ml, compared to the standard IC50 of 5.40 µg/ml. Additionally, the plant extract exhibited notable anti-inflammatory activity based on percent inhibition results. These findings suggest that *Trigonella foenum graecum* hydroethanolic extract hold considerable promise as an effective therapeutic agent with potent antioxidant and anti-inflammatory properties. Thus, hydroethanolic extract of fenugreek seeds has significant anti-inflammatory and anti-oxidant activities which are due to the presence of its active chemical constitutes.

Keywords: Anti-Inflammatory Activity, Anti-Oxidant Activity, *Trigonella Foenum Graecum*.

INTRODUCTION

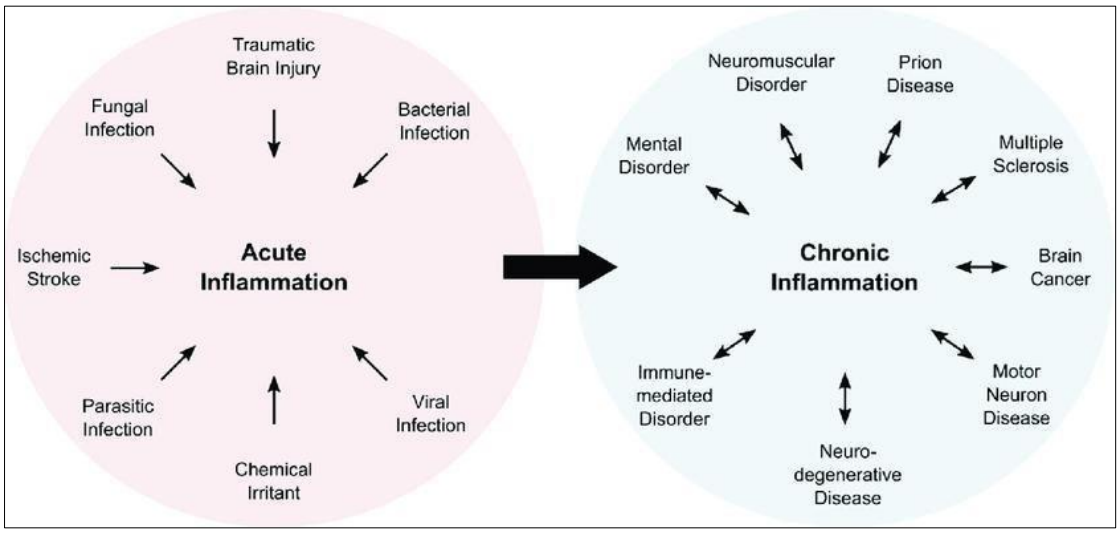
Inflammation is a biological response of the body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It's a normal and important process that allows the body to heal and fight off infection. The body releases chemicals that trigger an immune response to fight off infection or heal damaged tissue. Once the injury or infection is healed, the inflammatory process ends.

Inflammation is the body's defense mechanism against harmful stimuli, bacteria, toxins, and irritants. In addition to generating pain, heat, redness, swelling, and functional failure of the affected tissue, it also shifts vascular permeability, changes blood flow, and increases leucocyte migration to the inflammatory area. The main indicators of many disease processes, such as diabetes, cancer, arthritis, and other serious inflammatory illnesses, are typically pain and inflammation. Inflammation is the body's defense mechanism against harmful Antioxidants, antinociceptives, and anti-inflammatory medications are widely available, but they are claimed unreachable, expensive, ineffective, and sensitive to several adverse effects. Since over 80% of people globally rely on non-conventional medications for their everyday medical needs, especially in Asia and Africa, they hold a prominent place in healthcare systems.

The classic signs of inflammation include:

Redness (Latin: rubor): Caused by increased blood flow to the area.
Heat (Latin: Calor): Also due to increased blood flow.
Swelling (Latin: tumor): Caused by fluid accumulation outside blood vessels.
Pain (Latin: dolor): Resulting from tissue distortion and chemical mediators.
Loss of function: Can occur due to pain or swelling.

Types of Inflammation:
Acute Inflammation: A rapid, short-term response to injury or infection, often characterized by the classic signs above.
Chronic Inflammation: A prolonged, persistent inflammatory response that can damage tissues and lead to various diseases.



