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REVEAL THE ROLE OF GARLIC SILVER NANOPARTICLES AND GARLIC ZINC NANOPARTICLES ON CANDIDA ALBICANA ISOLATED FROM WOMEN WITH VULVOVAGINAL CANDIDIASIS

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Abstract: The current investigation aimed to reveal the role of garlic silver nanoparticles and garlic zinc nanoparticles on Candida albicana isolated from women with Vulvovaginal Candidiasis. 120 clinical samples were collected during the period from February to May 2024 from married female patients, collected from Salah al-Din General Hospital. Samples were collected for women with vaginal candidiasis whose ages ranged from (20-50 years). The initial diagnosis and examination for follow-up examinations were conducted with the assistance of a female gynecologist. The results of the microscopic and morphological examination of the total number of samples (120) showed that there were 69(57.5%) positive samples, while the number of negative samples was 51(42.5%). The molecular assay was positive for all DNA samples from the patients with Vulvovaginal Candidiasis, with Cq values ranging (24.402- 29.437) for ALS1 gene, (14.748-29.562) for HWP1 and (23.699-28.23) for ACT1. Results showed 9(90.0%), 10(100.0%) and 7(70.0%) of C. albicans isolates had ALS1, HWP1 and ACT1 gene respectively, which is one of the virulence factors in yeast C. albicanes. On the other hand, the results of the current study demonstrate the effectiveness of both AgNPs and ZnO NPs against Candida albicans. A concentration of 250 and 500 ppm was used for both AgNPs and ZnO NPs. It was found that the average diameter of the inhibition zone for 10 isolates tested was (8.04±3.47) and (21.25±3.18) for AgNPs. prepared with garlic, and the average diameter of the inhibition zone for 10 isolates tested was (8.99±2.24) and (19.57±2.54) for ZnO NPs prepared with garlic. It is concluded from the current study that both AgNPs and ZnO NPs prepared with garlic extract have inhibitory activity against Candida albicans.

Keywords: C. albicans; garlic; zinc nanoparticles; silver nanoparticles; vaginitis

Introduction

This study describes nanotechnology. In recent years, there has been a lot of interest in this technique. All particles with a diameter between one and one hundred nanometers that are composed of metal, carbon, metal oxides, or organic compounds are referred to as nanoparticles [1-2]. The health advantages of eating garlic have been linked to one of the most abundant classes of organosulfur compounds, which can be found in freshly sliced or crumpled garlic [3–4]. Garlic also contains a variety of bioactive phytochemicals, such as flavonoids, phenolics, organosulfur compounds, allyl thiosulfates, and vitamins. Garlic's possible health benefits are primarily due to its phenolics, which are the most significant component of the plant. These phenolics also have strong pharmacological effects and are found in large concentrations [5–6]. Metal oxide

nanoparticles have been synthesized using a number of physical and chemical techniques; however, there is currently an increasing demand to develop environmentally friendly nanoparticle synthesis methods that do not require the use of hazardous ingredients or significant energy usage [7-9]. Consumers' use of nanoparticles in a variety of industries, including the chemical, food, feed, health, and cosmetics, necessitates an environmentally responsible method of synthesizing them [10]. One of the plants used in the green synthesis of NPs is garlic (Allium sativum) [11–12]. Studies have demonstrated the antibacterial activity of garlic extract against gram-positive and gram-negative bacteria [13]. Furthermore, AgNPs green, which was developed using a variety of medicinal plant extracts, have strong antibacterial action against both gram-positive and gram-negative bacteria [14]. Zinc oxide (ZnO) is an inorganic substance that exhibits remarkable qualities in a variety of applications by itself [15]. A white powder that is water-insoluble is produced during the environmentally friendly manufacture of zinc oxide nanoparticles [16]. Different plants, including Cassia fistula and Melia Azadarch [17], garlic (Allium sativum) [18], Z. officinale (ginger), Aloe vera [19], and Lippia adoensis (koseret) [20], have been extracted in order to carry out green synthesis of ZnO NPs. Therefore, the current study aimed to reveal the role of garlic silver nanoparticles and garlic zinc nanoparticles on Candida albicana isolated from the vagina of women suffering from vaginitis

Methods

Sample collection

Between February and May of 2024, 120 clinical samples from female patients at Salah al-Din General Hospital were collected. Women between the ages of 20 and 50 who had vaginal candidiasis had samples taken. Assisted by a female gynecologist, the first diagnosis and evaluation for follow-up exams were carried out.

Direct microscopial examination

Swabs were used to collect the sample, which was then put on a glass slide, a drop of 10% KOH potassium hydroxide solution was added, and the slide cover was put on. Next, the sample was inspected under a microscope at 40X power to confirm the presence of yeasts.

