

PHARMACOGNOSTICAL PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION WITH SEEDS OF TRIGONELLA FOENUM - GRAECUM

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

By

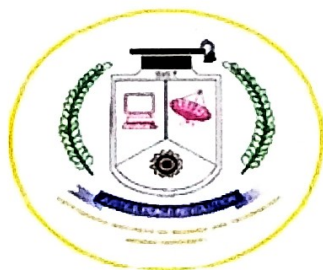
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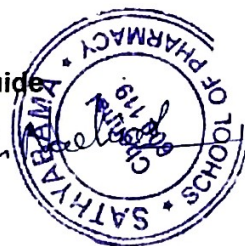
to the **SATHYABAMA Institute of Science & Technology** , Chennai – 600119 for the award of the degree of **Bachelor of Pharmacy** by Research is a bonafide record of research work carried out by him/her under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree, diploma, associateship, fellowship or any similar title and that it represents entirely an independent work on the part of the candidate.

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Declaration

I do hereby declare that the work presented in the thesis entitled "PHARMACOGNOSTICAL PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION WITH SEEDS TRIGONELLA FOENUM- GRAECUM", submitted by us to the SATHYABAMA Institute of Science & Technology, Chennai-600119 for the award of the degree of Bachelor of Pharmacy (2019-2023) by Research is a original record of research work carried out by me under the supervision of "Mrs. G. SHANTHINI NACHIYAR, Associate Professor, Department of Pharmacy". The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associateship etc.. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators

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ABSTRACT

Trigonella foenum-graecum Linn. (fabaceae), a spice seed used to flavour, color and texture of food and it is employed in various medicinal purposes in traditional systems. *Trigonella foenum-graecum* commonly known as fenugreek is a plant extensively used as source of anti diabetic compounds from its seeds

Trigonella Foenum Graecum (fenugreek) is a rich source of various essential compounds and such ingredients play an important role in treating diseases through their anti-diabetic activity. Furthermore, fenugreek seeds are a rich source of antioxidants and such ability prevents the pathogenesis of diseases through free radical scavenging activity. This research work summarizes the role of fenugreek seeds in health management with a special emphasis on modulating various physical and biochemical activities.

Keywords:

Trigonella Foenum- Graecum, Phytochemical, Pharmacognostic, Investigation Medicinal Plant , Anti- diadetic Activity..

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LIST OF ABBREVIATIONS

Abbreviation	Expansion
Pa	PARENCHYMA
VB	VASUCLAR BUNDLE
CT	CORTEX
E	EPIDERMIS
BC	BROWN CELL
CK	CORK
CU	CUTICLE
PCR	PRISMATIC CRYSTAL
PER	PERICYCLE
PI	PITH
V	VESSEL
LC	LYSIGENOUS CAVITY
MB	MEDULLARY BUNDLE
PH	PHLOEM
CHL	CHLORENCHYMA
SP	SPONGY PARENCHYMA
PAL	PALISADE PARENCHYMA
LE	LOWER EPIDERMIS
UE	UPPER EPIDERMIS
VE	VEIN
COLU	COLUMN CELLS OF SUB-EPIDERMIS

ENS	ENDOSPERM
XY	XYLEM
AG	ALEURONE GRAINS
ST	STOMATA
VI	VEIN ISLET
VT	VEIN TERMINATION

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CHAPTER - 1

INTRODUCTION

Despite the advances in Traditional medicine development, medicinal plants are still widely used for treating and preventing a variety of diseases due to their Traditional and nutraceutical values. *Trigonella foenum-graecum* L., are also commonly known as fenugreek, It belongs to the family of Fabaceae and is a self-pollinating annual herbaceous aromatic crop, which is commonly known as methi . Its origin is India and Northern Africa; however, it is now widely cultivated in Northern Africa, Europe, South Asia, Argentina, and Australia. Fenugreek is mainly produced in India, which accounts for 80% of the total world production .

Fenugreek seeds and leaves are used as a spice and ingredient in culinary preparation in several countries. It is used as a functional and traditional food, as well as in nutraceuticals and physiological application. Because of its high fibre, protein, and gum content, fenugreek has recently been utilized as a food stabilizer and emulsifying agent. Fenugreek is known to be one of the world's most ancient medicinal herbs, in relation to which the seeds and leaves are used as a treatment in various ailments . The leaves and seeds of *T. foenum-graecum* are extensively utilized to make extracts and powder for therapeutic applications in numerous investigations. There have been several preliminary animal and human trials that demonstrated fenugreek exhibiting hypoglycaemic, hypolipidemic, and hypocholesterolemic effects. *T. foenum-graecum* has also been reported to possess anti-fertility, anticancer, anti-parasitic, and antimicrobial effects . Fenugreek belongs to the Fabaceae family, *Trigonella* genus, and *Foenum-graecum* species

Fenugreek, *Trigonella foenum-graecum* L. is an annual crop from the family Leguminosae. The seed of this plant grown in South Asia, has been known to have health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases. The species name "*foenum-graecum*" means "Greek hay" indicating its

use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region, but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia. India is the leading fenugreek producing country in the world. Fenugreek has been used for centuries in folk medicine to heal ailments ranging from indigestion to baldness. Fenugreek is regarded as the oldest known medicinal plant in recorded history. In parts of Asia, the young plants are used as "pot herbs" and the seed as a spice or herbal medicine. *Trigonella foenum-graecum* is also employed as an herbal medicine in many parts of the world. Its leaves are used for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects. It is assumed to have a stimulating effect on the digestive process. Fenugreek seeds, which are described in the Greek and Latin Pharmacopoeias, are said to have anti-diabetic activity and hypocholesterolaemic effects. In addition, fenugreek has been reported to possess a curative gastric anti-ulcer action, anti-bacterial, anti-helminthic, anti-fertility effects and anti-nociceptive effects. Fenugreek seed contains both saponin and galactomannan polysaccharides which could be of use as natural antibacterial compounds.

Trigonella foenum graecum is one of the oldest medicinal plants, originating in India and Northern Africa. It is an annual plant, which grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. Its seeds are being used as spice and leaf as vegetable.

Leaves and seeds of *T.foenum-graecum* have been used extensively to prepare extracts and powders for medicinal uses. *T. foenum-graecum* is reported to have antidiabetic effects. The seeds of the *T. foenum-graecum* herb possess toxic oils, volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi. The potential uses of in vitro propagated plants as sources for new drugs are still largely unexplored. Based on several investigative studies, a compound produced in an in vivo plant could be produced at the same or different levels or not produced at all in an in vitro grown plant.

The seeds of fenugreek Fenugreek seeds are the most important and useful part of fenugreek plant. These seeds are golden-yellow in colour, small in size, hard and have four-faced stone like structure . Fenugreek seed is 3-6 mm long, 2-5 mm wide and 2 mm thick in geometry. Raw fenugreek seeds have maple flavour and bitter taste but by the process of roasting, their bitterness can be reduced and flavour can be enhanced. Fenugreek seeds are used as spices. The whole seed or its ground powder is used in pickles, vegetable dishes and spice powder. Dried seeds are used as condiments. Fenugreek seeds are gummy, fibrous, sticky and gummy in nature. Biologically, its seeds are endospermic in nature

Today Medicinal plants are found to use in different traits like pharmaceuticals, nutraceuticals, cosmetics and food supplements etc. Not only that, they are also used as traditional sources of medicine. Among the list of those important medicinal plants, Fenugreek is one of them. The seeds and plants are basically hot and dry and also they are suppurative, aperient, and diuretic. They have some useful aspect in dropsy, chronic cough, enlargement of the liver and the spleen. The leaves of Fenugreek is useful for both internal and external swellings and burns and also used to prevent the hair falling off The seeds are considered as carminative, tonic and aphrodisiac.

Fenugreek is used to ease child birth as well as it helps to increase the milk flow of mother. Egyptian women are still taking Fenugreek for menstrual pain and tourist use it as hilba tea to remove stomach problem. Not only that the plant is also recommended for use in dyspepsia with loss of appetite, in diarrhoea of puerperal women, and in rheumatism.

An infusion of seeds is given to small-pox patients as a cooling drink. Fenugreek seed contains various bioactive compounds like flavonoids (quercetin, rutin, vetexin), saponins (graecunins, fenugrin B, Fenugreekine), amino acids (isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine). As medicinal plant it shows its activity against allergies, appetite / loss of catarrh, bronchial, cholesterol, diabetic retinopathy, gas, gastric disorders, lung infections, mucus excessive, throat/sore, abscesses, anemia, asthma, boils, body odour, bronchitis, cancer, swollen eyes, fevers, gallbladder problems, heartburn, inflammation, sinus problems, ulcers, uterine problems etc. A study in India showed that Fenugreek

seed is used to reduce the blood sugar and other harmful fats. Not only therapeutic, Fenugreek also used as spices worldwide. The leaves are used as green leafy vegetables in the diet. Fenugreek seeds are bitter in taste and have been in use for over 2500 years. Fenugreek is a leguminous herb belonging to fabaceae, cultivated throughout the world specially in the Asia and North African countries. In different languages it has different names, as Fenugrec (French), Methi (Hindi), Bockshorklee (German), Fienogreco (Italian), Pazhitnik (Russian), Alholva (Spanish), Koroha (Japanese), Hulba (Arabian), Halba (Malaya), and K'u-Tou (China). India is the major producer of Fenugreek and its main consumers are culinary and medicinal users. In the indigenous system, it is effective against anorexia, and as gastric stimulant.

1.1 - BOTANICAL CLASSIFICATION OF TRIGONELLA FOENUM GRACUM

Domain	Eukarya
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Sub- Family	Trifoliae
Genus	Trigonella
Sub-Genus	Foenum graecum
Species	Trigonella Foenum- graecum.

