Original Research Article

Antifungal Activity of *Echinops echinatus* and *Fagonia cretica* from Cholistan Desert-Pakistan

ABSTRACT

Background: Fungal infections are getting worse due to their resistance against available antibiotics and have always remain a problem. Homoeopathic mother tinctures are part of therapy in clinical patients with less side effects and much efficient against pathogenic infections.

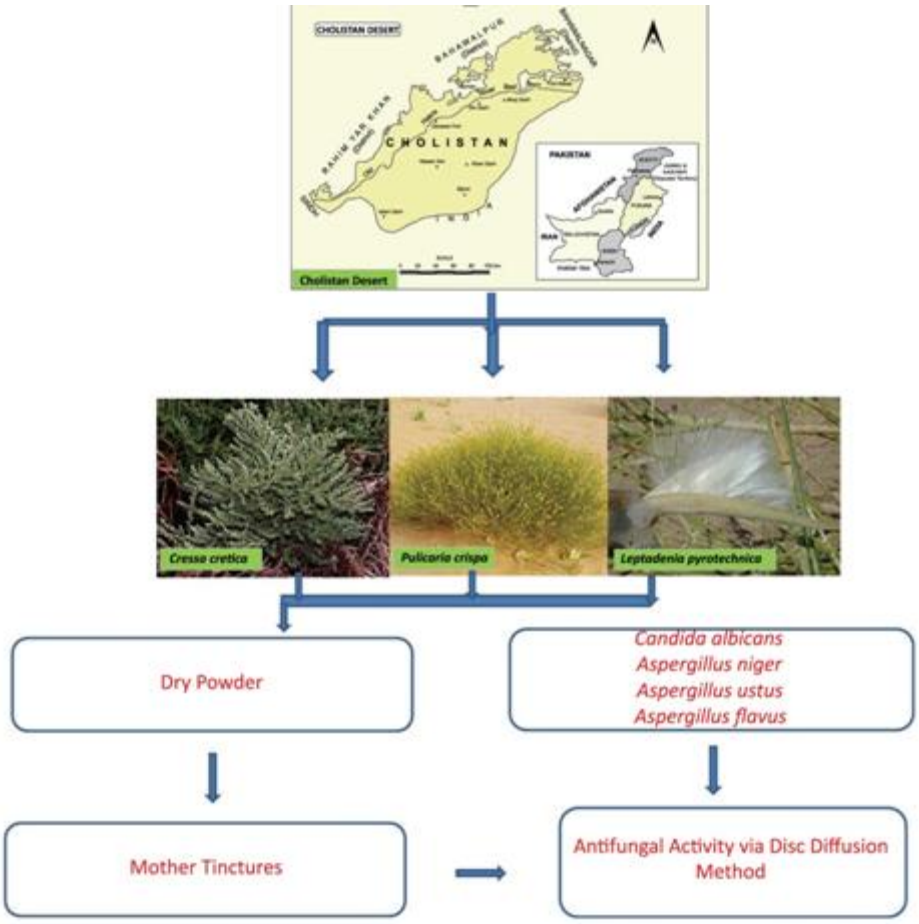
Methodology: *Echinops echinatus* and *Fagonia cretica* plant mother tinctures were performed and used to evaluate the anti-fungal potential of these plants against potentially pathogenic fungal species like *Candida albicans, Aspergillus ustus, Aspergillus niger* and *Aspergillus flavus* by agar disc diffusion method. Three doses were used (0.25 ml, 0.5 ml and 1 ml volume per disc) and zone of inhibition was observed in millimetres and compared with positive control Fluconazole (2 mg/ml) which is commercially available.

Results: The results indicated efficacy of *both mother tinctures showed remakarble activity against pathogens wi*th zone of inhibition ranging 17.33 – 30.33 mm.

Conclusion: Current work indicated anti-infective potential of plants which can be added in therapy of infections in future after confirmation in in-vivo models.

Keywords: Antifungal activity, Cholistan desert, homeopathic mother tincture, *Echinops echinatus, Fagonia cretica.*

GRAPHICAL ABSTRACT



Anti-Fungal Activity of *Echinops echinatus* and *Fagonia cretica*

INTRODUCTION

Cholistan is called as Rohi desert in local area, that covers 26,000 km^2, having length of 480 km and width of 32-192 km near Bahawalpur city, Pakistan. This desert is rich in various untested medicinal plants and contains 128 species of desert plants, which belong to 32 families. Local herbalists are using these plants to treat many ailments. Many plants contains tannins, sesquiterpenes, terpenoids, polyphenols, phenolics and flavonoids. They displayed antimicrobial properties against microrganisms (1).

