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Original article

Evaluation of antifungal activity of *Origanum vulgare* essential oil and its influence on *CDR*1 gene expression among *Candida albicans* in vulvovaginal candidiasis

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ABSTRACT

Background: The most prevalent mycosis in women with complex recurrence and tolerance to antifungals is vulvovaginal candidiasis (VVC), so more efficient therapies are needed. This study evaluated the antifungal efficiency of *Origanum vulgare* essential oil (*O. vulgare* EO) and its genetic influence on *CDR*1 efflux pump gene among azole resistant *Candida albicans (C. albicans)*. **Methods:** *C. albicans* was isolated from vaginal swabs. Antifungal effect of *O. vulgare* EO was determined in comparison to azole antifungal drugs against 30 vaginal *C. albicans* isolates using disk diffusion method. The MIC of fluconazole and *O. vulgare* EO was determined by microdilution method, while overexpression of efflux pump *CDR1* gene among azole resistant *C. albicans* and effect of *O. vulgare* EO on gene expression was detected by quantitative real time PCR. **Results:** *C. albicans* accounted for (76.9%) of the isolated species, with *C. glabrata* following with (7.7%). Of all cases, (34%) were recurrent VVC, of which (53.8%) were non-albicans *Candida*. (36.7%) of *C. albicans* isolates were resistant to one or more member of antifungal azoles, including (10%) isolates were resistant to all tested antifungal azoles. Regarding the group of tested azoles, resistant isolates were 16.7%, 13.4% and 10% for clotrimazole, itraconazole and fluconazole, respectively. (16.7%) of *C. albicans* were found to be resistant to fluconazole using the broth microdilution method. Disk diffusion testing revealed that all *C. albicans* isolates were sensitive to *O. vulgare* EO, with MIC values ranging from 0.125 to 0.007% v/v. (73%) of the isolates of azole-resistant *C. albicans* had overexpressed the *CDR*1 gene. In all azole-resistant *C. albicans* isolate with *CDR*1 gene over-expression, *O. vulgare* EO significantly reduced *CDR*1 gene expression (P-value=0.012). **Conclusion:** *O. vulgare* EO could act as an effective natural antifungal with potential utilization in the combat against azole resistant *C. albicans.*

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Introduction

One common genitourinary tract commensal organism is *Candida albicans (C. albicans).* Vulvovaginal candidiasis (VVC), a non-invasive infection, results from disruption of local host defense mechanisms that ordinarily limit *Candida* growth. One of the most typical causes of vaginitis is VVC. It is estimated that approximately 75% of women will experience VVC at least once in their lifetime, with 40 to 50% experiencing a repeat

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[1]. About 80 to 90% of VVC cases are caused by *C. albicans*; non-albicans *Candida* (NAC) species, usually *Candida glabrata*, only account for 10–20% of cases [2].

The most often prescribed class of antifungal medicines, azoles, frequently result in treatment failure because of the extensive usage of these frequently over-the-counter medications [3].

Azole ring found in azole antifungals prevents a variety of fungus from growing. The azole ring which has two nitrogens are known as imidazoles, including clotrimazole, ketoconazole and miconazole. The azole ring which contains three nitrogens are known as triazoles, including fluconazole and itraconazole. Azoles work by blocking the enzyme cytochrome P450 lanosterol 14-alpha-demethylase, which is encoded by the *ERG*11 gene, from converting lanosterol to ergosterol, the main sterol in the fungal cell membrane. Cell death results from ergosterol depletion because it weakens the cell membrane [4].

Upregulation of the ABC transporter family genes *Candida* drug resistance (*CDR*) 1 and *CDR2* as well as the multidrug resistance (*MDR*) 1 gene, which encodes a multidrug efflux pump, are the processes behind *C. albicans* resistance to azoles. Azole resistance is also linked to mutations in or overexpression of *ERG*11 gene [5].

Using natural alternatives, such as natural plant chemicals that act as natural antimicrobials, is one of the suggested strategies for managing drug-resistant pathogens [6]. Plants can yield highly volatile, fragrant oils called essential oils (EOs). Because of their volatility, they can be readily extracted using the steam distillation method from a variety of natural sources. EOs have been applied to food as preservatives, flavor enhancers, cosmetics, and medicinal ingredients [7]. Researches on the antibacterial, antifungal, antiviral, insecticidal, anticancer and antioxidant qualities of EOs have been conducted in the medical field. The primary advantage of these natural medicines is that prolonged use as medication does not result in an increase in resistance. Additionally, they frequently enjoy broad popular support, low toxicity, and minimal negative impacts on the environment [8].

There are 39 species in the *Lamiaceae* family's genus *Origanum* which known by mainly as "oregano," *Origanum vulgare (O. vulgare)* is a significant aromatic and medicinal plant of the *Origanum* genus. The phenolic components carvacrol and thymol, which are abundant in *O. vulgare*, are responsible for the high bactericidal and fungicidal activity against several infections [9].

