Evaluation of the antivirulence activity of ethyl acetate extract of *Deverra tortuosa* (Desf) against *Candida albicans*

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Objective
This study aimed to assess the efficiency of ethyl acetate (EtOAc) extract of *Deverra tortuosa* (Desf) against *Candida albicans*.
Materials and methods
The antifungal activity of the EtOAc extract of *D. tortuosa* was evaluated using the paper disk diffusion method. The minimum inhibitory concentrations were assessed using broth dilution methods. The antivirulence efficiency of the EtOAc extract was assessed through the evaluation of antibiofilm using the broth dilution method, reduction in fungal dimorphism using spider and GLcNAc media, and assessment of phospholipase activity using egg yolk emulsion medium. The time-kill assay of the EtOAc extract was assessed. Cytotoxicity evaluation of the EtOAc extract against normal cell line MRC-5 using MTT assay was done. The compounds in extract were analyzed using gas chromatography-mass spectrometry.
Results and conclusion
The inhibition zone of the EtOAc extract of *D. tortuosa* was 26 mm and minimum inhibitory concentrations was 12.5 mg/ml against *C. albicans*. The antibiofilm activity of the EtOAc extract showed inhibition of up to 52.2% at a concentration of 25 mg/ml. The EtOAc extract showed a complete reduction in fungal dimorphism and transition between yeast cell and hyphae at concentration of 25 mg/ml. The time-kill assay showed inhibition activity at different concentrations in a dose-dependent manner with –2.6 $\log^{10}$ CFU after 24 h at 25 mg/ml. Our results support the *in vitro* potential of *D. tortuosa* extract as anti-*C. albicans*. Gas chromatography-mass spectrometry indicated that 23 peaks were observed. Five (74.53%) antimicrobial compounds were present in considerable amounts, including 9-octadecenoic acid (Z)-, methyl ester/hexadecanoic acid, methyl ester/9,12-octadecadienoic acid (Z, Z)-, methyl ester/octadecanoic acid, methyl ester/phenol,2,4-bis (1,1-dimethyl ethyl)-, and another 18 compounds comprised 25.47%. Cytotoxic activity of EtOAc against MRC-5 cells showed little toxicity, with IC_{50} exceeding 249 μg/ml after 24 h of incubation.

Keywords:
anti-*Candida albicans*, antivirulence, cytotoxicity, *Deverra tortuosa* (Desf), natural product

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Introduction

Egypt is one of the main countries for the diversity of the genus *D. tortuosa* (Desf) DC, It is a perennial bushy desert medicinal and aromatic plant that belongs to the family Apiaceae. Anti-*Candida albicans* activity of *D. tortuosa* (Desf) extract in Egypt has poor review and studies. Therefore, this study aimed to evaluate the anti-*Candida* activity, antivirulence factor, cytotoxic potential, and detection of chemical compounds found in this plant extract.

C. albicans remains the most frequently isolated fungus from hospitalized patients [1] However, an increase in the proportion of infections caused by non-*C. albicans* has been observed recently [2]. Despite advances in antifungal therapy, *Candida* infections continue to have a major effect on mortality and morbidity, as well as on the duration and cost of hospitalization [3].

However, biofilm-producing *Candida* species are known to be more resistant to immune response and antimicrobial agents, which lead to treatment failure [4]. *C. albicans* colonizes host tissues and various indwelling medical devices [5] and readily develops biofilms on biotic and abiotic surfaces that are intrinsically resistant to conventional antifungal therapeutics and the host immune system. *C. albicans* can grow as oval budding yeasts, pseudohyphae, or true hyphae. For biofilm development, yeast cells initially attach to a surface, and this is then followed by germ tube formation and hyphal transition, and mature biofilms are typically

formed within 24 h. Biofilm formation greatly contributes to the pathogenicity of candidiasis [6].

Virulence factors, as well as the attenuated defense mechanism of the host, play a critical role in the development of these infections. Extracellular hydrolytic enzymes of *Candida* species facilitate adherence and tissue penetration, and therefore an invasion of the host [7].

Although there is a growing number of studies reporting virulence factors of other strains, *C. albicans* is the most commonly analyzed strain in which several virulence factors, including phenotypic changes, morphological dimorphism, adhesion molecules, hydrolytic enzymes (e.g. phospholipase, lipase, and aspartic proteinases), catalase, superoxide dismutase, and heat shock proteins, have been identified [8].

Plants contain a variety of important secondary metabolites such as tannins, alkaloids, and flavonoids, which possess *in vitro* antimicrobial properties [9]. Phytotherapy manuals have recorded various medicinal plants used for infectious disease treatment because of their availability, fewer adverse effects, and reduced toxicity [10].

A variety of herbal plants have been extensively used for the extraction of natural pure compounds used in the development of new and safe drugs. One of the most important therapeutic priorities in handling pain involves pain control; Apiaceae (Umbelliferae) is one of the important families in the flora of Egypt. The genus *Deverra* has two species (*D. tortuosa* and *Deverra triradiata*); these species are widespread in Wadis, especially the tributaries of Wadi El Natron [11]. *D. tortuosa* is a perennial bush belonging to the family Apiaceae; it grows naturally in sandy and stony plains [12]. The *Deverra* sp. is known in traditional local medicine for the treatment of hypertension, against constipation, and in the case of bites. The tender shoots and leaves are used as a condiment among the Bedouin local population. *Pituranthos tortuosus* (Desf.), recently known as *D. tortuosa* (Desf.). DC, is known in Arabic as Shabat El-Gabal and is used in folk medicine as diuretic, carminative, and analgesic. It is also used to relieve stomach pain and against intestinal parasites [13].

