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Research Article

Study of the activity of crude and nano cardamom extracts on some virulence genes of Candida albicans isolated from women with vaginal Candidiasis

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Abstract

The current study aimed to study the effectiveness of crude and nano cardamom extracts on some virulence genes of *C. albicans* isolated from married women with vaginal candidiasis. 80 clinical samples were collected during the period from November 2023 to January 2024 from married female patients, collected from Salah al-Din General Hospital. Samples were collected for women with vaginal candidiasis whose ages ranged from (17-45 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 (80) showed that there were 43(53.75%) positive samples. For Ag NPs that prepared in current study, The SEM image exposed that the formed nanoparticle was spherical in shape formed with the size range of 50-90 nm. The susceptibility test for *C. albicans* showed a different inhibition zone where the highest diameter of the inhibition for cardamom extract (250%) was 24 mm, while the diameter of the inhibition for cardamom extract (100% & 500%) was 12 mm. Regarding silver nanoparticles, the best inhibition diameter was 17 for a concentration of 250%. The study showed a decrease in the level of gene expression of ALS1, HWP1 and ACT1 at concentrations (10, 50, 100, 250 & 500%) compared to the sample not treated with the extract. It is concluded from the current study that each cardamom extract and silver nanoparticles prepared with cardamom have the ability to reduce the gene expression of some virulence genes in *C. albicans*

Keywords: C. albicans; cardamom; vaginal candidiasis.

INTRODUCTION

It has been common to isolate yeasts from fungal infections that belong to the Candida genus [1]. Twenty of the approximately 200 species of Candida that are known to exist are linked to infections in humans [2]. Human infections caused by Candida are thought to be a serious problem, particularly for hospitalized patients with serious underlying illnesses and immunocompromised ICU patients [3–4]. The main virulence factor of the opportunistic pathogenic fungus Candida albicans is the production of biofilms. Nevertheless, little research has been done on how antifungal drugs affect these virulence traits. Typical antifungal medications may inhibit the production of secreted hydrolases and the creation of biofilms [5]. An essential component of treating patients with fungal infections is testing for fungal susceptibility to commonly used antifungal medications [6]. The emergence of resistance to the most widely used antifungal therapies, which is also linked to the production of biofilms, has forced the development of new antifungal drugs [7]. Because of their antibacterial and antifungal qualities, especially with regard to food-borne bacterial illnesses, spices and aromatic herbs are becoming more and more popular in both industry and scientific research [8]. Plant extracts such as clove, cinnamon, garlic, mustard, onion, and oregano have been demonstrated to effectively treat a variety of bacterial diseases [9–10]. Most of the antibacterial compounds found in spices and herbs are found in their essential oil form. Traditionally used as a spice in cooking, the perennial aromatic plant cardamom (Elettaria cardamomum) is abundantly grown in Tanzania, Guatemala, southern India, and Sri Lanka. This plant is rich in flavonoids, alkaloids, terpenoids, anthocyanins,

and phenolic chemicals [11]. Since ancient times, cardamom has been used to cure a variety of illnesses, such as chronic jaundice, digestive problems, and asthma [12]. Numerous pharmacological actions of cardamom, such as antibacterial, anti-inflammatory, anti-cancer, and antioxidant properties, have been shown [13–15]. Therefore, the current study aimed to study the effectiveness of crude and nano cardamom extracts on some virulence genes of C. albicans isolated from married women with vaginal candidiasis.

Materials & Methods

Sample collection

80 clinical samples were collected during the period from November 2023 to January 2024 from married female patients, collected from Salah al-Din General Hospital. Samples were collected for women with vaginal candidiasis whose ages ranged from (17-45 years). The initial diagnosis and examination for follow-up examinations were conducted with the assistance of a female gynecologist.

Direct microscopial examination

The sample taken by swabs was placed on a glass slide, and a drop of 10% KOH potassium hydroxide solution was added to it, then covered with a slide cover, after which it was examined with a microscope under 40X power to confirm the presence of yeasts.