Picture 1: Types of inflammations

MATERIALS AND METHODS

Pharmacogenetic study of *Trigonella foenum-graecum*
Description of *Trigonella foenum-graecum*
Plant Botanical Name: *Trigonella foenum graecum*

Synonyms:

Buceras foenum-graecum (L.) All. Foenum-graecum sativum Medik. Foenum-graecum officinale Moench. Foenum-graecum officinale ssp. Cultum Alef. Folliculigera graveolens Pasq. Medicago foenu-graeca Ehz Krause. Telis foenum-graecum (L.) O.ktze. Trigonella graeca St.Lag. non Boiss. Trigonella ensifera Trautv.

Common Names:
English: Fenugreek French: Fenugrec Arabic: Hhelbah Dutch: Fenegriek

Vernacular Names:
Sanskrit: Methika Hindi: Methi
Kannada: Menthya Marathi: Methi

Collection and Authentication of Plant
The dried seeds of *Trigonella foenum-graecum* were purchased from local market and identified by pharmacognosist.

Processing of the Plant

Extraction of Seeds Seed powder of fenugreek (100 g) was extracted with suitable solvent (water and ethanol) (60–80°C) at room temperature for 4 days.

Parts Used: Seeds.

Seeds vary from rectangular to rounded in outline with a deep groove between the radicle and cotyledons, the length is 3.5-6 mm and the width 2.5-4 mm, light greyish, brown, olive green or cinnamon coloured, with a pronounced radicle that is half the length of the cotyledons.

Macroscopic Examination

Medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of such materials, and should be carried out before any further tests are undertaken. Visual inspection provides the simplest and quickest means by which to establish identity, purity and possibly quality. If a sample is found to be significantly different in terms of colour, consistency, odour or taste from the specifications it is considered as not fulfilling the requirements. Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface.

Preliminary Phytochemical Screening

The seed extract was subjected to preliminary phytochemical screening. Several tests for the identification of the chemical constituents present in the ethanol extract were carried out according to the method of Harborne (1984).

Steroids

Liebermann-Burchard Reaction: Freshly prepared LB reagent (5 ml acetic anhydride and 5 ml sulphuric acid) was added to the seed extract. Presence of steroids was confirmed by the development of a green colour.

Flavonoids

Shinoda Test: A few milligram of the extract was dissolved in a few ml of methanol and Magnesium powder was added, followed by 5M HCl. Flavonoids gave a pink colour.

Alkaloids

Mayer's test:

One or two drops of Mayer's reagent (1.36 g HgCl2 dissolved in 60 ml of distilled water and mixed with a solution of 5 g of KI in 10 ml of water) were added to the acidified plant extract. A white precipitate indicates the presence of alkaloids.

Dragendorff's Test:

Dragendorff's reagent (0.85 g of basic bismuth nitrate dissolved in 40 ml of water and 10 ml of glacial acetic acid, followed by addition of 8 g Potassium iodide dissolved in 20 ml water) was added to a little of the extract dissolved in ethanol. Alkaloids gave orange precipitate.

Saponins

About 0.5 g of the powdered sample was mixed with 5 ml of distilled water and shaken vigorously on cyclomixer (Remi equipments) and formation of persistent froth was taken as indication of saponins.

Tannins

5 g of powdered plant material is boiled with 100 ml water for 3 m. The extract is filtered and a clear filtrate is obtained. To 5 ml of the solution, 2 ml of 2 per cent gelatine solution is added. A curdy white precipitate indicates the presence of tannins.

Determination of Total Phenolic Content (TPC)

TPC in the extract of fenugreek seeds was estimated spectrophotometrically by the method of Singleton and Rossi (1965) using the Follin-Ciocateu's phenol reagent (Singleton and Rossi, 1965), with minor modifications: each 25, 50 and 100 µl of phenolic extract was oxidized with 400 µl of Follin-Ciocateu 10%. 2 to 5 min later, 500 µl of sodium carbonate (7.5%) were added to neutralise the reaction. After one hour of incubation at room temperature in dark, the absorbance was measured at 725 nm on UV-VIS spectrophotometer. The polyphenolic content was expressed in mg of gallic acid equivalents (GAE) per gram of dry seed, using a standard curve generated with gallic acid.