The rising resistance of yeast to traditional antifungals, as a result of their frequent and repeated usage, is one of the factors contributing to the increased incidence of candidiasis. When treating patients with symptomatic recurrent VVC, a highly prevalent illness, Azole-resistant *Candida* presents significant challenges and frustrates both doctors and patients. This results in the ongoing quest for novel tactical methods in the management and prevention of candidiasis [10].

Therefore, this study aimed to investigate the antifungal effect of *O. vulgare* EO against *C. albicans* isolated from patients of VVC in comparison to azole anti-fungal drugs and its influence on the expression of *CDR*1 efflux pump gene among azole resistant *C. albicans*.

Methods

Study design, settings and subjects

This cross-sectional study was conducted in Ain-Shams University Hospitals in the period from July 2022 to January 2023 and included thirty-nine cases with VVC. The patients were non-pregnant women of reproductive age complaining of itching and abnormal whitish vaginal discharge seeking medical advice at the outpatient clinics of Obstetrics and Gynecology Ain Shams University Hospitals. Two high vaginal swabs were collected aseptically from each patient. Vaginal swab samples were presented to "Medical Microbiology Laboratory of Ain-Shams University Hospitals". Informed consent was obtained from all the patients. The study has been approved by institutional ethical committee "No. FMASU MD 380\ 2019".

Isolation and identification of *Candida* species

One of the 2 swabs was used for direct Gram stain and direct wet mount microscopy while the second one was cultured on Sabauroud dextrose agar (HiMedia, India) with added chloramphenicol (0.5g/L) and subculture on selective chromogenic Hichrome *Candida* differential agar (HiMedia, India) for species identification [11]. Phenotypic identification of *C. albicans* isolates was done by conventional methods based on colonial morphology, microscopic examination of wet and gram-stained films and germ tube test. Isolates were preserved on "tryptone soya broth" with 15% glycerol at -80°C for future use [12].

Analysis of *Origanum vulgare* essential oil

Pure *O. vulgare* (oregano) EO (Aura cacia, Norway) was obtained by steam distillation of the stems, leaves and flowers. Spain was the country of origin. The chemical composition and physical properties of the oregano EO was obtained by gas chromatography-mass spectrometry (GC-MS).

Antifungal drugs and *Origanum vulgare* EO susceptibility testing using disc diffusion (DD) method

In accordance with the guidelines provided by Bona et al. [13] and the Clinical Laboratory Standards Institute (CLSI) M44, 2018 guidelines [14], the agar disc diffusion method was utilized to determine the EO's sensitivity. Strain suspensions (10^6 CFU ml^{-1}) were swabbed on Muller Hinton agar plates (HiMedia, India) supplemented with 2% glucose and 0.5 µg/ml methylene blue dye. Ten µl of *O. vulgare* EO was put to a filter paper disc (0.6 mm in diameter) that was placed on top of the agar surface. As positive controls, discs of 10 µg clotrimazole (CC), 25 µg fluconazole (FLC), and 8 µg itraconazole (IT) (HiMedia, India) were utilized. The negative control was 10 µl of pure dimethyl sulfoxide (DMSO) (Sigma-Aldrich in St. Louis, MO, USA) disc. Plates were incubated at 37°C for 48 hours. If the EO's sensitivity test produced an inhibitory ring greater than the one caused by clotrimazole (positive control; ≥100%), it is deemed positive. The isolates were classified as resistant (R), susceptible (S), and susceptible dose dependent (SDD) based on the size of inhibition zone for the selected antifungals, in accordance with the recommendations in the M27M44S document of the CLSI, 2022 [15].

Minimal inhibitory concentration (MIC) of fluconazole and *Origanum vulgare* essential oil

For each isolate of *C. albicans*, The MIC of the EO and fluconazole was determined by broth microdilution method utilizing 96-well microtiter plates on "Roswell Park Memorial Institute" broth media (RPMI 1640) supplemented with 0.2% glucose (Hi Media, India) according to CLSI M27, (2017) [16]. Zero point zero four ml of EO was dissolved in 1ml of DMSO to give final concentration of 40 ul/ml which was equivalent to 4% (v/v) [13]. Fluconazole and oregano EO were prepared at double fold dilutions as the following range: fluconazole from 64 to 0.125 µg/ml and oregano EO from 4 to 0·007% (v/v). 48-hour-cultured colonies on Sabauroud dextrose agar were used to prepare the yeast suspension in sterile distilled water to be adjusted at 0.5 McFarland, and RPMI 1640 (0.5 - 2.5 ×103 cell/mL) was used to prepare the inoculums. Each EO and fluconazole drug dilution (100 μl) was then inoculated with 100 μl of the yeast suspension. Growth control containing RPMI medium inoculated with *C. albicans* inoculum without any drug or oil while negative control containing RPMI medium with antifungal agent or oil and DMSO. All microtiter plates were incubated at 37°C for 24. Then, in-vitro susceptibility results and interpretation of MIC values to fluconazole were recorded according to CLSI M27M44S, 2022 guidelines. All isolates with a MIC of ≥ 8 µg/mL were defined as having resistance, ≤ 2 µg/mL as susceptible and 4 µg/mL as susceptible dose dependent (SDD) to fluconazole [15].

