Original Article

Microbial Biosystems 9(2) (2024) 2024.325202
10.21608/mb.2024.325202.1185

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Studies on *Candida albicans* causing vaginal and urinary tract inflammation: Prevalence, antifungal resistance, and natural product susceptibility

Hussein S. Salama[1*], Mohamed T. Shaaban[1] , Aymen S. Dawood[2], Azza M. Soliman[1], Mohamed N. Shaheen[1]

[1] *Botany and Microbiology Department, Faculty of Science, Menoufia University, Egypt.*
[2] *Obstetrics and Gynecology Department, Faculty of Medicine, Tanta University, Egypt.*

ARTICLE INFO

Article history
Received 1 October 2024
Received revised 17 November 2024
Accepted 29 December 2024
Available online 30 December 2024

Corresponding Editors
Osman, M. S.
Rizk, N.
Farouk, M.

Keywords
Apple cider vinegar,
C. albicans MS24 and MS44,
coconut oil,
Egypt,
garlic oil,
lavender oil,
tea tree oil.

ABSTRACT

Antifungal resistance in *Candida albicans* is one of the most harmful microorganisms that can cause inflammation in the vaginal and urinary tracts. To get around this problem, we tested how well some natural products worked against *C. albicans*. This study focused on isolating, identifying, studying the prevalence, and the susceptibility of *Candida* to some natural products. After isolation on specific media, we carried out morphological and biochemical identification of the recovered *Candida* isolates. We then estimated the antifungal resistance of the isolates against antifungal substances and their sensitivity to apple cider vinegar, garlic, lavender, coconut, and tea tree oils. We obtained only twelve *Candida* isolates out of fifty urine samples and vaginal swabs. The biochemical identification revealed that all isolates were *Candida* spp. The most antifungal-resistant isolates have an estimated ITS gene. After that, the susceptibility of six isolates to apple cider vinegar, tea tree oil, coconut oil, garlic oil, and lavender oil revealed the sensitivity of *Candida* to tested products. Finally, the ITS2 gene sequencing results confirmed the identification of *C. albicans* MS24 and *C. albicans* MS44 respectively. Our study concluded that *C. albicans* is considered one of the most common causes of vaginal and urinary tract inflammation. Also, natural products, especially tea tree oil and apple cider vinegar, have excellent antifungal activity against both *C. albicans* MS24 and MS44 respectively.

Published by Arab Society for Fungal Conservation

Introduction

Candida spp considered one of the most frequently observed agents in the diagnosis of vaginitis. Antifungal resistance of *Candida* observed and increased every year that causes more than 2,049,442 illnesses and 23,000 deaths per year in the United States. In 2016, about 6 million people in the world died due to fungal infections candidiasis caused mainly by *Candida albicans*, *C. glabrata* and *C. parapsilosis* (CDC, 2019). So, it is important to overcome antifungal resistance by using effective and save natural products to treat candidiasis.

Many natural products revealed large spectrum of biological activities against different microorganisms especially antifungal efficiency against *Candida spp*.

Phytochemicals have many classes, such as polyphenols, alkaloids, tannins, and terpenoids. Essential oils (EOs) contain natural volatile lipophilic chemicals extracted from specific plant parts in different ways such as distillation or cold extraction. EOs are enriched by many active constituents specific to the plant species and the used part, and this depends on the way they are extracted. EO's antifungal activity may be attributed to their ability to

***Corresponding author Email address*: husseinsalama@science.menofia.edu.eg (Hussein S. Salama)**

penetrate bacterial membranes, which affects the functional and lipophilic properties of the cell (Carvalho et al. 2018).

Many researchers have looked into the antimicrobial properties of apple cider vinegar (ACV). They discovered that ACV killed *E. coli*, *S. aureus*, and *C. albicans* by decreasing the production of cytokines and microbial proteins (Yagnik et al. 2018).

The current study's goals were to find out how common *Candida* spp. are in women with vaginal and urinary tract inflammation, figure out how resistant the isolates were to antifungals, and see how well some essential oils and apple cider vinegar worked as antifungal agents to get around the problem of antifungal resistance.

