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Study of Inhibitory Effect of *Echinops cephalotes* on *Candida* Spp. Isolated from Vulvovaginal Candidiasis Patients in Isfahan

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Abstract

Background: *Candida* spp. has been considered as the agents of acute and recurrent vulvovaginal candidiasis.
Objectives: The aim of current study was the evaluation of antifungal activity of *Echinops cephalotes* (Leaves and stem, manna) plant against species of *Candida* isolated from patients with vulvovaginal candidiasis.
Materials and Methods: In this research study identification of clinical isolates (50 cases) was inducted to the species level by means of conventional mycological methods, morophology on corn meal agar and chromogenic agar, germ tube production and biochemical methods. Antifungal activity of the ethanolic, methanolic and aqueous extracts of *E. cephalotes* was studied against isolated *Candida* using agar well diffusion and microdilution methods.
Results: *Candida* spp. which isolated from patients was *C. albicans*, *C. glabrata*, *C. tropicalis* and C. *parapsilosis*. The inhibition zone of ethanolic extract was 16.6, 13.3, 14, and 22 mg/mL respectively. Minimum inhibitory concentration (MIC) for most the cases were 15.6 mg/mL. The inhibition zone of aqueous extract was 16.8, 16.7, 15 and 15 mg/mL respectively. MIC for most the cases were 15.6-31.2 mg/mL. The inhibition zone of methanolic extract was 15.4, 13.2, 12 and 18 respectively. MIC for most of the cases was 7.8 mg/mL. Among the different extracts, ethanolic extract has the highest and aqueous extract has the lowest anti-*Candida* activity. Ethanolic, methanolic and aqueous extracts of trehala manna did not show any antifungal activity.
Conclusions: This research is the first study on antifungal activity of *E. cephalotes*. Hence, this plant may be used further as medicinal plant against *Candida* spp.

Keywords: Vulvovaginal Candidiasis, Extract, *Echinops cephalotes*, *Candida*

1. Background

Candida species have been introduced as commensal fungi which are the most common cause of invasive fungal infections in humans [1]. In healthy individuals *Candida* species are harmless members of the normal gastrointestinal, oral and vaginal microbial flora [2]. Candidiasis is an opportunistic fungal infection caused by many species of *Candida* that may affects different sites of the body [3]. Fungal infections also occur in chronic illnesses like diabetes and AIDS [4]. Vulvovaginal candidiasis (VVC) is a disease caused by overgrowing of yeasts in the mucosa of the female genital tract [2]. Approximately 75% of adult women will experience at least one episode of VVC during their lifetime, among which approximately 40 - 50% will experience further episodes and 5% will develop the recurrent type (RVVC) [5]. The majority of cases of vulvovaginal candidiasis are caused by *C. albicans*; however, non-albicans species such as *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* are common in certain populations [5]. A possible reason for more frequent isolation of non-albicans species from vulvovaginitis patients may be related with the overuse of topical azole agents available as over-the-counter preparations in the United States since 1992 [6]. This phenomenon emphasizes the importance of identification of the *Candida* species in the clinical settings. Herbal medicines have been used since ancient times as remedies for the treatment of a variety of diseases. In spite of advances in modern medication, plants still make an important contribution to health care [7]. Herbal extracts prepared from medicinal plants include different compounds with numerous biological activities confirmed by in vitro and in vivo studies, such as antibacterial, antifungal, antiviral, antiseptic, anti-inflammatory, antitumor, anti oxidative, anti allergic and diuretic properties [8].

The frequency of invasive fungal infections in one hand and resistance to antifungal therapy in other hand may lead to introduce new antifungal agents. In vitro antifungal susceptibility testing is now standardized internationally and is becoming essential in patient management and resistance surveillance [9]. Traditional plants are valuable source of novel antifungal [10]. *E. cephalotes* (Trehala

manna), is product of the hard work on insect of *Larinus* spp. on the species of *Echinops*. The genus belonging to the family of Compositae species. *Echinops* genus of Asteraceae family has 120 species distributed all over the world and according to Flora Iranica there are 54 species in different area of Iran. There is an exception of four *Larinus* species constructing trehala, whose larvae make capsule in the stems of the plant genus *Echinops*. The results showed that, although the *Larinus* insect was seen on the all of the species, they produce manna, only on four species, (*E. endotrichus*, *E. dichrous*, *E. tenuisectus* and *E. persepolitanus*). Morphologaical investigations indicated that manna produce species have spherical aggregated flowers and they have the same plant height [11, 12].