Determination of MIC by Resazurin-based 96-well plate microdilution method

After 24 hr incubation of the previously prepared microdilution plate, 30 µL of resazurin was added to each well and the plate was further incubated for 24 h to observe color change. Fungal growth was indicated by oxidation of resazurin from blue to pink. Therefore, the last unchanged blue or purple color well was recorded as MIC [17].

***CDR*1 efflux pump gene expression analysis**

Baseline *CDR*1 gene expression was determined in azole resistant (11) isolates relative to an azole sensitive isolate. In addition, each of azole resistant isolates with overexpression of *CDR*1 gene was treated with oregano EO at the subinhibitory concentration corresponding to each isolate. The effect of oil treatment on gene expression was determined and compared to base line gene expression in the tested (8) isolates before EO treatment.

Quantitative Real time Polymerase Chain Reaction (RT-PCR**)** was performed using PureLink™ RNA Mini Kit (Thermo-fisher, USA), GoScript™ Reverse Transcriptase kit (Promega, USA), DreamTaq Green PCR Master Mix (2X) kit (Thermo-fisher, USA) and 2 pairs of primers specific for *CDR1* genes. B-actin (*ACT1*) was used as a housekeeping gene to normalize levels of *CDR*1 transcripts. The sequences of primers used are described in **Table 1** [18, 19].

Suspensions in brain heart infusion broth were cultured overnight at 37 °C then were adjusted at 0.5 MacFarland by adjusting OD between 0.08 and 0.1 at a wavelength of 625 nm before being used

for RNA extraction [20]. RNA extraction and RT-PCR steps were done following the instruction of the kits' handbooks and according to the conditions mentioned by Jahanshiri et al. [19]. The results were determined by relative quantification according to Livak et al., determination of fold change was done by the relative threshold method using the ($2^{-\Delta\Delta CT}$) formula [21].

Statistical analysis

Version 27 of the Windows computer program, the Statistical Package for the Social Sciences (SPSS), was used to analyze data and provide descriptions of variables like number and percentage. When non-parametric data were discovered, the quantitative values were displayed as the median and inter-quartile range (IQR). The Wilcoxon Rank test was used to compare the quantitative data and non-parametric distribution of two paired groups. P-values were classified as non-significant if they were >0.05, significant if they were ≤0.05, and highly significant if they were ≤ 0.01.

Results

Isolation and identification of *Candida* species from VVC

Vaginal swab samples recovered from 39 VVC cases were cultured on chromogenic Hichrome Candida differential agar. The most common species of VVC were *C. albicans* (30/39, 76.9%), followed by *C. glabrata* (3/39, 7.7%), *C. krusei* (2/39, 5.1%), *C. parasilosis* (1/39, 2.6%) and *C. tropicalis* (1/39, 2.6%) while mixed infection presented (2/39, 5.1%) of all isolates; one with *C. albicans* and *C. tropicalis*, the other with *C. albicans* and *C. glabrata*. Patient's data were collected as regard age, risk factors and clinical diagnosis. The mean age was 33 years (ranging from 22-56 years). Recurrent vulvovaginal candidiasis (RVVC) represents 33% of all cases (13\39), of which NAC represent (53.8%, 7\13). The most common risk factors for VVC in the patients included in our study were oral contraceptives and IUD (60%) followed by behavioral factors (52.5%), comorbidity as diabetes (29%) and use of antibiotics (13%).

Analysis of *Origanum vulgare* essential oil

Gas chromatography coupled with mass spectrometry (GC/MS) was used to evaluate the chemical composition of *O. vulgare* EO. Carvacrol (72.72%), thymol (3.69%), p-cymene (7.5%), and γ-terpinene (6.13%) were the main components found in the extract.

Antifungal and *Origanum vulgare* essential oil susceptibility testing using disc diffusion (DD) method

Three different azole antifungal agents (clotrimazole, itraconazole and fluconazole) were evaluated by DD method against 30 isolates of *C. albicans*. The results showed percentage of resistant isolates to the tested azoles; it was found that 11 isolates (36.7%) were resistant or sensitive dose-dependent to one or more type of antifungal agents, including (3/30, 10%) isolates showed resistance to all tested antifungal agents. If an isolate was SDD to one drug and was sensitive to other drugs; this isolate was not included in the total number of resistant isolates. Regarding the group of tested azoles; resistant isolates were (16.7%), (13.3%) and (10%) for clotrimazole, itraconazole and fluconazole, respectively. Susceptibility to itraconazole, clotrimazole and fluconazole were 50%, 66.6% and 83.3% respectively. All *C. albicans* isolates (100%) were susceptible to *O. vulgare* EO confirmed by disk diffusion test with inhibition zones ranging from 30 to 50 mm as compared to clotrimazole zone of inhibition as shown in **(Figure 2&3)**.