Materials and Methods

Collections of samples, isolation and purification of fungal isolates

Fifty urine samples and fifty vaginal swabs were obtained from women patients suffering from vaginal and urinary tract inflammation at private gynaecological clinics and IUT clinics. Ethical approval related to sampling is the same according to our previous study (Soliman et al., 2024). The researchers collected all vaginal specimens using appropriate sterile swabs, each containing 5 mL of normal saline. We then transported the swabs and urine samples to the laboratory in cooled containers.

We spread vaginal swabs and urine samples on Cysteine Lactose Electrolyte-Deficient (CLED) agar and Sabouraud Dextrose Agar (SDA) medium that supplemented with chloramphenicol (250 mg/L). The plates were then kept at 30°C for 48 hours.

Morphological and biochemical identification of Candida isolates

Urine samples and vaginal swabs were put on slides and mixed with a drop of saline to look for clue cells and rule out trichomonas infection. The primary diagnosis of vaginal candidiasis was based on the presence of budding yeast cells and pseudo-hyphae in *Candida* species (Muzny and Schwebke, 2013). Next, we used biochemical tests to identify twelve different types of *Candida* spp., using Candida albicans ATCC 10231 as a positive control. The tests included the urease test, growth on SDA, fermentation of sugars, assimilation of sugars, and formation of germ tubes (Khadka et al., 2017).

Antifungal susceptibility test

We performed antifungal susceptibility testing by the disc diffusion method in accordance with CLSI guidelines and the manufacturer's instructions (Shaaban et al., 2023). The colonies were mixed with 5 ml of clean 0.85% saline, and the turbidity was set to get 1×10^5 to 1×10^6 cells/ml (0.5 McFarland standard). The colonies were then spread out on Muller-Hinton agar plates in a pattern. After that, the most frequently used antifungal agents in local markets tested (amphotericin B discs (20 mcg), fluconazole discs (25 mcg), and ketoconazole discs (10 mcg)) were added on agar surfaces, and the plates were incubated at 37°C for 24-48 h. These tests were repeated three times, and the most resistant isolates were selected for further identification and natural product treatments.

Genotypic identification of the most resistant Candida isolates

We conducted further testing on the *Candida* isolates most resistant to antifungals, primarily to identify their genotype using the ITS gene. We extracted the DNA in accordance with QIAamp DNA guidelines. The extraction process followed the instructions provided by the mini kit. We used oligonucleotide primer sequences to use PCR to find the ITS gene in pathogenic isolates. This produced a 109-bp product. We used *Candida albicans* ATCC 10231 as a positive control (Khadka et al., 2017).

Antifungal assay of essential oils and apple cider vinegar against the most resistant Candida isolates

All natural products under investigation were tested against six hard-to-kill types of *C. albicans* (S17V, S35V, S24V, S46V, S36U, and S44U). The oils used were apple cider vinegar (ACV), coconut oil (CNO), garlic oil (GO), tea tree oil (TTO), and lavender oil (LO). We estimated the antifungal activities using the disc diffusion method (Shaaban et al., 2023). Discs saturated with 20 µL of pure ACV, CNO, GO, TTO, and LO were added to Muller Hinton agar media, which had previously been inoculated by standard McFarland, and the plates were incubated at 37 °C for 48 h (Shaaban et al. 2023). Next, we evaluated the antifungal activity of these natural products by measuring the inhibition zone diameters in millimeters and we conducted this test in triplicate.

Confirmation of Genotypic identification using ITS2 sequencing

Molecular identification (Tarini et al., 2010) was performed for the selected $S_{24}V$ and $S_{44}U$ isolates (the most sensitive isolates to tested oil). The ITS2 regions were amplified from the previous fungi DNA using conventional PCR with primers ITS86-F (5′-GTGAATCATCGAATCTTTGAAC-3′) and ITS4-R (5′-TCCTCCGCTTATTGATATGC-3′), producing an amplicon of approximately 200 ~ 400 bp. The sequences of the selected isolates were uploaded to the NCBI website and the submission was conducted in GenBank.