Two medicinal plants obtained from Cholistan desert named as *Echinops echinatus* and *Fagonia cretica* were selected for antifunal activity. *Echinops echinatus* is related to the largest flowering plant families (Asteraceae) with about 3000 species, commonly known as “Brahmadandi” or “Utakatira”. It is an xerophytic herbaceous plant, widely distributed in desert regions. Triterpenoids, alkaloids (2), acylflavone glucoside (3), isoflavone glycoside, echinosie, 7-hydroxyisoflavone, kaempferol, kaempferol-3-O-alpha-L-rhamnoside, kaempferol-4’-

methylether, kaempferol-7-methylether and myrecetin-3-O-alpha-L-rhamnoside (4) have been isolated from *Echinops echinatus*. This plant diplayed some activities such as antifertility activity (5) and anti-inflammatory activity in rats (6). It is also used in treatment of sexual problems, leukorrhea, diabetes, eczema, skin papules, diarrhea, malarial fever, asthma, whooping cough, renal colic, heat stroke, jaundice, hysteria, dyspepsia, hoarseness of throat, polyurea, scrofula, migraine, heart diseases, joint pains, urinary infections and hemorrhoids. Its roots contains aphrodisiac properties as well as abortifacient. It is taken with honey to expel worms.

Antimicrobial activity of plant was also reported against *Alternaria tenuissima, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. Its antioxidant property was also reported in various *in-vitro* models. It also showed protective effect in prostate and liver. Its chloroform extract showed anti-irritant activity in *in-vivo* study (2). *Fagonia cretica* is related to family Zygophyllaceae (7). It has been used in eastern system of medicine for many ailments. It has been reported to contain various antioxidants (8). Plant contains natural compouds with potent DPP-4 inhibitory activity (9). This plant also showed antihaemorrhagic property against effect of black cobra venom (10). Plant also has antioxidant potential (11). Aqueous extract of plant showed antidiabetic activity (12). The aim of this study was to examine the antifungal activity of *Echinops echinatus* and *Fagonia cretica* against fungal strains. These plants were tested for antifungal potential against pathogens like *Aspergillus flavus, Aspergillus niger, Aspergillus ustus* and *Candida albicans.*

MATERIALS & METHODS

Collection of Plant Material: All plant samples were collected from Shikarwala toba, near Fort Moj Garh, Cholistan, Desert Bahawalpur. The plants sample were identified and authenticated by Dr. Shazia Anjum Director Cholistan Institute of Desert Studies, The Islamia University, Bahawalpur. The voucher specimens of *Echinops echinatus* (3513/CIDS/IUB) and *Fagonia cretica* (3515/CIDS/IUB) were deposited in the herbarium of CIDS, The Islamia University of Bahawalpur.

Preparation of Plant Material: Collected plant samples were crushed and washed to remove all the external dirt and unwanted material, and dried in open air under shade for 15 days and milled to fine powder in an electric grinder and stored in a well closed container (13).

Formation of Mother Tinctures: Powdered material of selected plants was used for preparation of Homeopathic mother tinctures. Mother tinctures were prepared by maceration process

mentioned in Homoeopathic pharmacopeia. Plant material one part and solvent nine parts were used for preparation of mother tinctures. Solvents used were ethanol and distilled water (70% ethanol and 30% purified distilled water) (13).

Maceration: It is process in which medicinal part of plant is softened in solvent for a specific period of time under standered temperature and pressure. 100g of each powdered plant material was placed in a 2 liter glass flask. Powder was soaked for 15 days in 900 ml of 70% ethanol. Flask was sealed and kept in cool dark place. Flask was shaken daily for 10 minutes. After 15 days, soaked material of each plant was filtered through several layers of muslin cloth by coarse filtration. Coarse filtrate was then filtered through a Whatmann filter paper. Mother tincture so obtained was collected in glass containers (13).

Fungal Strains: Three species of *Aspergillus* i.e., *niger, flavus* and *ustus,* were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS), University of Punjab Lahore. Whereas, *Candida albicans* strains were obtained from Microbiology department, Quaid-e-Azam Medical College (QMC) Bahawalpur, as well as from clinical isolates.