For fluconazole, MIC range was (64-0.125 µg/mL). Resistance rate of *C. albicans* to fluconazole determined by broth microdilution method was 16.7%, of which MIC were 8,16 and 32 µg/mL for 10%, 3.3% and 3.3% of isolates, respectively. While for *O. vulgare*, results demonstrated MIC ranging from 0.125 to 0.007 % v/v. Furthermore, *O. vulgare* EO was active at low concentrations ≤ 0·007% v/v in 46.7% of the strains.

Figure 4 shows resazurin based microbroth dilution test used to determine MIC of FLC and Oregano EO. FLC was diluted from 64 µg/mL to 0.125 µg/mL in columns from 1to10 of rows A, C, E, G. Oregano EO was diluted from 4% to 0.007% in columns from 1to10 of rows B, D, F, H. columns 11 and 12 represent the positive and negative controls respectively. Concentration in the last column showed no change of resazurin natural color (blue/purple) to the reduced form (pink) was taken as the MIC value.

Detection of overexpression of *CDR*1 efflux pump gene among azole resistant *C. albicans* and effect of *O. vulgare* EO treatment on gene expression

Over-expression of *CDR*1 gene was found in (73%, 8\11) of azole resistant *C. albicans* isolates relative to azole sensitive isolate. Fold change of

*CDR*1 gene expression was ranging from (0.92-7.56) with median 2-fold increase of gene expression. Furthermore, (100%, 8\8) of azole resistant isolates with *CDR*1 gene over-expression showed reduction in gene expression after *O. vulgare* EO treatment as shown in **Table 1.**

Table 2 shows that the median of cycle threshold (CT) of *CDR*1 gene was increased from 17.83 before treatment to 21.96 after treatment. the cycle threshold values are inversely proportionate with the concentration of DNA, increased cycle threshold means decrease in gene expression and vice versa. All azole resistant *C. albicans* 8 /8 (100%) isolates showed reduction in gene expression after oregano EO treatment.

There was a high statistically significant difference between gene expression before and after treatment with EO as shown in **Table 3 & Figure 5**.

Table 1. Sequence of primers used in molecular study

Primer name	Sequence 5′ to 3′	Reference
*CDR*1 forward	AAGAGAACCATTACCAGG	Monroy-Pérez et al. [18]
*CDR*1 reverse	AGGAATCGACGGATCAC	
B-actin (ACT1) forward	TTGGTGATGAAGCCCAATCC	Jahanshiri et al. [19]
B-actin (ACT1) reverse	CATATCGTCCCAGTTGGAAACA	

Table 2. The effect of Oregano EO treatment on expression of *CDR*1 gene among 8 azole resistant *C. albicans* isolates

Azole resistant *C. albicans*	CT *CDR*1 gene before EO treatment	Fold change of *CDR*1 gene before EO treatment	CT *CDR*1 gene after EO treatment	Fold change of *CDR*1 gene after EO treatment
Isolate N 1	15.1	1.77	20.59	0.042
Isolate N 2	18	1.49	23.19	0.036
Isolate N 3	18.72	2.49	28.1	0.14
Isolate N 4	18.06	2.26	23.7	0.12
Isolate N 5	17.02	2.67	20.71	0.51
Isolate N 6	15.5	3.53	20.72	0.69
Isolate N 7	17.66	3.63	20.29	2.04
Isolate N 8	26.43	7.56	28.2	0.098
Median	17.83	2.58	21.96	.130

Table 3. shows that sub-MIC of Oregano EO high significantly reduced *CDR*1 gene expression level in azole resistant *C. albicans* isolates.

	Fold change before EO	**Fold change after EO**	***Wilcoxon Rank test***	**P-value**	**Sig.**
Median (IQR)	2.58 (1.7) "1.49 – 7.56"	0.130 (0.589) "0.036 – 2.04"	2.521	0.012	HS

Figure 1. Differentiation of various species of *Candida* on Hichrome Candida differential agar. *C. albicans* (A), *C. tropicalis* (B), *C. krusei* (C), *C. glabrata* (D), *C. parapsilosis* (E), mixed *Candida* species (F).

