Journal of Healthcare and Life-Science Research
Vol. 3, No. 10, 2024 ISSN: 2181-4368

THE SYNERGISTIC ACTIVITY OF FLUCONAZOLE (FLC) RESISTANCE TO CANDIDA ALBICANS WITH A-LIPOIC ACID (ALA)

Annotation:

Fluconazole (FLC), a first-generation triazole, is one of the best widely used antifungal medications for Candida infections. To the best of our knowledge, this was the first study of in vivo and in vitro effect of the combinations of FCZ and/or A α-Lipoic acid (ALA) on VVC. This study was conducted on 100 women diagnosed with vaginitis by an Obstetrician and gynecologist in Basrah city, Iraq, between 10th August and 20th November 2023. For the diagnosis, swab samples were collected, packaged, and sent to the microbiology laboratories at the Basrah Teaching Hospital, where they were cultured on various media. Out of the (100) specimens collected from the participants women in this study, (75%) had grown on the media, only (50) of those specimens had Candida albicans growth, and only 30 (60%) of them had fluconazole-resistant Candida albicans and these isolates considered as the study population. In this study, the combination of fluconazole (FLC) with α-lipoic acid (ALA) was fully synergic, with FICI=0.3<0.1. Our findings suggest that the combination of fluconazole and α-lipoic acid (ALA) effectively synergizes against FLC-resistant isolates of C. albicans. This study also show a synergistic impact of ALA in combination with FLC against C. albicans in an in vivo setting. However, additional research is required to establish the fundamental mechanism behind this synergistic effect.

Keywords:

Candida albicans, Vulvovaginitis Candidiasis (VVC), Fluconazole (FLC), α-lipoic acid (ALA).

Information about theauthors

Maha Hassan Sahi
B.Sc. Pharmacy, College of Pharmacy, Basrah University

Dawood Chaloob Hilayail
PhD. Medical Mycology, Pharmacy College, Basrah University

Falah Hassan Shary
PhD. Clinical Biochemistry, Pharmacy College, Al-Maaqal University

Introduction

Candida albicans, which is a commensal organism that is found in 30–70% of healthy people's skin, gastrointestinal and genitourinary tracts, and oral and ocular flora (1,2), therefore, is the major agent of all distinct forms of candidiasis (3), especially Vulvovaginitis Candidiasis (VVC), which an infection of the vulva and vaginal mucosa by yeasts of the genus Candida (4) that is correlated with both infection and colonization and accounts for 80–90% of all cases (5–7). Most reproductive-aged women (75%) will experience vaginal candidiasis at least one episode, and 40%-50% will experience a recurrence (8). C. albicans is the most typical human pathogens, ranging from simple mucosal

infections to systemic (fetal) diseases with disseminated bloodstream infections that contribute significantly to a high death rate and account for more than 40% of all mortality rates (9,10).

Fluconazole (FLC) is one of the best widely used antifungal medications for Candida infections (11) due to FLCC's beneficial properties (bioactivity, bioavailability, and safety profile) (12). Women with VVC problems were studied by Sobel *et al.*, who compared the efficacy of single- and double-dose fluconazole courses. Microbiological and clinical responses were found to be superior with the two-dose regimen (13). Small quantity of fluconazole is processed by the liver, but 80-90 percent of the medicine is excreted unchanged by the renal, making this antibiotic particularly effective for treating Candida UTIs. The half-life in plasma is around 30 hours, and only 12% of the drug is bound to serum proteins (14).

Fluconazole is fungistatic rather than fungicidal; hence, medication in the presence of this antifungal allows acquired resistance to develop (15). Azole resistance in Candida spp. may be caused by alterations to the target enzyme or by a decrease in the drug's accessibility to the target. Additionally, repeated, extended, and overdosed antifungal medication treatments led to an increase in therapy failures and the establishment of several fluconazole-resistant C. albicans strains (16). The rise of azole-resistant isolates, particularly in cases of invasive infections, has been exacerbated by inappropriate drug dosing, overuse, and prolonged hospital stays (17,18).

In addition, due to harmful effects on human cells and undesirable effects, the present antifungal treatment has significant limitations (19,20). As the treatments used in candidiasis are not always targeted and correctly prescribed, Candida spp. have become increasingly resistant to standard antifungal medications (21). According to the CDC, around 7% of all Candida are fluconazole-resistance. In the United States (US), the incidence of fluconazole-resistant C. albicans is between 5% and 2% (15).

The increased rate of antimicrobial resistance seen in recent years can be traced to the overuse of these treatments in a highly linked global population (21–24). So, microbial resistance has been a cause for concern due to the fact that investment in antibiotic research has decreased significantly over time compared to that of expensive medications such as chemotherapeutics (25). Due to its improper use or the selection of strains with innate resistance mechanisms, antifungal medication resistance has developed as a significant problem that requires rapid attention (26). Abusing antibiotics makes the problem worse because it upsets the microbiome, which makes it easier for Candida albicans to grow (27).

The World Health Organization (WHO) states that over 80% of individuals use conventional medicines, mainly derived from plants and their byproducts, to combat infectious illnesses (28,29). Scientists have taken an interest in natural products from plants due to their antifungal capabilities (30). The development of azole resistance has prompted issue of combining these antifungals to enhance therapeutic effectiveness (31).

To the best of our knowledge, this was the first study of in vivo and in vitro effect of the combinations of FLC and/or ALA on VVC. This research could lead to the advancement of effective medications against pathogenic fungi (32). Compounds with diverse biological activities are widely found in nature, therefore, this research assessed the synergistic activity of FLC in combination with ALA at the lowest inhibitory concentration (MIC) with the largest inhibition zones against fluconazole-resistant Candida albicans responsible for VVC.

Methodology

Patients, Samples collection

This study was conducted on 100 women diagnosed with vaginitis by an Obstetrician and gynecologist in Basrah city, Iraq, between 10th August and 20th November 2023. For the diagnosis, swab samples

were collected, packaged, and sent to the microbiology laboratories at the Basrah Teaching Hospital, where they were cultured on various media.

Drugs and their Mechanism of action

Fluconazole (FLC)

Fluconazole (FLC) is an antifungal drug from the triazole family and one of the most regularly used antifungal agents of candidiasis. FLC can invade any part of the body, including the mouth, throat, esophagus, lungs, bladder, genital area, and blood (33,34). FLC interacts with 14-demethylase is a cytochrome P-450 enzyme that changes lanosterol into ergosterol (35). Fluconazole suppresses the production of ergosterol, which leads to an increase in the permeability of the fungal cells, therefore inhibiting endogenous respiration and suppressing yeast growth. It's also important to note that the loss of sterols happens at the same time that 14-methyl sterols build up in fungi. Because of a change in the ERG11 gene, lanosterol C14-α demethylase, can't bind to fluconazole as well. This makes the drug less effective (36).