2. Objectives

The aim of this study was the investigation of inhibitory effect of *Echinops cephalotes* (Leaves and stem, manna) on *Candida* spp. isolated from vulvovaginal candidiasis patients.

3. Materials and Methods

In this research study the aerial parts of plant were collected in spring 2011 from Ardestan in Isfahan province. The collected plants were identified in the agricultural and natural resources of the research center of Isfahan, Iran.

3.1. Preparation of Alcoholic and Aqueous Extracts

Leaves and stems of *E. cephalotes* were dried, powdered and stored in 4°C for further investigations. Fifty grams powdered plant materials were extracted in a soxhlet apparatus with 270 mL of each of solvent namely ethanol, methanol and aqueous for 8 hours, the alcoholic and aqueous extracts were evaporated at room temperature. Then 1gr of the dried plant extract was dissolved in 4 mL dimethylsulfoxide (DMSO) to obtain a final concentration of 250 mg/mL. Serial dilutions were performed, the extracts were tested at six concentrations that varied from 250 to 7.8 mg/mL [13].

3.2. Microorganisms and Identification

Clinical isolates of pathogenic yeast were obtained from 50 patients who visited in the pathological laboratory of Dr. Vahabi and Gharazi hospital in Isfahan for examinations of vaginal secretion. Randomly samples were selected on the basis of similar clinical. Cervical and vaginal specimens for the culture were collected with the aid of a disposable vaginal speculum (Vagispec, Brazil) and immediately spread on sabouraud dextrose agar medium «SDA» (Shaurlo, Spania) supplemented with 50 mg/mL of chloramphenicol (Sigma, USA). The dishes were incubated at 37°C for 48 - 72 hours, then a pool of the grown colonies were subcultured in CHROM agar *Candida* (CHROMagar Candida, France) to investigate the purity of the culture and the color of the colonies [14]. From this selective and differential medium the yeasts were identified according to biochemical tests and standard procedures including morphology, germ tube test and corn meal agar test [5].

3.3. Preparation of Inoculum

The fungal suspensions were prepared as per 0.5 McFarland turbidity standards. A 24 hours old culture was used for the preparation of fungal suspension. To prepare the inocula, yeast cell suspensions were adjusted such that it contained approximately 1.5×10^6 CFU/mL [15]. standard Mcfarland 0.5 containing barium chloride and sulphoric acid was used as turbidity control [16].

3.4. Antifungal Susceptibility Testing

The antifungal activities of extracts against *Candida* spp. isolated from patient with vulvovaginal candidiasis were determined using agar diffusion and micro-dilution methods. So Clinical and laboratories standards institute (CLSI) were used for measuring in vitro susceptibility of microorganisms to antimicrobial agents used in clinical settings [17].

3.5. Agar Diffusion Assay:

Twenty microliter of each test yeasts regularly was spread using a sterile glass spreader onto Sabouraud dextrose agar plates. The plates have been kept to dry and a sterile borer (6.4 mm in diameter) then used to punch holes in the agar medium. Subsequently, holes were filled with 150 μL of the plant extract at concentration of 7.8 - 250 mg/mL and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 25°C for 48 hours. The experiments were triplicate and the mean of the inhibition zone of each tested fungi was measured. Diameters less than 5 mm indicated no effect [10]. Sterile DMSO used as negative control. Fluconazole was used in the assay as positive control.