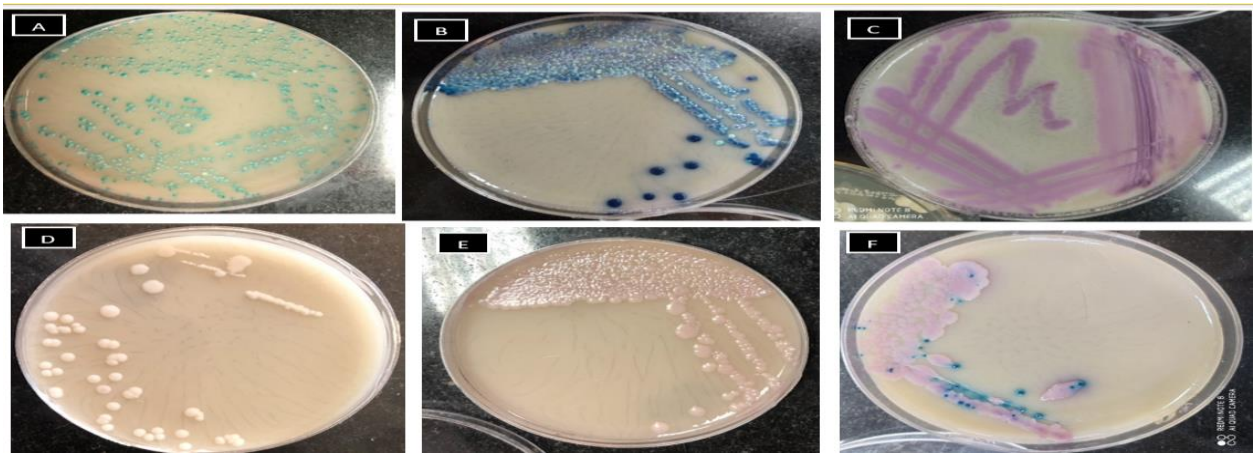


Figure 2. Azole resistant *C. albicans* isolates and susceptibility to Oregano EO (O) in comparison to clotrimazole (CC) which is the positive control for EO inhibition zone. DMSO is the negative control (C).

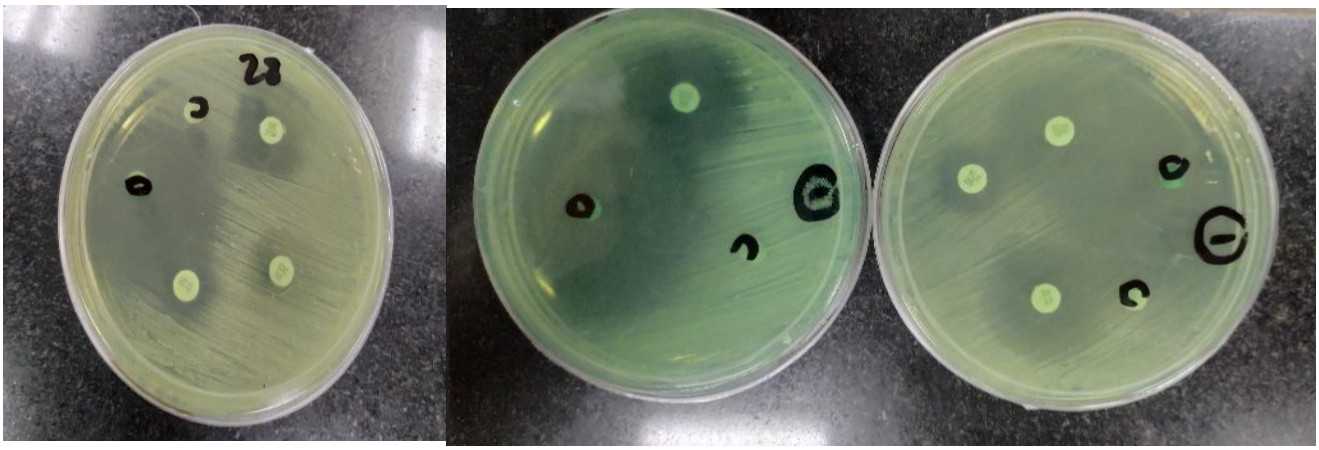


Figure 3. Results of antifungal susceptibility testing to the tested azoles and *O. vulgare* EO by DD method.