PHARMACOGNOSOSTICAL STUDIES

***In Vitro* Antioxidant Activity**

In vitro, antioxidant activity by DPPH radical scavenging assay was performed using ascorbic acid as standard. Briefly, the test solution (1 ml) was added with a 3 ml standard DPPH reagent (0.1 mmol/L prepared in methanol). Methanol was added to the DPPH solution as the control. The test tubes with the solutions were sealed and kept at room temperature in the dark for 30 minutes. Afterward, the absorbance was measured. The IC50 value was calculated using SPSS- 20 software 1

Following formula was used to calculate radical scavenging activity.

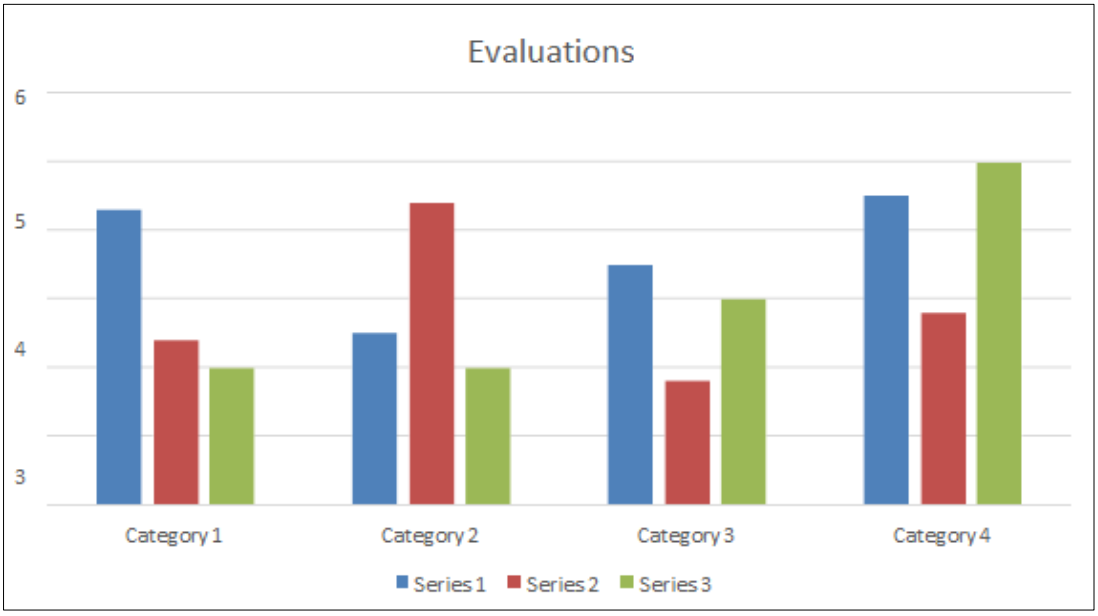
$aiasaegigatiit = (bsootro - bsosame)/(bsootro)\ 100$