The statistical analysis

Descriptive statistics was done using IBM SPSS statistics to evaluate the number and percentage of sensitive and resistant isolates to each antibiotic or extract. Using a post-hoc analysis, statistical significance only considered when p value ≤ 0.05. Statistical analysis results were represented on figure barres as a lowercase letter.

Results

Out of fifty urine samples and fifty vaginal swabs, A total 12 (15%) isolates of *Candida* were recovered. Nine *Candida* isolates (18%) obtained from vaginal swabs and three (7.5%) isolates from urine samples. Morphological and biochemical identification of the twelve isolates from vaginal swabs and urine samples were done using various tests as shown in table 1. The results indicated that the twelve isolates primarily identified as *Candida albicans*.

Table 1. Biochemical identification of 12 isolates from vaginal swabs and urine samples (F: fermentation positive; +: positive result; -: negative result).

Isolate	Germ tube	SDA with cyclohexamide	Urease test	Growth On SDA			Sugar fermentation					Sugar assimilation	
				Pseudohyphae	Chlamydospore	Glucose	Maltose	Sucrose	Galactose	Glucose	Maltose	Sucrose	Galactose
	Candida isolates from urine samples												
C. albicans ATCC 10231 (positive control)	+	+	-	+	+	F	F	-	F	+	+	+	+
S40U	+	+	-	+	+	F	F	-	F	+	+	+	+
S44U	+	+	-	+	+	F	F	-	F	+	+	+	+
S46U	+	+	-	+	+	F	F	-	F	+	+	+	+
	Candida isolates from vaginal swabs												
$S_{11}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{13}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{17}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{19}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{24}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{29}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{35}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{36}V$	+	+	-	+	+	F	F	-	F	+	+	+	+
$S_{46}V$	+	+	-	+	+	F	F	-	F	+	+	+	+

As shown in figure (1), antifungal resistance pattern indicated that 5 (41.67 %), 3 (25.00%) and 1 (8.33 %) *Candida* isolates were resistant to fluconazole, ketoconazole and amphotericin B, respectively. Statistical analysis revealed there were a significant difference between sensitive and resistant isolates in each antifungal treatment as revealed from Tukey's test. Also, statistical analysis revealed isolates No. $S_{17}V$, $S_{35}V$, $S_{24}V$, $S_{36}U$, $S_{40}V$, and $S_{44}U$ were the most antifungal resistant isolates.

Detection of ITS gene in the most six resistant isolates ($S_{17}V$, $S_{35}V$, $S_{24}V$, $S_{36}U$, $S_{40}V$, and $S_{44}U$) was performed using PCR and the amplified PCR products were electrophoresed via agarose gel, as shown supplementary figure. The results confirmed that the six isolates were *Candida albicans* when compared with the bands observed in ladder and *Candida albicans* positive control.

Antimicrobial activity of some natural products against selected antifungal resistant *C. albicans* isolates showed that all *Candida albicans* $S_{17}V$, $S_{24}V$, $S_{35}V$, $S_{36}V$, $S_{44}U$ and $S_{46}U$ showed sensitivity with apple cider vinegar, garlic oil, coconut oil, tea tree oil and lavender oil. Statistical analysis results showed no significant difference in sensitivity levels between the ACV, CNO, TTO, and lavender oil as the p-value more than 0.05. Whereas, there

was significant difference in sensitivity levels after GO tested. Also, significant difference between tested *Candida* isolates toward each tested natural products treatment represented on figure (2) barres.