Indirect examination

The media used was sabroid dextrose agar (SDA). Samples taken with cotton swab were grown on the medium, then the plates were incubated at a temperature of 37°C for a period of 24-48 hours.

Preparation of aqueous extracts

Cardamom was purchased from the market. Ground into fine powder in an electrical mixer. 100g of the finely powdered cardamom mixed with one liter of de- ionized water and kept in a water bath at 60 C for five hours, then filtered through filter paper. The extract was then left to dry at 40º C in hot air oven for evaporation of water. The extract was preserved in a refrigerator until use [16].

Phyto-assisted Synthesis of silver Nanoparticle

In order to create silver nanoparticles, silver nitrate (AgNO3) was utilized as a precursor. It was acquired from Himedia Laboratories in Mumbai, India. In a conical flask, 20 ml of an aqueous solution containing varying concentrations of AgNO3 (0.16, 0.18, 0.20, 0.22, and 0.24 gm) was mixed with approximately 5 ml of Elettaria cardamom seed extract. For 20 minutes, the mixture was heated to 80°C, causing the solution's color to shift from pale brown to dark brown. By analyzing the UV-vis spectrum of the reaction mixture (silver nitrate solution + seed extract) at different concentrations, the reduction of Ag+ ions to Ag0 were tracked [17].

Characterization studies

First, a UV-visible Spectrophotometer (Perkin-Elmer) with a wavelength of approximately 360–700 nm was used to assess the reduction of silver metal ions to silver nanoparticles at regular intervals. The solution mixture (seed extract and silver nitrate) was then centrifuged for 20 minutes at 15,000 rpm. This centrifugation procedure was then repeated three or four times, and the resultant solution was filtered using Whatman No. 1 filter paper before the pellet was dried in a hot air oven. Additionally, a Philips model CM 200 apparatus was used to perform scanning electron microscopy (SEM) on the dried particles in order to examine the morphological characteristics of the nanoparticles.

Determination of the inhibitory activity of aqueous and nano cardamom extract

The medium (SDA) was prepared, sterilized, cooled to 37–40°C, and then poured into Petri dishes in an amount of 15 ml per dish. I then made circular wall holes with a diameter of 6 mm for each dish, and after that, the fungi were grown and distributed uniformly over the culture medium. Finally, (50 microlitres) of extract at concentrations (10, 50, 100, 250, and 500%) were added to the holes prepared for them. This is how the inhibitory effectiveness of the aqueous and nano-extract was estimated, as stated by [18]. Following a 24-hour incubation period at 37°C, the diameter of inhibition was measured on the dishes, and distilled water was used to provide the positive control factor.

Preparing samples for molecular study

C. albicans was selected from the isolated yeasts based on the initial diagnosis, and the concentration (10, 50, 100, 250, 500%) was adopted for the molecular study. The sterile (SDB) media was prepared and cooled at a temperature of 37-40°C for 24 hours, and after it was activated, the sample was placed with extract (50µl) for each of the concentrations and incubated for 24 hours.

DNA extraction
The PrestoTM MiniGdna Yeast Kit was used, which was prepared by Geneaid Biotech Ltd and contains the following solutions: washing buffer, Lysis buffer, Elusion buffer, and binding buffer.

Polymerase chain reaction
Utilizing a Thermocycler apparatus from Singaporean Biosystem Applied, the polymerase chain reaction was conducted to ascertain the degree of genetic impact of crude and nano cardamom extract at concentrations (10, 50, 100, 250, 500%) on the ALS1, HWP1, and ACT1 genes in the chosen isolate C. albicans (Table 1).

Table (1): primers sequence and product size used in the present study.

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 testing
The qPCR-RT reaction was carried out using an American-made biosystem applied device, with the aim of determining the effect of the alcoholic extract of the cardamom plant on the gene expression of the ALS1, HWP1 and ACT1 genes at concentrations (100, 75, 50, 25) in the selected isolate C. albicans.