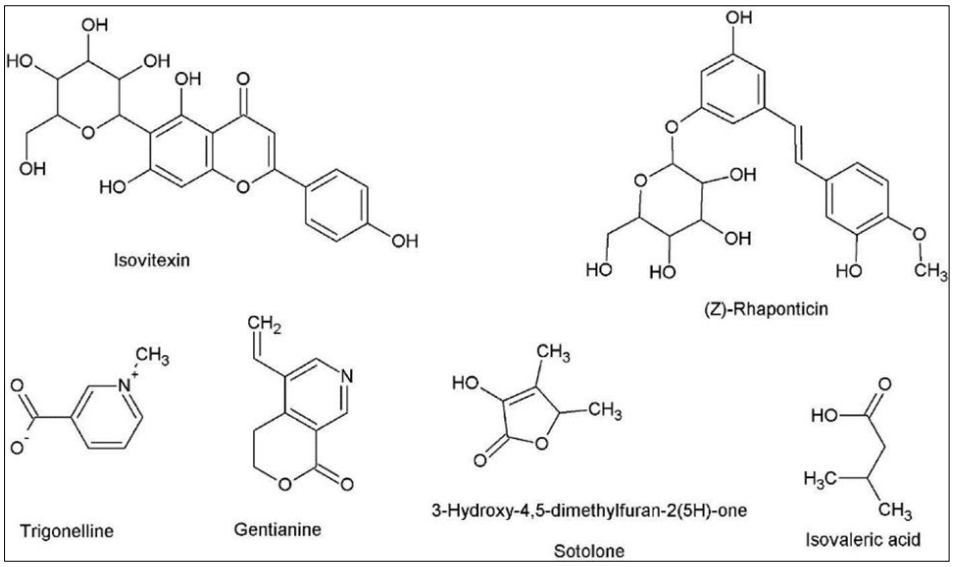
RESULT AND DISCUSSION PHARMACOGENETIC EVALUATION

Pharmacogenetic assessments guarantee the identification of botanicals and establish standardization criteria for preventing adulteration. These parameters are crucial for verifying the quality and purity of herbal drugs. The results revealed that all the physicochemical parameters are found to be within limits.

Table no 1: Determination of Proximate Analysis for powdered seeds of *Trigonella foenum graecum*

Parameters **Tests for extraneous material**	**Readings (per cent)**	**Mean (per cent w/w)**	**Inference (in per cent)**
Foreign Matter	1.04 0.96 1.19	1.06	Not more than 1.0
Sand & Silica	Absent Absent Absent	Absent	Should be absent
Physico-chemical analysis			
Loss on drying (LOD)	1.52 1.49 1.62	1.54	Not more than 2
Total ash	3.90 3.93 4.02	3.95	Not more than 4
Acid Insoluble Ash	0.47 0.48 0.41	0.45	Not more than 0.6
Water Soluble Ash	3.55 3.57 3.40	3.50	Not more than 4



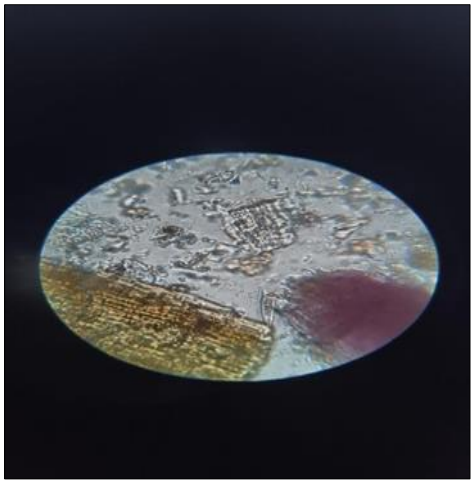


Extraction

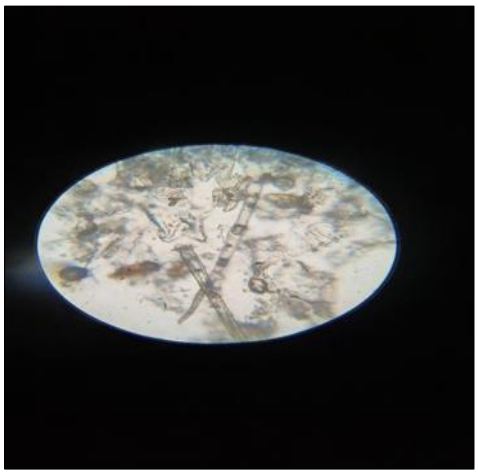
Extraction of *Trigonella foenum-graecum* was carried out by maceration technique and yield was found to be within limit.