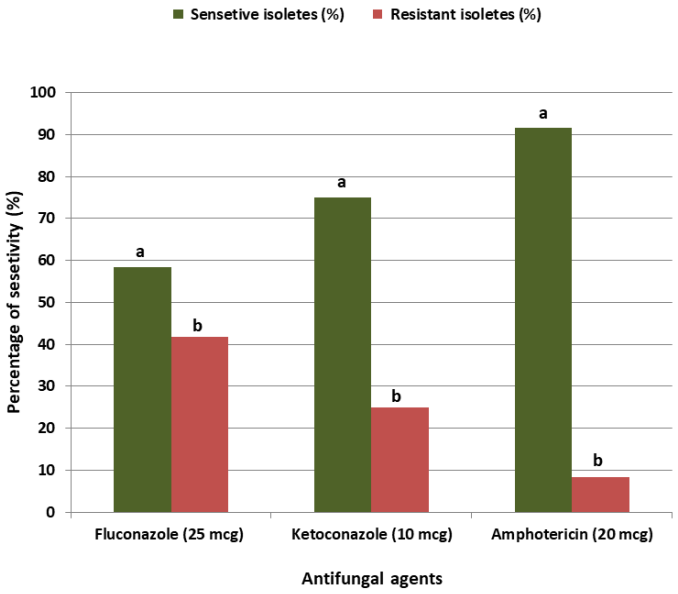

Fig 1. Antifungal susceptibility of fluconazole, ketoconazole and amphotericin against *Candida* isolates. Statistical analysis results were represented as lowercase letters; similar letters meaning no significant difference whereas different letters meaning the presence significant difference between compared treatments.

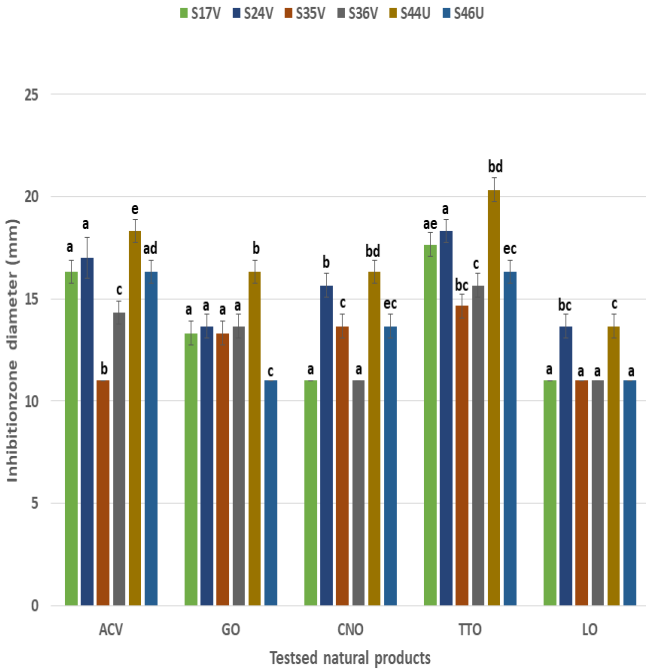

Fig 2. Antifungal activities of natural products (ACV: apple cider vinegar; GO: garlic oil; CNO: coconut oil; TTO: tea tree oil; LO: lavender oil) against six antifungal resistant tested *Candida* isolates. Isolated samples tested were $S_{17}V$ (No. 17 from vaginal swap), $S_{35}V$ (No. 35 from vaginal swap), $S_{24}V$ (No. 24 from vaginal swap), $S_{36}V$ (No. 17 from vaginal swap), $S_{44}U$ (No. 46 from urine sample) and $S_{46}U$ (No. 46 from urine sample). Statistical analysis results were represented as a lowercase letter on figure barres showing significant difference between compared isolates in each tested natural products; similar letters meaning no significant difference between compared isolates whereas different letters meaning significant difference between compared isolates.