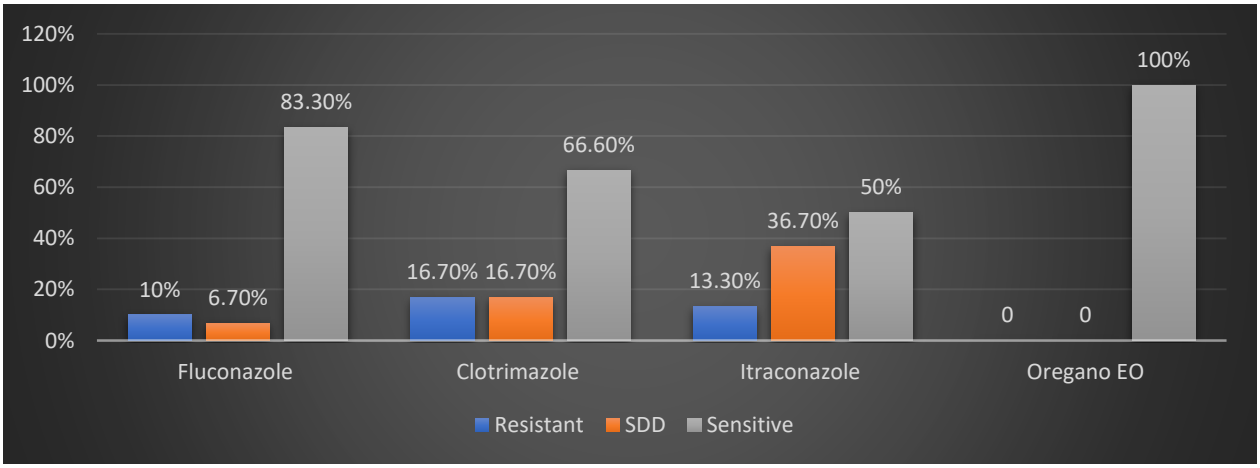


Figure 4. Resazurin based microbroth dilution test used to determine MIC of FLC and Oregano EO. FLC was diluted from 64 µg/mL to 0.125 µg/mL in columns from 1to10 of rows A, C, E, G. Oregano EO was diluted from 4% to 0.007% in columns from 1to10 of rows B, D, F, H. Columns 11 and 12 represent the positive and negative controls respectively. Concentration in the last column showed no change of resazurin natural color (blue/purple) to the reduced form (pink) was taken as the MIC value.

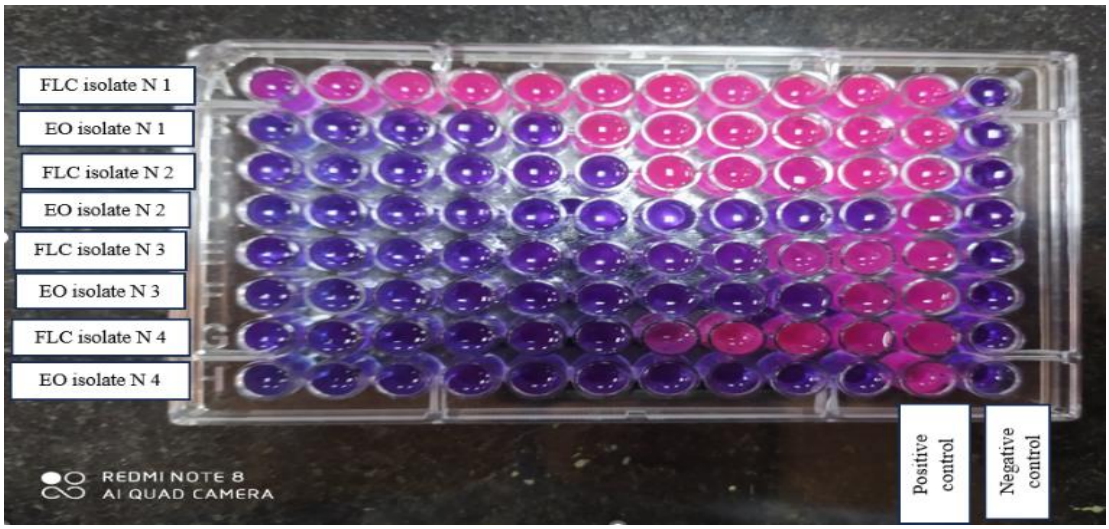
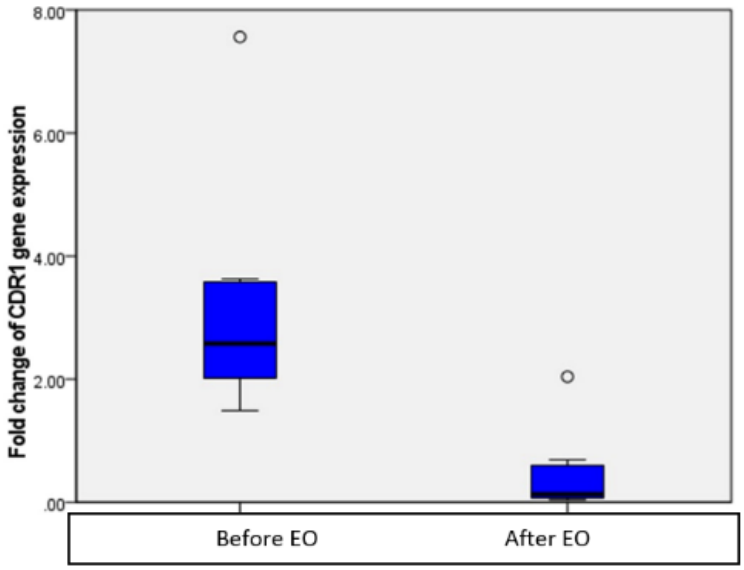


Figure 5. Fold change in *CDR*1 gene expression of azole resistant *C. albicans* isolates before and after EO treatment.