This study aimed at the investigation of *D. tortuosa* (Desf) extract activity against *C. albicans* and defining the minimum inhibitory concentrations (MIC), and also at studying the antivirulence activity of *D. tortuosa* (Desf) extract at different concentrations through antibiofilm formation assay, yeast-to-hyphal morphological transition technique, and phospholipase assay. Gas chromatography/mass spectrometric (GC-MS) techniques coupled with multi-variant analysis are widely employed to identify the chemical structures of most active compounds.

Materials and methods

Candida albicans strain

The *C. albicans* strain used in this study was a clinical isolate obtained kindly from Professor Dr Marwa Elsewaify, in the microbiology lab, at the National Cancer Institute (NCI), Giza, Egypt.

Collection of plant material

D. tortuosa (Desf) was collected from Degla valley protectorate, Elmaadi, Cairo, Egypt (N 29° 56' 16.5559" E 32°10'52.5384"] during March 2019 (Fig. 1) and identified by Prof. Dr Abdo Marei Hamed 'Professor of Plant Ecology, Botany and Microbiology Department, Faculty of Science, Al-Azhar University.'

Preparation of crude extract from *Deverra tortuosa* (Desf)

The aerial part of the plant was collected, washed, dried out of sun reach, and crushed into powder form. Overall, 10 g of plant powder was placed in a 500-ml conical flask containing 100 ml of the following solvent hexane, ethyl acetate (EtOAc), methanol, and water and tightly sealed and incubated at room temperature for 72 h under shaking (120 rpm at 37°C) (New Brunswick Scientific, Edison, New

Figure 1

Deverra tortuosa (Desf) plant.

Jersey, USA). After that, all mixtures were filtered through Whatman No. 1 filter paper and evaporated by using a rotary evaporator until crude extract of each solvent was obtained (all solvents were purchased from Sigma-Aldrich Co., 3050 Spruce Street Saint Louis, MO, United States).

Antibiotic sensitivity profile of *Candida albicans*

The *C. albicans* was subjected to the evaluation of antibiotic profile according to the Clinical and Laboratory Standards Institute guidelines [14]. Antibiotic panels used were itraconazole 50 µg, amphotericin B 20 µg, flucytosine 1 µg, miconazole 10 µg, and fluconazole 25 µg. The experiments were conducted in triplicate assays.

Antifungal activity of the crude extract against *Candida albicans* using disk diffusion assay

Stock culture of *C. albicans* was prepared at a concentration of 1×10^6 CFU/ml grown in Potato Dextrose Broth and 18-h age culture. One milliliter of the stock culture was added to the surface of Mueller–Hinton agar plates (MHA, Oxoid) and distributed evenly by a sterile cotton swab. The crude extracts were prepared at concentration of 100 mg/ml and loaded to filter paper discs 6 mm and left to dry for 1 h. The loaded discs were tested for its activity, and the inhibition zone diameter was measured in millimeter. The experiment was carried out three times [15].

Determination of the minimum inhibitory concentration of ethyl acetate extract

The MIC of EtOAc extract was performed according to El-Sherbiny *et al.* [16]. In brief, a cell suspension was prepared of *C. albicans* 1×10^6 CFU/ml in a morphine propane sulfonic acid-buffered RPMI 1640 medium (Sigma) supplemented with 0.2% (w/v) glucose. One hundred microliters aliquots of these cell suspensions were dispensed into 96-well microtiter plates. Tested EtOAc extract was tested in a twofold serial dilution. The extract was added to RPMI 1640 medium into wells at a final concentration ranging from 100 mg/ml–78 µg/ml. Wells containing negative control (medium+EtOAc extract at the tested concentrations) were performed to determine the differences in optical density (OD). The plate was incubated for 24 h at 37°C, and the absorbance was measured at 620 nm using a microplate reader (Biorad mod 680). MIC was defined as the lowest concentration of the EtOAc extract able to inhibit the visible growth of *C. albicans*.

Time-kill assay of most promising ethyl acetate extract obtained from *Deverra tortuosa* (Desf)

Overnight culture of *C. albicans* cells grown in yeast extract potato dextrose (YPD) medium was resuspended in RPMI 1640 medium at a concentration of 1×10^6 cells/ml. Cell suspensions were challenged with different concentrations of EtOAc extract at 37°C. At indicated time points, an aliquot from each treatment was taken and plated on YPD agars after serial dilution. After incubation for 24 h, numbers of CFU on YPD agars were counted. This assay was conducted in triplicate and repeated three times [17].

Effect of ethyl acetate extract from *Deverra tortuosa* (Desf) on yeast-to-hyphae transition morphology

Spider medium (nutrient broth 1%, mannitol 1%, K_2HPO_4, pH7.2) and N-acetylglucosamine (GlcNAc) medium (GlcNAc 0.4%, HK_2PO_4 0.3%, peptone 0.5%) were used to explore the effects of EtOAc extract on the yeast-to-hyphal morphological transition. The cell suspension of *C. albicans* at a concentration of 1×10^6 cells/ml in each medium was transferred into 96-well plates and then treated with the extract in DMSO at different concentrations (25, 12.5, and 6.25 m g/ml). After incubation at 37°C for 4 h, the morphologies of cells exposed to different concentrations of extract were examined under an inverted microscope [18].