In this study, we used pure powder of fluconazole (FLC) from Sama Al-Fayhaa for Pharmaceutical Industries (FPI), Iraq, and its chemical structure presented in the Figure 1 below:

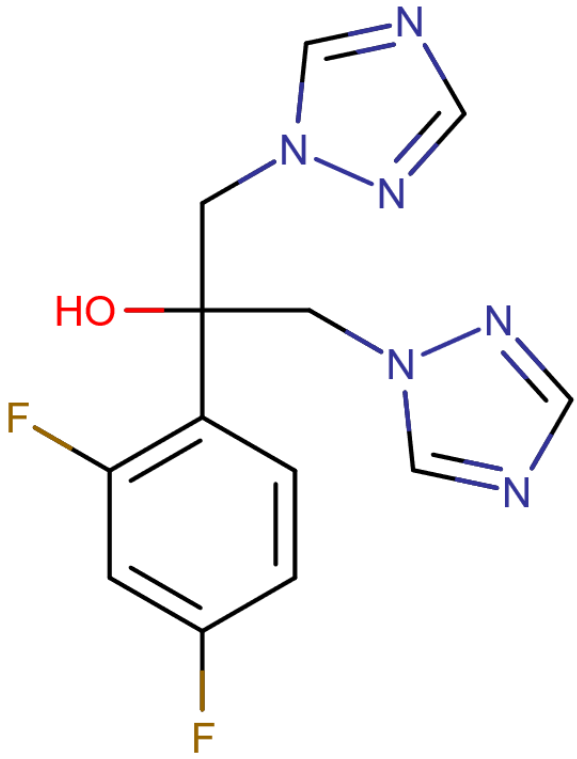

Figure 1 The Structure of Fluconazole ($C_{13}H_{12}F_2N_6O$)(37)**.**

α-Lipoic acid (ALA)

α-lipoic acid (ALA), which is a naturally occurring fatty acid, is a caprylic acid-derived antioxidant, is a compound usually found in mitochondria, essential for different enzymatic functions (38). Many people purchase alpha-lipoic acid as an herbal supplement. Many foods contain alpha-lipoic acid, including yeast, spinach, broccoli, red beets, carrots, potatoes, and organ meats like liver or kidney (39).

Briefly, ALA can create covalent connections with proteins and shows promise as a therapeutic agent. A-lipoic acid (ALA) has one chiral center and an asymmetric carbon, which makes it possible for it to have two optical isomers: R-lipoic acid and S-lipoic acid (Figure 2) (40). Therefore, ALA exists in the

S and R enantiomers, which are presented equally and regarded as mirror reflections of one another. The R form occurs naturally, whilst the S form is synthesized using chemical methods (41).

R-α-lipoic acid S-α-lipoic acid

Figure 2. The chemical composition of enantiomers of ALA (41)**.**

Figure 3. Kal Alpha Lipoic Acid 600mg, USA.

Preparation of Culture Media

All media, including Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth, Nutrient Broth, Blood Agar, MacConkey Agar, HiCrome™ Candida Differential Agar, and RPMI 1640 Agar, were made according to the manufacturer's instructions (HIMEDIA).

Cultivation of Microorganisms

This study cultivated 100 women's vaginal swabs in two broths, Nutrient broth, which used for cultivation and enrichment of less fastidious bacteria; and Sabouraud Dextrose Broth, which used for cultivation of yeasts, moulds and aciduric microorganisms from clinical and non-clinical samples; then incubated all tubes in incubation.

Cultural characteristics in Nutrient Broth detected after an incubation at temperature of 35-37°C for 18-48 hours; while in Sabouraud Dextrose Broth, cultural characteristics were detected after an incubation at temperature of 20-25°C for 3-5 days; turbidity liquid refers to growth.

After that, each isolate had given growth in broths, subcultured on Nutrient Agar, Blood Agar, MacConkey Agar, and Sabouraud Dextrose Agar (SDA); to identify the morphological characterization of microorganisms on media.

All of the tested isolates should be subcultured into Sabouraud Dextrose Agar (SDA) agar from sterile plates so that the purity and viability of the isolates may be guaranteed. The temperature must be kept at 37°C throughout the incubation period.

Only those isolates had given growth on SDA, and expected those were Candida, subcultured on HiCrome Candida Differential Agar, to differentiate Candida species.

The Candida spp. cultures were then transferred after 24 hours for the inoculum procedure, and 5 milliliters of sterile normal saline was used to suspend the colonies based on the CLSI M38-A2 report from 2002.

The resulting suspension needs to be well stirred for 15sec, and then the cell density (CD) needs to be adjusted using a spectrophotometer by adding adequate sterile saline to raise the transmittance to that of a (0.5) McFarland standard. This process should be repeated three times. Using this procedure, you will end up with a yeast stock suspension that has between 1×10^6 and 5×10^6 cells per milliliter.

After identifying these microorganisms, only fungus isolates were cultivated on RPMI 1640 Agar, which is used for the determination of the susceptibility of fungus to antifungal agents by determining MIC values for various antifungal agents.

Antifungal Susceptibility Testing (AFST)

AFST is suitable for analyzing Candida spp. directly from colonies cultivated on nonselective media using the gradient-strip technique (42). In order to determine the suitable range of antibiotic concentrations for the combination studies, the minimum inhibitory concentrations (MICs) of FLC, and ALA against Candida albicans were assessed using a broth microdilution method. This involved using dual serial dilutions in RPMI 1640 medium, following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI)'s approved standard M60 for yeasts. Three iterations of experiments were conducted, and the average MIC value was computed. The experiment was conducted using 96-well flat-bottomed microtitration plates, following the protocol described in a publication by Quan *et al.* (2006) (43). Following 15 seconds of agitation, the plates were placed in an incubator at a temperature of 35°C without any shaking. After 24 hours of incubation, readings were taken using both visual observation and optical density measurement at a wavelength of 630 nm.

Chequerboard microdilution assay

Assays were performed on 30 FLC-resistant C. albicans isolates using the approved standard M60 from CLSI. At first, the fungal suspension in RPMI 1640 medium was 10^3 CFU/ml, and the final values ranged from 0.128 to 75 ug/ml for FLC, and ALA. The plates were placed in an incubator at a temperature of 35°C for 24 hours. Following this, a visual assessment of the MICs was conducted, the optical density at a wavelength of 630 nm (OD630) was measured, and the background optical densities were subtracted from the values obtained for each well. Every individual sample was tested three times.

The chequerboard assay was used to evaluate the interaction of drug combinations (FLC-ALA). The data obtained through spectrophotometry were evaluated using the fractional inhibitory concentration index (FICI), which is based on the Loewe additivity (LA) theory (44).

FICI

FICI, which was defined as the sum of the MIC of each agent when administered in combination divided by the MIC of this agent when administered alone, is represented by the following equation:

$$\boldsymbol{FICI = FIC_A + FIC_B = \frac{C_{A_{comb}}}{MIC_{A_{alone}}} + \frac{C_{B_{comb}}}{MIC_{B_{alone}}}} \qquad \mathbf{1}$$

where MIC_A alone and MIC_B alone are the MICs of drugs A and B when acting alone and $C_{A_{comb}}$ and $C_{B_{comb}}$ are concentrations of drugs A and B at the is effective combinations, respectively. FICI values

of ≤0.5 indicated synergy, and values >4 indicated antagonism. A FICI value more than 0.5 but less than or equal to 4 was classified as indifferent (43,45).

Agar well diffusion

Candida albicans isolates were tested by agar diffusion assay, which was performed with 2% glucose supplemented RPMI 1640 agar by determining MIC values for various antifungal agents. Candida albicans was successfully suspended at the usual McFarland 0.5 concentration (1×10^6 CFU/mL). (46) Yeast species were cultured on 24-hour plate cultures to prepare the inoculum. A yeast suspension of 1×10^6 to 5×10^6 cells/mL was produced by suspending the colonies in 0.85% saline and comparing the turbidity to the 0.50 McFarland standard. A 100-mL aliquot of 10^6 CFU.mL^{-1} suspension was spread uniformly on yeast extract–peptone– dextrose agar plates. (43)

Analysis of Synergistic Potential with an Antifungal Agent In Vivo

Rats were shaved on the back, and two injection sites were chosen at random. The rats were then randomized into 5 groups with a total of 5 individuals in each group. Candida albicans stock suspension, which contained between 1 to 5 $\times10^6$ cells/ml, was injected into groups (A, B, C, D, and E), respectively. The procedures are described in more detail below:

(A) was treated with a Solution of (8µg/0.05ml) Lipoic acid (LA) with (4µg/0.05ml) of Fluconazole (FLC),

(B) was treated with a Solution of (16µg/0.05ml) Lipoic acid (LA) with (4µg/0.05ml) of Fluconazole (FLC),

(C) was treated with a Solution of (32µg/0.05ml) Lipoic acid (LA) with (4µg/0.05ml) of Fluconazole (FLC), and finally,

(D) Positive Control was treated with a Solution-Fluconazole (FLC) (4µg/0.01ml) only,

(E) Negative Control was blank group untreated (100 ml of DMSO).