Discussion

It has been suggested that *C. albicans* is a species that cause 80 to 95 percent of all VVC infections. Nonetheless, reports indicate that within the past 20 years, the number of cases brought on by *C. glabrata* has extremely grown [2]. *C. albicans* (76.9%) was the most frequent *candida* species in the current study that caused VVC, followed by *C. glabrata* (7.7%), *C. krusei* (5.1%), *C. parasilosis* (2.6%) and *C. tropicalis* (2.6%). The current study results were compatible with Egyptian studies. *C. albicans* was the dominate *Candida* spp. (62.4%), (63%) and (60.3%) associated with VVC in Menoufia, Assiut and Cairo, respectively [22-24]. According to a meta-analysis study conducted in Turkey, *C. albicans (*54.76%), *C. glabrata*

(24.04%), *C. krusei* (3.68%), *C. kefyr* (3.37%), and *C. tropicalis* (2.07%) were the most frequent *candida* species responsible for VVC, followed by other *Candida* species (12.29%) [1].

RVVC (≥3 episodes/year) represent 34% of all cases, of which NAC represent (53.8%). The high rate of recurrence is in agreement with Hösükoğlu et al. who reported 42% of the cases were RVVC. Also, 54.8% of RVVC was caused by NAC species [25]. According to Zaki and Denning study in Egypt, RVVC is estimated to occur in nearly 6% of adult women [26]. On the other hand, Kan et al. found RVVC rate 26% in 602 confirmed cases of VVC in China and (79%) were *C. albicans* [27]. The high occurrence of RVVC could be attributed to the fact that empirical therapy of VVC is practiced and fungal cultures and susceptibility testing are not routinely conducted in our country. Additionally, the emergence of *candida* that is resistant to a particular antifungal could lead to RVVC.

Three different antifungal agents were evaluated by disk diffusion method against 30 isolates of *C. albicans*. It was found that 11 isolates (36.7%) were resistant or susceptible dose dependent to one or more type of antifungal agents, including (10%) isolates showed resistance to all tested antifungal agents. Regarding the group of tested azoles; resistant isolates were 16.6%, 13.4% and 10% for clotrimazole, itraconazole and fluconazole, respectively. Significant increase in azole resistance of *C. albicans* isolates from VVC was reported in study from Egypt by Ajlan et al. who reported that for *C. albicans* isolates, the highest antifungal resistance rate was observed to itraconazole (43.4%) followed by miconazole (34%), clotrimazole (32.1%) and fluconazole (28.3%). While the lowest antifungal resistance rate was observed to voriconazole (13.2%) followed by nystatin (17%) [22].

Hassan et al. investigated eight conventional antifungal drugs against 128 isolates of *Candida* species, demonstrating a high prevalence of resistance isolates to the studied antifungals in Egypt. Of the isolates obtained, 67 were *C. albicans*. Resistance to the antifungal drugs was identified: itraconazole and nystatin (43.3%), ketoconazole (56.7%) and fluconazole (61.2%) [23]. File et al. in USA showed that of the 970 isolates of *C. albicans*, 71 (7.3%) with fluconazole resistant VVC were identified [28].

Yet, Liu et al. showed a low resistance rate; for fluconazole, itraconazole, and miconazole, the corresponding resistance rates to *C. albicans* were 1.1, 2.2, and 4.2% [29]. At a tertiary care hospital in Vietnam, the antifungal sensitivity of *C. albicans* was shown to be positive for the majority of the antifungals that were tested. Miconazole, caspofungin, and micafungin were all 100% effective against all 46 isolates of *C. albicans*. Amphotericin B, fluconazole, itraconazole, and voriconazole susceptibility rates were, in that order, 91.30%, 82.61%, 86.95%, and 95.65%, respectively [30]. The frequency of azole resistance varies, according to meta-analyses conducted in Africa. Using disc diffusion method, researchers discovered that *C. albicans* resistance to fluconazole is about 6.8% in Cameroon and 53.7% in Ethiopia [31]. However, the frequency of azole resistance reported by authors varies from study to study and may be connected to the susceptibility testing method in addition to the epidemiological resources.

MIC range of fluconazole was (64-0.125 µg/mL). Resistance rate of *C. albicans* to fluconazole determined by broth microdilution method was 16.7%, of which MIC were 8,16 and 32 µg/mL for 10%, 3.3% and 3.3%, respectively. In a recent study on pregnant females with VVC in Pakistan, Zaman et al. noticed that there was a higher rate of fluconazole resistance: 48.4% of the isolates of *C. albicans* had a MIC of 64 µg/ml for fluconazole resistance [32]. Hong et al. investigated total of 244 *C. albicans* isolates recovered from VVC patients in China and found that the susceptibility rate of fluconazole: 59.4% (145/244) with MIC mean of 2.01 µg/mL [33]. However, the resistance rates were low (1.7%) with MIC ranged from 0.5 µg/ml to 16 µg/ml for fluconazole in Brazil among samples from 166 patients of VVC [34]. Similarly in another study, among the 460 *C. albicans* isolated from vaginal samples in China, resistance rate to fluconazole was (6%) with MIC range 0.125–64 µg/mL determined by broth microdilution method [27].