Evaluation of the antibiofilm activity of ethyl acetate extract against *Candida albicans*

Biofilm formation of treated *C. albicans* with EtOAc extract in comparison with untreated cells was carried out by quantitative determination using a microplate method. The flat-bottomed 96 tissue culture plates were filled with 0.2 ml of 1×10^6 CFU/ml of *C. albicans* suspension in RPMI 1640 medium supplemented with 2% (w/v) glucose and adjusted to 1×10^6 CFU/ml. After 24 h incubation in aerobic condition at 37°C, the contents were aspirated and plates were washed twice with phosphate buffer saline (pH: 7.2). The plates were stained with 0.1% crystal violet for 2 min. The difference in color intensity was measured at 490 nm. The experiment was repeated three times, and the mean values of OD were then averaged. A three-grade scale was used to evaluate the strains slime-producing ability by comparing with OD of negative control or cutoff [19]. An aliquot of twofold serial dilutions of plant extract was prepared at the concentration of MIC and sub-MIC. RPMI 1640 medium containing 0.2% DMSO was employed as a negative control. RPMI 1640 medium without the extract was used as the nontreated well, and the medium with each concentration of the extracts was used as the blank control [20].

Effect of ethyl acetate extract on phospholipase activity of *Candida albicans*

Egg yolk emulsion agar was used to assess the effect of EtOAc extract on phospholipase production of *C. albicans*. The cell suspension of *C. albicans* (1.0×10^6 CFU/ml) was prepared, and 1 µl was added to the center of egg yolk emulsion agar supplemented with different concentrations of EtOAc extract (25, 12.5, and 6.25 mg/ml) and incubated at 37°C for 96 h to allow the precipitation zones (Pz) and colonies to form on agars. The Pz value was used to quantify enzymatic production. Pz=*d* colony/(*d* colony+precipitation), where d is the diameter of colony or precipitation [21].

Gas chromatography/mass spectrometry

The chemical composition of EtOAc extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, Texas, USA) with a direct capillary column TG–5MS (30 m×0.25 mm×0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/min to 230°C hold for 2 min, and then increased to the final temperature of 290°C by 30°C/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250 and 260°C, respectively. The method was based on Hashimoto [22] with some modification. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 40–1000 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Cytotoxicity assessment

Cytotoxicity of EtOAc extract was determined by MTT assay method [23] against one normal cell line MRC-5 ATCC CCL-171. The cells were used at a concentration of 1×10^5 cell/ml in Dulbecco's modified eagle medium. The cells were treated with different concentrations of EtOAc in a double-fold dilution starting from 2 mh/ml and incubated in CO_2 incubator for 72 hr at 37°C.

The percentage of cell inhibition was calculated by the following equation:.

$$\%\text{of Inhibition} = 1 - ((\text{AbsampleAbblank})/\text{AbcontrolAbblank})) \times 100$$

IC_{50} value (mean concentration required to kill 50% of the cell population) was calculated by plotting dose–response curve.

Statistical analysis

The data were expressed as the mean±SE value, which was calculated by using Minitab 18 software extended with a statistical package and Microsoft Excel 365.

Results

Anti-*Candida albicans* activity of *Deverra tortuosa* (Desf) plant extracts

The different solvent extracts assayed against *C. albicans* are shown in Table 1 and Fig. 2. The *Candida* isolate from clinical specimens was subjected to antibiotic sensitivity test, as shown in Table 2 and Fig. 3.

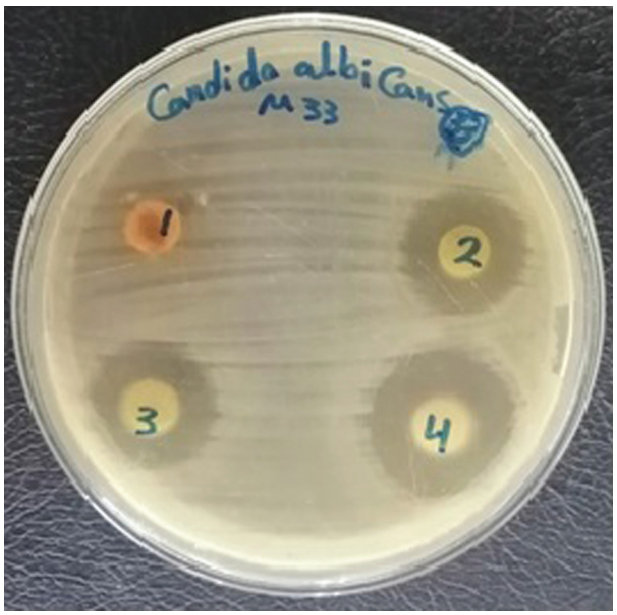
Determination of minimum inhibitory concentrations for most active extraction (ethyl acetate extract)

The MIC of EtOAc extract was determined using a standardized broth microdilution method (Clinical and Laboratory Standards Institute document M27-A2), as

Table 1 Anti-*Candida albicans* activity of *Deverra tortuosa* (Desf) plant extracts

Different extract	The diameter of the inhibition zone (mm)
Water extract	0
Methanol extract	19
Hexane extract	20
Ethyl acetate extract	26

