

An evaluation of the antifungal activity of some local medicinal plants against growth of *Candida albicans in vitro*.

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Abstract

The aim of this study was to evaluate the antifungal activity of the ethanolic extract of three local plants (*Elettaria cardamomum* , *Aloe vera*, *Thyme vulgaris*) against the growth of pathogenic *Candida albicans* in culture media. The antifungal activity was carried out by using agar well diffusion method. Ethanolic extracts of *Elettaria cardamomum* and *Aloe vera* inhibited the growth of *Candida albicans* isolates at all concentrations which tested in present study (25, 50, 100, 150, 200, and 400) mg / ml, while the extract of *Thymus vulgaris* showed no activity against tested *Candida albicans*

Introduction

Nosocomial fungal infections due to *Candida* species are an important cause of morbidity and mortality especially in immunocompromised patients. The use of available treatment options for invasive mycoses is limited due to narrow spectrum of activity, drug resistance, toxicity, and drug-drug interactions (1, 2). In view of this, there is a need to develop more effective and less toxic agents for the treatment of common as well as drug resistant fungal infections. Plants, as sources of medicinal compounds have continued to play dominant role in maintenance of human and animal health since ancient times. The World Health Organization estimates that plant extracts or

their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (3). Over 50% of all modern clinical drugs are of natural product origin (4). The Iraqi flora are rich in different medicinal plants uncommitted in most to any previous study, the possibility to find new antifungal agents is still widely ahead (5), therefore; in the present study we focus on the study of the *in vitro* effects of number of local medicinal plants against growth of *Candida albicans* and compare their result with the antifungal activity of standard anti-*Candida* drug (Clotrimazole, Nystatin) in culture media.

Materials and methods

Plant materials:

juice (Latex) of *Aloe vera* leaves had been used in this study which refers to a bitter yellow fluid extracted from the specialized areas of the inner leaf skin and is generally prepared as a powder (6). On the other hand the tested part of *Elettaria cardamomum* was fruits (air-dried), and dried leaves for *Thymus vulgaris*. All these plant materials were purchased from the local market and identified at the National Iraqi Institute for Herbs, Baghdad, Iraq.

Preparation of extracts:

The solid latex of *Aloe vera*, dried fruits of *Elettaria cardamomum*, and dried leaves of *Thymus vulgaris* were crushed and ground in a grinding machine to obtain fine powder

for each plant. Ethanolic extracts were accomplished according to the method of le Grand (7). Briefly 50 gm of each powdered plant sample was mixed with 250 ml of 96% ethanol. The mixture was kept for 2-5 days in tightly sealed containers at room temperature and shaken several times daily. This mixture was filtered through filter paper to remove the coarse plant materials. Further extraction of the residue was repeated 3-5 times until a clear supernatant extraction liquid was obtained. The filtrates of each tested plant were evaporated to dryness using a rotary evaporator at 40°C. The final dried samples were weighed and stored at -20°C until use.

Antifungals:

The following standard antifungal drugs were used as the antifungal control as such concentration in this study:

*Clotrimazole (Candistan[®]-solution, each 20 ml contains Clotrimazole 0.2 gm, The Arab Drug Company, Cairo-A.R.E.).

*Nystatin (Nystasyr[®]-drops, each 1 ml contains 100000 IU, Pharmasyr, Damascus, Syria).

Organisms:

Six isolates of *Candida albicans* were studied ; they were obtained from the Microbiology Laboratory and laboratories of unit of zoonotic diseases at the College of Veterinary Medicine, Al-Qadisiya University. All isolates were identified by germ-tube test (8), spore germination test (9), production of chlamydoconidia on corn meal agar (10). These isolates were maintained on Sabouraud's dextrose agar SDA (HIMEDIA Laboratories, Mumbai-India) at 4°C.