All *C. albicans* isolates (100%) were susceptible to *O. vulgare* (Oregano) EO confirmed by disk diffusion test with inhibition zones ranging from 30 to 50 mm as compared to clotrimazole zone of inhibition. Microbroth dilution test results demonstrated MIC ranging from 0.125 to 0.007 % v/v. Furthermore, Oregano EO was active at concentrations ≤ 0·007% v/v in 46.7% of the strains.

These outcomes concurred with those of Bona et al. who examined the impact of several EOs on *C. albicans*. The three EOs that worked best against *C. albicans* were oregano, winter savory, and mint. For every strain of *C. albicans*, the oils of oregano and winter savory proved to be efficacious. When compared to clotrimazole, the inhibition rate of 85% of isolates treated with oregano and winter savory oils was ≥200%. Moreover, 64% of the isolates had MIC of oregano EO less than 1% v/v. Further, 10% of the strains exhibited the activity of oregano EO at doses ≤0·0039% v/v [13]. Helal et al. reported encouraging results indicating that essential oils derived from medicinal plants in Saudi Arabia act efficiently in combating drug-resistant pathogenic bacteria and have potential clinical applications. The *Lamiaceae* family comprises all species, including *O. vulgare L.* EO, which demonstrated the strongest antifungal activity against drug-resistant *C. albicans*. The inhibition zones measured between 39 and 45 mm were observed, opposed to the 0 to 14 mm range of standard antifungal molecules, such as ketoconazole, and fluconazole [35].

Additionally, oregano plant extract and EO had the strongest antifungal activity against *C. albicans* in a study by Váczi et al. with MIC values of 4.9 mg/mL and 0.4 mg/mL, respectively [10]. According to Karpiński et al. findings, oregano and lemon balm essential oils have the strongest anti-*Candida* properties, with MIC values less than 3.125 mg/mL [36].

When Cid-Chevecich et al. investigated how *O. vulgare* EO affected reference strains of *C. albicans*, they discovered that the MIC for ATCC-90029 and ATCC-10231 was 0.01 mg/L and 0.97 mg/L, respectively. The investigation also revealed that *O. vulgare* EO and fluconazole and nystatin interacted synergistically [37].

However, because the composition of the EO utilized in this investigation can vary from that used in previous studies, comparing the MIC values from this study with those from past studies may not be valid. The variation across the methods under analysis is another crucial factor to note, even though it only shows a slight variation in the oil's in vitro efficacy. The results indicate that the EO of *O. vulgare* and its components are promising against yeasts, demonstrating inhibition at concentrations deemed low, even with the differences between EO concentrations, which consequently creates variability in the MIC.

Previous research has indicated that the most common mechanism of azole resistance is over expression of genes producing ABC membrane-transport proteins (CDR1 and CDR2) [38,39]. Over-expression of *CDR*1 gene was detected in (73%) of azole resistant *C. albicans* isolates relative to azole sensitive isolate. Fold change of *CDR*1 gene expression ranging from (0.92-7.56) with median 2-fold increase of gene expression. Furthermore, *O. vulgare* EO significantly reduced expression of *CDR*1 gene in all tested (100%) azole resistant *C. albicans* isolates with *CDR*1 gene over-expression (P-value=0.012).

This comes in accordance with research by Liu et al. which showed that 5 (41.6%) of the 12 azole resistant isolates had elevated *CDR*1 expression (3.78–10.13 fold increase compared to the average of susceptible isolates). There were greater amounts of *CDR*1 transcripts in four of the isolates [40]. Maheronnaghsh et al. conducted a study whereby they examined 74 *C. albicans* isolates. The results showed that the fluconazole-resistant isolates had considerably higher mean expressions of the *CDR*1 and *MDR*1 genes compared to the susceptible isolates. It was determined that the fold change for the *CDR*1 genes was (1.79) [41]. Voropaev et al. stated that high levels of *CDR*1 gene expression were discovered in 18 strains of *C. albicans* resistant to fluconazole and voriconazole isolated in the Moscow region: 89% and 78%, respectively [42].

On the contrary, a study conducted in Egypt by Sayed et al. , *CDR*1 gene was expressed in both susceptible and resistant isolates to fluconazole and terbinafine antifungals. This suggests that the gene is not selectively expressed by resistant *Candida* isolates [43].

To our knowledge, this is the first study report the genotypic influence of *O. vulgare* EO on *CDR*1 efflux pump gene expression in *C. albicans* in the literature, despite the fact that there are numerous studies on the effect of *O. vulgare* EO on *Candida*.

Conclusion

The current study's results are encouraging; we concluded that *O. vulgare* EO has an excellent inhibitory activity against *C. albicans* in vitro with genotypic effect on efflux pump gene *CDR*1. These positive findings enhance the herb's therapeutic qualities, offer a viable substitute for the management of candidiasis, and give hope for the battle against resistant *C. albicans*.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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