1.2 -PHYSICAL AND CHEMICAL PROPERTIES OF TRIGONELLA FOENUM GRACUM

Fenugreek (*T. foenum-graecum*) seeds sprout in three days once sown in suitable soil. The seedlings grow erect or semi-erect up to 30 to 60 cm of height . It is a self-pollinating annual leguminous bean that aids in soil nourishment and nitrogen fixation. Fenugreek plants are green in colour and

slender in shape, with yellow-brown pods bearing 10 to 20 seeds. Fenugreek seeds are brownish-yellow in colour, small, rhomboidal-shaped, and hard seeds are 3 to 6 mm long, 2 to 5 mm wide and 2 mm thick . Crude fenugreek seeds are known for their pleasantly bitter and maple flavour. They are glutinous, fibrous, and sticky in texture and biologically endospermic in nature.

1.3 - TRADITIONAL USES OF MEDICINAL PLANTS

The seeds also function as a preservative and are added to pickles, chutneys and other similar products (Vortex health, Fenugreek). In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages.

The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. Helpful in lowering fever, it is compared to quinine by some authorities. The seeds soothing effect makes them of value in treating gastritis and gastric ulcers. The seeds freshen bad breath and help restore a dulled sense of tastes.

Use fenugreek for head colds, influenza, catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis (Home remedies guide).

Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorb toxic material and eliminate it. The healing and soothing action creates a protective coating, like a lubricant, over inflamed areas. The slightly bitter properties of the seed are beneficial for digestion.

1.4 - BENEFICIAL ASPECTS OF FENUGREEK

Fenugreek seeds are rich with vitamin E. Fresh Fenugreek leaves are beneficial for indigestion, flatulence and in sluggish liver treatment. Regular use of fresh Fenugreek leaves paste helps hair grow, preserves natural color, keeps hair silky and also cures dandruff.

Fenugreek Seeds made in gruel, are given to nursing mothers to increase the flow of milk and also reduce the amount of calcium oxalate in the kidney which causes kidney stones. In animal studies, Fenugreek appeared to lowering the chance of developing colon cancer by blocking the action of certain enzymes. Traditional Chinese herbalists used Fenugreek for kidney problems.

Fenugreek is currently used as a source of the steroid diosgenin , one of its active constituent from which other steroids can be synthesized. Not only that Fenugreek has a broad range of pharmacological profile but also it has antidiabetic activity, antiplasmodic activity, hypolipidemic activity, immunological activity, antibacterial activity, anthelmintic activity, anti-inflammatory, analgesic activity and antioxidant activity

1.5 - ANTIDIABETIC INFLUENCE

It has been well recognized that dietary fiber offers substantial benefits to persons with diabetes mellitus. The mechanism of Fenugreek action in human is not properly understood but animal studies have revealed the ability of dietary fiber to delay gastric emptying, suppress release of the gastric inhibitory peptides and insulinotropic hormones .

Several researchers reported that treatment with a decoction of Fenugreek seed improved diabetes and suppressed glycosuria in mild diabetes and brought improvement in severe diabetic condition Fenugreek contains fiber to an extent of 51.7%, containing 19.2% mucilaginous fiber, and 32.5% neutral fiber, respectively.

It also contains an alkaloid named trigonelline, which has an effect on glycosuria. National Institute of Nutrition, India have demonstrated the beneficial effect of Fenugreek in both type-1 and type-2 diabetes with the help of different clinical trials on animals. There are so many reports available on the antidiabetic property of Fenugreek seeds.

CHAPTER - 2

LITERATURE SURVEY

A literature review is an evaluative report of studies found in the literature related to the selected area of research. The review describes, summarizes, evaluates and clarifies the literature available in the present research. It is a step towards further investigation on a particular work. It denotes with works derived from primary and secondary sources.

Review of Research Studies are presented under 2 headings:

- ✧ Studies related to Fenugreek Seeds,
- ✧ Studies related to Fenugreek Seed powder and diabetes mellitus.

STUDIES RELATED TO FENUGREEK SEED

Abu Saleh M. Moosa, (2006) studied the effects of fenugreek serum lipid profile in hypercholesteremic Type II diabetic clients, on administration of 25 gm of fenugreek seed powder orally twice daily for 3 weeks and 6 weeks .The findings revealed that there was significant ($P < 0.001$) reduction of serum total cholesterol, triacylglyceride and LDL-cholesterol in hypercholesteremic group. The study concluded that fenugreek seed powder would be considered as effective agent for lipid lowering purposes.

Pandian RS, Anuradha CV, Viswanathan P. (2002) the gastroprotective effect of fenugreek seeds were studied, on the basis of the effect of fenugreek seeds compared to omeprazole. The aqueous extract and a gel fraction isolated from the seeds showed significant ulcer protective effects. The cytoprotective effect of the seeds was due to the anti-secretory action but also due to the effects on mucosal glycoproteins.

The fenugreek seeds prevented the rise in lipid peroxidation induced by ethanol presumably by enhancing antioxidant potential of the gastric mucosa thereby lowering mucosal injury. Histological studies revealed that the 20 soluble gel fraction derived from the seeds was more effective than omeprazole in preventing lesion formation. These observations show that fenugreek seeds possess antiulcer potential.

M. Prasanna (2000) reported the hypolipidemic effect of fenugreek in hypercholesterolemia clients were studied. The Fenugreek seeds (FG) were powdered and was used for the study.

STUDIES RELATED TO FENUGREEK SEED POWDER AND DIABETES MELLITUS

Hiba A Bawadi (2009), *Trigonella foenum-graecum* has been reported to be beneficial for treating Type II Diabetes Mellitus. The study was conducted to investigate the postprandial hypoglycemic effect of fenugreek seeds on clients with Type II Diabetes Mellitus. Pretest - posttest control group design was used to test the hypothesis that fenugreek may have a hypoglycemic effect on blood sugar. One hundred sixty-six Type II Diabetes Mellitus clients were assigned into Fenugreek 0 (control group placebo drink), fenugreek 2.5 gm and fenugreek 5gm.

The participants were instructed to drink the extract and chew the seeds. Postprandial plasma glucose level was measured before and 2 hours after the administration of the treatment. The clients in fenugreek 5gm group showed the greatest decrease postprandial glucose with a pretest-posttest difference of 41 ± 6.1 mg/dl. Two hour plasma glucose dropped. Fenugreek seeds appear to have significant hypoglycemic activity in Type II Diabetes Mellitus clients

Bardia (2001) the Subjects were 20 Type II diabetes mellitus clients 2.5 g fenugreek seed twice daily for 30 days; the result showed that the fenugreek significantly reduced both fasting and postprandial blood glucose levels.

Raghuram TC (2001) 10 Type II diabetes mellitus clients (Aged between 38-54 yrs) were given the dose of 25g powdered fenugreek seed divided in to two equal

doses for 15 days. The result showed that the mean plasma glucose levels were significantly reduced. Area under the curve (AUC) reduced. Increase in glucose metabolic clearance rate was significant.

Sharma and Raghuram (2001) in an RCT, studied the effects of a diet enriched with fenugreek seeds on Type II diabetes mellitus clients at the National Institute of Nutrition Research, Hyderabad, India, using a randomized, crossover design in two trials.

These clients (33 percent female, ages 32 to 60) had diabetes from 2 months to 16 years. The second group consisted of five Type II diabetes mellitus clients (ages 35 to 58) who were also randomized to receive a fenugreek-enriched diet for either the first or second 20 days of the trial.

The researchers concluded that the addition of 100 g of fenugreek seeds to the daily diet of Type II diabetes mellitus clients could be effective supportive therapy in the prevention and management of long-term complications of diabetes.

CHAPTER - 3

AIM & SCOPE OF THE WORK

The use of herbal products is of global importance because of their low side effects, accessibility and affordability when compared with conventional medicine. *Trigonella foenum-graecum* is popular in indigenous systems of folk medicine and it is known to contain bioactive compounds such as trigonelline, isoorientin, orientin, vitexin, and isovitexin.

Fenugreek is used as a herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for the distinctive maple syrup smell of fenugreek.

Cuboid, yellow- to amber-coloured fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, dal, and spice mixes such as *panch phoron* and *sambar* powder. They are often roasted to reduce inherent bitterness and to enhance flavour.

This research work comprehensively summarizes the role of fenugreek seeds in health management with a special emphasis on modulating various physical and biochemical activities as well as in studies.

Based on the ethnomedical information and studies available, the present research work has been framed to carry out the following studies on the leaves of *Trigonella Foenum Gracum*

✧ **Phytochemical Evaluation** □

Preliminary phytochemical screening.edrrf

Estimation of Total Flavonoids Content.

✧ **Pharmacognostical Evaluation**

Macroscopy.

Anatomical Section of Leave, Stem, Seed.

Quantitative Microscopy of seeds.

✧ **Pharmacological evaluation**

In - Vitro Anti - Diabetic Activity Method

Non-enzymatic glycosylation of haemoglobin Assay.

CHAPTER - 4

EXPERIMENTAL WORK

4.1 - PHYTOCHEMICAL STUDIES

The leaves and stem of Feenugreek (*Trigonella foenum-graecum*) were collected and authenticated by Siddha Central Research Institute. The seeds and leaves were powdered and then subjected to the following preliminary phytochemical tests.

4.1.1 ORGANOLEPTIC EVALUATION

1. Nature - Coarse Powder
2. Colour - Light Brown
3. Odor - Odorless
4. Taste - Characteristic
5. Texture - Rough & Irregular

4.1.2 - GENERAL PROCEDURE OF PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE POWDER OF TRIGONELLA FOENUM GRAECUM

TEST FOR FLAVONOIDS

- a) . Shinado's Test :

a). The substance was shaken with 5 ml of alcohol and filtered. To the filtrate, a piece of magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes, Appearance Red color. Presence of Flavonoids

b). The substance was shaken with 5 ml of alcohol and filtered. To the filtrate, 10% sodium hydroxide solution or ammonia was added. Appearance Dark yellow colour, Presence of Flavonoids

c). The substance was shaken with 5 ml of alcohol and filtered. To the filtrate 1 ml of lead acetate solution was added, Yellow precipitate was formed. Presence of Flavonoids.