Synthesis cDNA
The method of measuring gene expression using the qPCR-RT technique requires converting single strands of RNA into complementary strands of DNA, and in order to accomplish this, the Easy Script first strand DNA synthesis kit, prepared by TRANS, was used, according to the following additions shown in Table (2).

Table (2): shows the additions needed to prepare cDNA

Component	Volume
RNA	7µl
Anchored oligo	1µl
ES Reaction mix	1µl
Easy script RT\RT Enzyme mix	1µl
Reaction mix	10µl

Steps of real-time polymerase chain reaction (PCR9_RT)
The mix super qPCR Green Perfectstar kit prepared by the company (TRANS) was used. The cDNA samples for each sample were prepared and placed inside a tube holder, and then the process of preparing the reaction mixture began according to the additions shown in Table (3).

Table (3): shows the additions of RT−qPCR

Component	Volume
C DNA template	1µl
Forward primer	0.5µl （Conc.: 10 Pmol）
Reverse primer	0.5µl （Conc.: 10 Pmol）
Perfect star Green9pcr super mix	10µl
RNase -free water	8µl

Results & Discussion

Isolation of *C. albicans*
The results of the microscopic and morphological examination of the total number of samples (80) showed that there were 43(53.75%) positive samples, while the number of negative samples was 37(46.25%), as shown in table (4) and figure (1). The results of the statistical analysis showed that there were no significant differences, P-Value = 0.174. Candidiasis is the second disease of vaginitis in women [19]. In the current study it has been found that about 53.75% of

women with vaginal candidiasis. The result is agreement with results that reported in Nigeria (62.2%) [20], Tanzania (42.9%) [21], Libya (43.8%) [19] and more than reported in Saudia Arabia (24%) [22], Nigeria (26%) [23].

Table (4): Distribution of positive and negative cultured cases

Procedures	Samples	Positive samples		P value
		No.	%	
Direct examined by 10% KOH	80	43	53.75	0.174
Culturing Procedure	80	43	53.75	

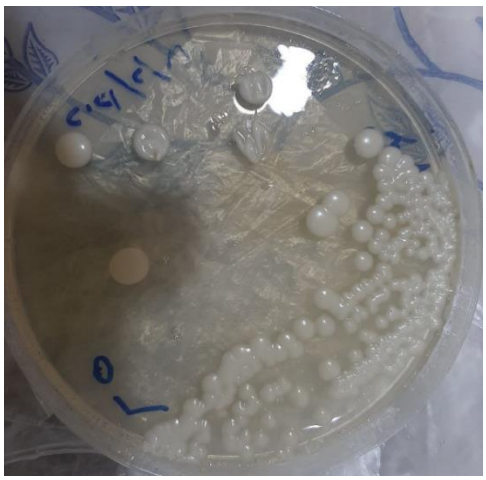

Figure (1): the colonies of C. albicans on SDA.

Properties of silver nanoparticles prepared by cardamom extract

Some properties of silver nanoparticles prepared with cardamom extract were studied for the purpose of confirming their formation.

UV–visible spectroscopy

An essential method to ascertain the emergence and stability of metal nanoparticles in aqueous solutions is UV–visible spectroscopy. The addition of different concentrations of metal ions causes the reaction mixture to change color. The silver nanoparticle's surface plasmon vibrations are excited, which results in these color changes [24]. Surface plasmon resonance (SPR), which is caused by a group of free conduction electrons generated by an interacting electromagnetic field, is acknowledged to be the cause of the dark brown color of silver colloid [25]. Between 440 and 480 nm is where the strong surface plasmon resonance band develops, and the peak's widening suggests that the particles are monodispersed.