Culturing of the swabs on agar plates

Using Sabouraud Dextrose Agar (SDA), 65 grams of SDA powder were dissolved in 1000 milliliters of distilled water in a flask with a pH fixed at 6.8. Following sterilization, 250 milligrams of the antibiotic chloramphenicol were added. The fungus were isolated and cultured using this medium. Following the media's preparation, swabs were screened under sterile circumstances on plates containing SDA. For 48 hours, the plates were incubated at 37 °C.

Bimolecular diagnosis

DNA extraction

For DNA extraction, isolate specimens weighing 0.25 g were employed. The Mo Bio Power Soil DNA Isolation Kit (Mo Bio Laboratories, California, USA) was used to extract DNA in accordance with the manufacturer's instructions. In 30 µl of C6 solution (10 mM Tris), the DNA was eluted. The extracted DNA was kept at -20°C until needed.

PCR procedure

Conventional PCR using 50µl of the PCR Mastermix was used to detect the ALS1, HWP1, and ACT1 sequences, whereas monoplex PCR using 20µl of the PCR Mastermix was used to detect the CNF1 sequence. Every primer was utilized at a 30 pmol/µl concentration. Table 1 contains a list of the primers used in the research that was presented.

Table (1): C. albicans genes PCR assayprimers

No.	Gene Name	Sequence		Band size
1	ALS1	F	AGCGGTTCTCATGAATCAGC	133
		R	CAGAAGAAACAGCAGGTGATGG	
2	HWP1	F	GACCGTCTACCTGTGGGACAGT	117
		R	GCTCAACTTATTGCTATCGCTTATTACA	
3	ACT1	F	TGTGTAAAGCCGGTTTTGCC	136
		R	TTGGATTGGGCTTCATCACC	

Real-Time PCR master mix preparation

Real-time PCR master mix was produced in accordance with business guidelines using a one-step reverse transcription method and a real-time PCR detection kit (AccuPower Rocket ScriptRT-PCR PreMix, Bioneer, Korea).

Real-Time PCR Thermocycler conditions

The RT-PCR Taq Man kit instructions and primer annealing temperature were followed for setting up the Real-Time PCR thermocycler.Thermal cycles were used to examine the Real-Time PCR, and the AccuPower® 2X GreenStarTM qPCR Master Mix instructions were followed. Additionally, the MiniOpticon Real-Time PCR equipment was used to calculate the degree Tm prefixes.USA/BioRad.

Real-Time PCR Data analysis

RT-PCR data analysis performed by calculation the threshold cycle number (CT value) that presented the positive amplification of gene in Real-time cycle number.

Preparation of garlic extract

50 grams of removed garlic crust were mixed with 200 milliliters of distilled water, and two phases were then identified. The initial unprocessed canvas and the subsequent nomination paper. Using a separating funnel, blend the filtrate in a quantity equivalent to the compound diethyl ether with the intention of Filtrate from fatty oils is purified by removing the bottom layer of the mixture and steaming it at 50 degrees Celsius in a rotary evaporator. This removes residues from diethylether. To use, keep the filtrate in opaque botteles [21].

Green and Chemical synthesize of the AgNPs

This procedure involved heating 50 milliliters of a 0.001 mol silver salt (AgNO3) solution as the starting material, then rapidly adding 5 milliliters of garlic extract while vigorously stirring. In this instance, the solution's hue quickly changed from pale yellow to light green. The mixture was then brought to a full boil for an additional ten minutes. Following this, the stirrer was rotated for a further fifteen minutes, allowing the mixture to settle to room temperature. Ultimately, a Gelman membrane filter with a 0.8 µm pore size was used to filter the mixture containing the artificially produced NPs [22]. NPs were diluted 200 times before being used in microbiological investigations. For chemically and environmentally produced NPs, the final concentrations of the liquids employed in susceptibility experiments were 250 and 500 ppm, respectively.

Synthesis of ZnO NPs

After making 200 ml of a 2 mM zinc chloride solution, it was stirred for 20 hours. The study's pH was adjusted, and pH 8 produced the best synthesis. As a result, 1 M NaOH solution was used to raise the pH of the solution to 8. Subsequently, 30 milliliters of the garlic skin extract solution was gradually added to the previously mentioned solution while stirring continuously. The reaction mixture's color altered after 30 minutes of incubation. After the incubation period verified the production of ZnO NPs, the solution was left to be agitated for four hours. The precipitate that produced was centrifuged at 7000 rpm for 15 minutes in order to separate it from the reaction solution. The pellet was then recovered after being repeatedly washed with distilled water and then ethanol to get rid of organic contaminants [23].