TEST FOR ALKALOIDS

The powdered plant material was boiled with 5 ml of dilute sulphuric acid and filtered. The filtrate was used for the following tests.

a). To the filtrate, a few drops of Dragendorff's reagent was added and shaken well. Formation of orange red precipitate, Presence of alkaloids.

b). To the filtrate, few drops of Wagner's reagent was added and shaken well. Formation of reddish brown precipitate, Presence of alkaloids.

c). To the filtrate, few drops of Mayer's reagent was added and shaken well. Formation of white precipitate, Presence of alkaloids.

TEST FOR PHENOL

a). To the alcoholic extract of the test substance a few drops of 5 % ferric chloride solution was added. Deep blue or black colour was formed, Presence of Phenol.

TEST FOR TANNINS

- a). To the aqueous extract of the test substance few drops of lead acetate solution was added. Formation of white precipitate, Presence of Tannins.

TEST FOR CARBOHYDRATES

- a). The substance was boiled with Fehling's solution I and II. Appearance of red coloration, Presence of Carbohydrates.

(Table - 4.1)

**PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE POWDER OF
TRIGONELLA FOENUM GRAECUM**

S:NO	TEST	RESULT
1.	TEST FOR FLAVONOIDS	
	a. Shinoda test	+
2.	TEST FOR ALKALOIDS	
	a. Dragendorff's reagent	+
	b. Wagner's reagent	+
	c. Mayer's reagent	+
3.	TEST FOR PHENOL	
	a. FeCl ₃ test	+
4.	TEST FOR TANNIS	
	a. Lead Acetate Test	+
5.	TEST FOR CARBOHYDRATES	
	a. Fehling's test	+
6.	TEST FOR VOLATILE OILS	-

(+) indicates positive reaction

(-) indicates negative reaction

(Table - 4.1. 2)

**PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE VARIOUS EXTRACTS
OF LEAF POWDER OF TRIGONELLA FOENUM GRAECUM**

S:NO	TEST	Ethyl Acetate	N- Butanol	Petroleum Ether	Ethanol
1.	TEST FOR FLAVONOIDS				
	a. Shinoda test	+	+	+	+
2.	TEST FOR ALKALOIDS				
	a. Dragendroff's reagent	+	+	+	+
	b. Wagner's reagent	+	+	+	+
	c. Mayer's reagent	+	-	+	-
3.	TEST FOR PHENOL				
	a. FeCl ₃ test	-	-	-	+
4.	TEST FOR TANNIS				
	a. Lead Acetate Test	+	-	+	-
5.	TEST FOR CARBOHYDRATES				
	a. Fehling's test	-	-	-	-
6.	TEST FOR VOLATILE OILS	-	-	-	-

(+) indicates positive reaction

(-) indicates negative reaction

4.2 - TOTAL FLAVONOID DETERMINATION

PRINCIPLE

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C4 keto group and other to C, or C5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavanoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids.

REAGENTS REQUIRED

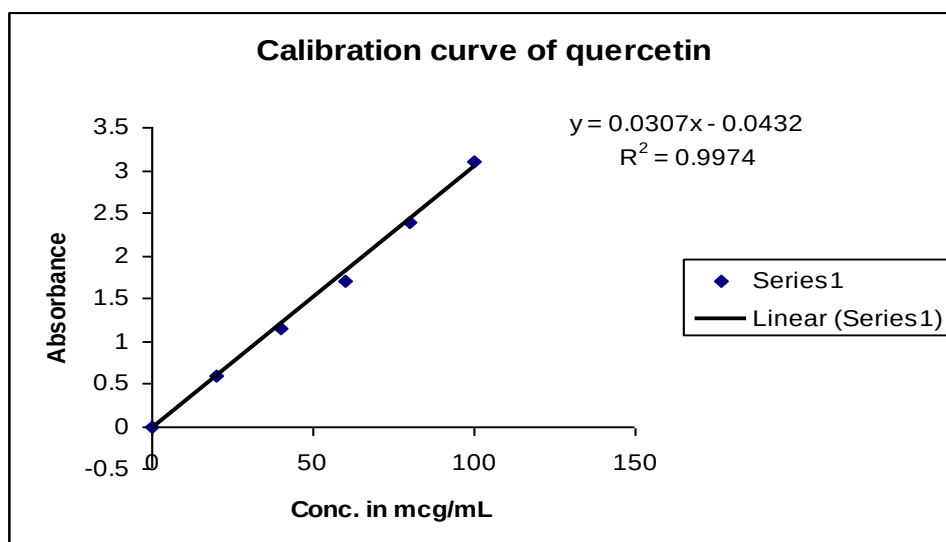
10% Aluminum chloride

1M Potassium acetate

PROCEDURE

1 mL of Ethanolic extract at concentrations 50µg/mL and 100µg/mL were taken and 0.1mL of aluminum chloride solution, 0.1mL of potassium acetate solution and 28mL of ethanol were added and the final volume was then made up to 5mL with distilled water. After 20 min the absorbance was measured at 415 nm. A calibration curve was constructed by plotting absorbance reading of quercetin at different concentrations. The sample without aluminium chloride was used as a blank.

The total flavonoid content in the extract was expressed as milligrams of quercetin equivalent per gram of extract and the results were tabulated.



ESTIMATION OF TOTAL FLAVONOID CONTENT OF *Trigonella foenum-graecum*

(Table - 4.2.1)

S: No	Conc. of quercetin in $\mu\text{g/ml}$	Absorbance at 415nm	Conc. of ethyl acetate extract in $\mu\text{g/ml}$	Absorbance at 415nm	Amt of total flavonoid content in terms mg quercetin equivalent/ g of extract
1	20	0.589 ± 0.01	50	0.0383 ± 0.001	467.21 ± 0.351
2	40	1.151 ± 0.04	100	0.0896 ± 0.006	486.36 ± 0.06
3	60	1.710 ± 0.09	150		
4	80	2.390 ± 0.03	-		
5	100	3.112 ± 0.03	-		
				Average	24.08

4.3 - PHARMACOGNOSTICAL STUDIES

4.3.1 - Macroscopy

(SOP No. PCOG-004-SOP): External feature of test sample was documented using Nikon D-5600 Digital camera.

4.3.2 - Microscopy

(SOP No. PCOG-005-SOP): Sample was preserved in fixative FAA for more than 48 hr. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin.

Transverse sections were photographed using Axiolab 5trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by scale bar.

4.3.3 - Quantitative microscopy

(SOP No. PCOG-007-SOP): The fresh leaf samples were boiled with saturated cholral hydrate solution and slides prepared.

Vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined.

4.3.1 - Macroscopy

The aerial part of the plant is moderately branched and weak.

The stem is erect, branched, green, smooth, herbaceous.

Leaves are petiolate, alternate, compound, trifoliate, fragrant and stipulate; seeds oblong flattened or irregularly rhomboidal, hard, a narrow depression dividing radicle pocket from cotyledons is seen, yellowish brown in colour with characteristic spicy odour and spicy mucilaginous, bitter taste (Fig - 4.1.1)



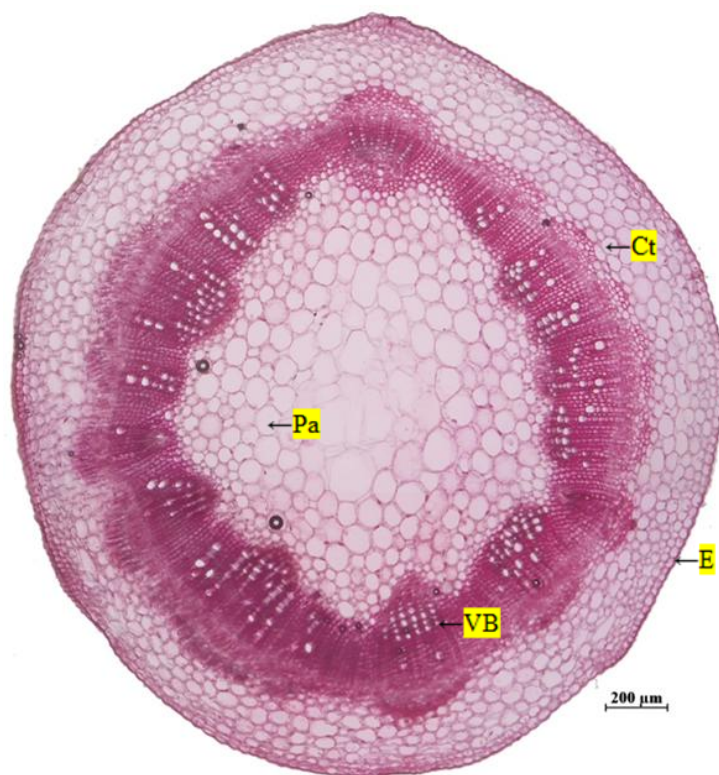
Twig of *Trigonella foenum-graecum* L. stem & leaves

(Fig - 4.3.1)

4.3.2 - Microscopy

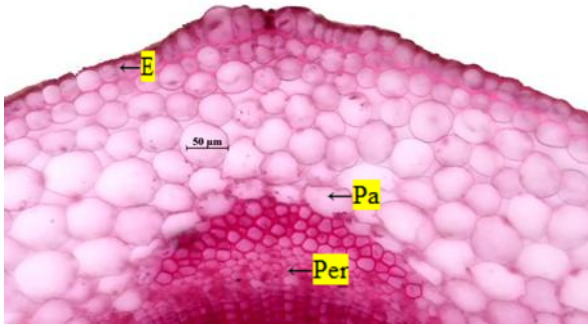
STEM

TS is oblong circular with uniseriate epidermis covered by a thin cuticle; a single layered collenchymatous hypodermis is present; cortical region is made up of 5 to 6 layers of thin walled parenchyma cells; collateral vascular bundles are arranged as a ring; discontinuous pericyclic fibres are seen surrounding the vascular region; a few prismatic crystals are seen randomly distributed in the pericyclic region; centrally placed thin walled parenchymatous pith is present in (Fig 4.2.1)