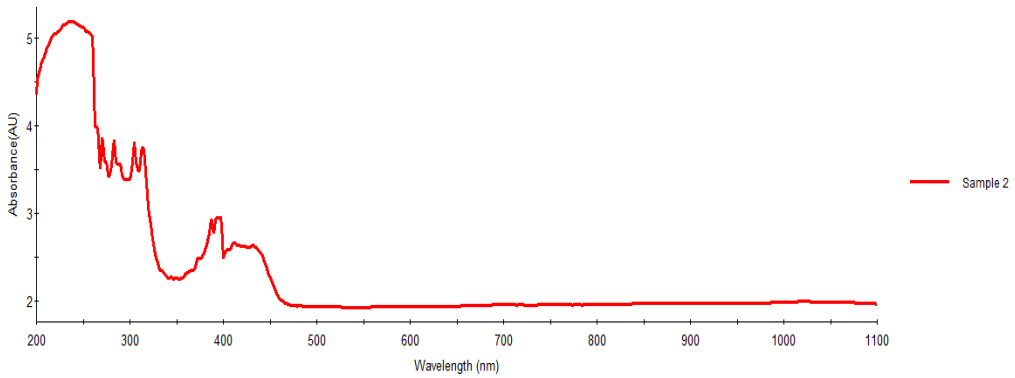

Figure (2): UV-Visible absorption spectra of biosynthesized silver Nanoparticle from *E. cardamomom* depicting peak at 410 nm.

Scanning electron microscope

A scanning electron microscope was used to characterize the morphology of the produced nanoparticles; the Philips model CM 200 instrument was used to capture this image. The majority of the produced silver nanoparticles had a consistent shape and were spherical in shape (Fig. 3). It is well known that a metal nanoparticle's form can significantly alter its optical and electrical characteristics [26]. The SEM picture revealed that the produced nanoparticle had a spherical form and ranged in size from 50 to 90 nm.

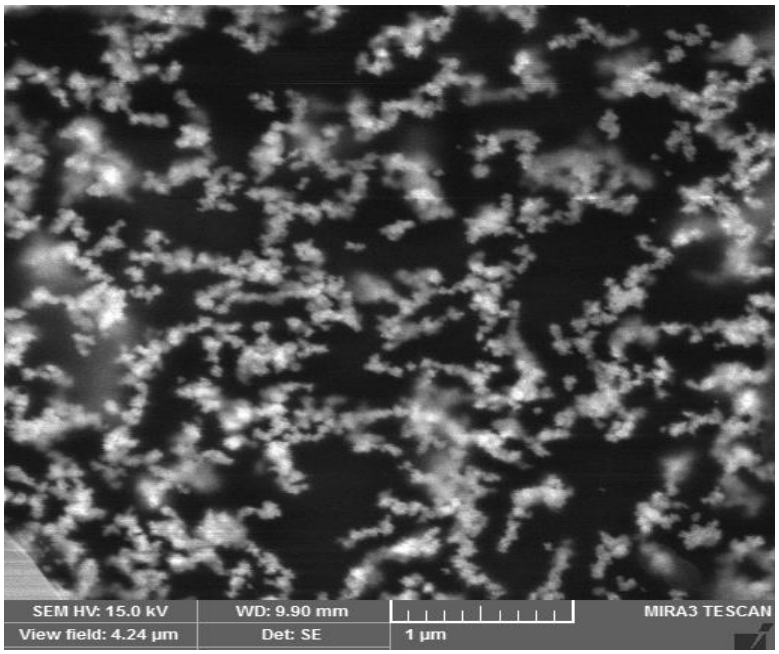

Figure (3): The SEM images of silver Nanoparticle synthesized from the E. cardamomom seed extracts at various magnification.

Susceptibility test

The susceptibility test for C. albicans showed a different inhibition zone where the highest diameter of the inhibition for cardamom extract (250%) was 24 mm, while the diameter of the inhibition for cardamom extract (100% & 500%) was 12 mm. Regarding silver nanoparticles, the best inhibition diameter was 17 for a concentration of 250%, as shown in table (3) and Figure (4).

Table (3): the diameter of inhibition zone of studied cardamom extract and Ag nanoparticles

Concentration	Inhibition zone (mm)	
	Cardamom extract	Ag nanoparticles
10%	10	5
50%	5	0
100%	12	5
250%	24	17
500%	12	13

Figure (4): the diameter of inhibition zone of studied cardamom extract and Ag nanoparticles.