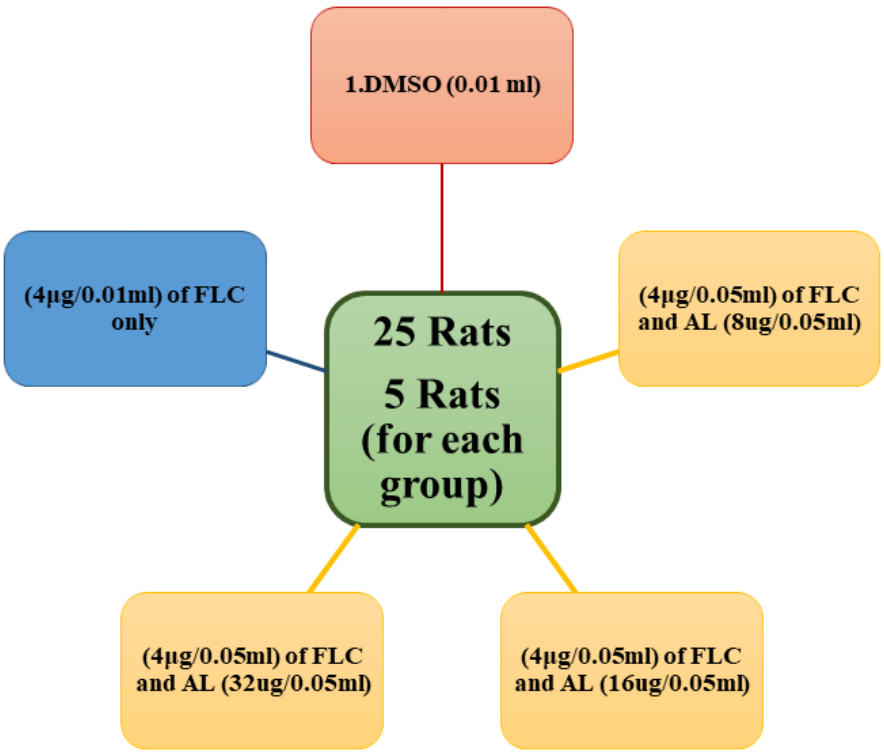

Figure 4. In Vivo Study Design (AL & FLC)

To compare the treated and untreated groups and to see how the wound surface changed over time, we weighed the rats on days 0, 3, 6, 9, 12, and 15 and measured the wound area using the rule technique (length by breadth).

For best results when inducing cutaneous candidiasis, we used Candida albicans stock solution. Using a hair removal shaving machine, the rats' back hairs are shaved, and a 2 cm^2 region is chosen for the application of the produced formulations. The Derma roller, a device rolled against the skin to develop micropores, is used the following day to create the pores. Transdermal application of medication has many advantages. Successful medicine delivery to the dermis was facilitated by penetrating the stratum corneum using this method. A cotton swab was used to apply the produced formulations to the skin of the rats. The ready-made mixtures were then swabbed into the skin of the rats. The rats received a single treatment once a day for six days. The positive control group was given simply Fluconazole (FLC), while the untreated group received nothing. After six days, the groups' responses were compared to those of a control group. To analyze the development of the wound surface and make a comparison between the treated and untreated groups, the wound area was determined using the rule technique (length multiplied by width), and the weight of the rats in each group was recorded on days 0, 3, 6, 9, 12, and 15 of the experiment.

Statically Analysis

Using Microsoft 365 Excel 2024 and SPSS (IBM version 26.0), the data were analysed. In this study, results were provided as mean ± SD and frequencies as percentages. The Chi-square test, and ANOVA test were used to compare distributed groups. Probability levels less than 0.05 were deemed physiologically significant, and p-values less than 0.01 were deemed extremely significant.

Results

As presented in the Figure 5, most of the participants women in this study were those aged between 20 and 30 years old (40%), illiterate (48%), students (47%) and those with low economic status (54%).

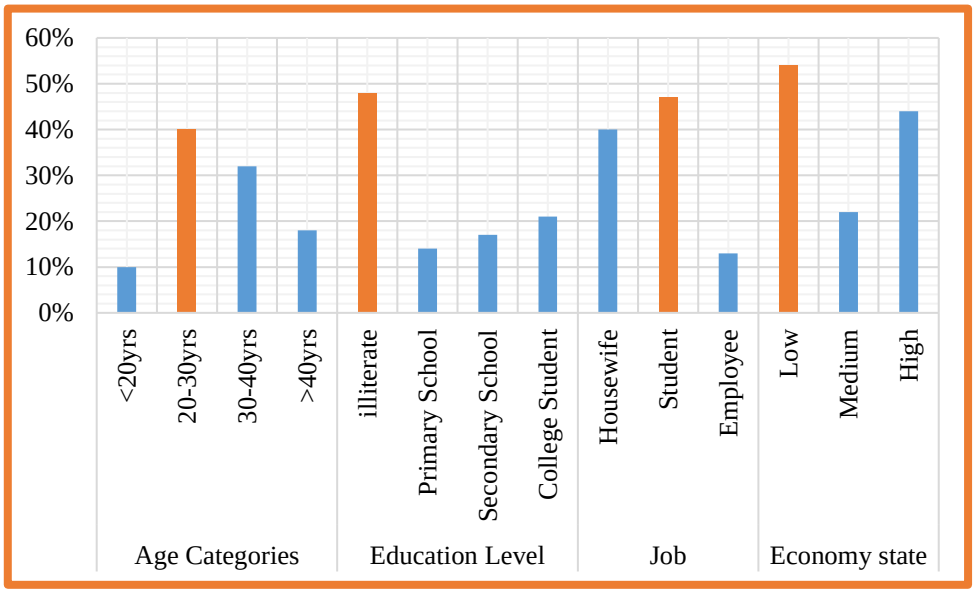

Figure 5. The Socio-demographic Characteristics of Participants Women in this study

As presented in the Table 1 and **Error! Reference source not found.**, out of (41) women in this study had past vaginitis with different causes, only (17%) of those had itching, (16%) had discharge, (23%) had smell, and (23%) had redness. While, out of (69) women in this study without past vaginitis, only (18%) of those were suffering from itching, (11%) suffered from discharge, (19%) suffered from a smell, and (20%) suffered from redness. The distribution between past vaginitis and symptoms of

infections (discharge, smell, and redness) were significant with p-values (0.02, 0.01, and 0.02, respectively) compared to (0.05), while it was not significantly with itching, with p-value (0.2).

Table 1. The Cross Tabulation between the Symptoms that Suffering Women and Past Vaginitis

Symptoms		Past Vaginitis		Total	Stat.	
		Yes	No		r	Sig.
Itching	Yes	17	18	35	1.276	0.259
	No	24	41	65		
Discharge	Yes	16	11	27	5.098	**0.024**
	No	25	48	73		
Smell	Yes	23	19	42	5.669	**0.017**
	No	18	40	58		
Redness	Yes	23	20	43	4.864	**0.027**
	No	18	39	57		
Total		**41**	**59**	**100**		

As presented in the Figure 6, out of the (100) specimens collected from the participants women in this study, (75%) had grown on the media, only (50) of those specimens had *Candida albicans* growth, and only 30 (60%) of them had *fluconazole-resistant Candida albicans* and these isolates considered as the study population.