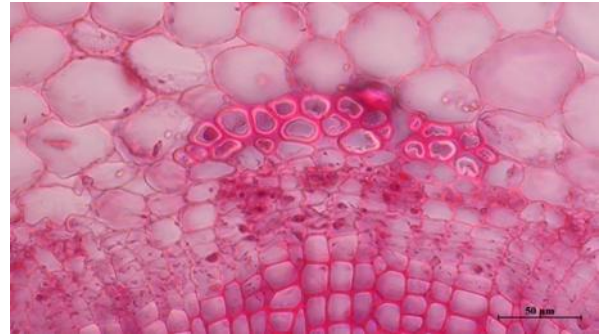


(Fig - 4.3.1) TS of *Trigonella foenum-graecum* stem

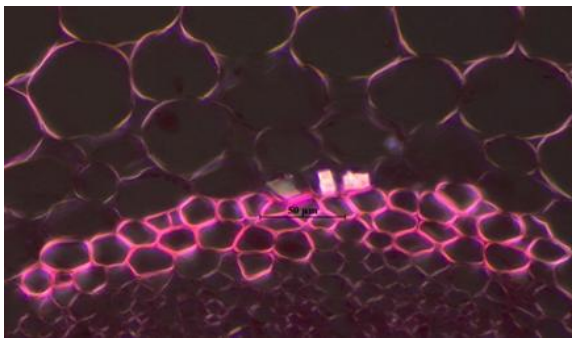
Pa- parenchyma; **VB** - vascular bundle **Ct** – cortex **E** – epidermis



(Fig - 4.3.2) Upper portion enlarged

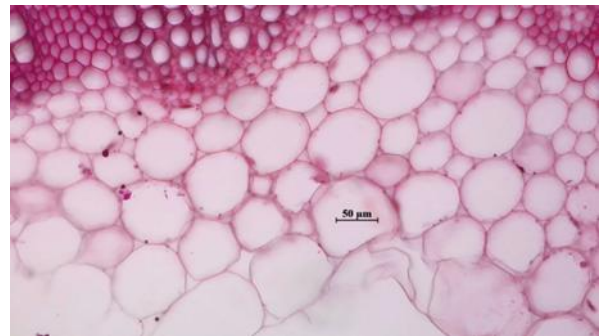


(Fig - 4.3.3) Vascular region enlarged



(Fig - 4.3.4)

Pericyclic fibre and crystal under polarizer

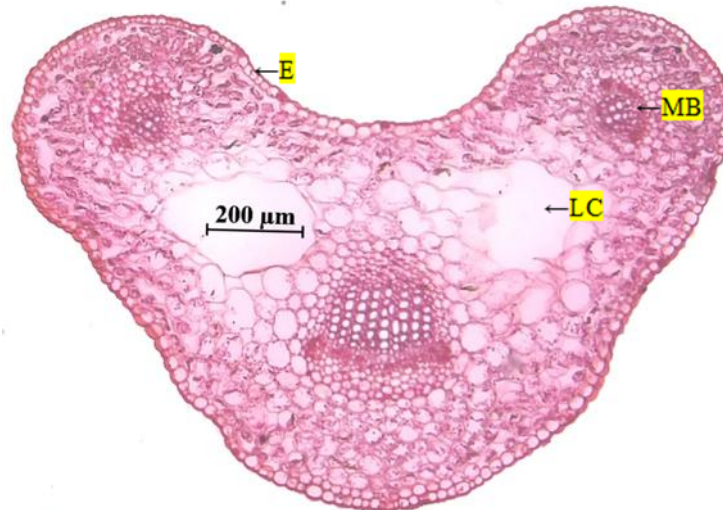


(Fig - 4.3.5) - Pith

BC - brown cell; **Ck** - cork; **Ct** - cortex; **Cu** - cuticle; **E** – epidermis; **Pa**- parenchyma;
PCr - prismatic crystal; **Per** - pericycle; **Pi** - pith; **V** – vessel;
VB - vascular bundle

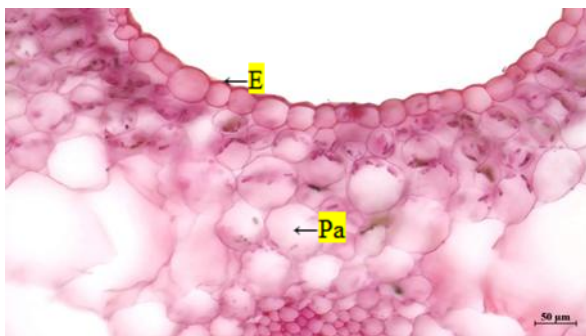
PETIOLE

TS is broad cordate in shape with a uniseriate epidermis surrounded by thin cuticle; ground tissue is made up of outer 2 layers of chlorenchymatous cells in the upper region and 4 to 5 layers in the lower region; followed by 3 to 5 layers of thin walled parenchymatous cells; a centrally placed bicollateral vascular bundle is present; small medullary bundles are seen below the winged region; 2 layered discontinuous pericycle is present surrounding the vascular bundles; two prominent lysogenic cavities are present in the inner ground tissue region in (Fig 4.2.6)

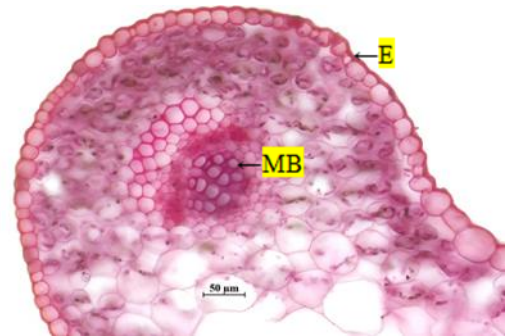


(Fig - 4.3.6) TS of *Trigonella foenum-graecum* petiole

E – epidermis **LC** - lysigenous cavity **MB** - medullary bundle



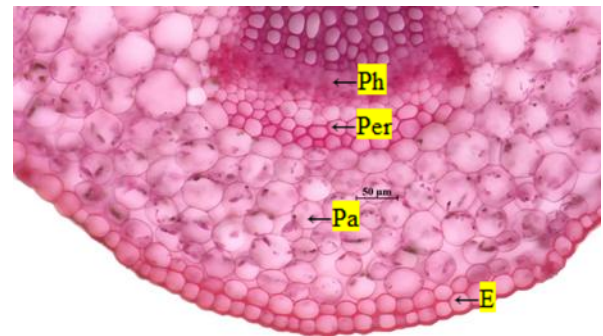
(Fig - 4.3.7) Upper portion enlarged



(Fig - 4.3.8) Winged portion enlarged



(Fig - 4.3.9) Vascular bundle



(Fig - 4.3.10) Lower portion enlarged

Cu - cuticle; **E** - epidermis; **LC** - lysigenous cavity; **MB** - medullary bundle;
Pa - parenchyma; **Per** - pericycle; **Ph** - phloem; **V** – vessel

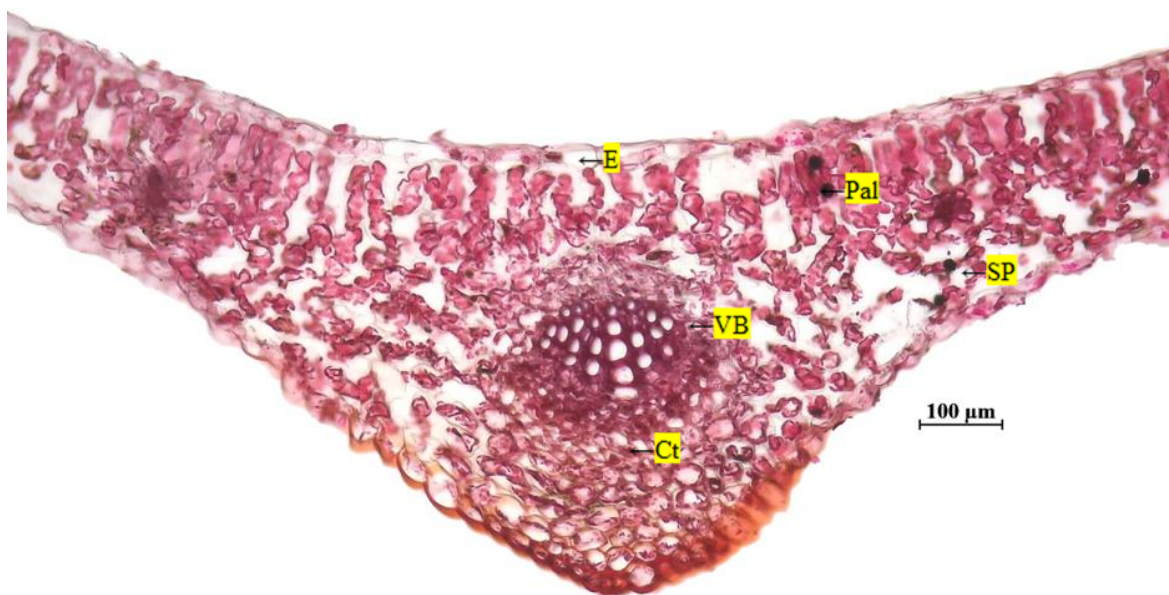
LEAF

Midrib :

TS shows single layered epidermis covered by thin cuticle; upper epidermis is followed by two layers of palisade cells; centrally located large vascular bundle is present surrounded by patches of pericyclic fibres; phloem is present below the xylem; 3 to 5 layers of chlorenchyma cells are present above the lower epidermis(Fig. 4.2.11).