Aqueous cardamom extract shown efficacy against Candida albicans in the current investigation. Cardamom seed antibacterial and antifungal properties have been documented in a number of earlier investigations [27–29]. It has been observed that cardamom contains a high concentration of medicinal substances, including vitamin C and flavonoids [30]. Strong antibacterial action of flavonoids has been demonstrated by a variety of methods, including bacterial membrane damage, decreased nucleic acid production, and decreased energy metabolism [31]. On the other hand, compared to cardamom extract, the efficacy of silver nanoparticles made from cardamom was reduced in treating candida. The size and form of the produced nanoparticles may have an impact on bacterial growth and impede it, as evidenced by the analysis of enzymes and cell leakage [32], which may account for the variance in inhibitory zone widths. These preparations have a wide range of uses in biotechnology and medicine, helping to manage harmful bacteria more effectively in aquatic environments by improving their dispersion. The ability of metal nanoparticles to interface closely with microbial membranes due to their tiny size and high surface to volume ratio has been linked to their bactericidal action, which is not solely caused by the release of metal ions in solution [33].

Gene expression of the 1ALS gene

The gene expression of the 1ALS gene was studied in C. albicans after treating it with different concentrations of cardamom aqueous extract and nanosilver. Table (4) shows the gene expression results for the samples treated with the hydroalcoholic extract of cardamom and nano-silver compared to the control sample. The study showed a decrease in the level of gene expression at concentrations (10, 50, 100, 250 & 500%) where the gene expression was (0.58962, 0.28604, 0.45578, 0.42122 & 0.11428, respectively) compared to the sample not treated with the extract, as showed in figure (5).

Table (4) folding values of ALS1 gene

	ct p	ct r	Δct p	Δct c	ΔΔct	Folding	Folding con.
10	28.554	28.548	0.006	0.854	-0.848	0.58962	1
50	28.543	29.437	-0.894	0.854	-1.748	0.28604	1
100	29.324	27.373	1.951	0.854	1.097	0.45578	1
250	27.827	25.786	2.041	0.854	1.187	0.42122	1
500	29.631	24.402	5.229	0.854	4.375	0.11428	1

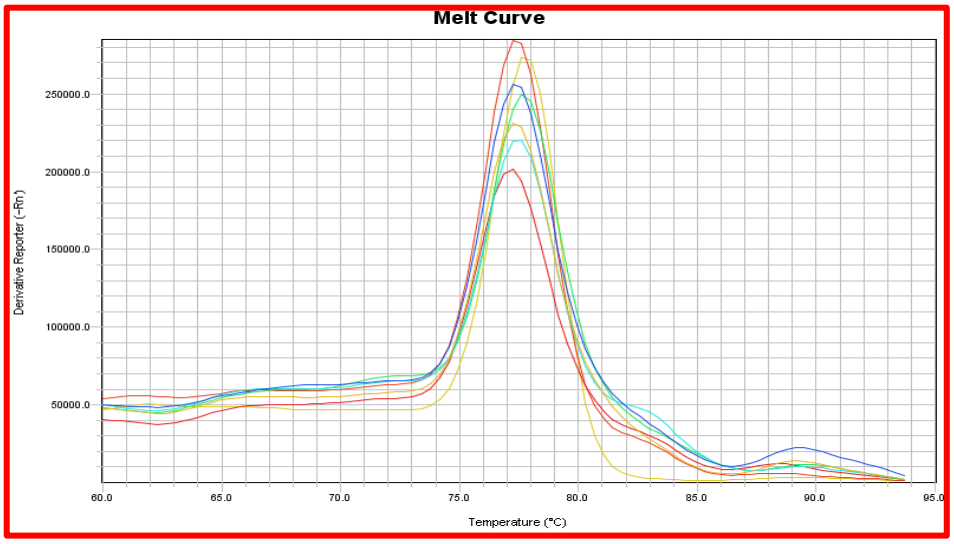

Figure (5): shows the curves of the CT threshold value for the ALS1 gene in the real-time RT−PCR reaction.