Antifungal susceptibility test:

A serial dilution of each extract was prepared for studying of their antifungal by using activity (depending on the agar well diffusion method at different concentrations and it was done by diluting 2 gm of each dry extract with 5 ml of 96% ethanol to obtain stock solution at a concentration of 400 gm/ml. From this stock solution various concentrations were made including: 200 gm/ml (consist of 2 ml of 96% ethanol and 2 ml of the stock solution at 400 gm/ml concentration), 150 gm/ml (prepared by adding 1 ml of 96% ethanol to 3 ml of the extract solution at concentration of 200 gm/ml), 100 gm/ml (it was made by adding 1 ml of 96% ethanol to 1 ml of the extract solution at a concentration of 200 gm/ml), 50 gm/ml (prepared by drawing 1 ml of the extract solution at a concentration of 100 gm/ml and adding to 1 ml of 96% ethanol), 25 gm/ml (done by mixing 1 ml of 96% ethanol with 1 ml of the extract solution at a concentration of 50 gm/ml). *Candida* isolates were subcultured in nutrient broth media (HIMEDIA Laboratories, Mumbai-India)

that was prepared by dissolving 13.00 gm of nutrient broth in 1000 ml of distilled water, shaking well and heated for several minutes using water bath to ensure complete dissolving, then sterilized for 15 minutes at 15 lb pressure in an autoclave. Several colonies of *Candida* were suspended with the aid of sterile cotton swab in sterile tube containing 10 ml of nutrient broth. After mixing, the tube was incubated at 37°C for 24 hours to produce a fungal suspension of moderate turbidity. Sabouraud dextrose agar (SDA) medium was prepared according to the manufacturer's instructions by dissolving 65 gm of the SDA in 1000 ml of distilled water then shaken, heated and autoclaved. This medium was poured aseptically at 45°C into sterilized Petri plates with the aid of sterile pipette of 20 ml capacity on the flat horizontal surface to a depth of 20 mm. After complete solidification, 7 wells were made aseptically with a diameter of 5 mm on the surface of each agar plate. Later, a sterile cotton swab was dipped into the fungal suspension and the surplus was removed by rotating the swab to the sides of the test tube used. The SDA media were inoculated by even streaking of the swab over the entire surface of each plate. Finally and after the inoculums were dried, 0.1 ml of each concentration of each extract was poured into the wells besides 0.1 ml of 96% ethanol which considered as a negative control on the same extract plate. Antifungals also used on each on different plate. These experiments were repeated on six plates for each extract and for each antifungal agent and mean calculated. All the plates were incubated at 37°C for 24-48 hours and following incubation, the diameter of zone of inhibition around each well was measured in millimeters with the help of ruler(11). The values are given as mean \pm SE and The data were analyzed by anova test with Least significant differences (LSD) at significant level of (P<0.05) by using SPSS (Version 10).

Results

In vitro anti-*Candida albicans* activity of three local plant extracts which were used in the present study and two standard antifungal drugs was showed in table (1) and (2). On the basis of this study, the extracts of three plant had been exhibited various antifungal activities against (6) isolates of *Candida albicans* according to the type of plant and the used concentrations in our study. Among the plants studied, the most active extract was that obtained from *Elettaria cardamomum* which gives zone of

inhibition with SE (8.38±1.6, 13.05±0.51, 16.11±0.52, 18.72±0.34, 19.5±0.18, and 20±0.37 mm) at a concentration of 25, 50, 100, 150, 200, 400 mg/ml respectively, while extract of *Aloe vera* showed moderate activity and gives inhibition zones about (10.6±0.13, 11.55±0.97, 12.66±0.2, 13±0.33, 13.11±0.36, and 13.77±0.24 mm) at a concentration of 25, 50, 100, 150, 200, 400 mg/ml respectively. *Thymus vulgaris* have no effect on all *Candida* isolates at all the tested six concentrations (figure: 1).

Table (1): Inhibition zones (mm) of *Candida albicans* growth produced by plant extracts in culture media.

