

Antifungal Effect of *Echinophora Platyloba*'s Extract against *Candida albicans*

Majid Avijgan^{a*}, Massoud Hafizi^b, Mehdi Saadat^b and Mohammad Ali Nilforoushzadeh^c

^aDepartment of Infectious and Tropical Diseases, Shahr-e-Kord University of Medical Sciences, Shahr-e-Kord, Iran. ^bShahr-e-kord University of Medical Sciences, Shahr-e-Kord, Iran. ^cIsfahan Skin and Leishmaniasis Diseases Research Center, Isfahan, Iran.

Abstract

The present study was undertaken to investigate the effectiveness of the ethanolic extract of *Echinophora platyloba* DC. on *Candida albicans*.

Using the agar dilution method, the growth condition of standard *Candida albicans* ATCC 10231, cultured on the media containing plant extracts at different concentrations (1, 2, 4, 8, 16, 32, 64, 128 and 256 mg/ml) was studied. The results were recorded twenty one days after the incubation period, maximum time for the growth of fungi.

Results showed that the extract of *Echinophora platyloba*, equal or above the concentration of 2mg/ml, effectively inhibits the growth of *Candida albicans*. In other words it shows growth on media containing 1mg/ml of the extract.

Results of the present study revealed a great promise in the application of *Echinophora platyloba* extract against *Candida albicans*. It is concluded that the plant studied could be a good antifungal source.

Keywords: *Candida albicans*; *Echinophora platyloba*; Ethanolic extract; Yeast; Traditional Medicine.

Introduction

Traditional Medicine, in some countries Like Iran has a major therapeutic role, and traditional healers have been using different plants to treat people for thousand of years. In Iran, in the last century, there has been a decline in the application of traditional medicine. However, fortunately the use of alternative medicines has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and

well being (1). Natural products have served as a major source of drugs for centuries, and about half the pharmaceuticals in use today are derived from natural products (2). One study has reported that 25 to 50% of current pharmaceuticals are derived from plants (3). Microbiologists and natural-product chemists are trying to discover more about phytochemicals, which could be developed for treatment of infectious diseases (3). There are some folkloric herbs still in use which are being used without any scientific evidence. one of them is *Echinophora platyloba* DC. It has ten species (4) and in Chahar mahal va bakhtiary province, its' main use is as a food seasoning (5) and also to prevent the fungal growth on some food like tomato paste and pickled cucumber. Among ten species, four of them are endemic in

* Corresponding author:

E-mail: avijgan@yahoo.com

Iran, including *E. orientalis*, *E. sibthorpiana*, *E. cinerea* and *E. platyloba* (4). A previous study, has shown the presence of components such as flavonoid, alkaloid and saponin (6). Another study showed a growth inhibitory effect with this extract, against some common dermatophytes (7). The lack of pharmacological and clinical data on the majority of herbal medicinal products is a major impediment to the integration of herbal medicines into conventional medical practices. For valid integration, pharmacological and clinical studies should be conducted on those plants lacking such data (8). Among them *Echinophora platyloba* could be considered.

Considering the folkloric application of this herb as a food seasoning (4) and also as an additive material to prevent the growth of fungal on some food like tomato paste and pickled cucumber, there is a lack of information on the antifungal action of *Echinophora platyloba*. This study is an attempt to determine the antifungal activity of *Echinophora platyloba*, on standard *Candida albicans* ATCC 10231.

Experimental

Material

The plants were collected from the southwestern parts of Iran (Shahr-e-Kord). A voucher specimen of plant was deposited at the Herbarium of the Faculty of Sciences, Isfahan University, Isfahan, Iran. The aerial parts were separated, shade-dried and crushed into powder using a pestle and mortar and kept in airtight light-protected containers.

Preparation of extract

The percolation method was used to obtain the crude extract (9). A quantity (100 gr) of powdered plant was soaked in 500 ml of 96% ethanol for 3 days with frequent agitation. Ethanolic extract was prepared by maceration of

the dried plant materials in 96% ethanol (the plant material to solvent ratio was 1:5). The mixture was filtered and the crude extract collected. The crude extract was then distilled at 48°C using a water bath. The semi-dried extract was collected for further drying in an oven at 37°C. The Final crude dry extract was equal to 10 gr per 100 gr of the plant.

Preparation of media

18×150 ml media tubes were used in this study. The method used for study was the agar dilution method (Macro-dilution). On the basis of company instructions, the base medium was made of Sabouraud dextrose agar (Merck, Germany). In contrast to the instructions, we added half the amount of distilled water, but later the remaining volume of water was added along with the antibiotics. After autoclaving the medium, antibiotics were added to make Sabouraud, Chloramphenicol and Cyclohexamide (SCC) medium. [Chloramphenicol (Lorestan Company, Iran) was used to prevent bacterial growth and Cyclohexamide (Oxoid, USA) to inhibit saprophytic fungal growth].

Preparation of dilution

At temperature of 37°C, 10 g of crude extract was mixed with 10 ml of distilled water to obtain concentration of 1000 mg/ml. On the basis of serial dilution method (Table 1), different concentrations of extract were achieved.

At this step, 5 ml of extract in various concentrations were added to 5 ml of the SCC medium to reach the final extract concentration (Table 2).

This mixture was placed into screwed tubes, was shaken and stored until a semi solid form obtained.

Microorganism

The microorganism used was *Candida*

Table 1. Difference concentrations of the extract.*

Tubes	1	2	3	4	5	6	7	8	9	10	11
Distilled water (ml)	8	4	4	4	4	4	4	4	4	4	8
Extract (ml)	8	4	4	4	4	4	4	4	4	4	0
Concentration (mg/ml)	≈ 512	256	128	64	32	16	8	4	2	1	0

* In order to obtain a serial dilution, half the total volume of each extract column (tube) was placed in the next extract column (tube), except for the 11th tube. The half volume of the 10th tube was discarded.

