Antimicrobial Activity Of Trigonella Foenum-Graecum Seed Extract And Curd Against against common fungal pathogens Candida *Albicans*, *Malassezia*, And *Aspergillus Niger*.

By: **Dr.Nakade Dhanraj,** Department of Microbiology, Elphinstone College,

Dr.Homi BhabhaUniversity.Mumbai -400032(MS)

Abstract:

Over the last two decades there has been a rise in the incidence of mycotic infections. superinfections arising from the overuse of antibiotics, and the use of glucocorticoid, diabetes and immunosuppressive drugs has greatly increased human susceptibility to fungal infections. In the last 20 years various antifungal antibiotics have become available, but only five are currently used to treat serious infections. As very few antifungal antibiotics are in clinical use there has been a change in the résistance pattern of pathogens. Infections are becoming increasingly common, and the occurrence of antibiotic resistant pathogens has increased. Thus discovery of new compound is required to combat fungal infection. Fenugreek has been reported since ancient times to have antifungal activity against various dermatophytes.

By considering this in the present study, aqueous and methanolic extracts of Fenugreek seeds were tested for their antifungal activities using agar well diffusion assay against Candida albicans, Malassezia ,and Aspergillus niger as test organisms.

Results indicated a significant difference in inhibition zone diameter between aqueous seed extract and methanolic seed extract. Methanolic seed extract was found to be most effective in inhibition zone diameter. These finding indicate the potential of the seeds of Trigonella foenum-graecum in treatment of fungal infection and may be used as potential remedy for prevention of dandruff and hair fall.

Key words: Antifungal activity, Aqueous extract, Methanolic extract, Fenugreek seeds, Trigonella foenum-graecum.

Introduction:

In the world, 30 % of the pharmaceutical preparations are manufactured from plants. Global market US $ 60.0 billion. Expected growth US $ 5.0 trillion by year 2050[1]. Medicinal species exist in India 7600/8000medicinal plants in Asia.Interestingly, Only 6% have been explored for their biological activities[2]. And Only 15% have been explored phytochemically for their constituents [3]

Fenugreek (*Trigonella*) is the oldest medicinal plant in the world[4]. Most popular species of this genus is *Trigonella foenum-graecum*, fenugreek is grown mainly as a spice crop in the recent times. It is used traditionally as Anti-inflammatory- Antiseptic- Antispasmodic- Appetite loss , Respiratory Problems- Colds and Flu, Bronchitis, Sore Throat- Diarrhea- Digestive Disorders- Dyspepsia- Female Health Maintenance- Skin Disorders- Skin inflammation- Sugar Control- Ulcers[5].

The plant contains a number of steroidal sapogenins, especially diosgenin found in the oily embryo[6]. Two furastanol glycosides, F-ring opened precursors of diosgenin have been reported, also hederagin glycosides. The alkaloid trigonelline, trigocoumarin, trimethyl coumarin and nicotinic acid are also present. Mucilage is a prominent constituent of the seeds. Trigonelline is the chief constituent of fenugreek[7].

By considering the above facts, we set our objectives for current work with the aim to determine the anti-fungal activity of Trigonella foenum-graecum (Leguminoseae) from Methanol and Aqueous extracts,against dermatophytes aAnd to study its Biological, Pharmacological investigations.

2.Materials and method:

- The Seeds of fenugreek plant were utilized for the experiment.
- Test Organisms for Bioassay:

Candida albicans(Causing Candidiasis), Malassezia (Causing Dandruff),and Aspergillus niger (Causing Aspergillosis)

Seeds Soaked in curd (Overnight) Grinded to form paste,Transferred to methanol(1:3) and Sterile Distilled water(1:3:) After Shaking for 10 Min. Methanol layer was collected:, Filtered and concentrated at Room temperature[8].

Antifungal assay:

Residue was diluted with sterile Distilled water to get the final concentration of the extract as 1.0 mg/m land Used for Bioassay[9].

Bioassay was done by Agar –Well diffusion method using Seeded Potato Dextrose Agar.

- The molten potato dextrose agar was inoculated with each fungal culture ($1*10^6$cells/ml for Candida albicans and $1*10^4$spores /ml for Malassezia spp,and Aspergillus niger) separately[10].
- Plates were poured and wells were made with standard cork borer.
- Each well was then inoculated with 0.1 ml of Extract and incubated at RT for 72 hours and measured for diameter of zone of inhibition as per CSLI guidelines[11,12,13].

MIC of Anti-fungal extract:

For susceptibility testing, the disc diffusion method according to Kirby-Bauer was used [14].

Serial dilutions of purified extract was prepared in Potato dextrose broth starting from 1ug/ml to 15ug/ml and inoculated with (Candida albicans $1*10^6$cells/ml and $1*10^4$spores /ml for Malassezia spp,and Aspergillus niger) separately(3 sets).

3.Result and Discussion:

This investigation confirmed the anti-fungal activity of Trigonella foenum-graecum seeds against ***Candida albicans, Malassezia spp,****and* ***Aspergillus niger*** *by comparing the MIC of the methanolic extract with that of CSLI Std.*

Name of the Pathogens	***Methanolic extract*** ***diameter of inhibitory zone(mm)***	***Aqueous extract*** ***diameter of inhibitory zone(mm)***
Candida albicans	*24(Std.>22)*	*21*
Malassezia spp.	*21(Std.>18)*	*17*
Aspergillus niger	*18(Std.>15)*	*15*

Fig: Activity against Candida albicans Fig:2 Activity against Messazzia spp.Activity against Aspergillus niger

- *MIC of Methanolic extract of Trigonella foenum-graecum L. was found to be 2 µg/mL* ***for Messazzia spp****. And* ***Candida albicans*** *And 5 µg/mL for****. Aspergillus niger****.*
- Results of the present study suggested that methanol and aqueous extracts contain more phytochemicals as compared to hexane and ethyl acetate extracts. Almost similar results were found in past work for the qualitative analysis of fenugreek seeds for the presence of phytochemicals in different extracts [17]. The knowledge of the extent and mode of action for antifungal activity of specific compounds, present in the plant extracts, may lead to the successful utilization of such natural drugs for the treatment of infections caused by pathogenic fungi[18,19,20]. Further identification and purification of active chemical constituents from the crude plant extracts will be helpful to develop drugs against pathogenic microorganisms.

Conclusion

In conclusion, this study demonstrates that Candida albicans is more susceptible to methanoloc extract. As per AST guidelines, e.g. from CLSI 2009 to EUCAST 1.3 (2018) guidelines, and can be used for treatment of treatment of dermatophytes. Further studies are needed to assess the effect of the extract on humans or animals.

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