Preparation of the Medium: 65g of Sabouraud's Dextrose Agar (SDA) medium was dissolved in 1000 ml of distilled water. Medium was sterilized by autoclaving at 121^{o}C for 15 minutes at 15 psi pressure (13).

Antifungal Assay: Experiments were performed three times under strict aseptic conditions. Antifungal tests were done by using disc diffusion method. Mother tinctures were applied on these discs drop by drop. Discs with Fluconazole were used as positive standards. These impregnated discs were then aseptically transferred into Sabouraud's Dextrose Agar (SDA) plates freshly inoculated with test organisms *Candida albicans, Aspergillus niger, Aspergillus flavus* and *Aspergillus ustus*. After autoclaving, media was poured into different petri plates and allowed to solidify. Fungi were then inoculated on agar plates by using sterilized culture swabs. Then, filter paper discs were placed in centre of petri plates by using sterilized forcep. Then, dropping of different concentrations of mother tinctures was done on discs. Three additional plates, one containing solvent, second containing positive control and third containing only fungi, were also maintained. Plates were incubated at 28^{o}C in incubator and observed after 48-72 hours. All experiments were done in triplicate. Antimicrobial activity was determined by

measurement of inhibition of zone around each paper disc. Antifungal activity of mother tinctures was evaluated by disc diffusion method (13).

Statistical analysis: Statistical analysis was performed with Graph Pad software, all data of experimental groups were expressed as mean ± SEM in triplicate experiments. For statistical analysis, group means were compared by one-way ANOVA and Bonferroni's test was used to identify differences between groups. The threshold for statistical significance was set at $P<0.05$. Endnote was used to insert references.

RESULTS

Anti-fungal activity of two different plants, *Echinops echinatus* and *Fagonia cretica*, was tested via disc diffusion method against pathogenic fungi which are involved in various infections of humans.

In the screening, *Echinops echinatus* showed strong antifungal activity with zone of inhibition of 17.33-30.33 mm. Figure 1-4 and table 1 are showing mean data of inhibition zone for three doses of mother tincture of *Echinops echinatus* against four fungal strains. All doses were found effective in comparison with positive control. Figure 5 is displaying comparison of plant activity in different fungi. In the case of mother tincture of *Echinops echinatus*, the best anti-fungal activity was obtained against *Aspergillus flavus*, considering the larger diameter of zone of inhibition, as compared to all other pathogens and this activity was higher for 1.0 ml dilution in comparison to the standard drug Fluconazole.

In the screening, *Fagonia cretica* showed strong antifungal activity with zone of inhibition of 19.00-37.00 mm while the highest antifungal activity was seen against *Candida albicans* and *Aspergillus ustus*. Figure 6-9 and table 2 are showing mean data of inhibition zone for three doses of mother tincture of *Fagonia cretica* against four fungal strains. All doses were found effective in comparison with positive control. Figure 10 is displaying comparison of plant activity in different fungi. In the case of mother tincture of *Fagonia cretica*, the best anti-fungal activity was obtained against *Candida albicans* in comparison to the standard drug Fluconazole.

DISCUSSION

Antifungal potential of plant mother tinctures were tested via disc diffusion method against fungi using Fluconazole as positive control. Antimycotic activity of mother tinctures was checked by using three different concentrations. Effects of plants in 0.25 ml and 0.50 ml on *Aspergillus niger* were same with mild difference and less significant. P value of both doses was

less than 0.03, while concentration of 1 ml of tinctures showed maximum zone of inhibition but level of antifungal activity was not significant in comparison with Fluconazole. *Echinops echinatus* showed fungitoxic effect on *Aspergillus flavus* was much considerable in concentrations of 0.25 ml and 0.50 ml and confirmed significant results. Whereas, activity against *Aspergillus ustus* was varied and lowest dose showed minimum inhibitory zone and high dose showed maximum effect. All doses were significant and level of significance ranged from 0.03 to 0.05. Activity of *Echinops echinatus* against *Candida albicans* was dose dependent and inhibitory in concentrations of 0.25 ml and 0.50 ml, which were more closer to Fluconazole. Generally, antifungal effect of *Echinops echinatus* was maximum on *Aspergillus flavus* (28.91%), moderate on *Candida albicans* (26%) and minimum on *Aspergillus niger* (23.34%) and *Aspergillus ustus* (25.78%). These results are related with those obtained by Mathur *et al*., which showed higher activity of *Achyranthus asper* against *Candida albicans* and *Aspergillus niger* (14) while current study revealed close antimycotic effect of *Echinops echinatus* against same species.