3.6. Microdilution Assay

The minimum inhibitory concentration (MIC) determination was carried out according to the national committee for clinical laboratory standards institute (M27-A) guidelines [18]. Extracts were dissolved in 100 μL sabouraud dextrose broth «SDB» (Shaurlo, Spania). The extracts were tested at six concentrations into sabouraud

dextrose broth that varied from 7.8 to 250 mg/mL. Microdilution method performed in sterile 96-well microplates. From each dilution, 100 μL volumes were distributed in microplates. All the wells were then filled with 10 μL of stock yeast culture. The following control wells were parallel prepared: wells contain only broth; other wells contain *Candida* strain without extract, and serial dilutions of fluconazole with the fungi at the recommended inhibitory concentrations. Absorbance was read at 630 nm, then wells were covered with sterile parafilm, and incubated at 35°C for 24 - 48 hours. Absorbance was reread at the same wave length [13, 19]. All tests were performed in triplicate. MICs are defined as the lowest drug concentration of the extract at which there was no visible growth of organisms [20].

3.7. Determination of the MFC (Minimum Fungicidal Concentration)

The MFC of *E. cephalotes* for the 50 strains were determined as described previously. Briefly, after the MIC for each strain was determined, 20 μL suspensions from each well of microtiter plates that prevented the visible growth of a microorganism were subcultured onto sabouraud dextrose agar plates. The MFC was defined as the lowest drug concentration at which less than three fungal colonies were observed after 48 hours of incubation at 35°C [21]. Statistical analysis was performed using SPSS-18 software.

The results at the level of $P < 0.001$ were analyzed. We observe ethics in various stages of research, particularly clinical research.

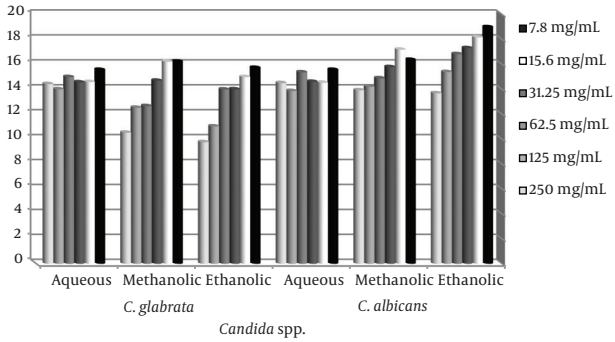

Figure 1. Average Inhibition Zone of Ethanolic, Methanolic and Aqueous Extracts Against *C. albicans* and *C. glabrata*

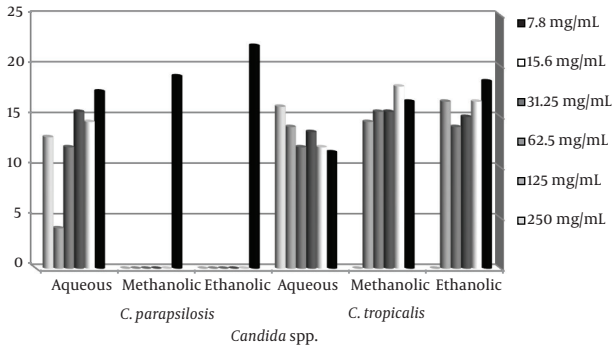

Figure 2. Average Inhibition Zone of Ethanolic, methanolic and Aqueous Extracts Against *C. parapsilosis* and *C. tropicalis*

4. Results

Candida spp. isolated from 50 patients with vulvovaginal candidiasis was C. albicans (50%), *C. glabrata* (46%), *C.parapsilosis* (2%), and *C.tropicalis* (2%). The antifungal activity was determined by measuring the diameter of zone of inhibition recorded [22]. The results are as shown in Figures 1 and 2.