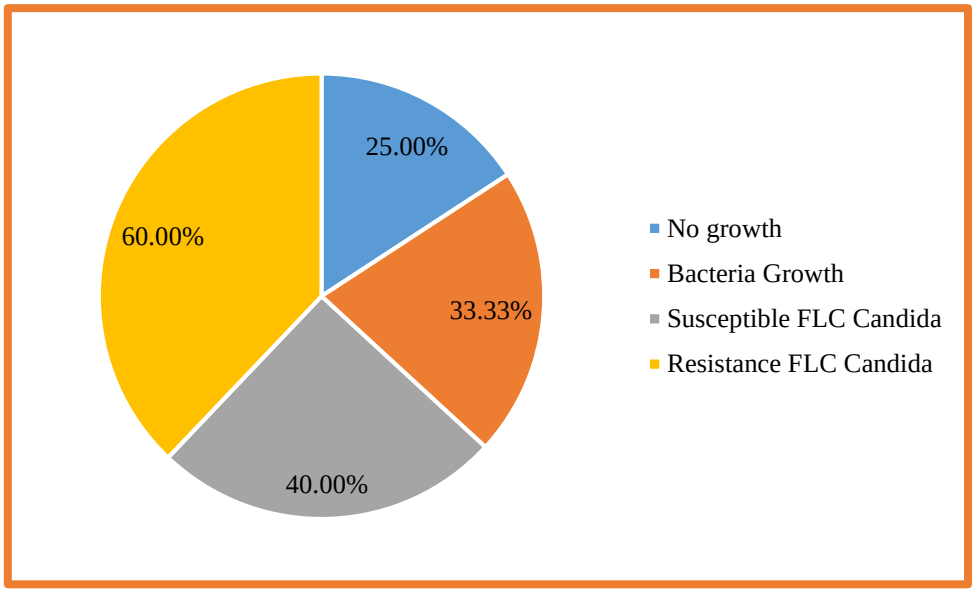

Figure 6. The Distribution of the Women Based on the Culture Results.

As presented in the Table 2 and Figure 7, the inhibition zone of fluconazole (FLC) against Candida albicans in this study was (13.53±1.83) mm when it was used in concentration (150µg), and it was the most zone length, while it was (4.77±1.48) mm when it was in (0.128µg), and FLC was resistant to Candida isolates in different concentrations. The inhibition zone of Lipoic acid (LA) against Candida albicans in this study varied between (16.27±1.93) mm when it was used in concentration (150µg), and (6.33±1.42) mm when it was in (0.128µg), and it was considered susceptible in more concentrated compared to lowest concentrated that was resistant.

While, the inhibition zone dimeters of the combination of both (FLC & LA) were raised in all used concentrations, which was (25.57±0.5) mm when they were used in concentration (150µg) as the most concentrated, and it was (14.3±0.79) mm when they were in (0.128µg), that was higher than the longest inhibition zone of FLC alone in the highest concentration (150µg).

Table 2. The Susceptibilities of C. albicans strains against FLC alone, AL alone, and in combination between them.

		FLC			AL			FLC & AL		
Conc.	**N**	**Mean**	**SD**	**SEM**	**Mean**	**SD**	**SEM**	**Mean**	**SD**	**SEM**
150 ug	**30**	13.53	1.83	0.34	16.27	1.93	0.35	25.57	0.50	0.09
75 ug	**30**	12.03	1.88	0.34	14.87	1.98	0.36	24.17	1.42	0.26
32 ug	**30**	11.03	2.08	0.38	13.77	1.85	0.34	24.03	0.81	0.15
16 ug	**30**	9.80	1.61	0.29	12.30	1.80	0.33	23.23	0.43	0.08
8 ug	**30**	8.57	1.36	0.25	10.90	1.86	0.34	19.07	1.08	0.20
4 ug	**30**	7.53	1.43	0.26	9.50	1.74	0.32	18.53	1.17	0.21
2 ug	**30**	6.83	1.42	0.26	8.37	1.81	0.33	17.23	0.73	0.13
1 ug	**30**	6.00	1.53	0.28	7.43	1.68	0.31	16.13	0.86	0.16
0.5 ug	**30**	5.33	1.35	0.25	6.90	1.61	0.29	15.50	0.86	0.16
0.128 ug	**30**	4.77	1.48	0.27	6.33	1.42	0.26	14.30	0.79	0.15
Total	**300**	**8.54**	**3.25**	**0.19**	**10.66**	**3.77**	**0.22**	**19.78**	**4.02**	**0.23**

The probability value for all three groups (FLC, AL, and FLC & AL) was 0.000, which was highly statistically significant compared to (0.001).

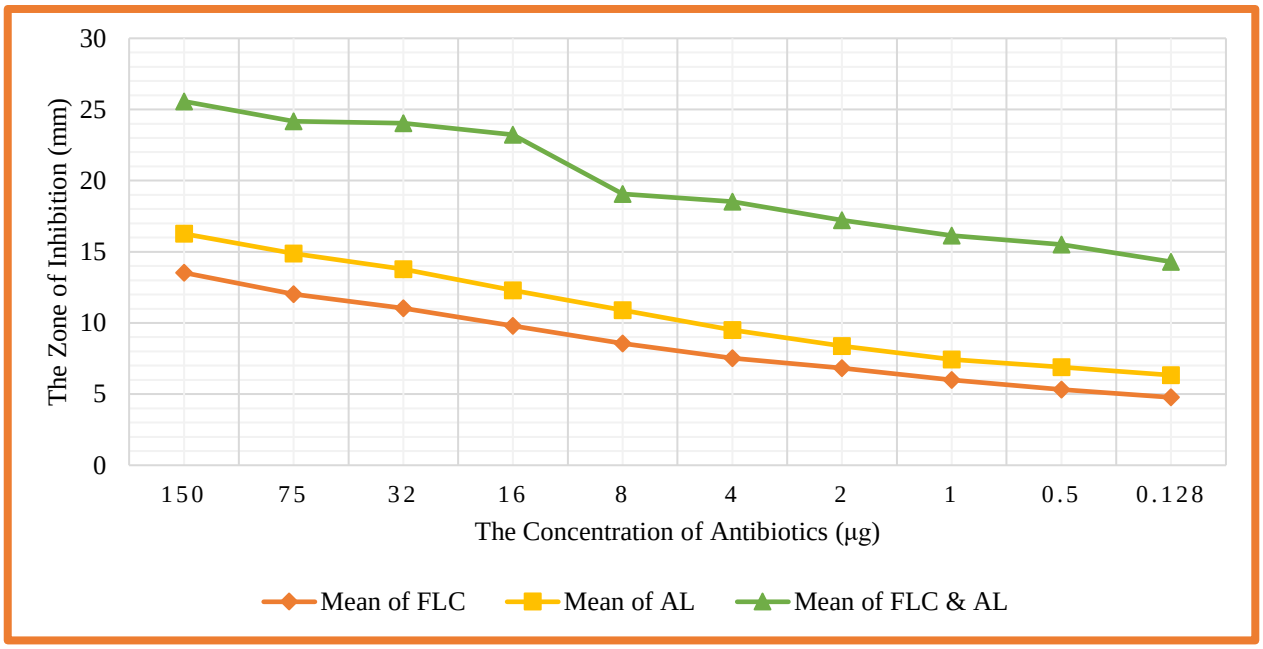

Figure 7. The Statistics of FLC and AL against Candida albicans

As presented in the Table 3 and Figure 8, the susceptibility of fluconazole (FLC) against Candida albicans isolates in this study in all used concentrations based on the inhibition zone diameter was the worst compared to other groups (ALA, & ALA with FLC). While the susceptibility of FLC combined with α-lipoic acid (ALA) was the best in all used concentrations. The effect of Antifungal Extracts (alone and/or both) was increased by increasing the concentration of antifungal extract.