Type of plant	Extract concentrations (mg / ml)					
	25	50	100	150	200	400
<i>Elettaria cardamomum</i>	8.38± 1.6 aA	13.05± 0.51 bA	16.11± 0.52 cA	18.72± 0.34 cA	19.5± 0.18 cA	20± 0.37 cA
<i>Aloe vera</i>	10.6± 0.13 aB	11.55± 0.97 aB	12.66± 0.2 aB	13± 0.33 bB	13.11± 0.36 bB	13.77± 0.24 bB
<i>Thymus vulgaris</i>	- aC	- aC	- aC	- aC	- aC	- aC

☒ Different small letters mean significant changes for vertical values at level ($p < 0.05$).

☒ Different capital letters mean significant changes for horizontal values at level ($p < 0.05$).

☒ Result for 6 isolates of *Candida albicans* with SE.

Table (2): Inhibition zones (mm) of *Candida albicans* growth produced by antifungal drugs in culture media when used as positive control.

Positive and negative control	Inhibition zone (mm)
Nystatin (100 IU / ml)	15.5±0.26
Clotrimazole (30 µg / ml)	18.8±0.61
Ethanol (96%)	7.2±2.61

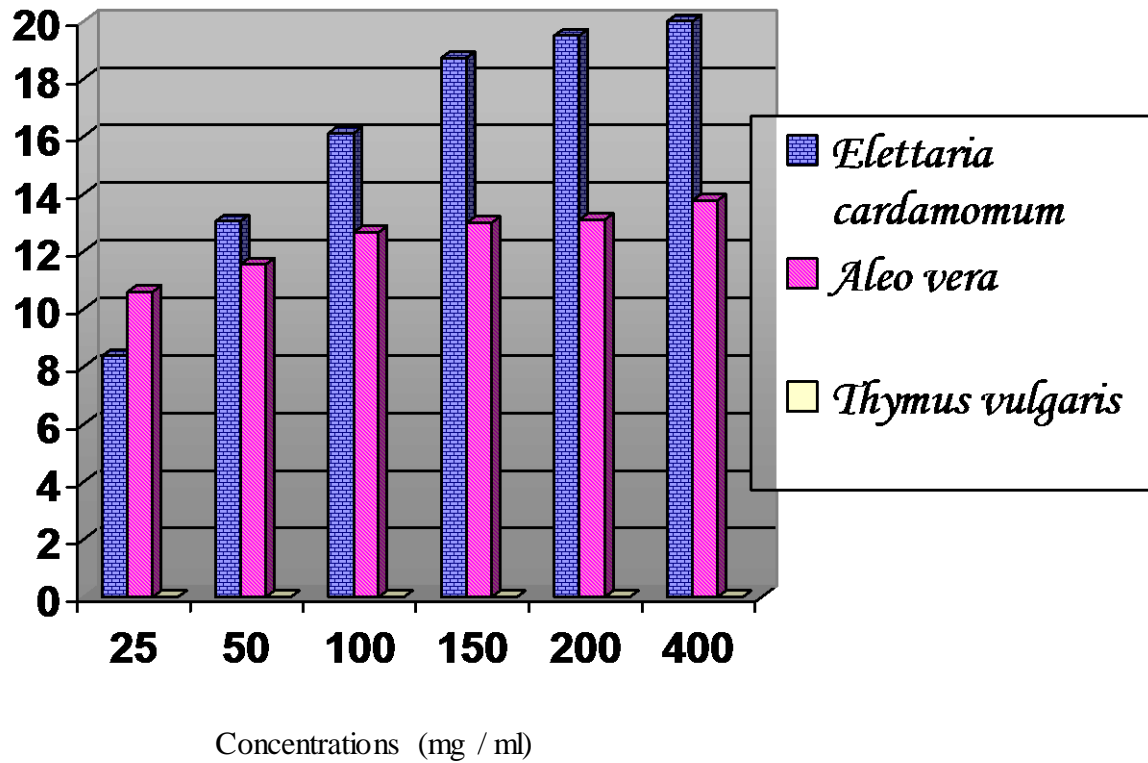


Fig. (1): Inhibition zones of *Candida albicans* exhibited by plants ethanolic extracts.