Figure 2

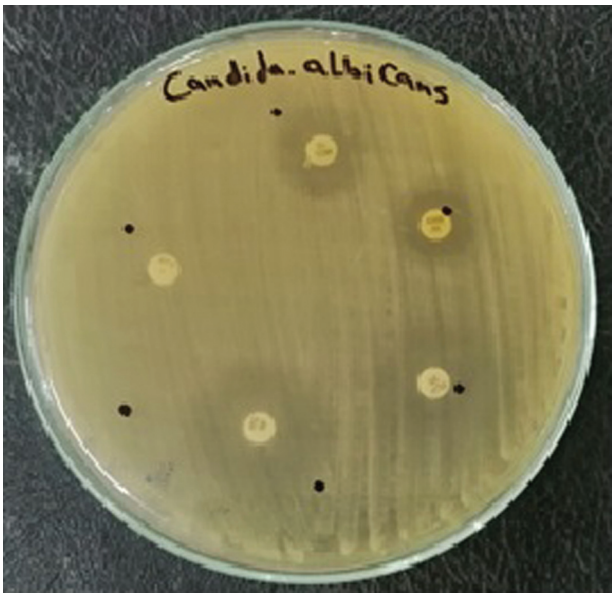


Anti-*Candida* activity of *Deverra tortuosa* (Desf) plant extracts.

Table 2 Antifungal susceptibility of *Candida albicans*

Antifungal	Abbreviation	Diameter of inhibition zone (mm)
Itraconazole	ITC	24
Amphotericin B	AMB	15
Flucytosine	AFY	0
Miconazole	MCL	18
Fluconazole	FLU	21

Figure 3



Antifungal susceptibility of *Candida albicans*.

described before. The MIC value against *C. albicans* was 12.5 mg/ml.

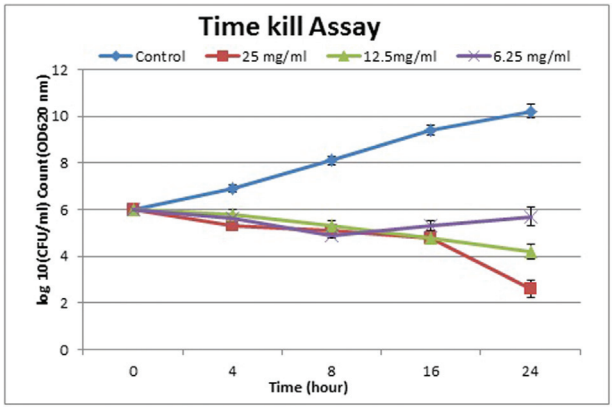
Time-kill assay

The data represented graphically in Fig. 4 refer to the inhibitory effect of different concentrations (25, 12.5, and 6.25 mg/ml) of plant extract correspondingly on the growth of *C. albicans*. The results indicated that all treated cultures were affected in a concentration-dependent manner, which means that the reduction in CFU count of *C. albicans* was increased by increasing the concentrations of plant extract in comparison with initial inoculum. Positive controls reflect the ideal growth behavior of *C. albicans* during 24 h of incubation.

Plant extract inhibits biofilm formation and development

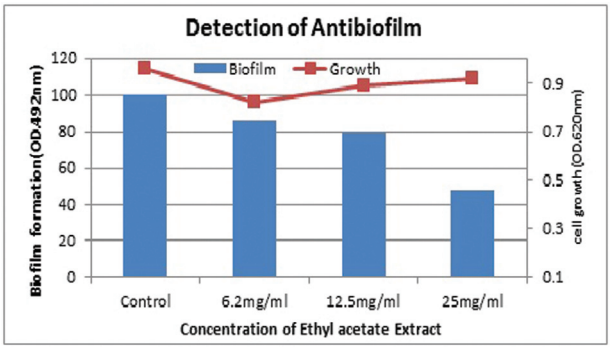
As shown in Fig. 5, EtOAc extract concentrations ranging from 6.25, 12.5, and 25 mg/ml suppressed 13.7–52.2% of biofilm formation, respectively, whereas this concentration band could only reduce the viability of cells in mature biofilms by less than 14.6%.

Figure 4



Time-kill assay of different concentrations of EtOAc extract on *Candida albicans*.

Figure 5



Antibiofilm effect of EtOAc extract on biofilm formation of *Candida albicans*.

Ethyl acetate extract inhibits yeast-to-hyphal transition of *Candida albicans*

To test the inhibitory effects of EtOAc on yeast-to-hyphal transition pattern, two types of hyphal-inducing media, namely, Spider and GlcNac medium, were used. As shown in Figs 6 and 7, EtOAc extract inhibited the morphological transition of *C. albicans*, although the extent of inhibition varied in different concentrations.

