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Antiacne activity of *Trigonella foenum graecum* Linn. Seed Extract

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Abstract

Acne vulgaris is a common skin disorder characterized by non-inflammatory and inflammatory lesions. This study has been taken to investigate *in vitro* antiacne activity of ethanolic and chloroform extract of *Trigonella foenum graecum* Linn. In this study, the selected plant extract were evaluated against the *Propionibacterium acnes*, *Staphylococcus epidermidis*. These bacteria are the main cause for acne. Agar well diffusion method is used for antiacne study and inhibitory activity is checked against *P. acnes* and *S. epidermidis* compared to the marketed tetracycline disc as positive control. From this study it was concluded that, *Trigonella foenum graecum* Linn. is highly effective against these bacteria and hence it can be used for treatment of acne vulgaris.

Keywords: *Acne vulgaris*, *Trigonella foenum graecum*, antiacne, *Propionibacterium acnes*, epidermidis

Introduction

Acne is a common disease of the Pilosebaceous units of the skin and topical treatment is recommended for the treatment of acne with Comedolytic, various anti-inflammatory agents, and antimicrobials. However, the topical application of these drugs leads to some side effects, adverse effects and also, there is an emergence of antibiotic resistance by acne causing bacteria [1]. A precise definition of acne vulgarism is, it can be defined as a chronic, self-limiting, inflammatory disease of Pilosebaceous unit, manifesting generally in adolescence with pleomorphic lesions like Comedones, papules, nodules and cysts.

The severity of acne can be divided into four groups as follows

- Group 1 (Mild): only Comedones.
- Group 2 (Moderate): Comedones, many papules, few pustules.
- Group 3 (Severe): pustules, nodules and abscesses.
- Group 4 (Cystic): Mainly cysts or abscess, widespread scarring [2].

Acne treatment in particular patients with oral retinoid can alter skin barrier function in treated patients. Most therapies used for the treatment of acne can induce epidermis changes altering the normal physiological skin barrier function. These alterations leads to some side effects manifesting in the skin of acne treated patients. Even if these alterations could be transient they could be a frequent reason for interrupting or stopping the treatment by the patient. An effective strategy for acne treatment should include a simple and effective therapy taking precaution to preserve skin barrier function and to reduce skin side effects of that particular treatment, which could help in improving patient adherence to treatments with a consequent better clinical outcome in terms of acne lesions resolution [3].

All forms of acne involve one or more of these pathophysiologic factors

- Hyper keratinization of the follicular epithelium with come done formation
- Increased sebum production
- Bacterial proliferation of *Propionibacterium acnes* (*P. acnes*)
- Local immune hypersensitivity causing inflammation.

Pathophysiology

The pathogenesis of acne vulgaris is multifactorial. The main important factor is genetics. Acne develops as a result of interplay of the following listed factors.

1. Follicular epidermal hyper proliferation with subsequent plugging of the follicle.
2. Excess sebum production.

3. The presence and activity of the commensally bacteria *Propionibacterium acnes*.
4. Inflammation.

Retention hyperkeratosis is the first recognized event in the development of acne vulgarism. The exact underlying cause of this hyper proliferation is not known. Currently, leading hypotheses have been proposed to explain why the follicular epithelium produces cells at a rapid rate that are retained in individuals with acne. First, androgen hormones have been implicated as the initial trigger. Comedones, the clinical lesion that results from follicular plugging, begin to appear around adrenarche in persons with acne in the T-zone area. Furthermore, the degree of comedonal acne in prepubertal girls correlates with circulating levels of the adrenal androgen dehydroepiandrosterone sulfate (DHEA-S). Additionally, androgen hormone receptors are present in sebaceous glands; individuals with malfunctioning androgen receptors do not develop acne [4].

Herbs are used medicinally in different countries by around 80% of people around the world, mostly in the developing countries. They are a source of many potent and powerful drugs, Principally due to the common belief that they are without any side effects along with being Economical and locally accessible. Fenugreek (*Trigonella foenum-graecum* L.) is an herb that grows anywhere around the world. Fenugreek is one of the oldest medicinal plants, originating in India and Northern Africa. Its dried seeds have a wide application as a flavoring additive and are good source of protein, fat, crude fiber, minerals and vitamins. In addition it has a broad spectrum of therapeutic properties. It has been used for numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health. Human and animal trials suggest possible hypoglycemic and anti hyperlipidemic properties of oral fenugreek seed powder [5].

