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Antifungal Activity of *Anastatica hierochuntica* L. extracts against different groups of fungal pathogens: An *in- vitro* test

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Abstract

The antifungal activity of Anastatica hierochuntica L. was studied using ethanol, methanol and aqueous extracts against fungal pathogens by the well diffusion method. Methanol extract (150mg/mL) showed maximum inhibition against Microsporum audouinii followed by ethanol extract (150mg/mL) against Candida albicans. The extracts showed same inhibition pattern when compared with standard antifungal agents such as amphotericin B and nystatin (100µg/mL). Preliminary phytochemical analysis revealed that the extracts contain alkaloids and phenolic content. The minimum inhibitory concentration of A. hierochuntica against Candida albicans was 22mg/mL, followed by Cladosporium sp (25 mg/mL). Aspergillus flavus and Epidermophyton floccosum were inhibited by 50mg/mL concentration; whereas, Fusarium sp. was inhibited by 100mg/mL and Microsporum audouinii by 150mg/mL. The results of the present study show that A. hierochuntica contain antifungal components which can be explored further for antifungal formulations to cure mycosis.

Keywords: *Anastatica hierochuntica* L.*;* antifungal activity; Saudi Arabia

1. Introduction

Traditional medicine is gaining attention in public health for management and control of infections. Isolation of novel antimicrobial component has been focused to overcome multidrug resistance. Some of these materials are having great potential due to its availability, less toxicity, lack of adverse effects and easy degradability [1]. Some of the compounds extracted from plants have been used to control bacterial and fungal pathogens [2]. Immuno-compromised individuals including AIDS, cancer and organ transplanted patients, are more susceptible to fungal infections [3]. The treatment for mycotic infections is limited despite of increased therapeutic options due to high toxicity and severity of infection. Recently many of the antifungal drugs used have become inactive due to the development of antifungal resistant strains [4]. Therefore, for effective management of mycosis, a search for novel antifungal agents is needed. Higher plants are good source of natural antifungal agents with novel mechanism action. Research has been conducted worldwide for elucidating the antifungal activity of different plant extracts [5].

Secondary metabolic components such as tannins, terpenoids, alkaloids, flavanoids, glycosides present in plant extracts are responsible for antimicrobial activity [6].

Anastatica hierochuntica L. is an herb that grows in Middle East Sahara, including the north African regions of Iran, Egypt, Israel, Palestine, Iraq, Pakistan and Jordan, commonly called as rose of Jericho. The plant is rich in minerals (Mg, Ca, Cr, Mn, Fe, Co, Cu and Zn), and phenolic components with antioxidant activity [7]. It is widely used during late pregnancy, for ease child birth, decreases uterine hemorrhage, and help in expulsion of dead fetus [8] and also in antepartum care. *A. hierochuntica* L. has been studied for its antimicrobial antioxidant and hypoglycemic properties. Additionally, the diseases such as respiratory disorders, depression, hypertension, digestive disorders, malaria, cardiac problems, infertility etc., are treated with *A. hierochuntica* [9, 10]. Even though, research on this medicinal plant has been carried out for long time, but the scientific evidence for antifungal activity and usage to cure mycosis are limited [11]. The aim of the present study was to determine the antifungal activity of *Anastatica hierochuntica* L. against different clinical pathogenic fungal isolates and its phytochemical analysis using different types of plant extracts.

2. Materials and Methods

2.1 Plant extracts

Plant samples collected from different areas of Kingdom of Saudi Arabia (KSA) were dried at room temperature and finely ground with a hammer mill. Each 20g of powdered plant material was extracted by maceration with 200mL methanol, ethanol or distilled water for overnight at room temperature before filtration. After filtration water and solvents were evaporated under reduced pressure until dryness. Approximately 0.1 g of the crude extract was dissolved in dimethyl sulphoxide (DMSO) to a final stock concentration of 50mg/mL or 100mg/mL. All crude extracts were kept at -20^0 C till use.