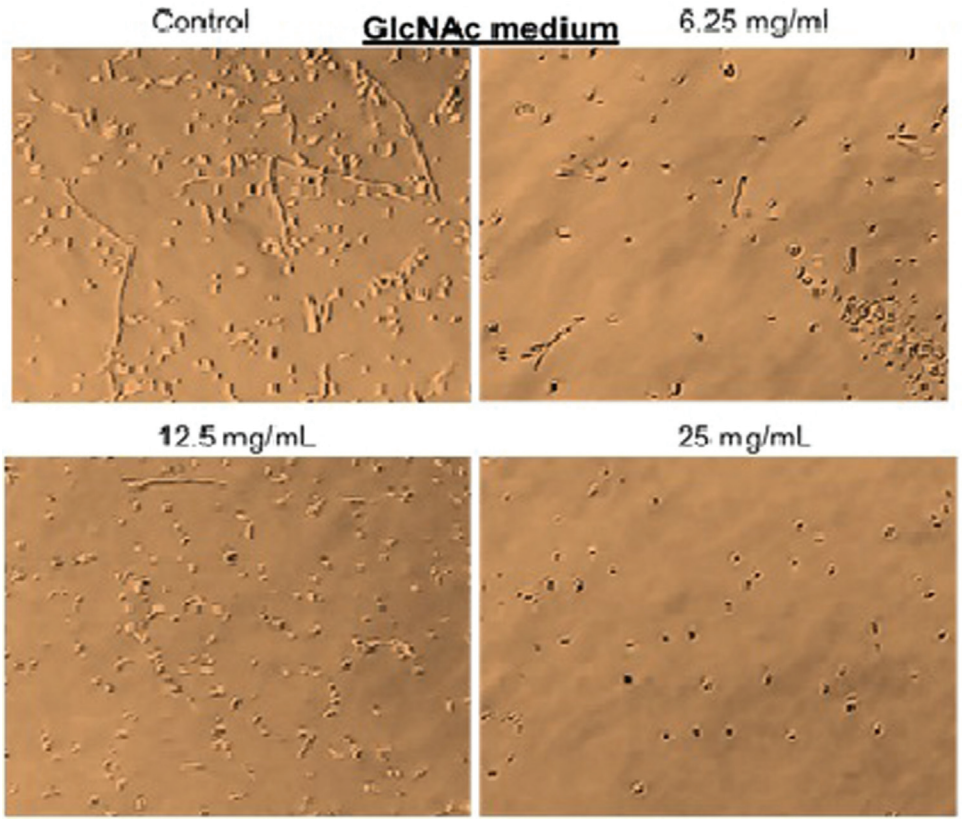
Phospholipase assay

To investigate whether EtOAc extract has an inhibitory effect on the production of *C. albicans* phospholipase, egg yolk emulsion agars were employed. In this assay, a smaller Pz value means a higher enzyme production. As shown in Fig. 8, EtOAc extract at different concentrations 25, 12.5, and 6.25 mg/ml showed no difference in the results of phospholipase production by *C. albicans*.

Cytotoxicity assay

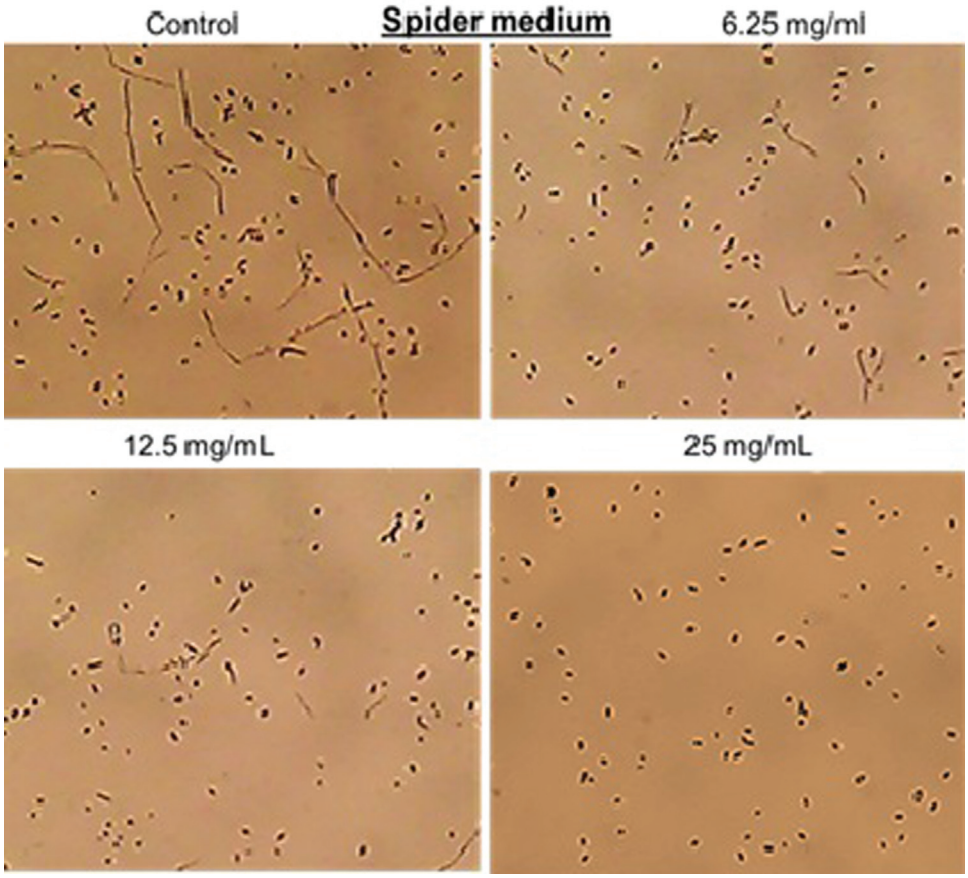
Cytotoxic activity of EtOAc extract of *D. tortuosa* against normal cell line MRC-5 ATCC showed

Figure 6



Inhibition of morphological transition in GlcNac media at different concentrations of EtOAc extract under inverted microscope.

Figure 7



Inhibition of morphological transition in spider media at different concentrations of EtOAc extract under inverted microscope.