Medicinal plants play an important role in the development of new therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics as a source of many valuable secondary metabolites which serves as plant defense mechanisms against different causative microorganism. There is a tremendous increase in search of antimicrobial plant extracts due to the fact that the resistance offered against antibiotic by the microorganism, in short the effective life span of any antibiotic is limited. One such valuable plant which has number of traditional uses is *Trigonella foenum graecum*. [6]

Medicinal plant represents a rich source of anti-microbial agents. Plants are used medicinally in different countries and are source of many potent and powerful drugs. A wide range of medicinal plants parts are used for extra as raw drugs and they possess varied medical properties, the vast majority of them have not been adequately evaluated [7].

Trigonella foenum graecum

Herbs have high medicinal value in Indian homes. Fenugreek (*Trigonella foenum-graecum*) is one of the most promising medicinal herbs and having nutritional value found on the continents of Asia, Europe, Africa and Australia. It is traditional remedy for treatment of various diseases. Fenugreek has a long history as both a culinary and medicinal herb in the ancient world. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. The Greeks and Romans used it for cattle fodder (Hence the Latin *foenum graecum* meaning

Greek hay). In ancient Rome, fenugreek was purportedly used to aid labor and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and edema of the legs. In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant.

It is an erect hairy annual herb of the bean family, reaching 30-60 cm in height that is of about three feet, which has three part leaves, the long slender stems bear tripartite, toothed, grey-green obovate leaves, 20-25 mm long. *Trigonella foenum-graecum* has long stalked leaves up to 5 cm long stipules triangular, lanceolate, leaflets about 2.5 cms long, obovate to obanceolate. The flowers are sessile axillary white or pale yellow in colour. The plant radiates a spicy odour which persists on the hands after touching [8, 9, 20].

Active constituents

Fenugreek seed contains 45-60% carbohydrates, 20-30% proteins and 5-10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.3 6%), choline (0.5%), and carpaine; the flavonoids Apigenin, Luteolin, Orientin, Quercetin, Vitexin, and Isovitexin; free amino acids, such as 4-hydroxyisoleucine (0.09%); arginine, histidine, and lysine; calcium and iron; saponins (0.6-1.7%); It also contain glycosides yielding steroidal sapogenins on hydrolysis (Diosgenin, Yamogenin, Tigogenin, Neotigogenin); It also shows the presence of cholesterol and sitosterol; vitamins A, B1,C, and nicotinic acid; and 0.015% volatile oils (n-alkanes and sesquiterpenes) [8].

Therapeutic uses

Fenugreek is a rich source of iron, silicon, sodium and thiamine. It also contains mucilagens which are important for soothing and relaxing injured tissues. Fenugreek seeds contain various alkaloids, including Trigonelline, Gentianine and carpaine compounds [9].

Mechanism of action

The mechanism of action of fenugreek is to delay gastric emptying, slow carbohydrate absorption and inhibit glucose transport. Fenugreek may also increase the number of insulin receptors in red blood cells and improve glucose utilization by peripheral tissues thus it shows anti-diabetic action on the pancreas and other sites. Fenugreek seed also contain amino acid that may also directly stimulate insulin secretion [9].

Traditional uses of medicinal plants

Chinese herbalists used fenugreek for kidney problems and conditions that affect the male reproductive tract. The seeds are also used as a preservative and are used in preparation of pickles, chutneys. The seeds or the extract are used in bakery products, frozen dairy products, meat products, condiments, candy etc. The nourishing seeds are also used in anorexia. Fenugreek seeds are used to lower the fever. The oil in the seeds is used as a skin softener and emollient.

They are also used to ease the labour pains (Indian food, Fenugreek). Fenugreek has a beneficial action on cleansing the blood [8].

Medicinal Uses

It plays an important role in digestion. It purifies blood and helps in flushing out the harmful toxins. It helps to dissolve excess mucus, thereby making the digestive organ refreshed and clean. Also it also improves memory power too. Helps to treat Diabetes & Reduce Cholesterol level.

Prevent hair loss: The Fenugreek seeds being high source of Protein are very useful in hair fall, also helps in thinning of hair and hair fall.

It also help to keeps the dandruff away and keeps the hair free of lice.

It also helps in losing body weight.

It is a excellent Antidote for skin problems: Fenugreek seeds prove to be an excellent beauty product that they help to prevent wrinkles, pimples, dryness and rashes.

It is also good remedy for health that is Fenugreek helps to attain hormonal balance in women and therefore, helps in enlargement of breasts. It helps to increase the lactation in lactating mothers.

It also helps to prevents Dandruff & Strengthens Hair [8].

The seeds are hot, with a sharp bitter taste; tonic, antipyretic, anthelmintic, increase the appetite, astringent to the bowels, cure leprosy, "Vata", vomiting, bronchitis, piles; remove bad taste from the mouth, useful in heart disease [10].