The stem and leaves extracts of *E. cephalotes* revealed strong activity with a 10 - 36 mm zone of inhibition against *Candida* spp. Alcoholic extracts of the plant were found to have maximum antifungal activity in comparison to aqueous extract. Ethanolic extract of *E. cephalotes* showed similar antifungal activity against *C. albicans* showing zone of inhibition viz. 35.5 mm (Table 1). Methanolic extract of *E. cephalotes* possessed potent antifungal activity among all the extracts against *C. albicans* showing zone of inhibition viz. 36 mm (Table 2). Aqueous extracts of *E. cephalotes* showed antifungal activity against *C. albicans* with diameter of inhibition zone of grow viz. 30.5 mm (Table 3). While ethanolic and aqueous extract showed stronger antifungal activity comparison to methanolic extract against *C. glabrata*. The results of the inhibition zone of grow the ethanolic, methanolic and aqueous of *E. cephalotes* extracts against *C. glabrata* are shown in tables 4, 5 and 6. In *C. tropicalis* isolate inhibition zone of grow the ethanolic and methanolic extracts in the range of 14 - 18 mm and aqueous extract in the range 11 - 16 mm respectively, as a result antifungal activity of ethanolic and methanolic extracts compared to aqueous extract is better. For *C .parapsilosis* in high concentrations of ethanolic and methanolic extracts, inhibition zone of grow is not show but in the concentration of 7.8 mg/mL zone of inhibition equivalent 22 mm was created, and inhibition zone of the aqueous extract in different concentrations of 12 - 17.5 mm was variable.

Thus in *C. parapsilosis* isolate the antifungal activity that of aqueous extract than the ethanolic and methanolic extracts is better. Ethanolic, methanolic and aqueous extracts of trehala manna not show any antifungal activity. In this study, fluconazole, as a positive control showed an-

Table 1. Antifungal Activity of Ethanolic Extract of *E. cephalotes* Against *C. albicans*

Con, mg/mL	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Ca_1	13.5	14.5	15	15.5	16.5	19	15.6	31.2
Ca_2	19	19.5	17	13.5	14	14	15.6	31.2
Ca_3	11.5	13	14	14	14	14	15.6	31.2
Ca_4	29	14	13	25	24	20.5	31.2	62.5
Ca_5	32.5	17.5	20.5	18.5	16	0	31.2	62.5
Ca_6	13.5	21	14	15.5	13	11.5	7.8	15.6
Ca_7	25	22.5	21.5	20.5	18.5	18	7.8	15.6
Ca_8	16	17.5	15	14.5	12.5	12	15.6	31.2
Ca_9	19.5	16	14.5	11.5	11	11	15.6	31.2
Ca_{10}	13.5	12.5	5	0	0	0	15.6	31.2
Ca_{11}	34.5	26.5	22	17.5	15.5	15	15.6	31.2
Ca_{12}	21	19.5	18	15	15	15.5	15.6	31.2
Ca_{13}	0	0	0	13.5	14	15	15.6	31.2
Ca_{14}	26	23	22	19	17	16.5	15.6	31.2
Ca_{15}	0	0	0	11.5	11.5	15.5	15.6	31.2
Ca_{16}	25.5	30.5	28.5	26	25.5	26.5	15.6	31.2
Ca_{17}	21	26	25.5	21	16.5	15	15.6	31.2
Ca_{18}	20.5	19.5	18.5	15.5	11.5	10.5	15.6	31.2
Ca_{19}	36	36	35.5	28.5	27	26.5	15.6	31.2
Ca_{20}	25.5	21	20	17.5	15.5	0	15.6	31.2
Ca_{21}	14.5	16.5	15.5	17.5	13	13.5	15.6	31.2
Ca_{22}	14.5	22.5	29.5	24	16	13.5	31.2	62.5
Ca_{23}	12.5	12	16	16.5	18.5	15	15.6	1.2
Ca_{24}	15.5	15.5	15	14	14.5	11.5	15.6	31.2
Ca_{25}	14.5	17.5	17	15	14	12.5	15.6	31.2
Ave	18.9	18.1	17.3	16.8	15.3	13.6	16.8	32.5

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.

tifungal activity with inhibition zone of 0 till 75 mm. Many of *Candida* species showed similar sensitivity to fluconazole and *E. cephalotes*. The MIC of the plant extracts varying between 7.8 - 250 mg/mL were tested.