Phytochemical Tests:

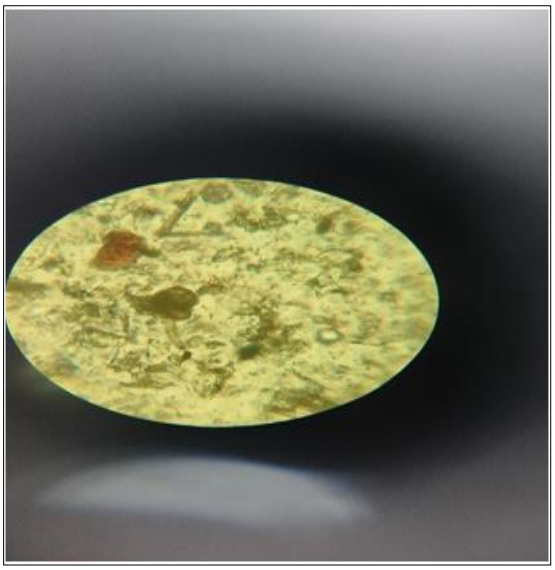
Powder Microscopical Characteristics:



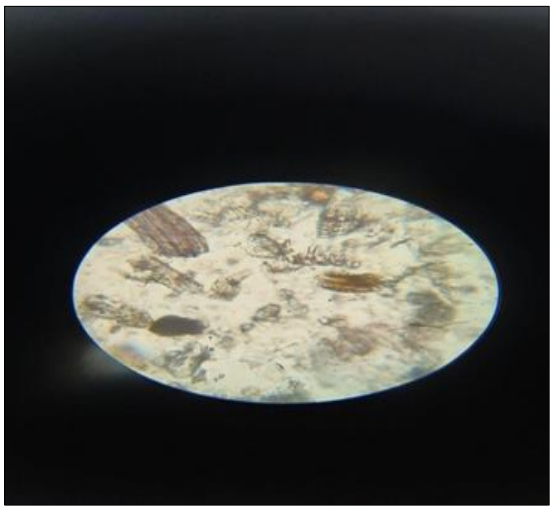
Picture 02: Phloem Fibers



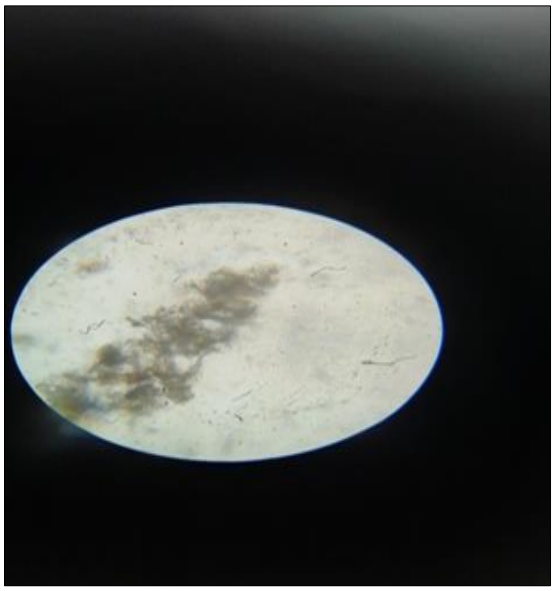
Picture 03: Calcium Oxalate Crystals



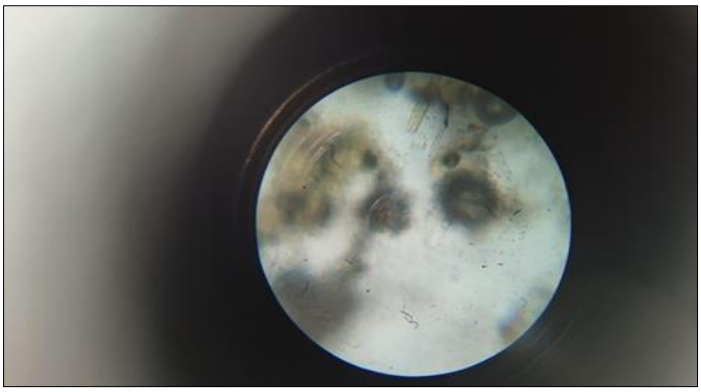
Picture 04: Proteins



Picture 05: Oil Globules



Picture 06: Starch



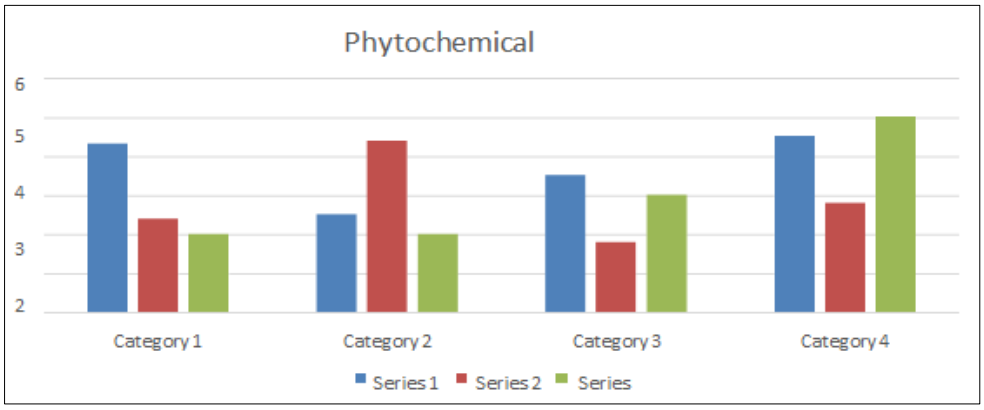
Picture 07: Microscopical Characters

Phytochemical Analysis

The phytochemical investigation tests were performed to determine the various phytoconstituents present in *Trigonella foenum-graecum* extract. The results revealed the presence of Steroids, Alkaloids, Flavonoids, Saponins and tannins.

Table no 02: Results of phytochemical screenings of successive extracts of seeds of *Trigonella foenum-graecum*

Phytochemial	Petroleum Ether	Methanol Extract	Benzene Extract	Chloroform Extract	Water: ethanolic extract
Steroids	+	-	+	+	+
Alkaloids	-	+	-	-	-
Flavonoids	-	+	-	-	-
Saponins	-	+	-	-	+
Tannins	+	+	-	-	+



Antioxidant Assay

Antioxidant activity was analyzed and compared to standard ascorbic acid. Remarkable free radical scavenging activity was observed. Table no. 3 showcases the IC_{50} values for the test and standard sample. Figure 1 represents the