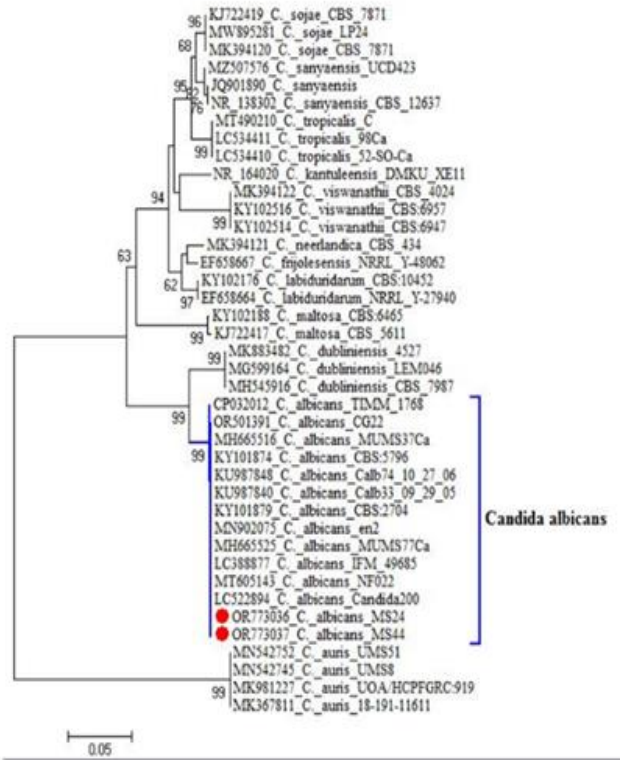

Fig 3. Phylogenetic tree analysis of *Candida albicans* MS24 *and Candida albicans* MS44.

Identification of the two most natural products sensitive clinical isolates were done by 18S rRNA sequence analysis as shown in figure (3). The amplified genes of *C. albicans* $MS_{24}V$ and $MS_{44}U$ had their partial nucleotide sequences submitted to GenBank along with accession numbers OR773036 and OR772947 for *Candida albicans* $MS_{24}V$ *and Candida albicans* $MS_{44}U$, respectively.

Discussion

Genital infections in women can lead to local discomfort during sexual relations and pain or pelvic inflammatory disease by causing vaginal discharge and mucosal ulceration. Recurring urinary tract infections are also

frequently seen in women with refractory vaginitis infection. In our study twelve isolates of *C. albicans* represent only 15 % from all hundred samples (fifty vaginal swabs and fifty urine samples) with 9 isolates obtained from vaginal swabs and 3 isolates from urine samples. These results were closely in agreements with (Dinç and Akyüz, 2023) who reported that 10.7 % Candida spp. Isolated from Vaginal Cultures in the Reproductive Period. Whereas, **Singh *et al.*** (Singh et al. 2016) observed the prevalence of *Candida* spp. in preterm pregnancy woman with 21 percentage of the total isolates. In the same trend Tortelli et al. (Tortelli et al. 2020) conducted associations between the vaginal microbiome and *Candida* colonization in women of reproductive age.

The results of antifungal sensitivity test indicated that, 91.67 % and 75.00 % of the isolates were sensitive to amphotericin and ketoconazole, respectively. Whereas, the level of isolates resistance to fluconazole was 41.67 % which indicated that resistance to antifungal azoles has been increased. These results were agreed with that of (Rajkowska et al. 2015) who reported that 85.1 % of *Candida* species isolates were resistant to fluconazole.

There has been an increased interest in last years in looking at antimicrobial properties of natural products from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential. The results detected that all *Candida* isolates showed sensitivity to tea tree oil (TTO). Whereas, five *Candida* isolates showed sensitivity to apple cider vinegar and garlic oil. Also, 66.6 % of *Candida* isolates showed susceptibility to coconut oil. While the least sensitivity (33.5 %) revealed with lavender essential oil. These results were agreed with the study of (Elgammal et al., 2020) who reported the efficiency of essential oils agents against *Candida albicans***.** Also, Yagnik *et al*. (Yagnik et al. 2018) concluded the efficiency of apple cider vinegar against *Candida albicans*.

Confirmation the identification of the most natural products sensitive two isolates $MS_{24}V$ and $MS_{44}U$ were done using 18S rRNA sequence analysis. The partial sequences amplified from two *C. albicans* isolates were confirmed as *C. albicans* MS24 and MS44 with accession number OR773036 and OR772947, respectively.