2.2 Fungal strains Pathogenic and opportunistic pathogenic fungal strains were isolated from clinical cases obtained from General Hospital of Shaqra city. These fungi were identified as *Candida albicans, Cladosporium sp, Aspergillus flavus, Fusarium sp., Microsporum audouinii* and *Epidermophyton floccosum*. Each organism was then

cultured on Sabouraud Dextrose Agar medium (SDA) incubated at 25^0 C for 7 days to obtain inocula for testing.

2.3 Determination of antifungal assay

Sterile filter paper discs of 6 mm diameter were impregnated with 0.1mL/disc of extract which have been dissolved in dimethyl sulphoxide (DMSO) and placed in duplicates onto SDA plates seeded with 0.2 mL of fungal suspension. The plates were then incubated at 37^0 C for 10-14 days [12, 13]. The zone of inhibition around each disc was measured in mm. To compare efficacy of the plant extract with synthetic antifungal antibiotics, antifungal susceptibility test was done using standard disc diffusion method. The antifungal agents used in the present study are Amphotericin B and Nystatin (100 µg/mL).

2.4 Minimum inhibitory concentration

The broth dilution method was performed to determine the minimum inhibitory concentration (MIC) of plant extracts as described by Espinel-Ingroff *et al.* [14], with slight modification. Different concentration (two times the final concentration) of plant extracts were made in 100% dimethyl sulfoxide (DMSO) (Sigma) and dispensed into sterile test tubes. The final concentration of plant extracts ranged from 50-150 (mg/mL). The conidial suspension (10^6 spores/mL) was diluted to a final concentration of $4.0x10^4$ to $5x\ 10^4$ CFU/mL and dispensed into the prepared test tubes. The test tubes were incubated at $25\text{-}28^0$ C for 48 h and inoculated into SDA plates to check the growth. The MIC was determined as the lowest concentration that inhibited at least 50% of the growth relatively with the control which does not have plant extracts.

2.5 Phytochemical analysis

The aqueous extracts of *Anastatica hierochuntica* L. were subjected to phytochemical analysis according to the methodology of Crombie *et al.* [15].

3. Results and discussion

Aqueous extract of *A. hierochuntica* 150 mg/mL showed maximum inhibitory activity towards *Microsporum audouinii,* whereas, the least activity was found in *Aspergillus flavus.* It is clear that the inhibitory activity of the extract increased with the concentration of the extracts. *Candida albicans and Cladosporium sp.,* were moderately inhibited by different concentration of the extracts. Earlier reports on antifungal activity states that 250mg/mL inhibited *Aspergillus niger* and *Penicillium digitatam* [16]. Compared to the

earlier reports the present study showed that lower concentration of the extracts inhibited the fungal growth. *Fusarium sp.,* and *Epidermophyton floccosum* were not significantly inhibited by the aqueous extracts (Table 1).

Table 1. Antifungal Activity of *Anastatica hierochuntica* Aqueous extracts against different fungal isolates*.

Extract type	Conc. (mg/mL)	Clinical isolates					
		Ca	*C*	*Af*	*F*	*Ma*	*Ef*
Aqueous	50	5.12 ± 0.15	5.31±0.16	4.08±0.75	6.01±0.35	8.00±0.33	6.62±0.34
	100	7.20±1.25	6.10±1.24	5.65±0.17	7.85±0.34	9.19±0.22	7.10±0.51
	150	12.22±0.33	12.76±1.31	10.67±1.01	14.20±1.77	18.32±0.55	14.38±0.45
Ethanol	50	9.14±1.61	6.76±0.55	5.22 ± 0.17	7.11±1.95	10.82±1.82	8.01±0.33
	100	11.30±0.44	7.29±0.45	6.03 ± 1.70	8.19±0.17	10.94±0.17	9.40±0.22
	150	20.04±0.18	15.26±1.27	15.28±1.44	16.00±1.02	19.29±0.44	17.40±0.42
Methanol	50	8.14±0.22	5.21 ± 0.46	4.16 ± 0.18	6.01 ± 0.31	9.29±0.33	7.10±0.71
	100	9.28±1.08	6.29±1.43	5.17 ± 1.26	7.44±1.16	9.17±1.76	7.88±0.44
	150	17.22±0.12	12.33±0.14	10.44±1.66	14.24±0.34	20.11±0.77	15.25±0.66
Amphotericinn	100 µg/ml	20.40±0.34	17.71±0.43	17.32±0.21	17.32±0.23	22.65±0.16	13.05±0.31
Nystatin	100 µg/ml	23.60±0.22	20.21±0.15	20.00±0.17	20.65±0.31	25.31±0.22	16.40±0.28