Table 3. The Average of The Zone of Inhibition (mm) of Studied Antibiotics based on the Different Concentration (µ)

Item	**150 ug**	**75 ug**	**32 ug**	**16 ug**	**8 ug**	**4 ug**	**2 ug**	**1 ug**	**0.5 ug**	**0.128 ug**
FLC	13.53	12.03	11.03	9.80	8.57	7.53	6.83	6.00	5.33	4.77
ALA	16.27	14.87	13.77	12.30	10.90	9.50	8.37	7.43	6.90	6.33
FLC & ALA	25.57	24.17	24.03	23.23	19.07	18.53	17.23	16.13	15.50	14.30

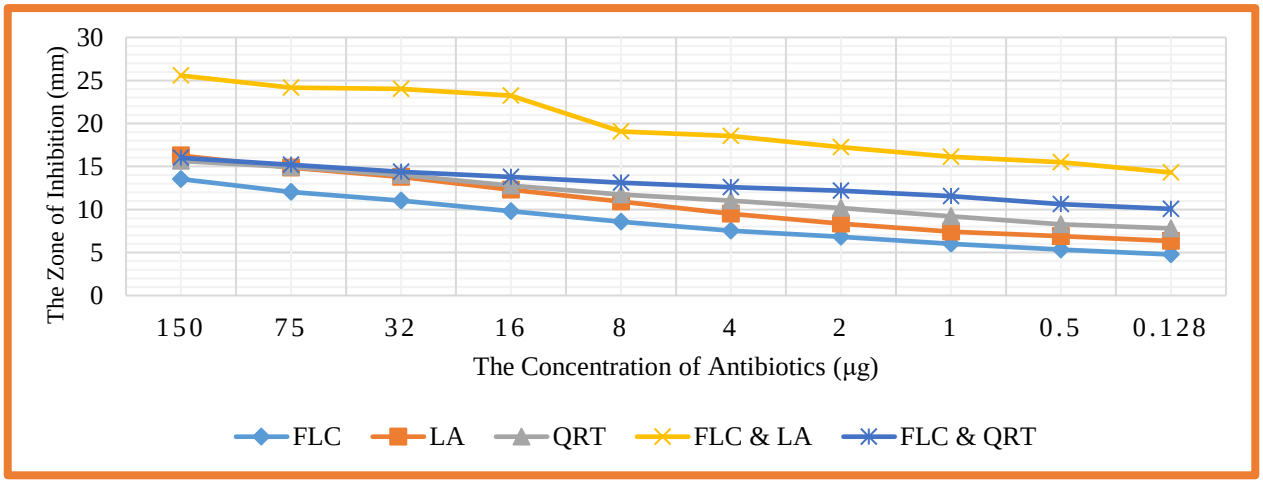

Figure 8. The Average of The Zone of Inhibition (mm) of Studied Antibiotics based on the Different Concentration (µg)

As presented in the Figure 9, the group of rats that received 4 µg of FLC combined with 32 µg of AL reached 100% healing within 15 days of treatment compared to those 4 µg of FLC combined with 8 µg of AL reached 74 % within 15 days of treatment, this relation was statistically significant. As a result of the infection, the rats' wounds became acute, suppurative, and vividly red. During the treatment, which lasted from 0 to 15 days, our findings revealed that the groups with varied doses of AL in conjunction with FLC underwent an exceptionally speedy recovery process. In contrast, the group of rats treated with FLC only showed a marginal recovery and a substantial loss of weight. The untreated rats (the control group) did not recover and instead experienced excessive weight loss, which ultimately led to their death.

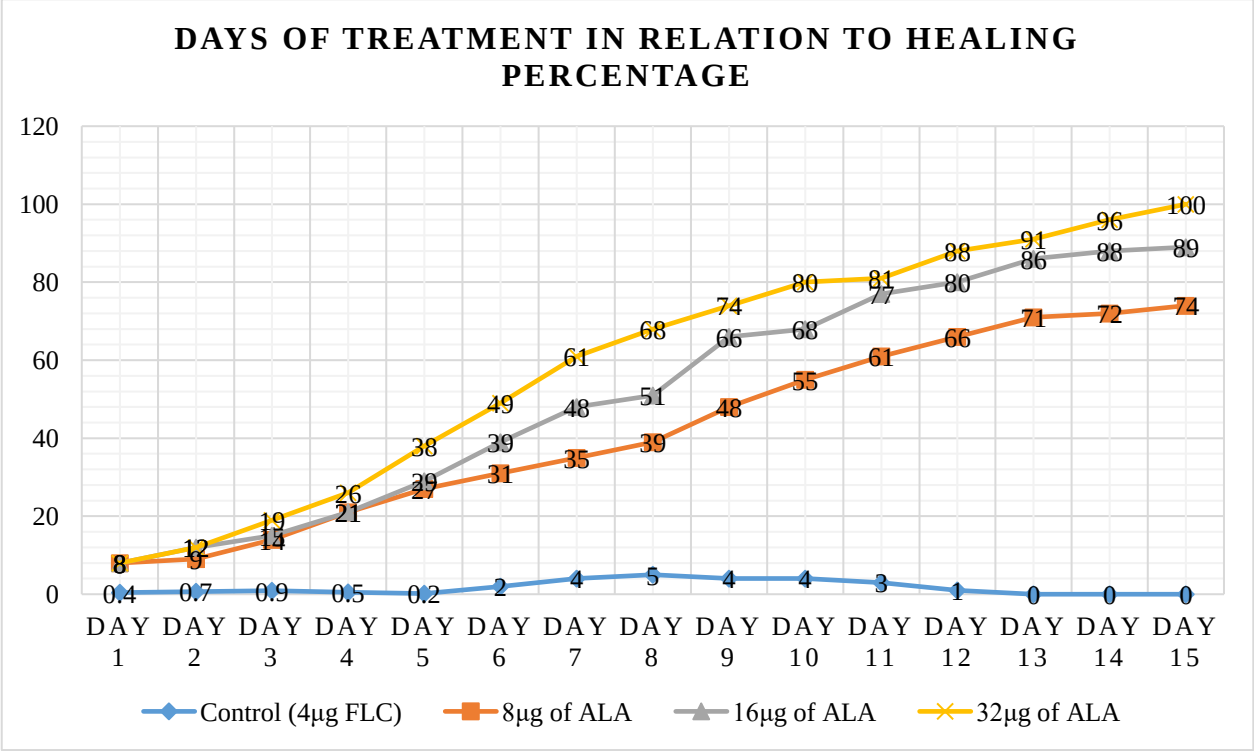

Figure 9. The healing time of the different groups receiving different concentrations of LA & (4 µg) of FLC in relation to healing percentage.

As presented in the Table 4, the combination of fluconazole (FLC) with Lipoic acid (LA) was fully synergic, with FICI=0.3<0.1.

Table 4. The Fractional Inhibitory Concentration (FIC) Index of the Studied Antibiotics

Item	MIC
FLC	4
LA	16
FLC and LA	1
FIC	0.3
Full Synergy	

Discussion

The incidence of Vulvovaginitis Candidiasis (VVC), an infection of the vulva and vaginal mucosa by yeasts of the genus Candida (4) that causes irritation, pruritus, and vaginal discharge (47), has skyrocketed in recent years. VVC is the leading cause of women seeking gynecological care worldwide (48). Most reproductive-aged women (75%) will experience vaginal candidiasis at least one episode, and 40%-50% will experience a recurrence (8).

In this study, out of one hundred adult women with pre-diagnosed with vaginitis by an obstetrician and gynecologist, 75 (75%) of them had VCC, and only (50) of those specimens had *Candida albicans* growth. Another study by Hedayati, M. T. *et al.* (49) found among 234 women with vaginitis, 66 (28.2%) had VVC, about a quarter of these people had RVVC. In the United States, another investigation by Foxman *et al.* found that 6.5% of women over the age of 18 reported experiencing at least one episode of VVC, and 8% experienced at least four such episodes in the year prior to the survey (50). It is interesting that regional differences exist in the spread of Candida species (5). Possible causes for the disparity include incorrect diagnosis of pathogens, poor administration, antibiotic resistance, inadequate dosing, treatment by laypeople, neglect of proper hygiene, and infection of the digestive tract (49).