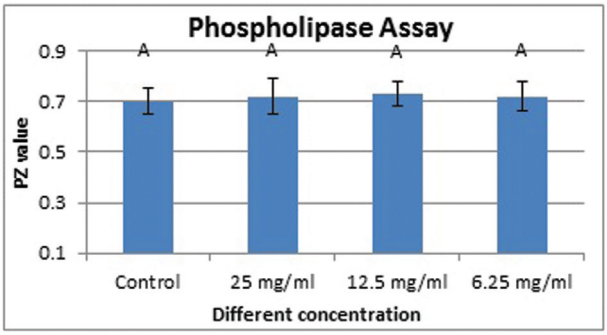
little toxicity, with IC_{50} exceeding 249 μg/ml after 24 h of incubation (Fig. 9).

Chemical compounds of ethyl acetate extract of *Deverra tortuosa* (Desf) using gas chromatography-mass spectrometry

The GC-MS analysis of EtOAc extract of *D. tortuosa* (Desf) contained a mixture of volatile compounds. A total of 23 peaks were observed (Fig. 10). Five antimicrobial compounds were present in considerable amounts (74.53%), and another 18 compounds comprised 25.47%, in range 0.62–2.84% for each compound, as described in Table 3. Chemical constituents were identified using spectrum data-based software (NIST 11.0) installed in GC-MS. The high anti-*Candida* activity of EtOAc extract from *D. tortuosa* collected might be attributed to a wide range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons.

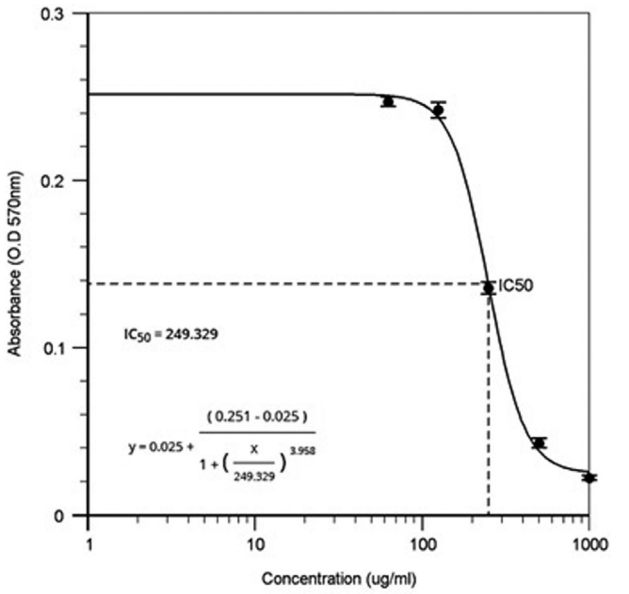
The GC-MS analysis of the EtOAc extract of *D. tortuosa* (Desf) revealed that the main chemical constituents were organic compounds, including 9-octadecenoic acid (Z)-, methyl ester (28.39%); hexadecanoic acid, methyl ester (20.12%); 9,12-octadecadienoic acid (Z, Z)-, methyl ester (16.35%); octadecanoic acid, methyl ester (4.98%); and phenol, 2,4-bis(1,1-dimethylethyl) (4.69%), as shown in Table 3.

Figure 8



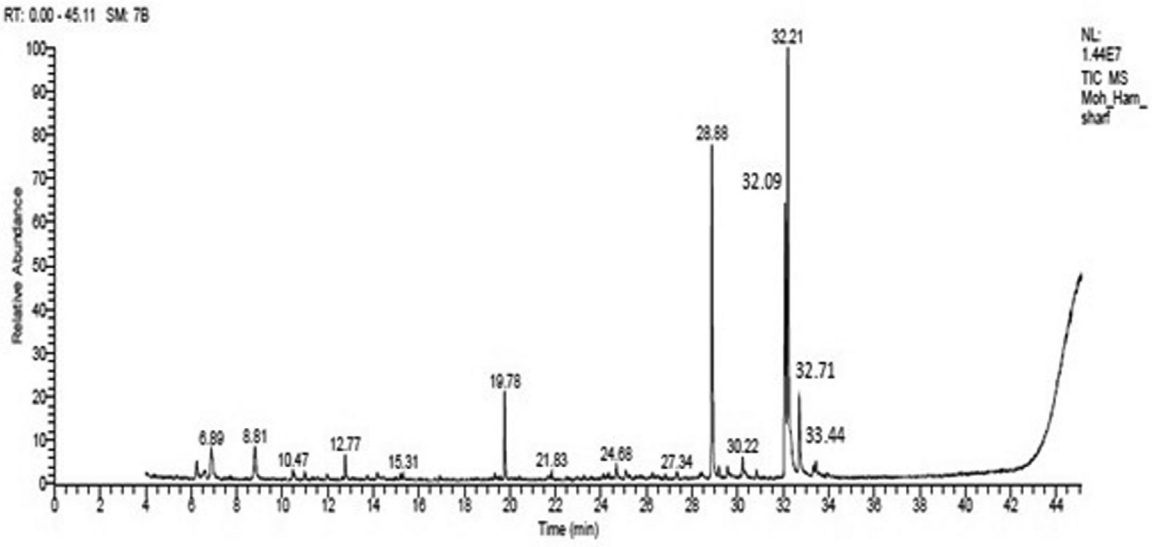
Effect of EtOAc extract on production of phospholipase secreted by *Candida albicans*. In the phospholipase assay, a larger precipitation zones value indicates less enzyme production.