Fungitoxic activity of *Fagonia cretica* was tested against fungi in concentrations of 0.25 ml, 0.50 ml and 1 ml, significant results were observed on *Candida albicans, Aspergillus niger* and *Aspergillus flavus*. Antifungal activity of 1 ml dose was significant in *Candida albicans as* compared to positive control, whereas fungitoxic effect of *Fagonia cretica* on other species were considerable and less significant. As a whole, maximum antimycotic effect of *Fagonia cretica* tincture was observed on *Candida albicans* (26%) and *Aspergillus ustus* (27.66%) and minimum on *Aspergillus flavus* (22.46%) and *Aspergillus niger* (23.83%). Similar study of screening natural plant extracts against different fungal pathogens was well recorded in literature by Avasti *et al*. (2010) where aqueous extract of *Trachyspermum ammi* showed 27.76 % inhibitory effect against *Aspergillus niger*, while in present study, mother tincture of *Fagonia cretica* proved 23.83% antimycotic results which is close to reported experiments (13, 15).

CONCLUSION

The current results showed that plants possessed antifungal activity against all the tested pathogenic fungal strains. The isolation of active constituents showing antifungal activity can be more useful in future. Further studies of these plants can be performed in in-vivo models.

COMPETING INTERESTS DISCLAIMER AND FUNDING:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table- 1: Composition of the Sabouraud's Dextrose Agar (SDA) medium

Sabouraud's Dextrose Agar (SDA) medium (1000 ml)	
Ingredients	**Amount**
Dextrose	40.0 gm
Peptone	10.0 gm
Agar	20.0 gm
Distilled water	q.s to 1000 ml
Ph	5.6

Table 2 Antifungal activity of *Echinops echinatus* mother tincture

Concentrations	***Aspergillus niger*** **Zone of Inhibition (mm)**	***Aspergillus flavus*** **Zone of Inhibition (mm)**	***Aspergillus ustus*** **Zone of Inhibition (mm)**	***Candida albicans*** **Zone of Inhibition (mm)**
0.25 ml	18.33 ± 0.88**	17.33 ± 0.88**	17.66 ± 0.88**	21.00 ± 1.15**
0.50 ml	20.33±1.76 **	20.33 ± 1.20 **	20.66 ± 0.88**	23.66 ± 2.02*
1.00 ml	22.33 ± 1.76*	27.66 ± 1.33	24.66 ± 1.76*	25.00 ± 2.08
Fluconazole (2 mg in 1 ml)	27.33 ± 0.881	28.66 ± 0.881	30.83 ± 1.364	30.33 ± 0.333

*≤ 0.05, **≤ 0.01

Table 3 Antifungal activity of *Fagonia cretica* mother tincture

Concentrations	***Aspergillus niger*** **Zone of inhibition (mm)**	***Aspergillus flavus*** **Zone of inhibition (mm)**	***Aspergillus ustus*** **Zone of inhibition (mm)**	***Candida albicans*** **Zone of inhibition (mm)**
0.25 ml	21.33 ± 1.452**	19.00 ± 0.57**	27.66 ± 0.66	29.00 ± 1.45
0.50 ml	24.66 ± 0.88	22.33 ± 1.33 *	30.66 ± 0.88	31.66 ± 0.88
1.00 ml	29.00 ± 1.154	27.33 ± 1.45	33.66 ± 0.333	37.00 ± 1.154**
Fluconazole (2 mg in 1 ml)	27.33 ± 0.881	28.66 ± 0.881	30.83 ± 1.364	30.33 ± 0.333

*≤ 0.05, **≤ 0.01

Figure Legends:

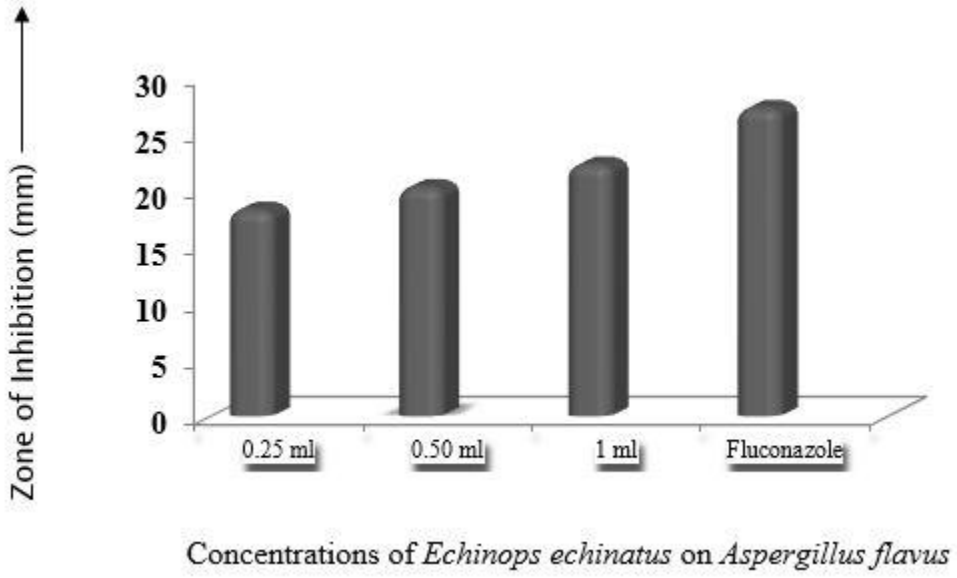

Figure 1: Effect of different concentrations of *Echinops echinatus* on *Aspergillus niger.*

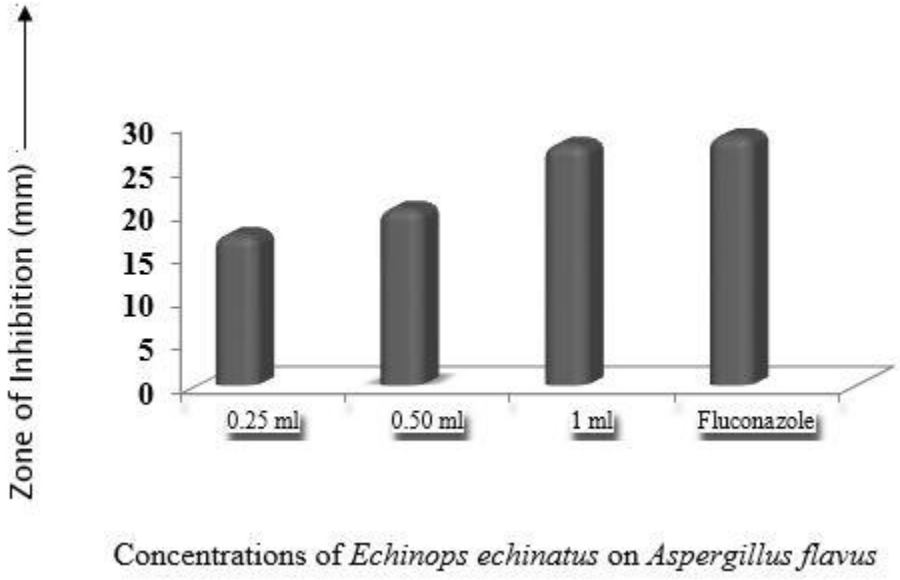

Figure 2: Effect of different concentrations of *Echinops echinatus* on *Aspergillus flavus.*