Gene expression of the HWP1 gene

The gene expression of the HWP1 gene was studied in C. albicans after treating it with different concentrations of cardamom aqueous extract and nanosilver. Table (5) shows the gene expression results for the samples treated with the hydroalcoholic extract of cardamom and nano-silver compared to the control sample. The study showed a decrease in the level of gene expression at concentrations (10, 50, 100, 250 & 500%) where the gene expression was (0.13469, 0.02876, 0.10296, 0.2145 & 0.78003, respectively) compared to the sample not treated with the extract, as showed in figure (5).

Table (5): folding values of HWP1 gene

	ct p	ct r	Δct p	Δct c	ΔΔct	Folding	Folding con.
10	28.605	27.767	0.838	4.55	-3.712	0.13469	1
50	14.748	27.581	-12.833	4.55	-17.383	0.02876	1
100	27.895	28.201	-0.306	4.55	-4.856	0.10296	1
250	27.29	25.071	2.219	4.55	-2.331	0.2145	1
500	29.562	25.653	3.909	4.55	-0.641	0.78003	1

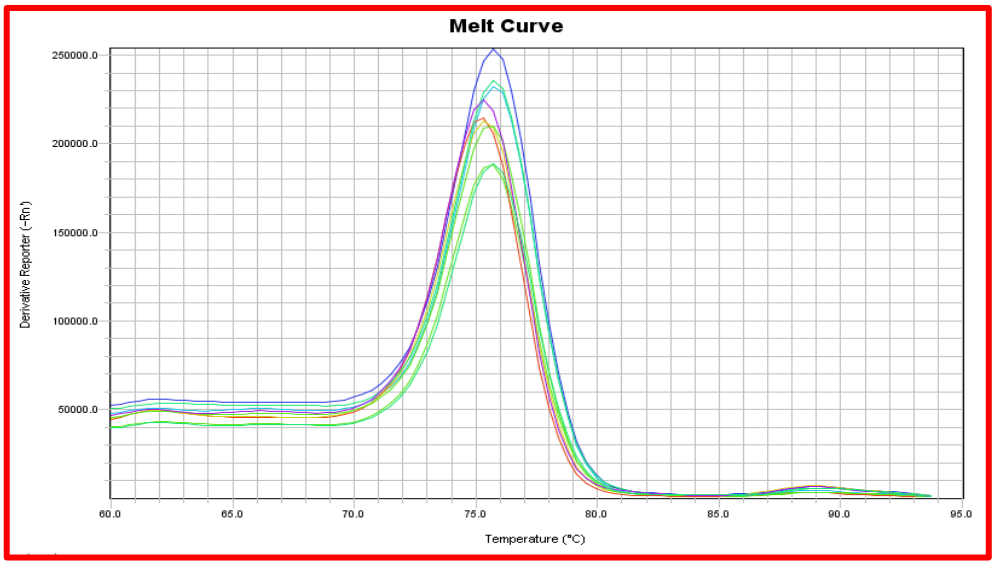

Figure (6): shows the curves of the CT threshold value for the HWP1 gene in the real-time RT−PCR reaction.

Gene expression of the ACT1gene

The gene expression of the ACT1gene was studied in C. albicans after treating it with different concentrations of cardamom aqueous extract and nanosilver. Table (6) shows the gene expression results for the samples treated with the aqueous extract of cardamom and nano-silver compared to the control sample. The study showed a decrease in the level of gene expression at concentrations (10, 50, 100, 250 & 500%) where the gene expression was (0.91074, 0.68493, 0.36469, 0.92421 & 0.13846, respectively) compared to the sample not treated with the extract, as showed in figure (6).