Tested effect AgNPs and ZnO NPs on C. albicans

Determine the antifungal activity of AgNps was followed by agar well method was done by method [24].

Result and Discussion

The results of the microscopic and morphological examination of the total number of samples (120) showed that there were 69(57.5%) positive samples, while the number of negative samples was 51(42.5%), as shown in table (2).

Table (2): Distribution of positive and negative cultured cases

Procedures	Samples	Positive samples	
		No.	%
Direct examined by 10% KOH	120	69	57.5

Out of all the Candida species found in women's vaginal swabs, Candida albicans was the most frequently found species linked to vulvovaginitis. This result is consistent with other studies [25–26] that found Candida albicans to be the most common spp. causing vaginal candidiasis, with a frequency of 61.2–70.82 percent. This may be because Candida albicans is a normal component of the vaginal flora in women of reproductive age [27], which causes Candida infection opportunistically due to altered host conditions, where the fungus proliferates more quickly and causes vaginal candidiasis [25]. The prevalence of Vulvovaginitis Candidiasis (VVC), an infection caused by yeasts of the genus Candida [28] that produces vaginal discharge [29], irritation, and pruritus, has increased dramatically in recent years. The primary reason why women seek gynecological care globally is the VVC [30].

The molecular assay was positive for all DNA samples from the patients with Vulvovaginal Candidiasis, with Cq values ranging (24.402- 29.437) for ALS1 gene, (14.748-29.562) for HWP1 and (23.699-28.23) for ACT1 (Table 2). Results showed 9(90.0%), 10(100.0%) and 7(70.0%) of *C. albicans* isolates had ALS1, HWP1 and ACT1 gene respectively, which is one of the virulence factors in yeast *C. albicanes*, Figures (1, 2 & 3).

Table (2): Data of the samples with positive qPCR assay

No.	ALS1	HWP1	ACT1
1	28.554	28.605	27.641
2	28.543	14.748	26.852
3	29.324	27.895	26.276
4	27.827	27.29	25.935
5	29.631	29.562	28.23
6	28.548	27.767	26.172
7	29.437	27.581	26.662
8	27.373	28.201	26.727
9	25.786	25.071	25.556
10	24.402	25.653	23.699