Our results revealed a strong antifungal activity of methanolic extracts of *E. cephalotes* with MIC values varying from < 7.8 to 7.8 mg/mL and moderate antifungal activity of ethanolic extract with MIC values 15.6 mg/mL and less activity of aqueous extract with MIC values 31.2 mg/mL. *C. albicans* and *C.glabrata* were the most sensitive strains to the tested extracts. The lowest MICs were obtained with methanolic and aqueous extracts for *C. albicans* (< 7.8 mg/mL) and the highest MICs were obtained for aqueous extracts on *C. tropicalis* (31.2 mg/mL).

5. Discussion

According this research, *E. cephalotes* extract had strong antifungal activity and the development of new therapeutic agents, as they inhibited the growth of *Candida*. Subsequently determination of *E. cephalotes* fractionations is nessessary to identify antifungal compounds. As a result the plant *E. cephalotes* can play an important role in the treatment of infectious diseases, particularly vulvovaginal candidiasis. Some researchers were reported

Table 2. Antifungal Activity of Methanolic Extract of *E. cephalotes* Against *C. albicans*

Con, mg/mL	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Ca_1	24	12	13	14	15	17.5	7.8	15.6
Ca_2	16.5	18	15.5	13	13	13.5	7.8	15.6
Ca_3	26	11	12	12.5	12.5	12.5	7.8	15.6
Ca_4	12.5	17	17.5	15.5	24.5	20.5	7.8	15.6
Ca_5	14.5	17.5	15.5	0	0	0	7.8	15.6
Ca_6	19	19	22	18.5	18	15	>7/8	7.8
Ca_7	13	24.5	22.5	20.5	20	20	7.8	15.6
Ca_8	24	20	18.5	16.5	14.5	13	7.8	15.6
Ca_9	11.5	12.5	13	18	17.5	18	7.8	15.6
Ca_{10}	19	14	0	0	0	0	7.8	15.6
Ca_{11}	24	20	20.5	16	5	14	7.8	15.6
Ca_{12}	12	18	15	18	16	16.5	7.8	15.6
Ca_{13}	11.5	0	0	15.5	16.5	8.5	7.8	15.6
Ca_{14}	14	23	22.5	19.5	15.5	20	7.8	15.6
Ca_{15}	15	0	0	16	14	13	15.6	31.2
Ca_{16}	16.5	30.5	30.5	25.5	25	26.5	31.2	62.5
Ca_{17}	15	27.5	24	19.5	19	20.5	7.8	15.6
Ca_{18}	12.5	16	16	15	12	12.5	7.8	15.6
Ca_{19}	14.5	36	35.5	22.5	26	26	7.8	15.6
Ca_{20}	12.5	6.5	0	0	0	0	15.6	31
Ca_{21}	18.5	21.5	18.5	19.5	19	19.5	7.8	15.6
Ca_{22}	12	18.5	18	13	13	14	15.6	31.2
Ca_{23}	22.5	14	12.5	15.5	15	14.5	15.6	31.2
Ca_{24}	11.5	16	16.5	15.5	11	0	15.6	31.2
Ca_{25}	17	16.5	15.5	12.5	13	12.5	7.8	15.6
Ave	16.3	17.1	15.7	14.8	14.2	13.9	10.4	20.2

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.

that the genus of Echinops are consist of flavonoids, alkaloids, saponins, phytosterols, polyphenols, carotenoids, sesquiterpene lactones/alcohols, lignans, acetylenic and thiophene compounds and essential oils. Flavonoids have two main roles in plants, in leaves, protecting it from fungal pathogens and UV-B radiation [23]. Although the findings of this research can be due to the existence of compounds contained in the plant *E. cephalotes*, including cellulosic materials, starch and sugar trehalose. *E. cephalotes* plant with there sugar compounds include trehalose from ancient times until now, attention [24]. Based on obtained results in current study, it seemed that *E. cephalotes* have useful medical components that identification it, is valuable. Darwish and Aburjai observed effectiveness of methanol extract of *E. polyceras* against *C. albicans* with an MIC value of 6.3 mg/mL [13]. Abd-Ellatif et al. found positive results for methanol extract of *E. spinosissimus* with zone of inhibition 13 mm against *Candida* species [25]. *Machocho* showed strong antifungal activity of crude extract of *E. hispidus* and the other effective compounds were polyacetylene thiophenes which gave an average inhibition of 23 mm [26]. Fokialakis et al. studied on antifungal activity of thiophenes from the dichloromethane extract of the radix of *E. ritro* that according to the result compounds 5-(3-buten-1-ynyl)-2, 2-bithiophene; R-terthienyl and 2-[pent-1, 3-diynyl]-5-[4-hydroxybut-1-ynyl] thiophene had significant