Table (6): folding values of ACT1gene

	ct p	ct r	Δct p	Δct c	ΔΔct	Folding	Folding con.
10	27.641	26.172	1.469	0.92	0.549	0.91074	1
50	26.852	26.662	0.19	0.92	-0.73	0.68493	1
100	26.276	26.727	-0.451	0.92	-1.371	0.36469	1
250	25.935	25.556	0.379	0.92	-0.541	0.92421	1
500	28.23	23.699	4.531	0.92	3.611	0.13846	1

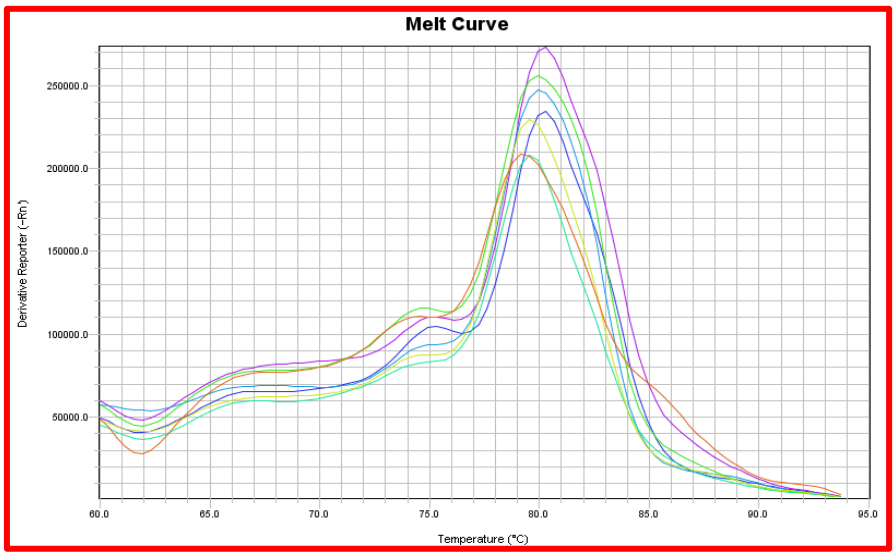

Figure (7): shows the curves of the CT threshold value for the HWP1 gene in the real-time RT−PCR reaction.

Our results suggested that the expressions of the ALS1 gene, cardamom aqueous extract, and nanosilver are positively correlated. It appears plausible to suggest that biofilm contributes to resistance in isolates since all of the C. albicans isolates expressing the ALS1 were sensitive to cardamom aqueous extract and nanosilver, and their adherence was stronger than the control group (without any expression). This result is consistent with the findings of other studies that identified ALS1 as the primary overexpressed gene in biofilm formation and adherence, both of which are critical for drug inhibition to reach the fungal microcolonies [34–35]. Using reverse transcriptase-PCR (RT-PCR), Green et al. [36] assessed the expression patterns of genes in the ALS gene family in clinical oral, vaginal, and urine tissues. They demonstrated that at both inoculation densities and all times, the ALS1 gene was expressed. Additionally, they stated that the clinical strains of these samples showed comparable patterns of gene expression. In the current work, ALS1 and HWP1 gene expression was shown to decrease following the use of cardamom aqueous extract and nanosilver. The use of cardamom aqueous extract and nanosilver in the current study resulted in a decrease in ACT1 gene expression, which is in line with the findings of a previous study [37] that reported that treating different strains of Candida albicans with these neolignans caused a significant decrease in the expression of genes activated by this pathway and the MAPK cascade pathway (RAS1, EFG1, TEC1, CDC35, ECE1, HWP1, ALS3), which is crucial in hyphal cell adhesion. Furthermore, in strains treated with magnoliol and honokiol, exogenous cAMP reinstated the production of hyphal segments. The present study observed a decrease in ACT1 gene expression following the application of cardamom aqueous extract and nanosilver. This finding is in line with the findings of Erfaninejad et al. [38], who reported a significant reduction in ACT1 expression in C. albicans biofilm treated with Peganum harmala (P = 0.0068). The catalase gene, ACT1, is crucial for improving resistance to oxidative stress [39] and is needed for C. albicans to detoxify from reactive oxygen species (ROS) [40].

Conclusions

It is concluded from the current study that both cardamom extract and silver nanoparticles prepared with cardamom have the ability to reduce the gene expression of some virulence genes in Candida albicans, and that the cardamom extract was better than the nanoparticles.

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