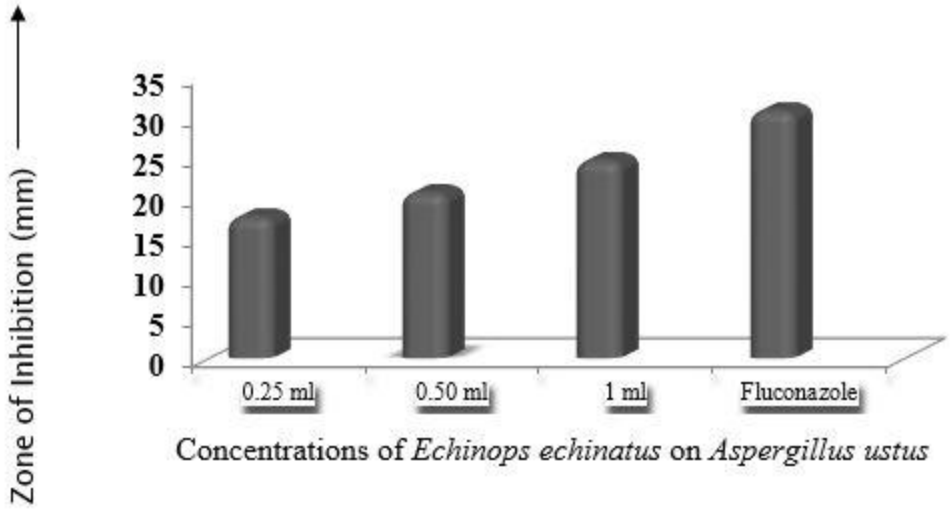

Figure 3: Effect of different concentrations of *Echinops echinatus* on *Aspergillus ustus.*

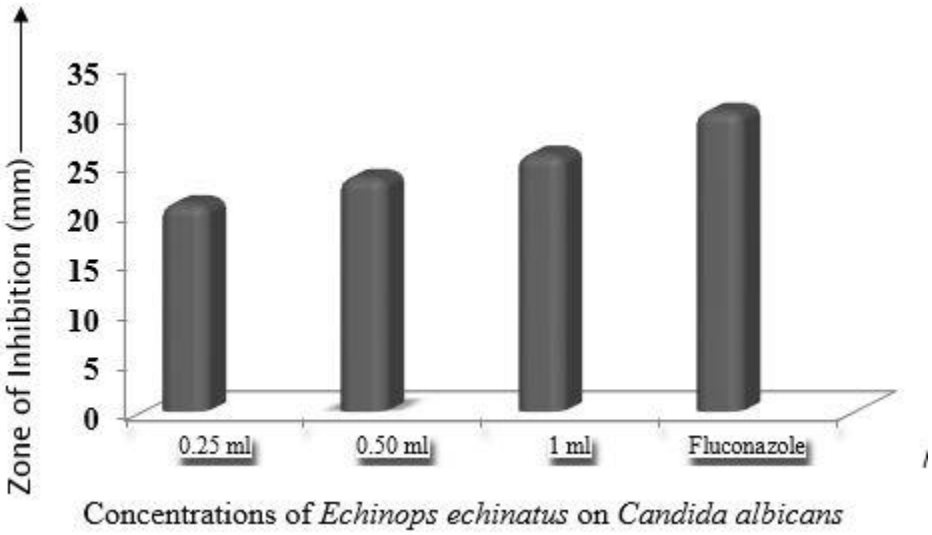

Figure 4: Effect of different concentrations of *Echinops echinatus* on *Candida albicans.*

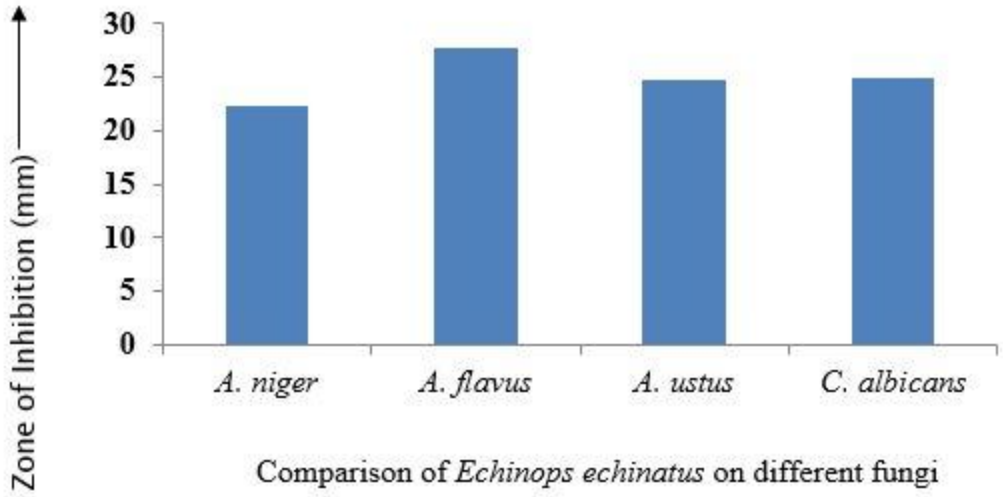

Figure 5: Antifungal activity of mother tincture of *Echinops echinatus* on different strains of fungi.