*Data are presented as mean ±SD of zone of inhibition (mm); inhibition zones are the mean of three replicates; *Ca= Candida albicans, C= Cladosporium sp, Af= Aspergillus, flavus F = Fusarium sp., Ma =Microsporum audouinii, Ef =Epidermophyton floccosum*.

The ethanol extract of *A. hierochuntica* showed antifungal activity towards all the fungi tested. The growth of *Microsporum audouinii* is inhibited by 150 mg/mL whereas; lower concentration 100 mg/mL did not have any significant effect on growth inhibition when compared to 50mg/mL. Similarly all of the fungal isolates were inhibited by 150 mg/mL, in which *Cladosporium* sp., *Aspergillus flavus*, *Fusarium* sp., had less effect towards the extract. Methanol extract also showed significant antifungal activity towards *Microsporum audouinii* and *Candida albicans*. Increasing concentration of methanol extract had an increased antifungal activity. However, the methanol extract had a minimum inhibitory activity to other fungal isolates tested.

The minimum inhibitory concentration of *A. hierochuntica* ethanolic extract was tested for all of the clinical isolates (Table 2). A low concentration of 22 and 25 mg/mL of the extract was

sufficient for inhibiting the growth of *Candida albicans* and *Cladosporium* sp. respectively. Methanol extracts of various plant materials such as roots and leaves showed antifungal activity to fungal pathogens [17]. Despite of less solubility in water the methanol extracts may penetrate into the fungal cells and interrupts the cellular metabolism, function and disrupting the cellular components leading to the inhibition of fungal growth [18]. *Aspergillus flavus* and *Epidermophyton floccosum* required 50 mg/mL as minimum inhibitory concentration. *Fusarium sp. was* inhibited by 100 mg/mL, whereas, *Microsporum audouinii* required high concentration of 150 mg/mL as minimum inhibitory concentration.

Table 2. Minimum Inhibitory Concentration (MIC) of the ethanolic extracts of *Anastatica hierochuntica* plant against tested fungi

Test organism	MIC (mg/mL)
Candida albicans	22
Cladosporium sp	25
Aspergillus flavus	50
Fusarium sp.	100
Microsporum audouinii	150
Epidermophyton floccosum	50

Table 3. Phytochemical analysis of *Anastatica hierochuntica* samples

Constituent	Level*
Alkaloids	+++
Phenolic contents	+++
Carbohydrates	++
Reducing sugars	+++
Tannins	++
Aminoacids and proteins	++
Polysaccharides	+
Volatile oils	±

*:+ = low concentration, ++ = medium concentration, +++ = high concentration,
±= traces, - = not detectable

4. Conclusion

The most interesting feature of the present study is that all of the extract had significant antifungal activity that inhibits serious fungal pathogens. Further studies can be focused on novel phytochemical extraction from these plants and formulation of antifungal drugs after *in vivo* studies. Finally, we may conclude that due to increasing resistance of current and old antibiotics, it may be suggested that the use of plant-based drugs will support to overcome the resistance developed by microorganisms.

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