results of the assay Natural antioxidants be found abundantly in various food and medicinal plants. Safety concerns have emerged over time despite the widespread use of synthetic antioxidants. Numerous studies have been published, highlighting a potential link between prolonged consumption of these antioxidants and various health problems, including skin allergies, gastrointestinal issues, and, in certain instances, an elevated risk of cancer.

Table 3: Results of antioxidant assay

Standard			Sample		
Conc (µg/ml)	%RSA	IC50 (µg/ml)	Conc (µg/ml)	%RSA	IC50 (µg/ml)
5	47.14		5	25.22	
10	55.75		10	34.85	
15	61.52		15	42.44	
20	72.85		20	52.90	
25	80.50	5.40	25	61.19	18.64

1. Std % inhibition, 2. Sample % inhibition

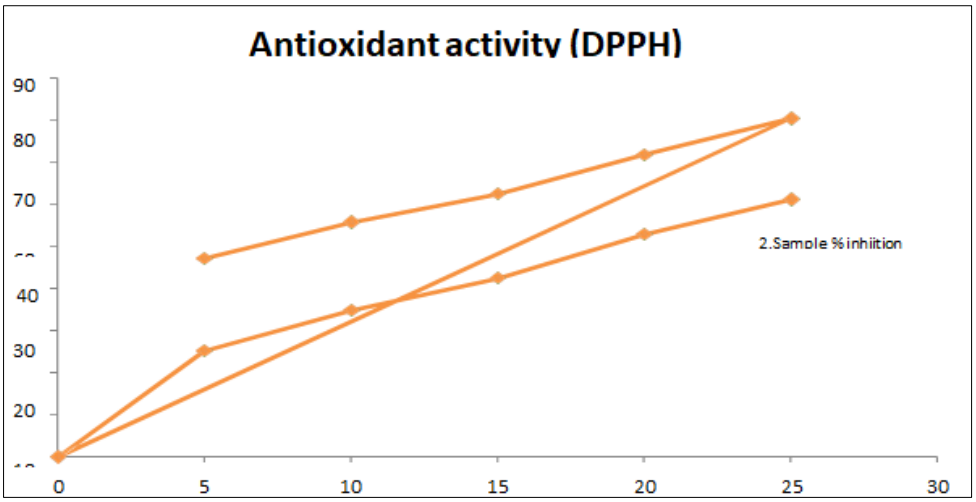


Figure 1: Antioxidant activity

Anti-Inflammatory Assay

The quest for alternative treatments that can disrupt the inflammatory process has emerged as a crucial focus in scientific investigations, particularly exploring natural compounds and minimizing the adverse effects of traditional drugs. In the present research work, the anti- inflammatory assay was performed by egg albumin method using diclofenac as a standard drug and it has shown promising results the results are depicted in Table 4 and Figure 2.