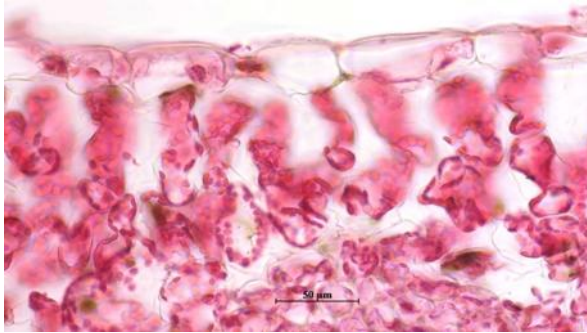
Lamina :

TS is dorsiventral and shows single layered wavy epidermis covered by indistinct cuticle; mesophyll is differentiated into outer two layers of palisade followed by inner 3 to 4 layers of spongy parenchyma cells; veins are seen traversing through the mesophyll (Fig. 5).



(Fig - 4.3.11) TS of *Trigonella foenum-graecum* midrib

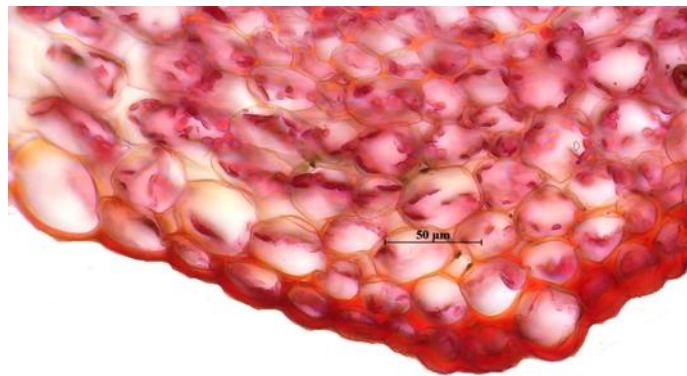
Ct - cortex; **E** - epidermis; **Pal** - palisade parenchyma; **SP** - spongy parenchyma;
VB - vascular bundle



(Fig - 4.3.12) Upper portion enlarged

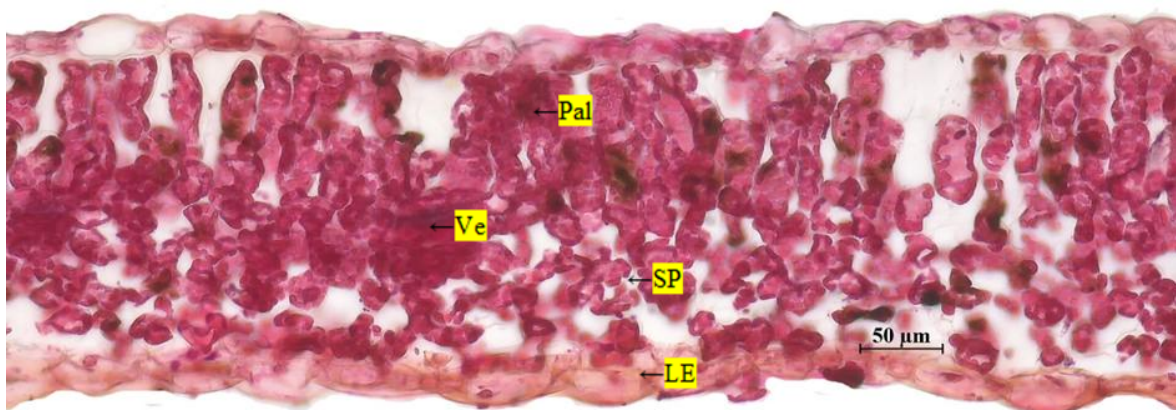


(Fig - 4.3.13) Vascular bundle

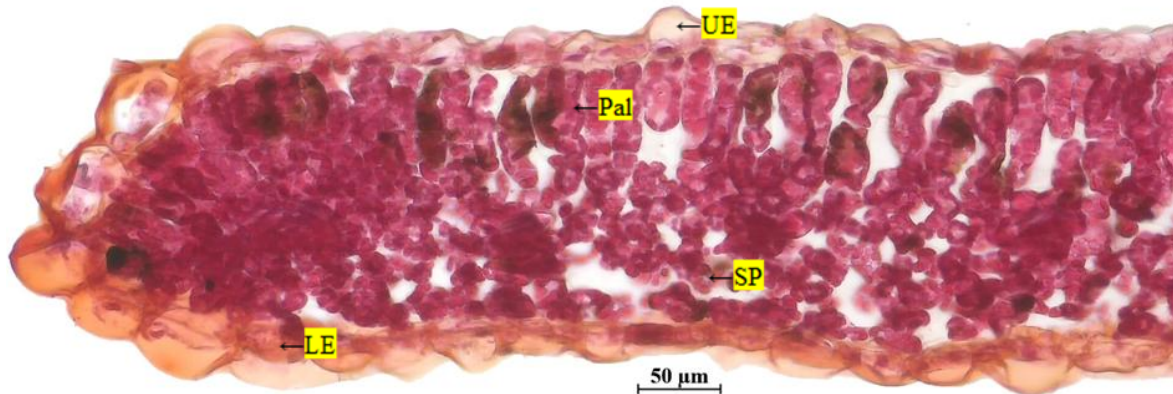


(Fig - 4.3.14) Lower portion enlarged

Chl - chlorenchyma; **Ct** - cortex; **Cu** - cuticle; **E** - epidermis; **Pal** - palisade parenchyma; **Ph** - phloem; **SP** - spongy parenchyma; **V** - vessel;
VB - vascular bundle



(Fig - 4.3.15) TS of *Trigonella foenum-graecum* lamina



(Fig - 4.3.16) TS of margin

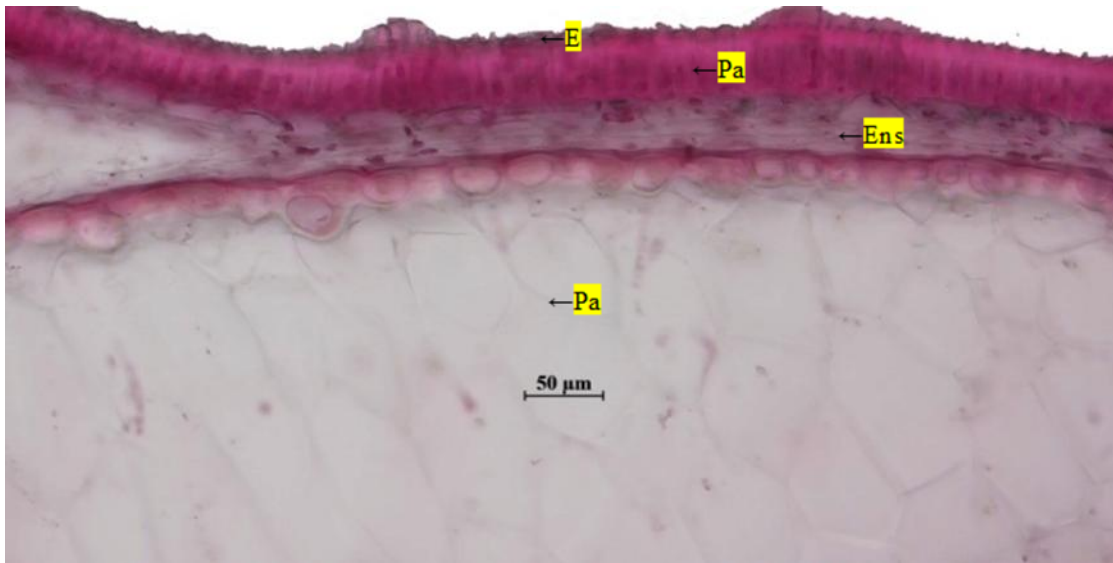
LE - lower epidermis; **Pal** - palisade parenchyma; **Sp** - spongy parenchyma;
UE - upper epidermis; **Ve** – vein

SEED

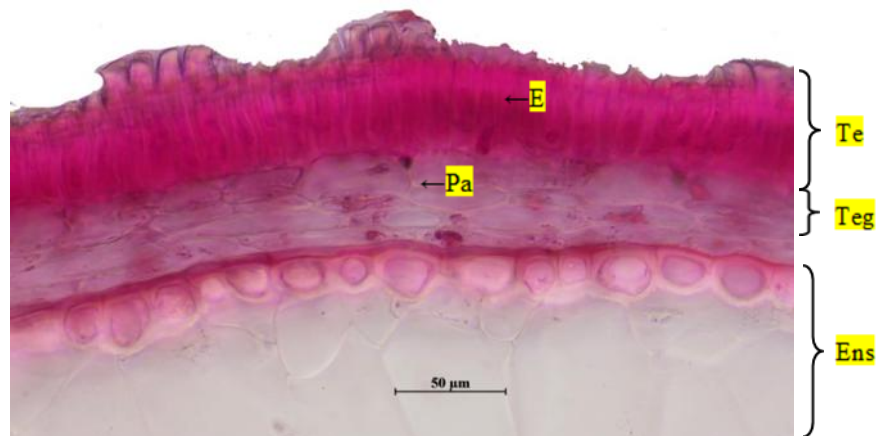
TS shows outermost layer of testa made up of thick-walled cylindrical palisade like cells covered with thick cuticle; below it a sub epidermis made up of columnar cells are present; a narrow thin zone of tangentially elongated parenchyma cells follows; outer endosperm cells are filled with aleurone grains and the inner endosperm cells are made up of large parenchymatous cells filled with mucilage.

Cotyledon consists of upper and lower epidermis; 4 to 5 layers of palisade cells are seen below the upper epidermis; on the dorsal side 7 to 10 layers of spongy parenchyma cells are seen which are filled with oil globules and aleurone grains; small vascular bundles are present in the mesophyll region.