Table 3. Antifungal Activity of Aqueous Extract *E. cephalotes* Against *C. albicans*

Con(mg/mL)	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Ca_1	15.5	14.5	17	16	4.5	15.5	15.6	31.2
Ca_2	19	16	19.5	17.5	14.5	12	7.8	15.6
Ca_3	21	21.5	24.5	26	21.5	25.5	7.8	15.6
Ca_4	18.5	19.5	19	21	19.5	18.5	15.6	31.2
Ca_5	22.5	22.5	19.5	19.5	21.5	22	7.8	15.6
Ca_6	16.5	19	21.5	19.5	15.5	15	<7.8	7.8
Ca_7	21	22.5	20	20.5	17.5	15.5	7.8	15.6
Ca_8	13.5	13.5	14.5	20.5	20.5	21	31.2	62.5
Ca_9	22	15	18	16.5	14.5	20.5	7.8	15.6
Ca_{10}	20	16.5	15.5	0	0	0	7.8	15.6
Ca_{11}	25.5	23.5	21.5	18	19.5	21	7.8	15.6
Ca_{12}	24	21.5	25.5	21.5	20	20	15.6	31.2
Ca_{13}	13	12.5	12.5	17.5	0	0	15.6	31.2
Ca_{14}	31	30.5	30.5	26	25.5	24	15.6	31.2
Ca_{15}	17	17.5	15.5	21.5	16.5	12	15.6	31.2
Ca_{16}	27.5	30.5	21	21.5	21.5	25.5	15.6	31.2
Ca_{17}	15.5	18	19.5	26.5	21	20.5	15.6	31.2
Ca_{18}	15.5	15.5	15	11.5	20.5	23	15.6	31.2
Ca_{19}	14.5	15.5	15.5	14.5	14.5	16	31.2	62.5
Ca_{20}	17	17	16.5	7.5	18	13	31.2	62.5
Ca_{21}	14.5	16.5	14.5	15.5	14.5	12	31.2	62.5
Ca_{22}	19.5	14.5	19	0	0	0	31.2	62.5
Ca_{23}	0	5	3.5	17	11	14.5	31.2	62.5
Ca_{24}	15.5	14.5	14.5	11.5	16	16	31.2	62.5
Ca_{25}	10.5	12.5	11.5	10	9.5	0	31.2	62.5
Ave	18	17.8	17.8	16.6	15.1	15.3	18.5	35.9

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.

activity against *Colletotrichum* species and *Fusarium* [27]. Muithya and Machocho reported that copmopund of polyacetylene thiophenes; 2-Octyl-5-(3, 4-dihydroxybut-1-enyl) thiophene of *E. hispidus* showed no antifungal activity but compounds of [4-[5-(penta-1, 3-dieynyl) thien-2-yl] but-3-ynyl alcohol and 2-(penta-1,3-diynyl)-5-(3, 4 dihydroxybut-1-ynyl) thiophene of *E. hispidus* in the family of Asteraceae exhibited very strong antifungal activities against *C. neoformance* [28]. Hymete et al. reported the flower extract of *E. ellenbeckii* showed inhibitory effect against *C. albicans* and leaf and stem extracts of *E. longisetus* inhibited the growth of *S. aureus*. The *aureus* (pl. *aurei*) was a gold coin of ancient Rome valued at 25 silver denarii. The *aureus* was regularly issued from the 1st century BC to the beginning of the 4th century AD, when it was replaced by the solidus in a dose dependent manner [29]. Toroglu et al. reported the ethyl acetat extracts of leaves of *E. vicosus* showed antifungal activity against *M. pusilus* with 11 mm inhibition zone and the ethanol extract of flower of *E. microcephalus* presented antifungal activity against *M. pusilus* with 7 mm inhibition zone [23]. Data obtained from other studies demonstrated positive results for these plants as well. Although these result is not exactly accordance with ours. There may be many factors such as the different species of *Echinops*. Fluconazole, which is commonly used for treatment of various fungal infections, considered as a fungistatic agent