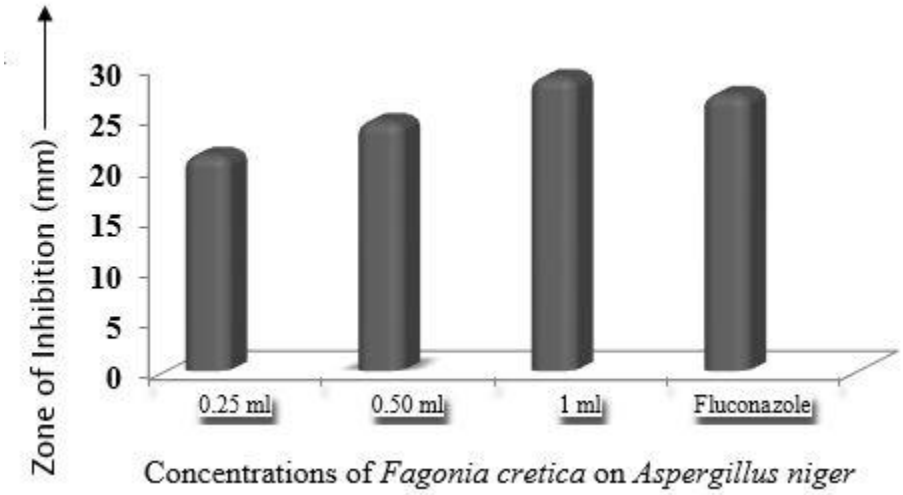

Figure 6: Effect of different concentrations of *Fagonia cretica* on *Aspergillus niger.*

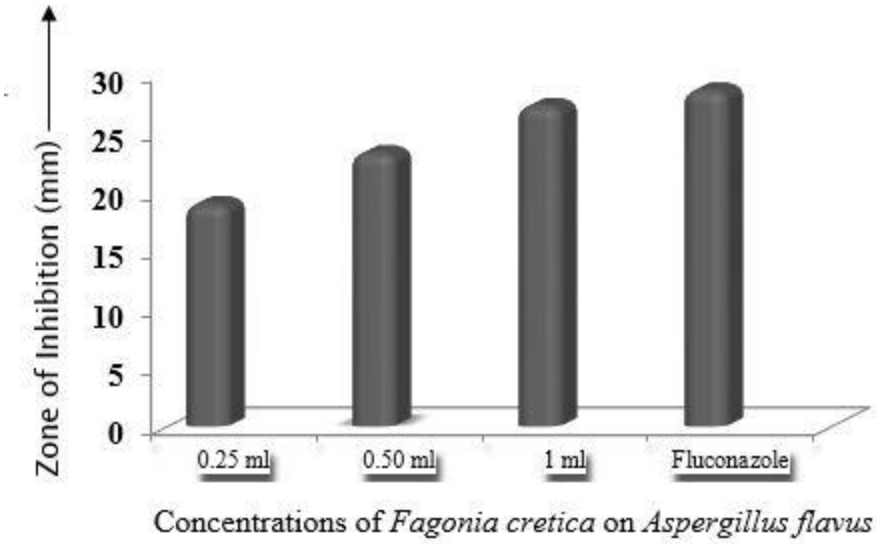

Figure 7: Effect of different concentrations of *Fagonia cretica* on *Aspergillus flavus*

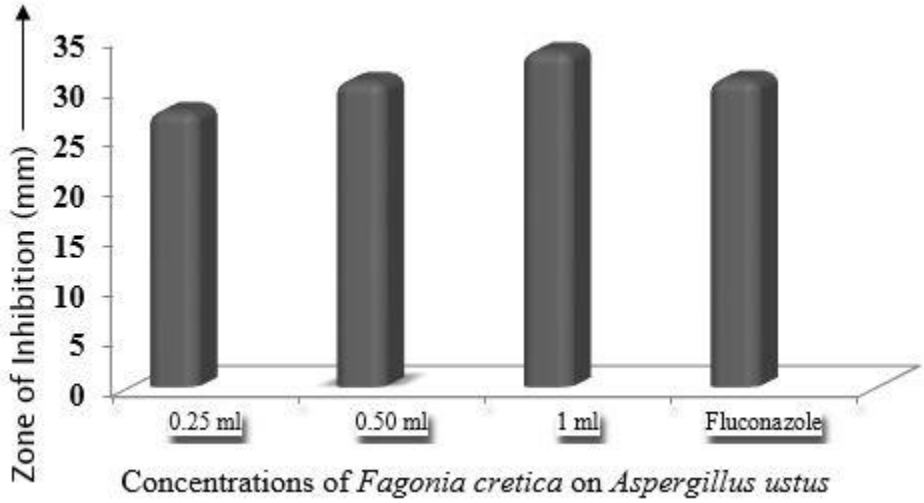

Figure 8: Effect of different concentrations of *Fagonia cretica* on *Aspergillus ustus*