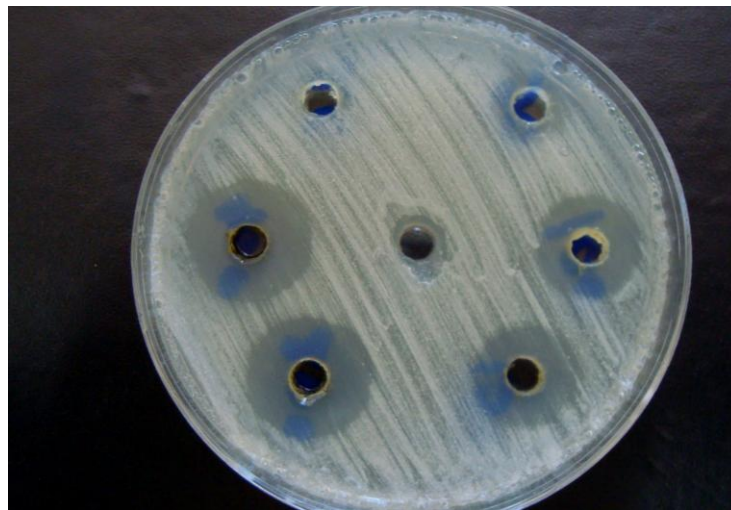


Figure (2): Inhibition zones of *Candida albicans* growth on SDA produced by ethanolic extract of *Elettaria cardamomum*, the peripheral six wells contained extract concentrations (25, 50, 100, 150, 200, 400 mg/ml) whereas the central well contained 0.1 ml of 96% ethanol.



Fig. (3): Inhibition zones of *Candida albicans* growth on SDA resulting from the use of ethanolic extract of *Aloe vera*, the peripheral six wells contained extract concentrations (25, 50, 100, 150, 200, 400 mg/ml) while the central well contained 0.1 ml of ethanol at a concentration of 96%.

Discussion

Antifungal susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology. Number of reports is available showing efficacy of various plant extracts as antifungal agents (12, 13, 14). *Candida* species becoming increasingly important as opportunistic fungal pathogens (15,16), that frequently cause oral infections in immunocompetent and immunocompromised individuals (17) due to the suppression of local as well as systemic defense mechanisms (18), it can also contaminate the failed root canal treated teeth (19). *Candida albicans* remains the most common infection-causing fungus, about 45% of clinical infections are caused by this pathogen (20). despite serious environmental implications associated with the excessive use, chemical fungicides still remain the first line of defense against fungal pathogens. More over, these fungicides when ingested by human beings and animals through food and water cause various ailments in the body. Search of natural fungicidal principle, from the plant sources would definitely be a better

alternative to these hazardous chemicals (21). Al-Bayati (22) and Mahmoudabadi *et al* (10) demonstrated that ethanolic extracts of medicinal herbs inhibit growth of *Candida albicans*. The present study showed that similar extracts from *Elettaria cardamomum* and *Aloe vera* at the concentrations of 400, 200, 150, 100, 50, and 25 mg/ml for each extract have promising antifungal activity against six isolates of *Candida*, while ethanolic extract of *Thymus vulgaris* at the same concentrations produced no effect on the growth of this yeast, and also the solvent control (96% ethanol) showed very limited anticandidial activity. We had found that there was proportional relationship between the prepared concentrations of each plant extract and the diameter of zones of inhibition (Figure 2,3). Ethanolic extract of *Elettaria cardamomum* was more effective in inhibition of *Candida* growth in comparison to *Aloe vera* extract. Little researches were available about the effect of cardamom *in vitro*. Cardamom contains protein, fat, carbohydrate and fibers. The therapeutic properties are due to volatile oils