Table 4. Antifungal Activity of Ethanolic Extract of *E. cephalotes* against *C. glabrata*

Con, mg/mL	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Cg_1	24.5	21.5	21	23.5	19	21	15.6	31.2
Cg_2	25.5	20.5	18.5	18	16.5	16	15.6	31.2
Cg_3	10	9.5	0	0	0	0	15.6	31.2
Cg_4	13	13	14	15	16	16	15.6	31.2
Cg_5	13.5	10.5	0	0	0	0	7.8	15.6
Cg_6	24.5	21.5	22	21	10	16	7.8	15.6
Cg_7	14	11.5	12	11	10	0	31.2	62.5
Cg_8	24	21.5	21.5	20	13.5	12.5	15.6	31.2
Cg_9	23.5	22	19	15.5	11	12.5	31.2	62.5
Cg_{10}	14	15	15.5	17.5	15.5	17.5	15.6	31.2
Cg_{11}	5.5	10	15.5	16.5	0	0	15.6	31.2
Cg_{12}	14	14.5	13.5	12	12.5	10.5	7.8	15.6
Cg_{13}	13.5	10.5	10	0	0	0	15.6	31.2
Cg_{14}	17.5	17.5	13.5	13	10	0	15.6	31.2
Cg_{15}	14.5	15.5	14	15.1	18.5	16	15.6	31.2
Cg_{16}	13	13.5	10	12	0	0	15.6	31.2
Cg_{17}	12	12.5	15.5	16	14.5	13	15.6	31.2
Cg_{18}	17.5	16.5	17.5	18	14.5	13	15.6	31.2
Cg_{19}	12.5	12.5	13.5	14.5	11.5	10	15.6	31.2
Cg_{20}	21	22	21	19	19	24.5	7.8	15.6
Cg_{21}	11	10.5	11.5	14	13	13.5	15.6	31.2
Cg_{22}	12.5	12	10.5	14	13	12.5	7.8	15.6
Cg_{23}	10.5	10.5	12.5	16	15.5	0	15.6	31.2
Ave	15.7	14.9	14	13.9	11.0	9.76	15.2	30.5

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.

and there are reports of emerging resistance among clinical isolates of *C. albicans* [30]. In this context, natural products have been used historically and continue to be the focus of researches on antifungal agents. The main source is to be found in plants. Many plant extracts have biological activity both in vitro and in vivo [31]. Difference of susceptibility to fluconazole and *E. cephalotes* may be due to the indiscriminate use of fluconazol in patients with vulvovaginit candidiasis. This research is the first study on antifungal activity of the *E. cephalotes* against *Candida* spp. isolated from patients with vulvovaginal candidiasis. Hence, this plant might be a promising material for controlling *Candida* spp. and may used further as medicinal plant against *Candida* spp. Also the results from the present study have reported the scientific basis for traditional uses of the genus *Echinops* in the treatment of some illness. Since the identifying of active phytochemical compounds has conducted by researchers, more investigation should be done in vitro and in vivo for safety. After that stage, it can be produced commercially.

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Footnotes

Authors' Contribution: Sara Heshmati: responsible for writing and undertake research process, Mahboobeh Madani: supervisor professor, Leila Amjad: consultant professor.