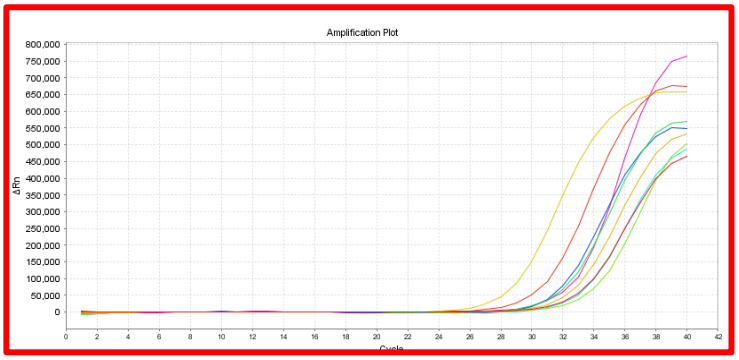

Figure (1) Amplification plot in Real-Time PCR for ALS1 gene in *C. albicans*

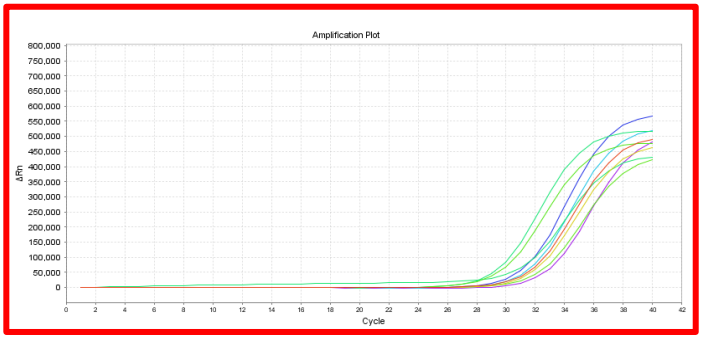

Figure (2): Amplification plot in Real-Time PCR for HWP1 gene in *C. albicans*

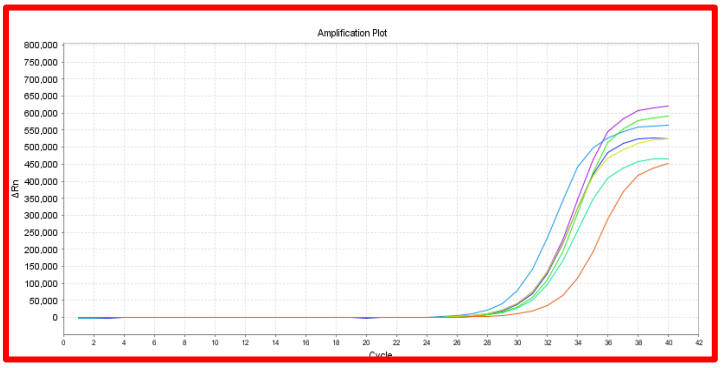

Figure (3): Amplification plot in Real-Time PCR for ACT1 gene in *C. albicans*

The ALS1 gene was selected for this investigation because it is a member of the ALS family and encodes a cell surface glycoprotein that is linked to adherence to host surfaces [31]. Previous research [32–33] has used a variety of techniques, such as transcript profiling, traditional RT-PCR, and northern blot analysis, to report differential expression of the gene. However, none of these techniques yield quantifiable information. These findings concur with Rajendran et al.'s study [34], which assessed the expression of genes linked to biofilm in C. albicans biofilms and discovered that isolates with high biofilm had higher levels of HWP1, ACT1, and ALS1. Despite the fact that high BF has higher expression levels of ALS3 and HWP1 than low BF. Rajendran et al.'s most recent study [35] built upon their earlier research, showing that high BF enhanced hyphal

specific gene expression, which in turn raised HWP1 and ALS1. Comparable to the biofilm formation process, the proportion of up-regulated genes linked to hyphal development and cell adhesion in the LBF and HBF was the same, making up a total of only 4% of the up-regulated genes in each group. However, the current study's findings show that AgNPs and ZnO NPs are equally effective against Candida albicans. For both AgNPs and ZnO NPs, concentrations of 250 and 500 ppm were employed. It was found that the average diameter of the inhibition zone for 10 isolates tested was (8.04±3.47) and (21.25±3.18) for AgNPs. prepared with garlic, and the average diameter of the inhibition zone for 10 isolates tested was (8.99±2.24) and (19.57±2.54) for ZnO NPs prepared with garlic.

Table (3): inhibition zone (nm) of AgNPs and ZnO NPs on Candida albicans

No. of isolates	AgNPs (nm)		ZnO NPs (nm)	
	250 ppm	500 ppm	250 ppm	500 ppm
1	7.21	18.37	10.26	21.63
2	4.11	20.66	9.13	19.58
3	2.14	19.57	5.05	23.81
4	8.31	21.48	8.19	16.68
5	10.47	27.55	13.41	19.63
6	9.67	18.13	10.22	17.32
7	14.2	25.17	11.31	15.49
8	10.43	23.48	8.31	21.84
9	8.05	19.17	7.35	20.31
10	5.77	18.92	6.65	19.45
Average	8.04±3.47	21.25±3.18	8.99±2.24	19.57±2.54

The antifungal efficacy of garlic-biosynthesized AgNPs and the sensitivity of Candida albicans were investigated in vitro. As the concentration of silver nanoparticles grew, so did the inhibitory effect of AgNPs [36]. There was a minimum fungicidal concentration (MFC) of 100 ppm and an effective fungicidal concentration (EC50) of 1 ppm. AgNPs have been shown to be effective antifungal agents in the past. Similar to the situation of, 24 reported that, when using the agar dilution method, biosynthesized with a size of 23 nm were shown to have antifungal efficacy against experimental yeast samples in the 1 to 100 ppm range [37]. In a study, Hosseini et al. looked into the zinc oxide NPs' ability to suppress the growth of C. dubliniensis biofilm. In order to stop the growth of C. dubliniensis biofilm, they demonstrated that the inhibitory power of biofilm in the presence of zinc oxide NPs and fluconazole was more than twice the MIC [38]. Using the E-test, Aleš Panáček et al. assessed the antifungal efficacy of AgNPs against isolates of Candida albicans, Candida tropicalis, and Candida parapsilosis from blood specimens. According to their findings, AgNPs successfully stopped the tested Candida species from growing [39]. Additionally, a great deal of research has documented how AgNPs affect many Candida species, most notably Candida albicans [40–41]. Findings corroborated the research of [42],Two species of yeast are effectively inhibited by the nanoparticle particles' antifungal properties (Candida albicans, Saccharomyces cerevisiae) observed using electron microscopy's scan The structure of the yeast's membrane changed when it interacted with the silver nanoparticles, as evidenced by holes that were found on the membrane's surface and which caused the cell to die. It was also observed that the process of buds was inhibited as a result of the membrane collapsing, which prevented the yeast from growing [43].

Conclusion

It is concluded from the current study that both AgNPs and ZnO NPs prepared with garlic extract have inhibitory activity against Candida albicans. The results also showed that silver

nanoparticles prepared with garlic were more effective and effective compared to zinc nanoparticles in terms of their effect and inhibition of the growth of Candida albicans.

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