Figure 9



Cytotoxicity of EtOAc extract determined by MTT assay.

Figure 10



The GC-MS analysis of the EtOAc extract of *Deverra tortuosa* (Desf). EtOAc, ethyl acetate; GC-MS, gas chromatography-mass spectrometry.

Table 3 Antimicrobial compounds, retention times, and percentages of (ethyl acetate) extract of *Deverra tortuosa* (Desf) analyzed by gas chromatography-mass spectrometry

N	*R* time	Compound name	%
1	6.25	Cyclotetrasiloxane, octamethyl-	2.33
2	6.89	Cyclohexanol,1-methyl-4-(1-methylethenyl)-, acetate	2.84
3	8.81	Undecane	2.82
4	10.47	Cyclopentasiloxane,Decamethyl-	0.92
5	11.97	Benzaldehyde, 2,5-dimethyl-	0.66
6	12.77	Carvone	1.43
7	15.31	1,2-Dimethyl-4-(Dimethoxymethyl)Benzene	1.57
8	19.78	Phenol, 2,4-bis(1,1-dimethylethyl)-	4.69
9	21.83	Octadecane, 6-methyl-	0.93
10	24.12	1-Chlorooctadecane	0.70
11	24.68	Tetradecanoic acid, methyl ester	1.68
12	26.83	Tetradecanoic acid, 12-methyl-, methyl ester	0.67
13	27.34	Quinindoline	0.85
14	28.43	9-Hexadecenoic acid, methyl ester, (Z)-	0.62
15	28.88	Hexadecanoic acid, methyl ester	20.12
16	29.19	9-Heptadecene-4,6-diyn-8-ol, (Z)-	0.63
17	29.56	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	1.25
18	30.22	Hexadecanoic acid, ethyl ester	1.82
19	32.09	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.35
20	32.21	9-Octadecenoic acid (Z)-, methyl ester	28.39
21	32.71	Octadecanoic acid, methyl ester	4.98
22	33.33	Linoleic acid ethyl ester	1.05
23	33.44	10-Octadecenoic acid, methyl ester	2.7

Discussion

From antifungal susceptibility tests, the result showed that the anti-*Candida* activity of EtOAc extract which contains the decanoic acid derivative has the highest anti-*Candida* activity with the diameter of inhibition zone 26 mm. Another paper by Wei *et al.* [24] reported that hexadecanoic acid methyl ester has antibacterial properties, which affected different pathogenic microorganisms, such as *Aeromonas hydrophila*, *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Klebsiella* sp., *Pseudomonas aeruginosa*, *Salmonella* sp., *Vibrio alginolyticus*, *Vibrio cholera, and Vibrio parahaemolyticus*, with MIC range from 31.25 to 125 mg/ml, and this result is far from our result of MIC against *C. albicans* of 12.5 mg/ml, which may be owing to the different organisms that have been experimented.Moreover, phenol, 2,4-bis (1,1-dimethylethyl)-, which is found in EtOAc extract of *D. tortuosa* (Desf), may be produced as a secondary metabolite of the plant as a response to infect with pathogenic microorganism, and this phenol, 2,4-bis(1,1-dimethylethyl)- has antifungal activity against *C. albicans*. This agrees with Zhao *et al.* [25] who reported that phenol, 2,4-bis (1,1-dimethylethyl)- exhibited a broad range of antimicrobial activity against all tested organisms in their research.

EtOAc extract of *D. tortuosa* (Desf) suppressed biofilm formation from 13.7–52.2% according to the concentration of plant extract. The main compound found in the extract is 9-octadecenoic acid (Z)-, methyl ester, and this compound reported has antibiofilm activity against *P. aeruginosa* ATCC 9027 without affecting its growth, as reported by Singh *et al.* [26]. Moreover, another compound phenol,2,4-bis(1,1-dimethylethyl)- found in the extract is similar to the report of Padmavathi *et al.* [21], which found that phenol,2,4-bis(1,1-dimethylethyl)- can modulate the secreted EPS of *Serratia marcescens*, which in turn could facilitate the disruption of biofilms, as well as favoring the diffusion of antimicrobials into the cell aggregates, resulting in the eradication of persistent biofilms.

In this current study, phospholipase assay gave nonsignificant results. Because the result of control and the treated groups are approximately equal in Pz and in the phospholipase test, a larger Pz value indicates lower enzyme production.

Zhao *et al.* [25] reported that phenol,2,4-bis(1,1-dimethylethyl)- exhibited fungicidal potential at higher concentrations. Various antibiofilm assays and morphological observations revealed that phenol,2,4-bis(1,1-dimethylethyl)- inhibited and disrupted biofilms of *C. albicans* via the possible inhibition of hyphal development, and this result is similar to our result of EtOAc extract, which inhibited the morphological transition of *C. albicans* with variation according to the concentration of plant extract.