Table 2. Final concentrations of the extract within the media

Tubes	1	2	3	4	5	6	7	8	9	10	11
SCC media (ml) medium	5	5	5	5	5	5	5	5	5	5	5
Milliliter of each column from table 1	5	5	5	5	5	5	5	5	5	5 ml of distilled water	5
Final concentration of extract in the medium	256	128	64	32	16	8	4	2	1	0	256
Addition of the fungal suspension	+	+	+	+	+	+	+	+	+	+	-

albicans ATCC 10231, purchased from the Biotechnology Research Center of Iran.

Anti-fungal activity of plant extract

Some fresh cultured colonies (cultured colonies less than one week after passage on the new medium) of *Candida albicans* were added to sterile normal saline to obtain 0.5 McFarland turbidity standard. Finally 200 microlitres of this fungal suspension were added to the media and cultured (with streak method) using a sterile loop and then incubated at room temperature in a closed condition (at the hood) and observed weekly for 21 days.

The 10th tube was made without any extract (control for media) and the 11th tube without a fungal suspension (control for contamination).

The concentration of extract in the first tube, which inhibits the growth of *Candida albicans*, was recorded as the minimal inhibitory concentration.

Results

On the basis of this study, *Candida albicans* is sensitive to a minimum dilution of 2 mg/ml of ethanolic extract of *Echinophora platyloba*. There is an overt growth of yeast in the control tube (10th tube) and the tube containing

1 mg/ml of extract, but no growth of any fungi in the other tubes (dilutions of 2, 4, 8, 16, 32, 64, 128 and 256 mg/ml) and the control tube for contamination (11th tube) (Table 3).

All the concentrations extract (except 1mg/ml) at 2, 4, 8, 16, 32, 64, 128 and 256 mg/ml demonstrated growth inhibitory activity against *Candida albicans*. The concentration of 2 mg/ml is minimum inhibitory dilution of this study.

Discussion

There is no previous report on evaluation of this plant concerning its' activity against *Candida albicans*. Although, the antibacterial effect of another species of this family (*E. sibthrobica*) has been reported (5). This study was designed to evaluate the activity of *Echinophora platyloba* extract against *Candida albicans*.

The potential of this extract to inhibit *Candida albicans* ATCC 10231 growth in at least an MIC of 2 mg/ml concentration revealed a reasonable effect against *Candida albicans*.

The studied plant is one of its' four species, being endemic of Iran, which grows in many parts of the country (10).

One study has reported that this plant contains saponin, alkaloid and flavonoid (6). Fungal agents are susceptible to extract's

Table 3. Final result of fungal growth in each extract concentration within the media

Tubes	1	2	3	4	5	6	7	8	9	10	11
SCC media (ml) medium	5	5	5	5	5	5	5	5	5	5	5
Milliliter of each column from table 1	5	5	5	5	5	5	5	5	5	5 ml of distilled water	5
Final concentration of extract in the medium (mg/ml)	256	128	64	32	16	8	4	2	1	0	256
Addition of the fungal suspension	+	+	+	+	+	+	+	+	+	+	---
Growth of <i>Candida albicans</i>	---	---	---	---	---	---	---	---	+++	+++	---

components like saponins. Some steroidal saponin, like CAY-1, have shown to be a potent fungicide against *Candida albicans* by disrupting the membrane integrity of the fungal cells, at concentrations below the threshold of mammalian cell toxicity (11, 12). Also, in one study on saponins obtained from *Hedera taurica*, the antibacterial and antifungal action of saponins was investigated (13). This study revealed that saponins H1 and H2 had no antimicrobial effect, but saponin Sx1 possessed in vitro antifungal activity with respect to *Candida albicans*, *C. krusei* and *C. tropicalis* (13). Further more, the isolated alkaloid from *Schizozygia coffaeoides* has a potent antifungal effect in comparison to other components (14). Considering that this plant has saponins, flavonoids and alkaloids, these findings could be related to the effect of these components.

Thymus vulgaris and *Populus candicans* essential oils, which are not usually considered to be particularly antifungal, have been found in a study to be highly inhibitory at normal therapeutic concentrations (15). A similar finding was noted in our study, showing the effective antifungal effect of *Echinophora platyloba* against *Candida albicans*.

Herbal medicines, as independent or complementary drugs, are helpful in the treatment of some diseases. For example the therapeutic effect in patients treated with a combination of *Agastache* lotion and western medicine was significantly better in curing skin tinea and genital candidiasis than when they were used alone (16). The essential oil of *Agastache rugosa*, combined with ketoconazole, may be particularly useful against *Blastoschizomyces capitatus*, a rare pathogenic fungus documented to cause severe and fatal mycoses in immunocompromised patients (17). There are some active component extracts, such as the *Ginseng radix* extract and the extract obtained from the Japanese herb, *Juzen-taiho-to*, which had been demonstrated to enhance the anti-*Candida* activity of macrophages in vitro and prolong the survival time of *C. albicans*-infected ones (18).

On the basis of folklore application of this herb, which is used as a food seasoning and also to prevent fungal growth in tomato paste, as well as the results of this study, it is confirmed that

this plant has an effective role in the inhabitation of fungal growth specially against *Candida albicans*.

In conclusion, the studied plant could provide some activity against *Candida albicans*, however, it is not known that which component of the extract is responsible for this effect. Further studies using isolated constituents instead of the whole extract should be carried out.

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