Indonesian Journal on Health Science and Medicine

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.80

Evaluation of The Effect of Aqueous Garlic Extract Against Candida Albicans and Candida Krusei Isolated From The Vagina

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Abstract. The research sought to identify a plant-based antifungal alternative that demonstrates an anti-candida effect. The study focused on isolating and identifying vaginal candida while also testing garlic aqueous extracts for their effectiveness against the pathogenesis of Candida infections. Twenty Candida isolates were collected from 25 vaginal swabs taken at private clinics. The diagnostic results indicated a 60% infection rate for Candida albicans and 40% for Candida krusei. The garlic aqueous extracts demonstrated varying levels of inhibition that rose with higher concentrations (100, 500, 1000 µg/ml), with the maximum inhibition diameter observed at 1000 µg/ml, measuring 33mm for C. albicans and 29mm for C. krusei. The inhibitory effective concentrations of the aqueous extracts were similar to those of widely used antifungals (fluconazole and nystatin).

Highlights:

1. Identified Candida albicans (60%) and Candida krusei (40%) from vaginal swabs.
2. Garlic aqueous extract showed dose-dependent antifungal activity against Candida species.
3. 1000 µg/ml extract had inhibition comparable to fluconazole and nystatin.

Keywords: Aqueous garlic extract, vaginal candida, antifungal

Introduction

The Candida genus is a component of the normal human flora. Numerous studies show that Candida yeast colonies can be found in the mouths of 20% to 40% of healthy individuals, as well as in the vagina, respiratory tract, and other areas [1] The yeast changes from a beneficial organism to an opportunistic pathogen due to the virulence factors that Candida has, which include adherence to epithelial cell surfaces, enzyme production that breaks down fats and proteins, and the development of the germ tube[2]. It exploits the body's compromised immune system to become pathogenic, which is referred to as an opportunistic fungus.

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Candida leads to various ailments, such as common mucosal skin infections, which encompass vaginal infections and certain persistent mucosal skin infections [3, 4] It also leads to infections of the skin and nails. Candida can also lead to systemic infections, affecting the blood, heart, brain, lungs, bones, and more[5]. Numerous antifungals have been utilized to decrease fungal infections in affected individuals, yet the improper and frequent use of antifungals has resulted in the development of strains resistant to these medications[6].

The resistance of pathogenic strains to antifungal agents results from various mechanisms they possess, including genetic ones that code for antibiotic resistance mechanisms. Pathogens possess various methods to resist antifungals, such as altering the permeability barrier and modifying the target site, among others, all of which are encoded by genetic factors found in fungi[7].

Certain plant extracts have been traditionally utilized to address fungal infections, including those caused by Candida fungi. For example, garlic has sulfur compounds like allicin, known for its antifungal effects[8].

Thus, garlic may serve as a powerful remedy for Candida. Nonetheless, further studies are required to validate these findings and identify the ideal doses and suitable techniques for using garlic to treat Candida.

Methods

Samples collection:

In the current study, 25 clinical samples were collected from women attending private clinics in Qurna by taking a vaginal swab from those infected with vaginal candidiasis.

Isolation and identification:

The samples were cultured on potato dextrose agar. The plates were incubated at 37°C for 24 hours[9]. The Candida spp. were identified based on direct microscopic examination as well as growth features on CHROMagar[10].

Preparation of aqueous garlic extract

Garlic Allium sativum was collected from local markets. The cloves of garlic were transferred to the laboratory, washed with sterile distilled water. Then it was placed on Whatman (England) filter papers and let at room temperature until it dried. The dried

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garlic cloves were ground using a Mixer Grinder to obtain the powder. A 20 g of the powder was mixed with 400 mL of distilled water and left the suspension with continuous stirring on a magnetic stirrer for 24 hours at room temperature. The extract was filtered using medical gauze and then with Whatman filter papers. Then the filtrate was placed in a Petri dish and dried using a lyophilizer(freeze dryer, LFFD C11, UK) . The process was repeated several times to obtain a sufficient amount of the plant extract, then it was collected and stored in the refrigerator at 4 °C until use.

Effect of the plant extract against Candida spp

The diffusion plate method was used to evaluate the effect of different concentrations (1000, 500, 100 µg/ml) of aqueous garlic extract against 3 isolates each of C. albicans and C. krusei. The method includes spreading actively tested isolate on a potato dextrose agar plate with a sterile cotton swab. 100µl from each concentration were placed in 6 mm pore (were made by sterile cork borer). The plates were incubated at 37°C for two days to observe and measure the diameter of the growth inhibition zone in mm. [11, 12]. sterile distilled water was used as a negative control and two types of antifungals (Fluconazole and Nystatin(as a positive control. The experiment was conducted with three replicates for each species.

Result and Discussion

The findings yielded 20 isolated (80%) from 25 vaginal swabs that were cultured on potato dextrose agar (Fig 1). The colonies manifested as white or cream-hued, circular or oval formations, ranging from small to large sizes, featuring a smooth, wrinkled, or velvety texture. In this context, Ellis et al. (2007) noted that Candida spp. This outcome aligns with the observations made by Singh et al. (2013) [13]regarding the formation of colonies that are shiny cream-colored, smooth, and circular, attributed to the presence of favorable culture conditions. The biochemical identification through CHROMagar Candida yielded varied colors (Fig 2).