Conclusion

Our research findings revealed that *Candida albicans* considered one of the most causing vaginal and urinary tract inflammations in females. Also, natural products especially tea tree oil and apple cider vinegar have excellent antifungal activity against *Candida albicans* MS24 and *Candida albicans* MS44. However, further clinical trials needed for further applications of these oils.

Availability of data and material

The manuscript has no associated data

Funding

No funding

Use of artificial intelligence (AI)

AI was used with caution in this article, primarily to improve its readability.

Conflicts of interest

The authors have no conflict of interest.

References

CDC. (2019). *Antibiotic resistance threats in the United States, 2019*. https://doi.org/10.15620/cdc:82532

Dinç K. Akyüz S. (2023). Agents Isolated from Vaginal Cultures in the Reproductive Period and Their Antibiotic Sensitivities (Vaginal Culture and Antibiotic Sensitivitie). *European Journal of Therapeutics*, *29*(1), 55–59. https://doi.org/10.58600/eurjther-131

Elgammal E. El Gendy A E N. Elgamal A E B. (2020). Mechanism of action and bioactivities of Cinnamomum zeylanicum essential oil against some pathogenic microbes. *Egyptian Pharmaceutical Journal*, *19*(2), 162. https://doi.org/10.4103/epj.epj_63_19

Khadka S. Sherchand J B. Pokhrel B. M. Parajuli K. Mishra S K. Sharma S. et al. (2017). Isolation, speciation and antifungal susceptibility testing of Candida isolates from various clinical specimens at a tertiary care hospital, Nepal. *BMC Research Notes*, *10*(1), 218. https://doi.org/10.1186/s13104-017-2547-3

Rajkowska K. Kunicka-Styczyńska A. Pęczek M. (2015). Hydrophobic properties of Candida spp. under the influence of selected essential oils. *Acta Biochimica Polonica*, *62*(4), 663–668. https://doi.org/10.18388/abp.2015_1096

Shaaban M T. Zayed M. Salama H S (2023). Antibacterial Potential of Bacterial Cellulose Impregnated with Green Synthesized Silver Nanoparticle Against S. aureus and P. aeruginosa. *Current Microbiology*, *80*(2), 75. https://doi.org/10.1007/s00284-023-03182-7

Singh S. Swain S. Das L. Das P C. Sahoo S. (2016). Isolation and characterization of organisms in high vaginal swab culture in preterm pregnancy (28-37 week). *Int J Reprod Contracept Obstet Gynecol*, *5*(11), 3853–3858.

Soliman A M. Shaaban M T. Dawood A S. Shaheen M N. Salama H S (2024). Microbiological studies on Escherichia coli and Klebsiella pneumoniae causing vaginal and urinary tract inflammation ; prevalence , antibiotics-resistance and natural products susceptibility. *Microbial Pathogenesis*, *196*(October), 107000. https://doi.org/10.1016/j.micpath.2024.107000

Tarini N M A. Wahid M H. Ibrahim F. Yasmon A. Djauzi S. (2010). Development of multiplex-PCR assay for rapid detection of Candida spp. *Medical Journal of Indonesia*, 83. https://doi.org/10.13181/mji.v19i2.387

Tortelli B A. Lewis W G. Allsworth J E. Member-Meneh N. Foster L. R. Reno H E. Peipert J F. Fay J C. Lewis A L. (2020). Associations between the vaginal microbiome and Candida colonization in women of reproductive age. *American Journal of Obstetrics and Gynecology*, *222*(5), 471.e1-471.e9. https://doi.org/10.1016/j.ajog.2019.10.008

Yagnik D. Serafin V. Shah A. (2018). Antimicrobial activity of apple cider vinegar against Escherichia coli, Staphylococcus aureus and Candida albicans; downregulating cytokine and microbial protein expression. *Scientific Reports*, *8*(1), 1732. https://doi.org/10.1038/s41598-017-18618-x