whose main constituents are cineole, terpinol, and limonene (23). A chemical investigation revealed that the action of non-saponifiable lipid fraction of cardamom consisted mainly of waxes and sterols. In the sterol fraction β -sitosterone and γ -sitosterol are newly reported. Phytol and traces of eugenyl acetate were also identified for the first time (24). So this can indicate that the inhibitory effect of ethanolic extract of cardamom upon *Candida* growth could be attributed to its active constituents properly sterols since we had been used ethanolic extract not the volatile oils. *Aloe vera* had also shown bactericidal and antifungal activity *in vitro* (25,26) and *in vivo* (27). *Aloe vera* leaves produce two substances, a gel and a juice or latex (28) that had been widely used in therapy. *Aloe vera* leaves contain a range of biologically active compounds, the best studied being acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and various lectins (29,30,31). Agarry *et al* (32) had been found that the growth of *Candida albicans* was also inhibited by *Aloe vera* leaf extract but was not affected by the gel, this positive effect could be related to its active constituent mainly the anthraquinone glycoside that present in the leaf juice (latex) or extract but not in the gel (33), making this study resembling ours in their positive results coming from the use of ethanolic extract of *Aloe vera* latex. On the other hand the present study had revealed that ethanolic extract of *Thymus vulgaris* was ineffective against *Candida albicans* at all the used concentrations as seen by the absence of zones of inhibitions on the agar surface. Clotrimazole and nystatin are antifungal drugs, so they were used as positive control in our study whose its results revealed that clotrimazole was more effective than nystatin, where as Sehgal *et al*

(34) in a study done on the efficacy of *Calotropis procera* latex on *Candida* growth in comparison to three standard antifungals drugs were found that nystatin was more effective when compared with the used clotrimazole and griseofulvin. Clotrimazole is belonging to azole antifungal agent, it interact synergically *in vitro* with zeamatin as shown in systemic therapy against murine candidiasis (35), and with nikkomycin Z in therapy of a *Candida* vaginitis model (36). Azoles have direct effect on the fatty acids of cell membranes (37), they inhibit ergosterol biosynthesis through their interactions with the enzyme lanosterol demethylase, which is responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane, leading to the depletion of ergosterol in the membrane (38,39). Our results agreed with Shadomy (40) in producing inhibitory effect against *Candida albicans in vitro*, but Jabra-Rizk *et al* (41) demonstrated that *Candida* cells in biofilm had developed resistance against azoles especially fluconazole and clotrimazole *in vivo*, but the formation of biofilms can be inhibited by azoles (42). Nystatin was chosen as the control against *Candida albicans* as it combines with the fungal cell membranes exhibiting both fungicidal and fungistatic activity *in vitro* (43, 44). Many studies were demonstrated the anti *Candida* activity of nystatin through the effect on the biosynthesis of fatty acids and phospholipids causing abnormal growth of *Candida albicans* (45,46) by increasing membrane fluidity (47). We conclude that *Elettaria cardamomum* and *Aloe vera* represent untapped source of potentially useful anti-*Candida*, they are worthy for future clinical study and provide a basis for the further investigations and development of other plant extracts with potent antifungal activity and low host toxicity.

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تقييم الفاعلية المضادة للفطريات لبعض النباتات الطبية المحلية ضد نمو خميرة

المبيضات البيضاء في الزجاج

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الخلاصة

هدفت هذه الدراسة الى تقييم الفعالية ضد- فطرية لمستخلصات ايثانولية محضرة من ثلاث نباتات محلية (الهيل والصبر والزعر) ضد نمو ستة عزلات من خميرة المبيضات البيضاء المرضية في الأوساط الزرعية والتي أنجزت بواسطة طريقة الانتشار في حفر الأكار. لقد أظهرت المستخلصات الأيثانولية المحضرة من الهيل والصبر تأثيراً تثبيطياً واضحاً ضد نمو عزلات خميرة المبيضات البيضاء وبكافة التراكيز المختبرة في دراستنا الحالية وهي ٢٥ ، ٥٠ ، ١٠٠ ، ٢٠٠ ، ٤٠٠ ملغم/أمل، في حين لم يظهر مستخلص الزعر الأيثانولي أي تأثير يذكر ضد نمو الخميرة المذكورة.