As per the manufacturer's guidelines, the findings noted 12 samples (60%) out of 20 as green for C. albicans and 8 samples (40%) as pink and fuzzy for C. krusei. The CHROMagar medium includes reactive substances known as chromogens. Chromogens interact with enzymes found in various Candida fungi species to generate distinct colors (Marinho et al., 2010)[14].

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Fig 1: Candida spp on potato dextrose agar

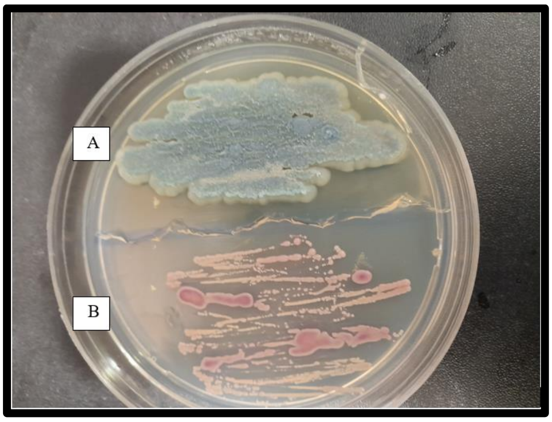

Fig 2: Candida spp on CHROMagar medium , A: C. albicans, B: C. krusei

The results of the garlic aqueous extract (figure 3 and table 1) demonstrated varying effectiveness, with a notable difference in impact based on concentration. The 1000 µg/ml concentration exhibited the greatest inhibitory capacity, achieving an inhibitory diameter of 33 mm for C. albicans and 29 mm for C. krusei. A prior investigation by Bommanavar and associates (2017) [12] suggested that varying levels of garlic extract are the most potent in inhibiting several Candida species.

Garlic is regarded as one of the plants that possess natural substances with antifungal characteristics. It is recognized for its natural antifungal compounds like allicin, agenin, and organic sulfoxides [15].

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Fig. 3: The inhibition zone of Candida spp by aqueous extract of garlic

Table 1: the average of inhibition diameters of 6 isolates of Candida by the aqueous extracts of garlic.

No.	*Candida* spp	Control	The average of inhibition zone diameter (mm) – The concentration (µg/ml)		
			100	500	1000
1	*C. albicans* (1)	0	22	26	28
2	*C. albicans* (2)	0	22	26	32
3	*C. albicans* (3)	0	23	25	33
4	*C. krusei* (1)	0	22	25	29
5	*C. krusei* (2)	0	16	19	21
6	*C. krusei* (3)	0	20	24	27
Average of inhibition diameter for each concentration	*C. albicans*	-	22.33	25.66	31
	C. krusei	-	19.33	22.66	25.66

L.S.D for concentrations = 0.432, L.S.D for candida spp= 1.063

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The impact of antifungals on Candida spp. revealed variable effects depending on the concentration, as demonstrated in Tables 2 and 3. The findings showed a notable difference among the concentrations. The peak inhibition zone was noted at a concentration of 1000 µg/ml for the antifungal fluconazole, measuring 26 mm on C. albicans and 20 mm on C. krusei. Nystatin showed 21mm against C. albicans and 20mm against C. krusei. This result aligns with the study by Lyu et al. (2016)[16], which indicated that fluconazole is more effective in treating candidiasis.

Another study conducted by Khalandi et al. (2020)[17] noted a strong impact of fluconazole on Candida. The findings were likewise comparable to those obtained by Abdullah, A. (2022)[4]. This occurs as nystatin interacts with steroid components within the cell membrane, leading to the leakage of the fungal cell contents and resulting in its demise [18]. Regarding fluconazole, it targets the fungal cell membrane by hindering the function of ergosterol found in that membrane[19].

Table 2: the average of inhibition zone diameters of 6 isolates of Candida by fluconazole.

No.	*Candida* spp	Control	The average of inhibition zone diameter (mm) The concentration (µg/ml)		
			100	500	1000
1	*C. albicans* (1)	0	14	16	20
2	*C. albicans* (2)	0	16	17	26
3	*C. albicans* (3)	0	13	18	24
4	*C. krusei* (1)	0	14	17	20
5	*C. krusei* (2)	0	15	18	20
6	*C. krusei* (3)	0	13	19	20

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Average of inhibition diameter for each concentration	*C. albicans*	-	14.33	17	32.33
	C. krusei	-	14	18	22.66

L.S.D for concentrations = 0.372, L.S.D for Candida spp= 0.772

Table 3: the average of inhibition zone diameters of 6 isolates of Candida by nystatin.

No.	*Candida* spp	Control	The average of inhibition zone diameter (mm)		
			The concentration (μg/ml)		
			100	500	1000
1	*C. albicans* (1)	0	10	12	18
2	*C. albicans* (2)	0	14	15	21
3	*C. albicans* (3)	0	11	13	19
4	*C. krusei* (1)	0	12	18	21
5	*C. krusei* (2)	0	14	19	20
6	*C. krusei* (3)	0	11	14	19
Average of inhibition diameter for each concentration	*C. albicans*	-	11.66	13.33	
	C. krusei	-	12.33	17	

L.S.D for concentrations = 4.241, L.S.D for Candida spp= 2.093.

Conclusion

The present research shows considerable antifungal efficacy of aqueous garlic extracts against Candida spp. The water extracts demonstrated strong inhibitory effects

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against Candida spp. The effects were similar to the usual antifungals. This excerpt examined the hopeful medicinal benefits of garlic as a natural antifungal substance.

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