Its seeds and green leaves are used in food as well as in medicinal applications. As it provides natural food fiber and other required nutrients in the body and also helps in nitrogen fixation and soil enrichment [11].

Materials and Methods

The Study was conducted at the department of pharmaceutics, Dayanand College of pharmacy, Latur, Maharashtra, India.

Collection of sample

The seeds of *Trigonella foenum graecum* were collected from the local market of Latur, Maharashtra India. For authentication of plant, herbarium was prepared and sent to Dayanand science college, Latur.

Preparation of extract of fenugreek plant material

The ethanol and chloroform extract are prepared of fenugreek plant material for which, weighed 15 gm of seeds are powdered, add 250 ml of chloroform and ethanol separately to it, kept the mixture for 7 days and filtered it with muslin cloth, filtrate was allowed for hot extraction process on water bath at 40°C [11].

The extract so obtain was used for qualitative analysis for the presence of Saponins, Flavonoids, Steroids, Terpenoids, Tannins, Alkaloids, Phenols, Glycosides [12].

Table 1: Preliminary phytochemical screening of the ethanolic and chloroform extracts of *Trigonella foenum graecum*

Test	Fenugreek ethanolic Extract	Fenugreek chloroform extract
Carbohydrate	+	+
Protein/amino acid	+	+
Fats/ waxes	-	-
Glycoside	+	+
Flavonoides	-	-
Alkaloids	-	-
Terpenes	+	+
Steroids	+	+
Saponins	+	-
Phenolics /Tannins	-	-
Volatile oil	+	+

+ (presence)

- (Absence)

Organism used

Microorganisms used for the study were obtained from the (MTCC) Microbial Type Culture Col-lection (MTCC), Institute of Microbial technology (IM-TECH), Chandigarh, India. The test organisms included *P. acne* and *S. epidermidis* which were used in the present study. The bacteria were grown in nutrient broth (Himedia, M002) at 37±1oC and maintained on nutrient agar at 4oC.

Antiacne activity of fenugreek plants

The antiacne activity of fenugreek plants was determined against the *P. acne* and *S. epidermidis* at two different concentrations of each extract by the agar well diffusion method. Well diffusion method was measured the zone of inhibition to know the antimicrobial activity of fenugreek [12].

All procedures involved in this preparation were done under strict aseptic conditions to avoid contamination of the extracts. All glassware including beaker, volumetric flask, dropper, measuring cylinder, pipette, conical flask, laboratory glass bottles were autoclaved at 121°C for 20 minutes prior to use.

Preparation of different concentration of extracts

Two different concentrations of chloroform, ethanolic extracts 100µg/ml, 200 µg/ml were prepared from extract by using DMSO.

Preparation of culture media

Dehydrated media and standard antimicrobial drugs (Discs) were purchased from Hi-Media Laboratories Ltd, India. All the media were prepared in sterile glass petriplates (4 mm thickness) according to the manufacturer's instructions [13].

Screening of anti-acne activity of the Plant extracts

Antibacterial test was performed by agar well diffusion method. A freshly grown culture was diluted with 15-20 mL of Mueller-Hinton agar and 5% of blood was poured on glass Petri plates of same sizes and allowed to solidify. Agar surface of each plate was streaked by using sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL of each extract taken in the concentration of 100 µg/mL, 200 µg/mL the plates were allowed to stand for 30 min. Then the plates were incubated at 37 °C for 48 hrs under anaerobic conditions. Only DMSO served as control [14, 19].

Similarly freshly grown culture of *s. epidermidis* was diluted with 15-20 mL of Mueller-Hinton agar on glass petri plates of same sizes and allowed to solidify. Agar surface of each plate was streaked by using sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size and 100 µL of each extract taken in the concentration of 100 µg/mL, 200 µg/mL. The plates were allowed to stand for 30 min. Then the plates were incubated at 37 °C for 24 hrs under aerobic conditions.

Only DMSO served as control. The zone of inhibition formed around the wells was measured. Plates were prepared in triplets and the mean diameter of the zone of inhibition was noted [15, 16, 17].