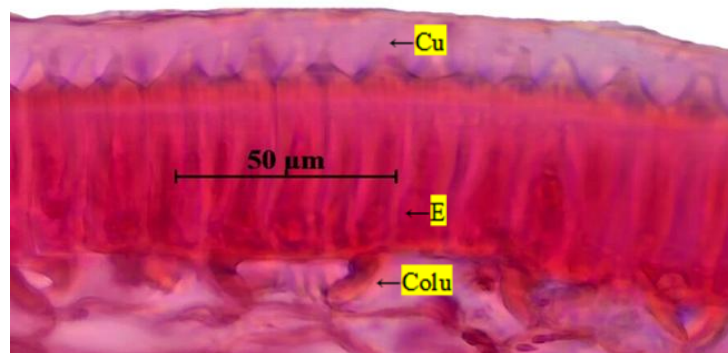
Radicle TS is circular in outline; the single layered epidermis is present covered by cuticle followed by broad cuticle made up of thin walled parenchyma cells filled with aleurone grains; vascular region is present in the centre



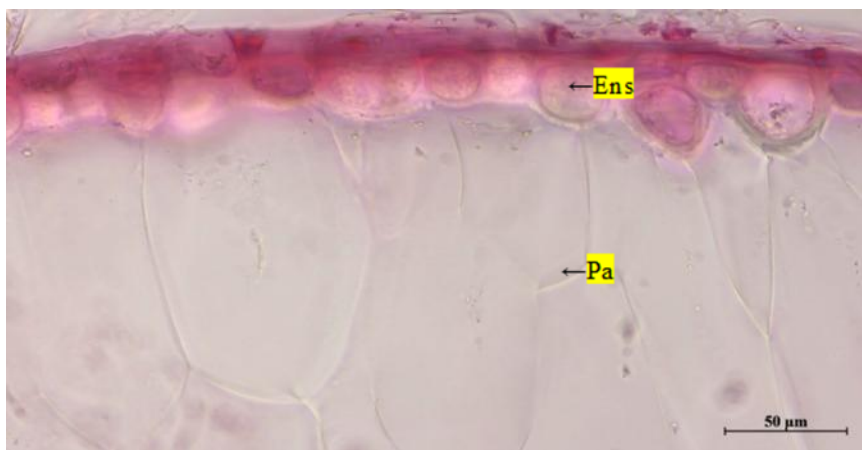
(Fig - 4.3.17) TS of *Trigonella foenum-graecum* seed



(Fig - 4.3.18) TS of seed coat enlarged view

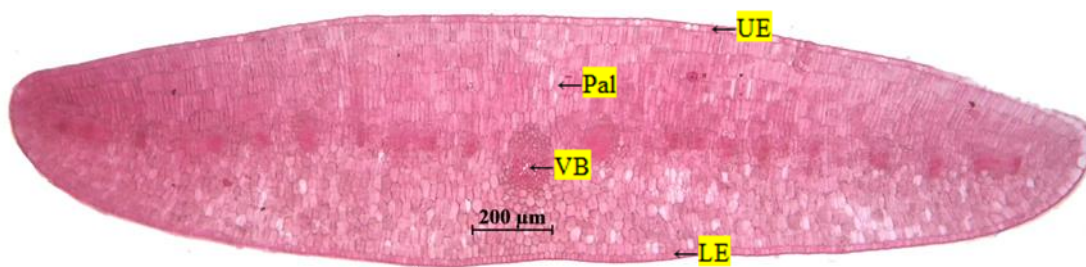


(Fig - 4.3.19) Testa and tegmaen enlarged view

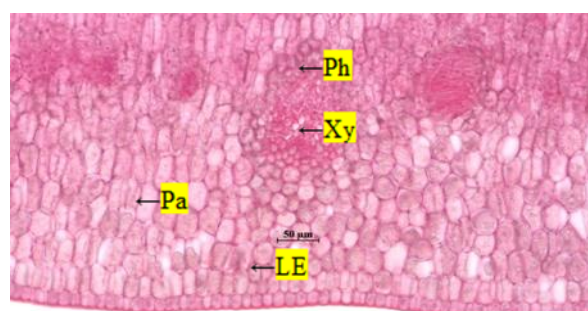
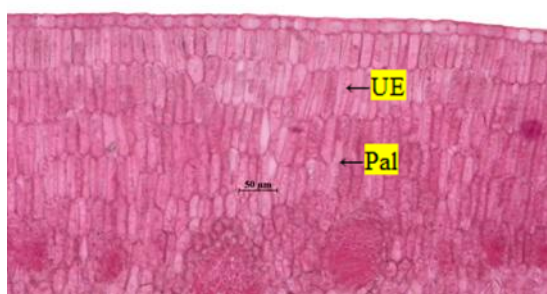


(Fig - 4.3.20) Endosperm enlarged view

Colu - column cells of subepidermis; **Cu**- cuticle; **E** - epidermis; **Ens**- endosperm;
Pa – parenchyma

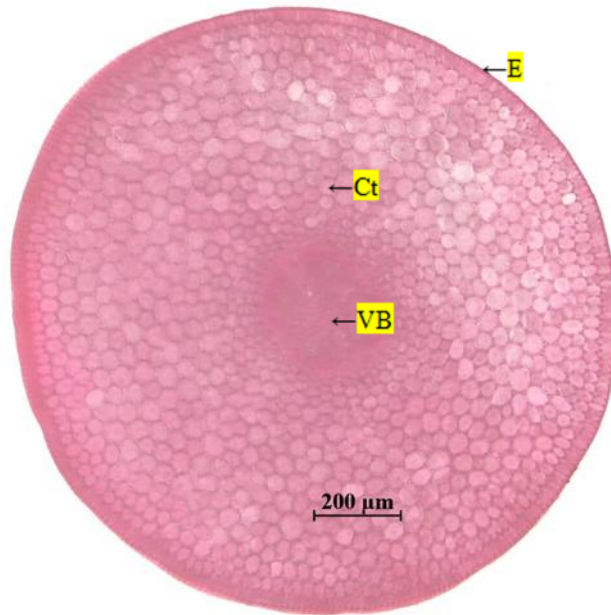


(Fig - 4.3.21) TS of *Trigonella foenum-graecum* cotyledon

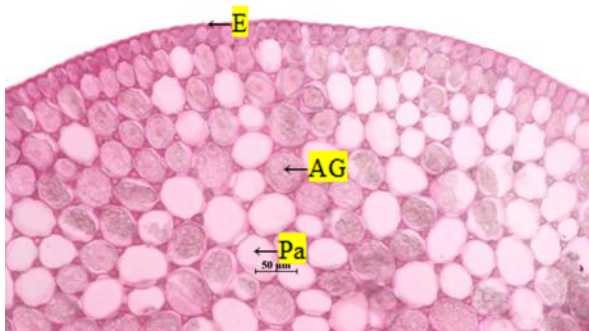


(Fig - 4.3.22) Upper portion enlarged (Fig - 4.3.23) Lower portion enlarged

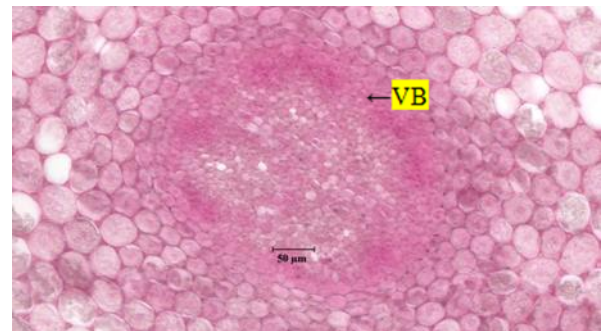
LE - lower epidermis; **Pa** - parenchyma; **Pal**- palisade layer; **Ph**- phloem;
UE- upper epidermis; **VB**- vascular bundle; **Xy**- xylem



(Fig - 4.3.24) TS of *Trigonella foenum-graecum* radicle



(Fig - 4.3.25) Outer region enlarged



(Fig - 4.3.26) Vascular bundle enlarged

AG - aleurone grains; **Ct**- cortex;**E** - epidermis;**Pa** - parenchyma;
VB- vascular bundle

4.2.3 - QUANTITATIVE MICROSCOPY

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded . The leaf is amphistomatic with anomocytic stomata; the epidermal cells were wavy.

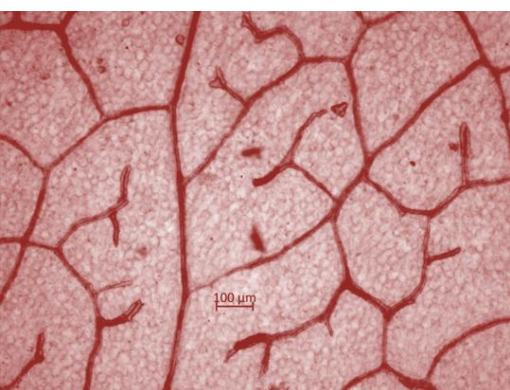
Table -4.2.3 Quantitative microscopy of *Trigonella foenum-graecum* leaf

Parameters	Upper epidermis (/mm ²)	Lower epidermis (/mm ²)
Epidermal number	100 - 110	110 -120
Stomatal number	40 - 45	45 - 55
Stomatal index	28 -31	30 -31
Palisade ratio	25 - 30	



Upper Epidermis

Lower Epidermis



Vein islets and terminations

E - epidermis; **St** - stomata; **VI** - vein islet; **VT** - vein termination

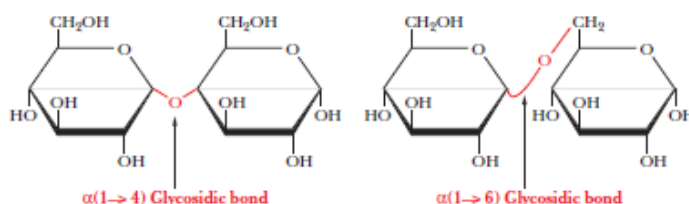
4.4 PHARMACOLOGICAL EVALUATION

Alpha amylase Inhibitory Activity

In vitro Antidiabetic Potential of *Trigonella foenum graecum*

Alpha amylase Inhibitory Activity

Alpha amylase is a most important digestive enzyme, present in pancreas which catalyzes the **hydrolysis of the alpha-1, 4 glycosidic linkages of starch**. This is responsible formost of starch digestion in humans.