VVC is symptomatic and manifests with inflammatory symptoms in the absence of additional causal agents. (51) Itching, hyperemia, leucorrhea, burning, vaginal discomfort, vaginal and vulvar erythema, abnormal white vaginal discharge (which may be minimal or thick, watery, or cottage cheese-like (52), and edema and erythema or swelling of the vulva and the area outside it are the mainly common clinical symptoms of VVC (53–55). In this study, 59% of women were diagnoses with vaginitis as the first episode based on their symptoms. A study in Australia found that 37% of asymptomatic women under the age of 50 with a VVC history were vaginally infected with Candida (56). Out of (41) women in this study had past vaginitis with different causes, only (17%) of those had itching, (16%) had discharge, (23%) had smell, and (23%) had redness. While, out of (69) women in this study without past vaginitis, only (18%) of those were suffering from itching, (11%) suffered from discharge, (19%) suffered from a smell, and (20%) suffered from redness. In the study by Hedayati, *et al.*, erythema accompanied by itching was the most prevalent symptom in individuals with VVC. Researchers from the University of Michigan also identified itching as the most prevalent symptom of VVC (49).

Treatment for fungal diseases typically takes a long time. The majority of drugs that kill or suppress fungal pathogens are also hazardous to the host because both humans and fungi are eukaryotes (57). Eliminating risk factors is necessary. It is important to keep the affected area dry (58). Since more than 20% of women may have yeast as part of their natural vaginal flora and are asymptomatic, don't need treatment (59). As a result, treating vaginal candidiasis without symptoms is not advised (60). In asymptomatic hosts, neither the CDC recommendations nor the Canadian Guidelines on Sexually Transmitted Infections recommend therapy regardless of a positive culture result (47,57). The Canadian Society of Obstetricians and Gynecologists says that therapy is not necessary unless the condition gets worse if there are no symptoms, but a lab test shows that Candida is present (61).

Antifungal medications have a broad spectrum of effects (62). Considerations for selecting a topical antifungal medication include its price, sensitivity to the medication, convenience of application, and

taste. Furthermore, whether a patient has simple or difficult VVC affects the treatment plan (63). During 2 to 3 days, the symptoms often go away, with short-term therapy using local azoles for up to 3 days, 80 to 90% of the time, this treatment is successful (52).

Unchecked antifungal use has supported to the rapid emergence of resistance in strains isolated from Candida spp., highlighting the necessity for new options for fungi treatment to reduce the adverse effects of present antifungals and improve their efficacy (64,65). In this study, we used agar (disc) diffusion, and 30 of 50 (60%) of *Candida albicans* isolates were *fluconazole-resistant Candida albicans*. In the study by Mohamadi, J., *et al.*, the resistance of C. albicans isolated from vaginitis to eight antifungal drugs revealed that fluconazole resistance was the highest at 79%. This disparity in outcomes may be attributable to distinct age groups and sampling (66).

α-lipoic acid (ALA), which is a naturally occurring fatty acid, has therapeutic potential, and many people purchase it as an herbal supplement (40). The study by Huang, *et al.*, (2010) (67) reported that linoleic acid (LA) has antimicrobial activities against C. albicans. Another study by Shebi and Ezhilarasan (2020) found that Alpha-lipoic acid has a potent inhibitory effect on the growth of C. albicans at low concentrations, suggesting its potential as a therapeutic alternative for treating C. albicans infection (68). To the best of our information, there have been no earlier publications on the study of fluconazole (FLC) in combination with α-lipoic acid (ALA), so we can't compare our results with others.

In this study, the combination of fluconazole (FLC) with α-lipoic acid (ALA) was fully synergic, with FICI=0.3<0.1, while another study by Wang, *et al.*, (2022) showed no synergistic effect between ALA and FLC, ALA (at 0.01 mM) even slightly grew the FLC's MIC values, which might be due to low ALA concentrations promoting the C. albicans growth with biofilm status, while GLA at 0.1mM inhibit the biofilm formation (69). The possible antibiofilm effects of essential fatty acids (EFAs) may be attributed to their ability to impact the adhering surface, alter cell-membrane fluidity, decrease extracellular polysaccharide or hyphae production, and regulate quorum-sensing systems (70). This difference between this study may be due to the ability of Wang, *et al.*, (2022)' isolates to produce the mature biofilm.

Conclusion

Our findings suggest that the combination of fluconazole and α-lipoic acid (ALA) effectively synergizes against FLC-resistant isolates of C. albicans. This study also show a synergistic impact of α-lipoic acid (ALA) in combination with FLC against C. albicans in an in vivo setting. However, additional research is required to establish the fundamental mechanism behind this synergistic effect.

References

1. Wang Y. Looking into Candida albicans infection, host response, and antifungal strategies. Virulence [Internet]. 2015 Jan 1 [cited 2021 Jun 14];6(4):307–8. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=kvir20
2. Ghaddar N, Anastasiadis E, Halimeh R, Ghaddar A, Dhar R, Alfouzan W, *et al.* Prevalence and antifungal susceptibility of Candida albicans causing vaginal discharge among pregnant women in Lebanon. BMC Infect Dis [Internet]. 2020 Jan 13 [cited 2021 Jun 14];20(1):1–9. Available from: https://doi.org/10.1186/s12879-019-4736-2
3. Filler SG, Sheppard DC. Fungal Invasion of Normally Non-Phagocytic Host Cells. PLoS Pathog [Internet]. 2006 Dec 29;2(12):e129-. Available from: https://doi.org/10.1371/journal.ppat.0020129
4. Criseo G, Scordino F, Romeo O. Current methods for identifying clinically important cryptic Candida species. Vol. 111, Journal of Microbiological Methods. Elsevier B.V.; 2015. p. 50–6.
5. Mendling W, Brasch J. Guideline vulvovaginal candidosis (2010) of the german society for gynecology and obstetrics, the working group for infections and infectimmunology in gynecology

and obstetrics, the german society of dermatology, the board of german dermatologists and the german speaking mycological society. Mycoses [Internet]. 2012 Jul [cited 2021 Jun 14];55(SUPPL. 3):1–13. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1439-0507.2012.02185.x

6. López-Martínez R. Candidosis, a new challenge. Clin Dermatol [Internet]. 2010;28(2):178–84. Available from: https://www.sciencedirect.com/science/article/pii/S0738081X09002569
7. Török E, Moran E, Cooke F. Oxford handbook of infectious diseases and microbiology2016 . [cited 2021 Jun 14]; Available from: https://books.google.com/books?hl=ar&lr=&id=h4GuDQAAQBAJ&oi=fnd&pg=PT13&dq=Török,+E.,+Moran,+E.,+%26+Cooke,+F.+(2016).+Oxford+handbook+of+infectious+diseases+and+microbiology.+Oxford+University+Press.&ots=Bf_wnUVJ6g&sig=i2fBT8BnyOkQUcxms3lRyPC4LR8
8. Sobel JD. Genital candidiasis. Vol. 42, Medicine (United Kingdom). Elsevier Ltd; 2014. p. 364–8.
9. Cui J, Ren B, Tong Y, Dai H, Zhang L. Synergistic combinations of antifungals and anti-virulence agents to fight against Candida albicans. Virulence [Internet]. 2015 Jan 1 [cited 2021 Jun 14];6(4):362–71. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=kvir20
10. Lohse MB, Gulati M, Johnson AD, Nobile CJ. Development and regulation of single- and multi-species Candida albicans biofilms. Nat Rev Microbiol. 2018 Jan 3;16(1):19–31.
11. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, *et al.* Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-Year Analysis of Susceptibilities of *Candida* Species to Fluconazole and Voriconazole as Determined by CLSI Standardized Disk Diffusion. J Clin Microbiol. 2010 Apr;48(4):1366–77.
12. Arendrup MC, Patterson TF. Multidrug-Resistant Candida: Epidemiology, Molecular Mechanisms, and Treatment. J Infect Dis. 2017 Aug 15;216(suppl_3):S445–51.
13. Sobel JD, Kapernick PS, Zervos M, Reed BD, Hooton T, Soper D, *et al.* Treatment of complicated Candida vaginitis: Comparison of single and sequential doses of fluconazole. Am J Obstet Gynecol. 2001 Aug 1;185(2):363–9.
14. Moudgal V, Sobel J. Antifungals to treat *Candida albicans*. Expert Opin Pharmacother. 2010 Aug 10;11(12):2037–48.
15. Berkow E, Lockhart S. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist. 2017 Jul;Volume 10:237–45.
16. Kullberg BJ, Arendrup MC. Invasive Candidiasis. New England Journal of Medicine. 2015 Oct 8;373(15):1445–56.
17. Liao X, Qiu H, Li R, Guo F, Liu W, Kang M, *et al.* Risk factors for fluconazole-resistant invasive candidiasis in intensive care unit patients: An analysis from the China Survey of Candidiasis study. J Crit Care. 2015 Aug 1;30(4):862.e1-862.e5.
18. Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. Mycoses. 2015 Jun;58:2–13.
19. Epstein JB, Dawson JR, Buivids IA, Wong B, Le ND. The effect of a disinfectant/ coolant irrigant on microbes isolated from dental unit water lines. Special Care in Dentistry. 2002 Jul;22(4):137–41.