Results

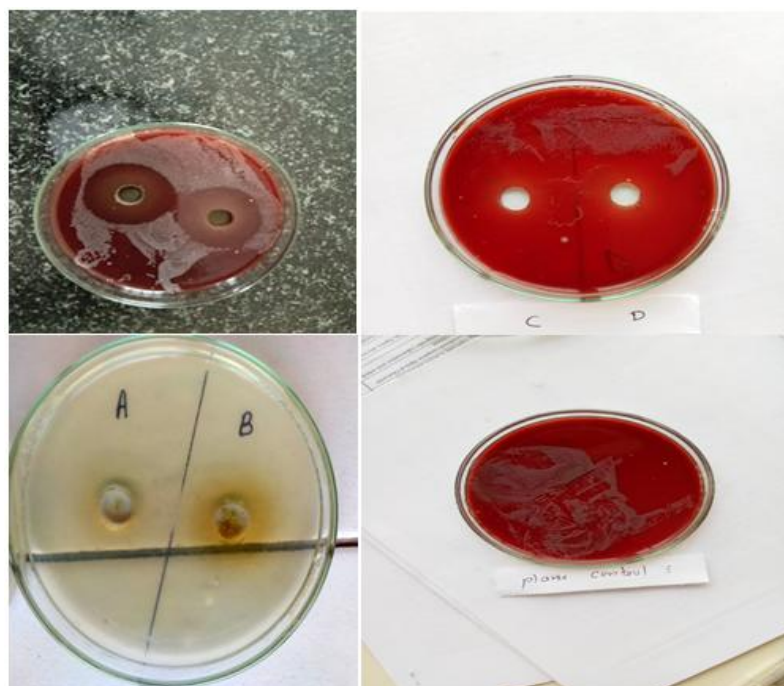


Fig 1: Antiacne activity of various fractions of seed extract

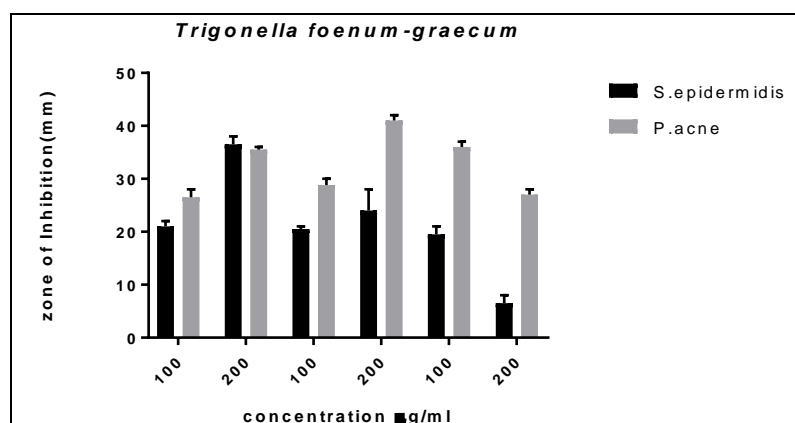


Fig 2: Graph showing antiacne activity of various fractions of seed extract.

Table 2: Zone of inhibition of various fractions of seed extract.

Sr. No.	Concentration	Staphylococcus epidermidis	Propionibacterium acne
F1A	100	14.500 ± 2.121	9.500 ± 3.535
F1B	200	22.000 ± 1.414	12.000 ± 1.414
F2C	100	27.500 ± 1.414	24.000 ± 8.485
F2D	200	40.000 ± 1.414	23.000 ± 2.828
F3E	100	47.000 ± 2.828	16.500 ± 2.121
F3F	200	64.500 ± 5.656	39.500 ± 2.121

Discussion

Medicinal plants are rich sources of chemical constituents with huge therapeutic benefits. Several researches have been done over the years in order to investigate pharmacological activity of different plants in the treatment of various diseases. Crude ethanol and chloroform extract of the *Trigonella foenum graecum* L. seed was subjected for phytochemical screening, which identified presence of Carbohydrates, proteins, fats, glycosides, steroids etc.

Antibiotic resistance is a major concern and investigation of new agents from plants could play a major role in meeting the demand for new antimicrobial agents with improved safety and efficacy. In the present investigation, we have shown that

the ethanol and chloroform seed extract of the *Trigonella foenum graecum* L. exhibited highest antimicrobial activity. The plant extract was tested against *P. acne* and *S. epidermidis*. DMSO was used as a control drug. 64.500±5.656 highest zone of inhibition was recorded against, *P. acne* by ethanolic extract of fenugreek at concentration of 200 µg/mL. The antibacterial activity of the plant is remarkable considering the importance of this organism^[18].

Conclusion

The result of the present study showed the presence of wide spectrum of antibacterial activities against *P. acne* and *S. epidermidis*. Therefore it can be used as antibacterial

supplement and for the development of new therapeutic agent. Thus this study demonstrates the antiacne activity of ethanolic and chloroform extract of *Trigonella foenum graecum* L. which is effective in the treatment of acne vulgaris.

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