Inhibitors of α -amylase (hydrolyzing enzyme) have been useful as oral hypoglycemic drugs **for the control of hyperglycemia especially in patients with Type 2 Diabetes Mellitus**.

This activity can be measured *in vitro* by **hydrolysis of starch** in presence of α -amylase enzyme.

Plant extracts possess the inhibitory effect of these enzymes that may lead to reduction in post prandial hyperglycaemia in diabetic condition

The *in vitro* anti-diabetic activity of, *Trigonella foenum graecum* seeds in all the two extracts (ethyl acetate & water) were determined **by estimating the inhibitory activity of the enzyme α -amylase** which involves the breakdown of starch to produce maltose and then to glucose by modified method of Bernfeld.

The α -amylase inhibitory property of *Trigonella foenum graecum* seeds was determined by the modified method of Bernfeld with varying concentrations

ranging from 0.2, 0.4, 0.6, 0.8, 1.0 mg /ml in comparison with standard acarbose (20-100 µg) and IC50 values were calculated.

Experimental procedure for α-amylase inhibition assay

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min.

After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent.

The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm.

Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Calculation of 50% Inhibitory Concentration (IC50)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I \% = \frac{(Ac-As)}{Ac} \times 100$$

whereas,

Ac = Absorbance of the control

As = Absorbance of the sample.

α -Amylase Inhibition of aqueous extract of *Trigonella foenum graecum*

Table - 4.4.1

Concentration (μ g/ml)	IC50 Value 50% Inhibitory Concentration
0	0
25	25
50	28
75	38
100	49
125	52

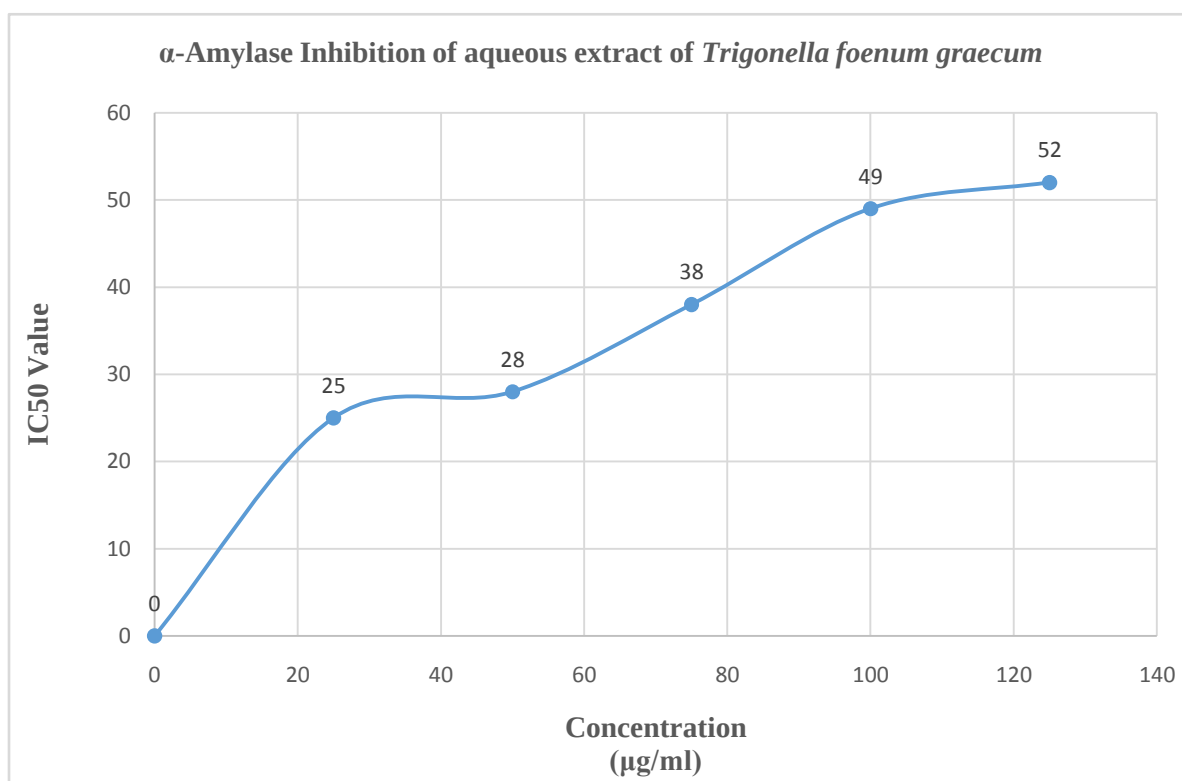


Fig - 4.4.1

α -Amylase Inhibition of ethyl acetate extract of *Trigonella foenum graecum*

Table - 4.4.2

Concentration (μ g/ml)	IC50 Value 50% Inhibitory Concentration
0	0
25	32
50	43
75	51
100	66
125	80

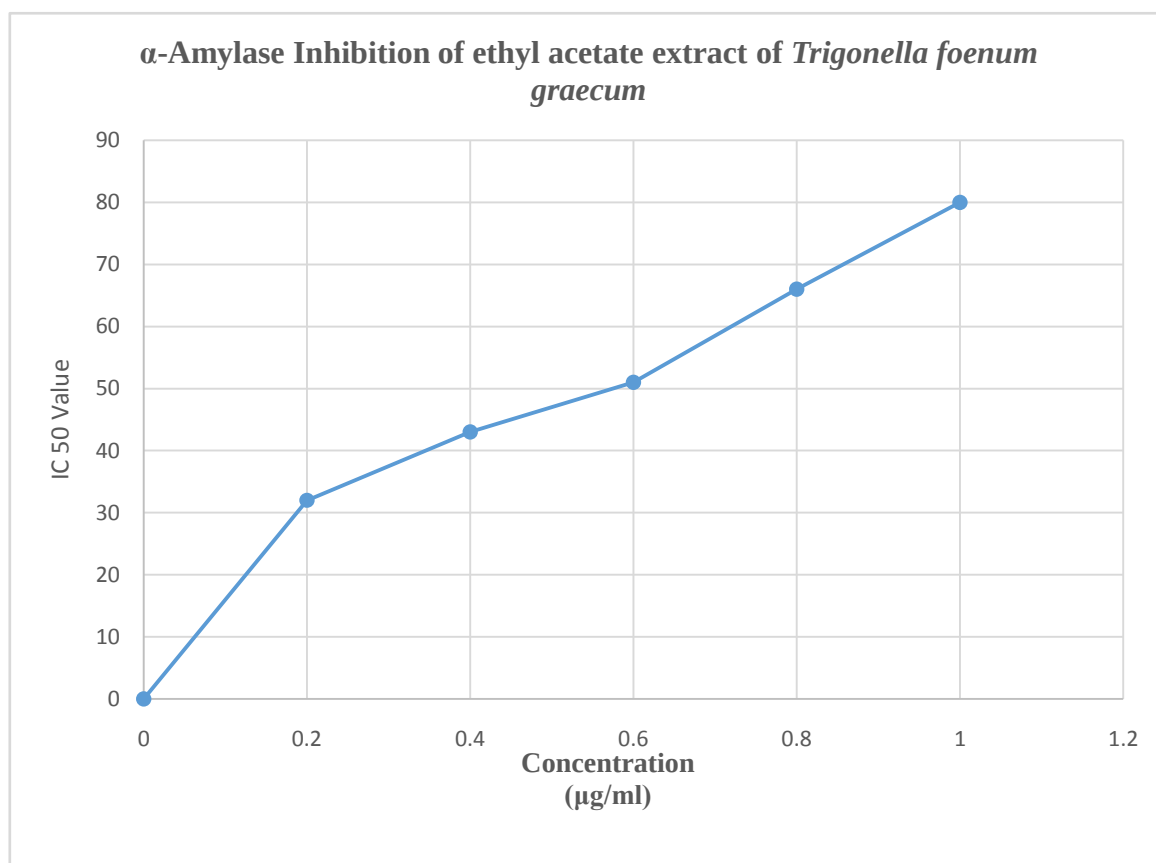


Fig - 4.4.2

α -Amylase Inhibition of standard Drug Metformin (Positive control)

Table - 4.4.3

Concentration (μ g/ml)	IC ₅₀ Value 50% Inhibitory Concentration
0	0
25	25
50	38
75	48
100	57
125	72