Table no. 4: Results of anti-inflammatory assay

Standard		Sample	
Conc (µg/ml)	**% Inhibition**	**Conc (µg/ml)**	**% Inhibition**
100	58.90	100	56.14
200	64.24	200	62.73
300	72.43	300	71.48
400	82.71	400	80.62
500	93.90	500	87.46

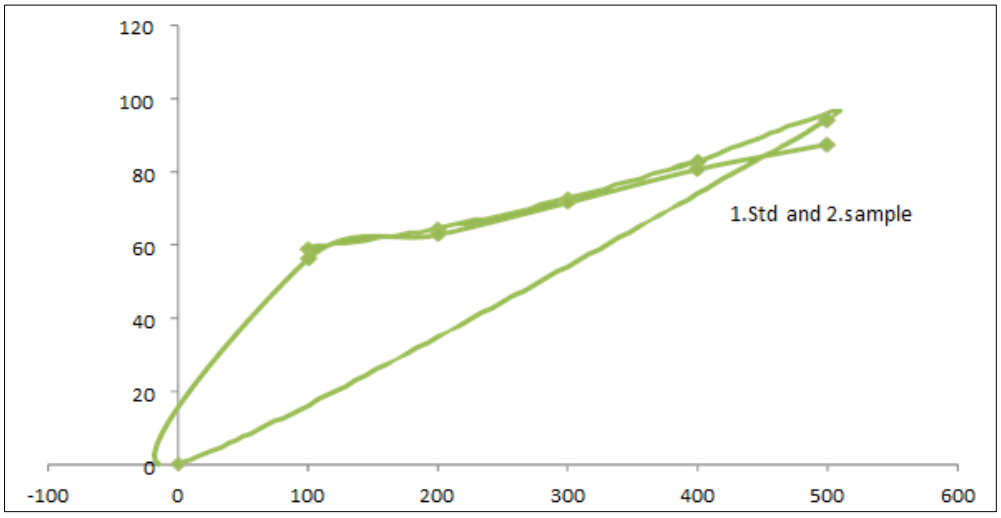


Figure 2: Anti-inflammatory activity

CONCLUSION

Ethnomedically, the seeds of *Trigonella foenum-graecum* were used by local people for the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Trigonella foenumgraecum seed contains both saponin and galactomannan polysaccharides which could be of use as natural antibacterial compounds. Inspite of numerous medicinal uses attributed to this plant, there is very less pharmacognostical report on the macroscopical and other physicochemical standards required for the quality control

of the crude drugs The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. In the present study the seeds were characterized macroscopically in terms of shape, size, colour and odour and the To further analyse the *Trigonella foenum-graecum* seed extract it was subjected to preliminary phytochemical screening. Several tests for the identification of the chemical constituents present in the ethanol extract were carried out according to the method of Harborne (1984). The presence of steroids, alkaloids, flavonoids, saponins and tannins in the seed extracts was studied and the presence of steroids and tannins was found in the petroleum ether extract, the methanol extracts showed the presence of alkaloid, flavonoids, saponins and tannins whereas the benzene and chloroform extracts only showed the presence of steroids also, the distilled water extract showed the presence of steroid, saponins and tannins. This comparative and multidisciplinary approach to the study of *Trigonella foenumgraecum* does help in understanding its identification, taxonomical determination, medicinal importance and scientific documentation in depth. *In vitro* antioxidant assay was performed using DPPH assay and results revealed good scavenging properties of prepared extract Even, it is evident in the results that, it showcased better anti- inflammatory potential as compared with standard diclofenac.

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