Conclusion

Our results showed that EtOAc extract of *D. tortuosa* (Desf) has anti-*Candida* activity with inhibition zone 26 mm and MIC 12.5 mg/ml, and also the extract has antivirulence activity against biofilm formed by *C. albicans* up to 52.2%. The EtOAc extract showed a complete reduction in fungal dimorphism and transition between yeast cell and hyphae at a concentration of 25 mg/ml. The time-kill assay showed inhibition activity at different concentrations in a dose-dependent manner with −2.6 log10 CFU after 24 h at 25 mg/ml. The GC-MS analysis of the EtOAc extract of *D. tortuosa* (Desf) revealed that the main chemical constituents were organic compounds, including 9-octadecenoic acid (Z)-, methyl ester

(28.39%); hexadecanoic acid, methyl ester (20.12%); 9,12-octadecadienoic acid (Z,Z)-, methyl ester (16.35%), octadecanoic acid, methyl ester (4.98%); phenol, 2,4-bis(1,1-dimethylethyl)- (4.69%), and another 18 compounds comprised (25.47%). Cytotoxic activity of EtOAc extract against mammalian cells showed little toxicity with IC_{50} exceeding 249 µg/ml after 24 h of incubation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Lohse MB, Gulati M, Johnson AD, Nobile CJ. Development and regulation of single-and multi-species *Candida albicans* biofilms. Nat Rev Microbiol 2018; 16:19–31.

2 Gong X, Luan T, Wu X, Li G, Qiu H, Kang Y, *et al.* Invasive candidiasis in intensive care units in China: Risk factors and prognoses of *Candida albicans* and non–*albicans Candida*infections. Am J Infect Control 2016; 44:59–63.

3 Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis 2014; 2014:541340.

4 Demirbileki M, Timurkaynak F, Can F, Azap O, Arslan H. Biofilm production and antifungal susceptibility patterns of *Candida* species isolated from hospitalized patients. Mikrobiyol Bul 2007; 41:261–269.

5 Sardi JO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Giannini MM. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013; 62:10–24.

6 Ramage G, Martínez JP, López-Ribot JL. *Candida* biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res 2006; 6:979–986.

7 Galocha M, Pais P, Cavalheiro M, Pereira D, Viana R, Teixeira MC. Divergent approaches to virulence in *C. albicans* and *C. glabrata*: two sides of the same coin. Int J Mol Sci 2019; 20:2345.

8 Gültekin B, Eyigör M, Tiryaki Y, Kırdar S, Aydın N. Investigation of antifungal susceptibilities and some virulence factors of *Candida* strains isolated from blood cultures and genotyping by rapid-PCR. Mikrobiyol Bul 2011; 45:306–317.

9 Lewis K, Ausubel FM. Prospects for plant-derived antibacterials. Nat Biotechnol 2006; 24:1504.

10 Lee SB, Cha KH, Kim SN, Altantsetseg S, Shatar S, Sarangerel O *et al.* The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms. J Microbiol 2007; 45:53–57.

11 Azzazy MF. Morphological studies of the pollen grains of Wadi El-Natrun plants, West Nile Delta, Egypt. Plant Syst Evol 2011; 294:239–251.

12 Boulos L. Flora of Egypt VII. Cairo, Egypt: Al Hadara Publishing; 2000.

13 El-Mokasabi FM. Floristic composition and traditional uses of plant species at Wadi Alkuf, Al-Jabal Al-Akhder, Libya. Am Eurasian J Agric Environ Sci 2014; 14:685–697.

14 Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts: approved guideline, 2nd ed. CLSI; 2009.

15 Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—a review. J Am Coll Nutr 2006; 25:79–99.

16 El-Sherbiny GM, Moghannem SA, Sharaf MH. Antimicrobial activities and cytotoxicity of *sisymbrium irio* L extract against multi-drug resistant bacteria (MDRB) and *Candida albicans*. Int J Curr Microbiol App Sci 2017; 6:1–13.

17 Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, *et al.* Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010; 54:4678–4683.

18 Yang L, Liu X, Zhong L, Sui Y, Quan G, Huang Y, *et al.* Dioscin inhibits virulence factors of *Candida albicans*. Biomed Res Int 2018; 2018:4651726.

19 Stepanovic S, Vukovi D., Hola V. Quantification of biofilm in microtiter plates: overview of testing conditions and practical 252 recommendations for assessment of biofilm production. APMIS 2007; 115:891–899.

20 Chusri S, Sompetch K, Sompetch M, Mukdee S, Srichai T, Maneenoon K, *et al.* Inhibition of *Staphylococcus epidermis* biofilm formation by traditional Thai herbal recipes used for wound treatment. Evid Based Complement Alternat Med; 2012; ID 159797, 8 pages.

21 Padmavathi AR, Bakkiyaraj D, Thajuddin N, Pandian SK. Effect of 2, 4-di-tert-butylphenol on growth and biofilm formation by an opportunistic fungus*Candida albicans*. Biofouling 2015; 31:565–574.

22 Hashimoto K, Urakami K, Fujiwara Y, Terada S, Watanabe C. Determination of residual solvents in pharmaceuticals by thermal desorption-GC/MS. Anal Sci 2001; 17:645–648.

23 Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L. Cell viability assays 'assay guidance manual [Internet]'. Bethesda, MD: Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2013.

24 Wei LS, Wee W, Siong JY, Syamsumir DF. Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Med Iran 2011; 49:670–674.

25 Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural sources and bioactivities of 2, 4-di-tert-butylphenol and its analogs. Toxins 2020; 12:35.

26 Singh VK, Kavita K, Prabhakaran R, Jha B. Cis-9-octadecenoic acid from the rhizospheric bacterium *Stenotrophomonas maltophilia* BJ01 shows quorum quenching and anti-biofilm activities. Biofouling 2013; 29:855–867.