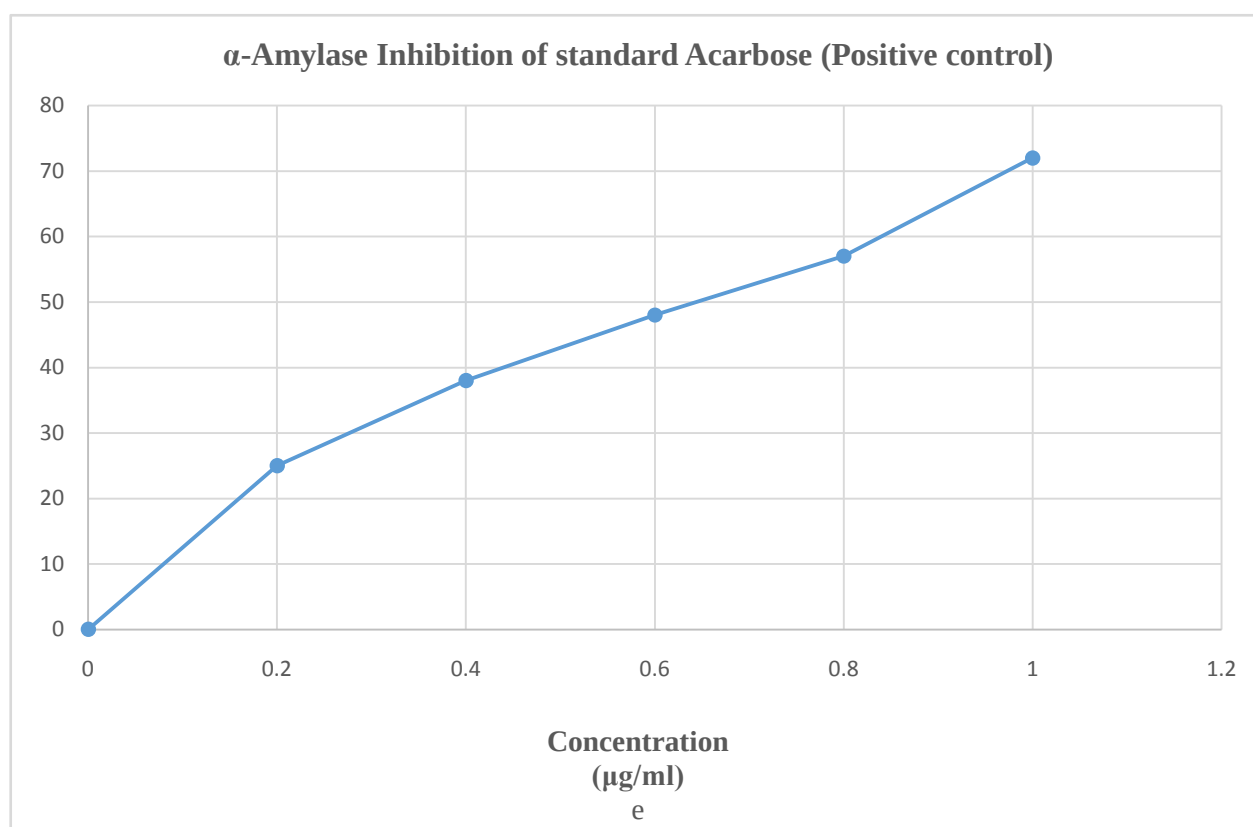


Fig - 4.4.3

There was an increased inhibition by *Trigonella foenum graecum* seeds extracts in dose dependant manner. As the concentration of the inhibitor increased, the amylase enzyme activity was decreased.

It was concluded that the ethyl acetate extract showed better inhibitory activity compared with the standard and aqueous extract of *Trigonella foenum graecum* seeds.

Inhibition was found to be dose dependant by standard drug metformin which is a commercially available alpha-amylase inhibitor for the treatment of Diabetes.

CHAPTER - 5

RESULT AND DISCUSSION

✧ **Phytochemical Evaluation**

The preliminary phytochemical screening procedure of the aqueous and Trigonella Foenum- Graceum extract of fenugreek seeds showed the presence of carbohydrates, alkaloids, flavonoids, whereas phenol, tannins, and volatile oil were absent.

✧ **Estimation of Total Flavonoids Determination**

The linear regression equation was found to be $y=0.0307x-0.0432$ while the correlation was found to be 0.9974. The amount of flavonoid content present in the extract in terms mg quercetin equivalent/g of extract was found to be 24.08 by using the above linear regression equation.

✧ **Pharmacognostical Evaluation**

Macroscopy :

The aerial part of the plant is moderately branched and weak. The stem is erect, branched, green, smooth, herbaceous. Leaves are petiolate, alternate, compound, trifoliate, fragrant and stipulate; seeds oblong flattened or irregularly rhomboidal, hard, a narrow depression dividing radicle pocket from cotyledons is seen, yellowish brown in colour with characteristic spicy odour and spicy mucilaginous, bitter taste (Fig - 4.1.1)

Anatomy of Stem, Leaves and Seeds

Stem :

TS is oblong circular with uniseriate epidermis covered by a thin cuticle; a single layered collenchymatous hypodermis is present; cortical region is made up of 5 to 6 layers of thin walled parenchyma cells; collateral vascular bundles are arranged as a ring; discontinuous pericyclic fibres are seen surrounding the vascular region; a few prismatic crystals are seen randomly distributed in the pericyclic region; centrally placed thin walled parenchymatous pith is present

Leaf :

Midrib

TS shows single layered epidermis covered by thin cuticle; upper epidermis is followed by two layers of palisade cells; centrally located large vascular bundle is present surrounded by patches of pericyclic fibres; phloem is present below the xylem; 3 to 5 layers of chlorenchyma cells are present above the lower epidermis(Fig. 4.2.11).

Lamina

TS is dorsiventral and shows single layered wavy epidermis covered by indistinct cuticle; mesophyll is differentiated into outer two layers of palisade followed by inner 3 to 4 layers of spongy parenchyma cells; veins are seen traversing through the mesophyll (4.2.12)

Seeds :

TS shows outermost layer of testa made up of thick-walled cylindrical palisade like cells covered with thick cuticle; below it a sub epidermis made up of columnar cells are present; a narrow thin zone of tangentially elongated parenchyma cells follows; outer endosperm cells are filled with aleurone grains and the inner endosperm cells are made up of large parenchymatous cells filled with mucilage

Cotyledon consists of upper and lower epidermis; 4 to 5 layers of palisade cells are seen below the upper epidermis; on the dorsal side 7 to 10 layers of spongy parenchyma cells are seen which are filled with oil globules and aleurone grains; small vascular bundles are present in the mesophyll region

Radicle TS is circular in outline; the single layered epidermis is present covered by cuticle followed by broad cuticle made up of thin walled parenchyma cells filled with aleurone grains; vascular region is present in the centre (4.2.17)

Quantitative Microscopy of seeds :

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded . The leaf is amphistomatic with anomocytic stomata; the epidermal cells were wavy

✧ Pharmacological Evaluation :

There was an increased inhibition by *Trigonella foenum graecum* seeds extracts in dose dependant manner. As the concentration of the inhibitor increased, the amylase enzyme activity was decreased.

It was concluded that the ethyl acetate extract showed better inhibitory activity compared with the standard and aqueous extract of *Trigonella foenum graecum* seeds.

Inhibition was found to be dose dependant by standard drug metformin which is a commercially available alpha-amylase inhibitor for the treatment of Diabetes.

Phytochemical investigation reveals the presence of flavonoids, and role of flavonoid in diabetic, The results reveals that Alpha - amylase Inhibition activity was high with 125 mg /ml of Ethyl acetate Trigonella foneum, compared with aqueous extract of 125mg /ml .

Trigonella foenum Exhibits towards Reduction in Glucose level, which plays a vital role equivalent to synthetic molecule in curing the diabetic conditions.

In Future the study would be conducted in animals (In- vivo studies) to prove the betterment of fenugreek seeds towards reducing the glucose level in Patients Suffering with diabetics without any Adverse side effects .

Organic Drugs are first born in India to cure Various Diseases,.among these life threatening diseases such as diabetes ,some herbal plants are used traditionally in olden days to cure diabetes.Patients suffering from diabetes may affect with skin allergy,cornea encephalopathy etc...

CHAPTER - 6

SUMMARY AND CONCLUSION

The present study entitled the “Pharmacognostic, Phytochemical and Pharmacological evaluation of the Seeds of *Trigonella Foenum- Graceum* focuses on a plant which is commonly available throughout India and traditionally used in treatment of various diabetics .

Studies on the Seeds of *Trigonella Foenum- Graceum* are still lacking. Hence to exploit its potential use prompted the present study to investigate the seeds of this plant with clear scientific protocol.

The chapter on Literature Review deals with the information regarding the pharmacognostical, phytochemical and pharmacological evaluation of the Seeds of *Trigonella Foenum- Graceum*

✓ The chapter on **Pharmacognostical studies** highlights on

Macroscopical features were studied and the adherence of general characters to the family *Citrullus lanatus* was found. □

Microscopical study reveals the presence of actinocytic stomata, multi cellular uniseriate unbranched epidermal trichomes. Vascular system of the midrib is multistranded, a large abaxial median bundle, two adaxial bundle. All the bundles are bicollateral having phloem strand both outer and inner side of the xylem. The epidermal cells are small elliptical or rectangular and thin walled. Spongy parenchyma cells small and spherical. Palisade zone consist of single layer of cylindrical cells, loosely arranged.

Quantitative microscopical studies namely stomatal number, stomatal index, vein islet number, vein termination number, ash value, extractive value, loss on drying value etc.,

Also studied cell powder microscopy, fluorescence analysis of powder and the results helps in achieving a trouble-free identification and authenticity of the plant leaf or in powder form in future.

✓ The chapter on **Phytochemical Evaluation** deals with

Preliminary phytochemical screening reveals the presence of carbohydrate, alkaloids, flavanoids, Tannis Volatile oil, Phenol etc. □

TLC & HPTLC studies showed the presence of flavanoid, In HPTLC studies the extract was compared with standards and the presence of quercetin, were formed.

✓ The chapter on **Pharmacological studies** focuses

The antidiabetic activity of extract of Seeds of Trigonella Foenum- Graceum was carried out by) Alpha amylase inhibition assay method. The extract showed better alpha-glucosidase inhibition property

The extract exhibit significant inhibition of glycosylation as compared with the standard drug Metformin. Decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases.

The extract showed greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug Metformin.

Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these leaf extracts, the glucose uptake was found to increase in a dose dependent manner

Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. The result showed extract of trigonella Foenum- Graceum significant activity as compared to a standard drug.

The in vitro assays of the present study indicated the extract of Trigonella Foenum -Graceum possess good anti diabetic activity.

□

Trigonella Foenum -Graceum is popular in indigenous system of folk medicine. The seed extract of Trigonella Foenum -Graceum contain ,bioactive compounds such as trigonelline, isoorientin, orientin, vitexin, and isovitexin The extract may serve as a lead medicinal plant to synthesise varioussynthetic drugs to treat various life threatening disease.

CHAPTER - 7

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