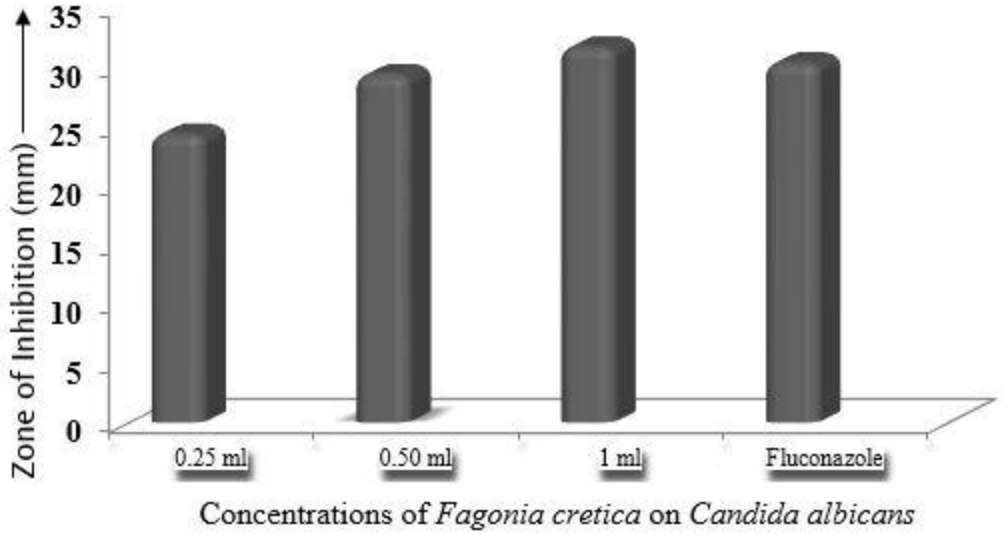

Figure 9: Effect of different concentrations of *Fagonia cretica* on *Candida albicans*

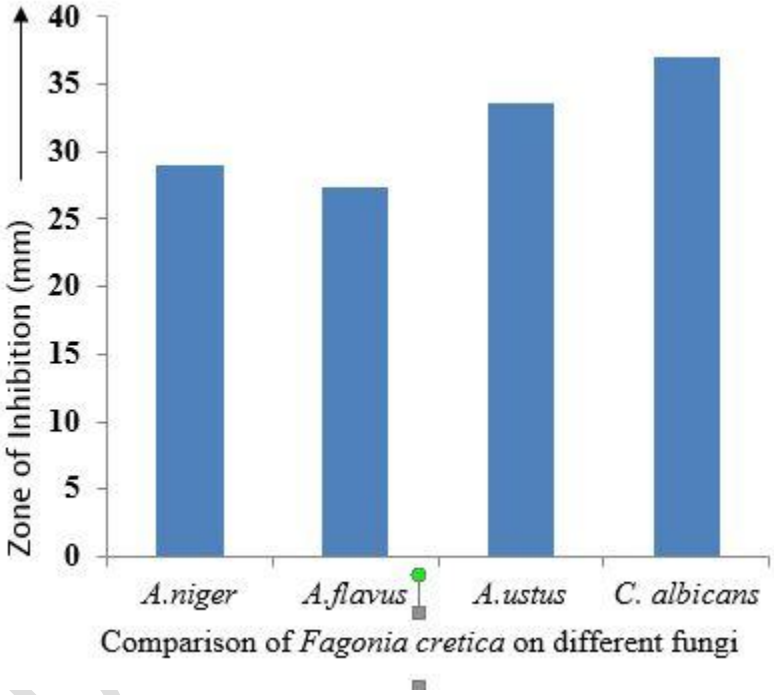

Figure 10: Antifungal activity of mother tincture of *Fagonia cretica* on different strains of fungi.