20. Gableri IG, Barbosa AC, Velela RR, Lyon S, Rosa CA. Incidence and anatomic localization of oral candidiasis in patients with AIDS hospitalized in a public hospital in Belo Horizonte, MG, Brazil. Journal of Applied Oral Science. 2008;16:247–50.
21. Nasiri S, Mahmoudvand H, Shakibaie M, Mousavi SAH, Sepahvand A. Antifungal effects of zinc nanoparticles green synthesized by Lavandula angustifolia extract, alone and combined with nystatin against Candida albicans, a major cause of oral candidiasis. Journal of HerbMed Pharmacology,. 2022;
22. Michael CA, Dominey-Howes D, Labbate M. The Antimicrobial Resistance Crisis: Causes, Consequences, and Management. Front Public Health. 2014 Sep 16;2.
23. HOMSIANG W, KAMONKHANTIKUL K, ARKSORNNUKIT M, TAKAHASHI H. Effect of zinc oxide nanoparticles incorporated into tissue conditioner on antifungal, physical, and mechanical properties. Dent Mater J. 2021;40(2):481–6.
24. Mohamadi J, Havasian MR, Panahi J, Pakzad I. Antifungal drug resistance pattern of Candida. spp isolated from vaginitis in Ilam-Iran during 2013-2014. Bioinformation [Internet]. 2015 Apr 30 [cited 2023 Jan 26];11(4):203. Available from: /pmc/articles/PMC4479047/
25. Bax R, Green S. Antibiotics: the changing regulatory and pharmaceutical industry paradigm. Journal of Antimicrobial Chemotherapy. 2015 May 1;70(5):1281–4.
26. Ying Y, Zhao Y, Hu X, Cai Z, Liu X, Jin G, *et al. In Vitro* Fluconazole Susceptibility of 1,903 Clinical Isolates of *Candida albicans* and the Identification of *ERG11* Mutations. Microbial Drug Resistance. 2013 Aug;19(4):266–73.
27. Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, *et al.* Antibiotic Exposure as a Risk Factor for Fluconazole-Resistant Candida Bloodstream Infection. Antimicrob Agents Chemother. 2012 May;56(5):2518–23.
28. Rocha da SC, Batista de ANJ, Rosana de SC, Silvestre FN, Serpa SL, Ferreira MHI, *et al.* Synergistic Effect of the Flavonoid Catechin, Quercetin, or Epigallocatechin Gallate with Fluconazole Induces Apoptosis in Candida tropicalis Resistant to Fluconazole. Antimicrob Agents Chemother [Internet]. 2014 Feb 19;58(3):1468–78. Available from: https://doi.org/10.1128/aac.00651-13
29. Kaur R, Dhakad MS, Goyal R, Haque A, Mukhopadhyay G. Identification and antifungal susceptibility testing of Candida species: a comparison of Vitek-2 system with conventional and molecular methods. J Glob Infect Dis. 2016;8(4):139.
30. Geraldi A, Wardana AP, Aminah NS, Kristanti AN, Sadila AY, Wijaya NH, *et al.* Tropical Medicinal Plant Extracts from Indonesia as Antifungal Agents against Candida Albicans. FBL. 2022;27(9):274-null.
31. Guo N, Wu X, Yu L, Liu J, Meng R, Jin J, *et al. In vitro* and *in vivo* interactions between fluconazole and allicin against clinical isolates of fluconazole-resistant *Candida albicans* determined by alternative methods. FEMS Immunol Med Microbiol. 2010 Mar;58(2):193–201.
32. Lamoth F. Novel Therapeutic Approaches to Invasive Candidiasis: Considerations for the Clinician. Infect Drug Resist [Internet]. 2023 Dec 31;16(null):1087–97. Available from: https://www.tandfonline.com/doi/abs/10.2147/IDR.S375625
33. Rex JH, Rinaldi MG, Pfaller MA. Resistance of Candida species to fluconazole. Antimicrob Agents Chemother [Internet]. 1995 [cited 2024 Feb 13];39(1):1. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC162475/
34. Galgiani JN, Ampel NM, Blair JE, Catanzaro A, Geertsma F, Hoover SE, *et al.* 2016 Infectious Diseases Society of America (IDSA) Clinical Practice Guideline for the Treatment of

Coccidioidomycosis. Clinical Infectious Diseases [Internet]. 2016 Sep 15;63(6):e112–46. Available from: https://doi.org/10.1093/cid/ciw360

35. Spampinato C, Leonardi D. Candida infections, causes, targets, and resistance mechanisms: Traditional and alternative antifungal agents. Biomed Res Int. 2013;2013.
36. Rad KK, Falahati M, Roudbary M, Farahyar S, Nami S. Overexpression of MDR-1 and CDR-2 genes in fluconazole resistance of Candida albicans isolated from patients with vulvovaginal candidiasis. Curr Med Mycol. 2016;2(4):24.
37. Kaduk JA, Gindhart AM, Blanton TN. Crystal structure of fluconazole polymorph V, C13H12F2N6O. Powder Diffr [Internet]. 2018 Dec 1 [cited 2024 Feb 13];33(4):330–1. Available from: https://www.cambridge.org/core/journals/powder-diffraction/article/abs/crystal-structure-of-fluconazole-polymorph-v-c13h12f2n6o/0EF98E968233490354CFCE845DE70BFE
38. Packer L, Cadenas E. Lipoic acid: energy metabolism and redox regulation of transcription and cell signaling. J Clin Biochem Nutr. 2010;48(1):26–32.
39. CANSIZ D. VITAMIN-LIKE SUBSTANCES. A Guide to Vitamins and Their Effects on Diseases. 2023;126.
40. Brookes MH, Golding BT, Howes DA, Hudson AT. Proof that the absolute configuration of natural α-lipoic acid is R by the synthesis of its enantiomer [(S)-(–)-α-lipoic acid] from (S)-malic acid. J Chem Soc Chem Commun. 1983;(19):1051–3.
41. Salehi B, Berkay Yılmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, *et al.* Insights on the Use of α-Lipoic Acid for Therapeutic Purposes. Biomolecules 2019, Vol 9, Page 356 [Internet]. 2019 Aug 9 [cited 2024 Feb 13];9(8):356. Available from: https://www.mdpi.com/2218-273X/9/8/356/htm
42. de Sousa ESO, Cortez ACA, de Souza Carvalho Melhem M, Frickmann H, de Souza JVB. Factors influencing susceptibility testing of antifungal drugs: a critical review of document M27-A4 from the Clinical and Laboratory Standards Institute (CLSI). Brazilian Journal of Microbiology. 2020;51:1791–800.
43. Hua Q, Ying-Ying C, Zheng X, Jing-Xia Z, Ping-Hui G, Xiao-Feng Q, *et al.* Potent In Vitro Synergism of Fluconazole and Berberine Chloride against Clinical Isolates of Candida albicans Resistant to Fluconazole. Antimicrob Agents Chemother [Internet]. 2006 Mar 1;50(3):1096–9. Available from: https://doi.org/10.1128/aac.50.3.1096-1099.2006
44. Shujuan S, Yan L, Qiongjie G, Changwen S, Jinlong Y, Lin M. In Vitro Interactions between Tacrolimus and Azoles against Candida albicans Determined by Different Methods. Antimicrob Agents Chemother [Internet]. 2008 Feb 1;52(2):409–17. Available from: https://doi.org/10.1128/aac.01070-07
45. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. Vol. 52, Journal of Antimicrobial Chemotherapy. Oxford University Press; 2003. p. 1.
46. Bastami F, Salahshoori A, Shirani F, Mohtashami A, Sharafkhani N. Risk Factors of Depression on the Elderly: A Review Study. joge [Internet]. 2016 Dec 1;1(2):54–65. Available from: http://joge.ir/article-1-102-en.html
47. Al-Kobaisi MF. Jawetz, Melnick & Adelberg's Medical Microbiology 24th Edition. Twenty-Six. Timothy M, editor. Vol. 7, Sultan Qaboos University Medical Journal [SQUMJ]. The McGraw-Hill Companies, Inc; 2007. 671–715 p.
48. Farage MA, Miller KW, Sobel JD. Dynamics of the Vaginal Ecosystem—Hormonal Influences. Infectious Diseases: Research and Treatment [Internet]. 2010 Jan 20 [cited 2021 Jun 14];3:IDRT.S3903. Available from: http://www.la-press.com.