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Table 5. Antifungal Activity of Methanolic Extract of *E. cephalotes* against *C. glabrata*

Con, mg/mL	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Cg_1	24	24	24	29.5	18.5	24	7.8	15.6
Cg_2	26	22.5	21	18.5	16.5	16.5	31.2	62.5
Cg_3	12.5	15.5	13.5	13	12	11	15.6	31.2
Cg_4	14.5	14.5	15.5	15.5	15.5	15.5	7.8	15.6
Cg_5	13	14.5	0	0	0	0	7.8	15.6
Cg_6	24	21	14	15.5	15.5	0	7.8	15.6
Cg_7	11.5	12	11.5	12.5	12.5	0	15.6	31.2
Cg_8	19.5	18.5	19	13	13	13	7.8	15.6
Cg_9	24	24	24	12.5	14	14.5	7.8	15.6
Cg_{10}	12.5	16	15	15.5	14	11.5	7.8	15.6
Cg_{11}	12	17	17	9.5	0	0	7.8	15.6
Cg_{12}	14	14	0	0	0	0	15.6	31.2
Cg_{13}	15	13	13	10	0	0	7.8	15.6
Cg_{14}	16.5	15.5	14.5	13	12.5	12	15.6	31.2
Cg_{15}	15	13.5	13	12	10.5	10.5	15.6	31.2
Cg_{16}	12.5	13	12.5	0	0	0	7.8	15.6
Cg_{17}	13	14	15.5	14	13.5	13.5	15.6	31.2
Cg_{18}	19	18	18.5	12.5	11	10.5	15.6	31.2
Cg_{19}	12	12	14.5	12.5	12.5	10.5	15.6	31.2
Cg_{20}	22.5	20.5	20.5	16	6.5	13	15.6	31.2
Cg_{21}	12	11	11	15	15	14	7.8	15.6
Cg_{22}	17	16	16	15	14	11	7.8	15.6
Cg_{23}	11.5	13	14.5	16	15	0	15.6	31.2
Ave	16.2	16.2	14.6	12.6	10.5	8.73	12.2	24.4

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.

Table 6. Antifungal Activity of Aqoues Extract of *E. cephalotes* against *C. glabrata*

Con, mg/mL	7.8	15.6	31.25	62.5	125	250	MIC	MFC
				Inhibition Zone, mm				
Cg_1	20.5	20.5	21	20	19.5	21	< 7.8	7.8
Cg_2	26	21.5	21	20	15.5	20.5	15.6	31.2
Cg_3	11	13	13.5	16.5	14.5	15.5	15.6	31.2
Cg_4	17.5	17.5	16.5	14	16	21	15.6	31.2
Cg_5	16	19.5	20	15.5	4	12.5	15.6	31.2
Cg_6	21	18.5	15	4.5	13	19	15.6	31.2
Cg_7	18	15.5	15	17	18.5	15	7.8	15.6
Cg_8	14.5	14.5	15	13	12	10.5	15.6	31.2
Cg_9	19.5	20.5	27	23	16.5	21	15.6	31.2
Cg_{10}	22.5	19	17	17	17	13	15.6	31.2
Cg_{11}	12.5	12	10.5	13	12.5	4	31.2	62.5
Cg_{12}	16	16	12.5	10.5	3.5	11	31.2	62.5
Cg_{13}	18	18	15.5	16	17.5	15.5	31.2	62.5
Cg_{14}	18	11	11	15.5	14.5	15	31.2	62.5
Cg_{15}	14.5	17	7.5	19	21	16	31.2	62.5
Cg_{16}	10.5	12	6	13	15	16	31.2	62.5
Cg_{17}	15.5	16.5	17	15.5	16	15.5	31.2	62.5
Cg_{18}	0	0	0	15	15.5	15	31.2	62.5
Cg_{19}	19	16	18	15	15	4.5	15.6	31.2
Cg_{20}	16.5	16.5	14.5	14	15	20	15.6	31.2
Cg_{21}	21.5	16.5	16	10	12.5	12	15.6	31.2
Cg_{22}	11.5	6.5	12	19	14	13.5	7.8	15.6
Cg_{23}	0	0	13.5	14.5	13.5	4.5	15.6	31.2
Ave	18	17.8	17.8	16.6	15.1	15.3	20.5	39.7

Abbreviation: Ave, average; Con, concentration; Ca_{1-25}, *Candida albicans* spp; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concenterration.