49. Hedayati MT, Taheri Z, Galinimoghadam T, Aghili SR, Cherati JY, Mosayebi E. Isolation of different species of Candida in patients with vulvovaginal candidiasis from Sari, Iran. Jundishapur J Microbiol. 2015;8(4).
50. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel J. Urinary tract infection: self-reported incidence and associated costs. 2000;10:509–15.
51. Achkar JM, Fries BC. Candida infections of the genitourinary tract [Internet]. Vol. 23, Clinical Microbiology Reviews. 2010 [cited 2021 Jun 14]. p. 253–73. Available from: https://journals.asm.org/journal/cmr
52. Burkman RT. Berek & Novak's Gynecology. JAMA [Internet]. 2012 Aug 1;308(5):516–7. Available from: https://doi.org/10.1001/jama.308.5.516
53. Mtibaa L, Fakhfakh N, Kallel A, Belhadj S, Belhaj Salah N, Bada N, *et al.* Les candidoses vulvovaginales : étiologies, symptômes et facteurs de risque. J Mycol Med [Internet]. 2017 Jun 1 [cited 2021 Jun 14];27(2):153–8. Available from: http://dx.doi.org/10.1016/j.mycmed.2017.01.003
54. Ilkit M, Guzel AB. The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: A mycological perspective. Crit Rev Microbiol. 2011 Aug 20;37(3):250–61.
55. Anderson MR, Klink K, Cohrssen A. Evaluation of Vaginal Complaints. JAMA [Internet]. 2004 Mar 17;291(11):1368–79. Available from: https://doi.org/10.1001/jama.291.11.1368
56. Watson CJ, Fairley CK, Grando D, Garland SM, Myers SP, Pirotta M. Associations with asymptomatic colonization with candida in women reporting past vaginal candidiasis: An observational study. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2013 Jul 1;169(2):376–9.
57. Seeniammal S. Clinicomycological study of vulvovaginal candidiasis [Internet] [M.D.DEGREE EXAMINATION]. 2015. Tirunelveli Medical College, Tirunelveli; 2015 [cited 2021 Jun 14]. Available from: http://repository-tnmgrmu.ac.in/id/eprint/7490
58. Sastry A, Bhat S. Essentials of medical microbiology2018 . [cited 2021 Jun 14]; Available from: https://books.google.com/books?hl=ar&lr=&id=Xq52DwAAQBAJ&oi=fnd&pg=PA1&dq=Sastry,+A.+S.,+%26+Bhat,+S.+(2018).+Essentials+of+medical+microbiology.+JP+Medical+Ltd.&ots=dT5DQ8Boj-&sig=Uy_PVwqv9Er3PkjZpIiNt7c-bFA
59. Farr A, Kiss H, Holzer I, Husslein P, Hagmann M, Petricevic L. Effect of asymptomatic vaginal colonization with Candida albicans on pregnancy outcome. Acta Obstet Gynecol Scand [Internet]. 2015 Sep 1 [cited 2021 Jun 14];94(9):989–96. Available from: https://obgyn.onlinelibrary.wiley.com/doi/full/10.1111/aogs.12697
60. Sobel JD. Vulvovaginal candidosis. Vol. 369, Lancet. Elsevier B.V.; 2007. p. 1961–71.
61. van Schalkwyk J, Yudin MH, Allen V, Bouchard C, Boucher M, Boucoiran I, *et al.* Vulvovaginitis: Screening for and Management of Trichomoniasis, Vulvovaginal Candidiasis, and Bacterial Vaginosis. Journal of Obstetrics and Gynaecology Canada. 2015 Mar 1;37(3):266–74.
62. Greenwood D, Slack RCBR, Barer MMR, Irving WLW. Medical Microbiology E-Book: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. With STUDENT CONSULT Online Access [Internet]. Eighteenth. Elsevier Health Sciences; 2012 [cited 2021 Jun 14]. 795 p. Available from: www.elsevierhealth.com
63. Pappas PG, Kauffman CA, Andes D, Benjamin Jr DK, Calandra TF, Edwards Jr JE, *et al.* Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(5):503.

64. El-Houssaini HH, Elnabawy OM, Nasser HA, Elkhatib WF. Correlation between antifungal resistance and virulence factors in Candida albicans recovered from vaginal specimens. Microb Pathog. 2019 Mar 1;128:13–9.
65. Sardi J de CO, Gullo FP, Freires IA, Pitangui N de S, Segalla MP, Fusco-Almeida AM, *et al.* Synthesis, antifungal activity of caffeic acid derivative esters, and their synergism with fluconazole and nystatin against Candida spp. Diagn Microbiol Infect Dis. 2016 Dec 1;86(4):387–91.
66. Mohamadi J, Motaghi M, Panahi J, Havasian MR, Delpisheh A, Azizian M, *et al.* Anti-fungal resistance in candida isolated from oral and diaper rash candidiasis in neonates. Bioinformation. 2014;10(11):667–70.
67. Huang CB, George B, Ebersole JL. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. Arch Oral Biol [Internet]. 2010;55(8):555–60. Available from: https://www.sciencedirect.com/science/article/pii/S0003996910001317
68. SHEBI S, EZHILARASAN D. Antimicrobial Effect of Alpha Lipoic Acid Against Oral Microorganisms. The journal of contemporary issues in business and government. 2020;26(2):1778–85.
69. Wang S, Wang P, Liu J, Yang C, Wang Q, Su M, *et al.* Antibiofilm Activity of Essential Fatty Acids Against Candida albicans from Vulvovaginal Candidiasis and Bloodstream Infections. Infect Drug Resist [Internet]. 2022 Jan 1;15(null):4181–93. Available from: https://www.tandfonline.com/doi/abs/10.2147/IDR.S373991
70. Kumar P, Lee JH, Beyenal H, Lee J. Fatty acids as antibiofilm and antivirulence agents. Trends Microbiol. 2020;28(9):753–68.