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Effect of piperine on antifungal-resistant clinical isolates of *Candida albicans* and *PLB1* expression

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Abstract

Candida albicans is the most common cause of oral, cutaneous and vaginal Candidiasis which the incidence ranging up to 50% among those patients. Phospholipase proteins, genes like *PLB1* act as virulence genes that code for *C. albicans* colonization and biofilm formation. Piperine is alkaloids content located in the seed of black pepper (*Piper nigrum*), and it has antimicrobial effects. This study aimed to study the effectiveness of piperine on resistant isolates of yeast isolated from patients with candidiasis. One hundred clinical samples were swabbed from oral and vaginal candidiasis patients who attended Baqubah teaching hospital in Iraq for a period of fourteen months, extending from December 2023 to January 2025. The samples are cultured using Sabouraud dextrose agar medium (SDA) at 37°C for 2-3 days. The identification of *C. albicans* was done by culturing it on HiChrom Candida agar medium, Vitek and SDA at 42°C for 2 days. Serial dilutions of Piperine, which were (2.5, 5, 10, 20, 40, 80, 160 and 320) µg/ml, were made for the treatment of the resistant isolates of *C. albicans* toward fluconazole (FLU) (25 µg) and nystatin (NY) (100 IU) antifungals. Real-time PCR technique was used for *PLB1* gene expression of *C. albicans* isolates before and after treatment with piperine. Forty-two isolates were identified as Candida species (42.0%). According to the cultural and biochemical detection methods, sixteen (16 out of 42) isolates were identified as *C. albicans*. A statistically significant difference was observed between study groups of genders concerning the causes. Based on antifungal susceptibility testing, five isolates were resistant to two studied antifungals, with a percentage of 31.25%. The MIC of piperine (40 µg/mL) was affected on *C. albicans* isolates with ascending inhibition zones (5, 8, 9, 14 and 16) mm. The results of the RT-PCR technique revealed that *PLB1* gene expression of resistance *C. albicans* isolates was reduced after treatment with piperine extract. This study concluded that these findings are primarily the detection of the piperine effect against the *C. albicans* isolated from patients with candidiasis. So, in the future, another study must be conducted with large samples of candidiasis and isolates of *C. albicans.*

Keywords: *Candida albicans, PLB1,* Candidiasis, Fluconazole, Nystatin.

1. Introduction

The *Candida* genus represents the commonest aetiology of invasive fungal infections, especially among patients with immunosuppression (Pappas *et al.,* 2018). Several predisposing conditions like old age, decreased immunity, DM, AIDS, as well as the use of antibiotics for a long period, led to candidiasis especial that caused by *Candida albicans* (Millsop and Fazel, 2016). The clinical significance of *Candida* spp. extends beyond classical candidiasis, as these yeasts have also been detected in contaminated dental water systems and other healthcare environments, highlighting their importance as opportunistic human pathogens (Hikal et al., 2023).

Candidiasis includes oral, cutaneous and vaginal, the Incidence of *Candidiasis* has ranged up to 50% among those patients. Although *C. albicans* is the most prevalent species, non-*albicans Candida* species like *C. tropicalis*, *C. krusei*, *C. glabrata, C. parapsilosis*, and *C. auris* become a consequential clinical challenge (Sharifi *et al.,* 2023).

The high rates of azole resistance reported for *C. albicans* make treatment extremely problematic. In candidiasis, azoles are the first-line treatment for most *Candida* species; however, the increasing use of antifungals results in the frequent emergence of azole resistance. Almost all *C. albicans* clinical isolates are shown to be highly resistant to azole drugs, especially miconazole, with some strains resistant to all three classes of commonly used antifungals. This presents a huge challenge for researchers in terms of completely understanding the molecular mechanism of azole resistance to develop more efficient drugs (Jangit *et al.,* 2023).

Piperine is alkaloid found in the seed of black pepper (*Piper nigrum*), and it is also extracted from other *Piper* spp like *P. longum* and *P. retrofractum*. However, the highest concentrations of piperine are found in *P. nigrum* due to its higher fruit yield that responsible for alkaloid biosynthesis (Tiwari *et al.,* 2020). Piperine was used for influenza, rheumatism, and migraine treatment. Also, it has antimicrobial, antioxidant activity and anti-inflammation (Gorgani *et al.,* 2017).

Secretions of phospholipase proteins, such as *PLB1*, which acts as a virulence gene coding for *C. albicans* colonization by degradation of host cell phospholipids that promotes tissue invasion and biofilm formation (Priya and Shunmugiah, 2014).

2. Materials and Methods

Sample collection and isolation

One hundred samples were swabbed from persons infected with (oral and vaginal). Candidiasis patients who attended Baqubah Teaching Hospital during the period from December 2023 to January 2025, the inclusion criteria of the collected samples depended on whether they had previously been treated with antifungals and classified as recurrent candidiasis. The samples consisted of 53 oral and 47 vaginal swabs. All samples were collected from males and females (50 each) of different ages, ranging from 1-70 years old. The ethical approval and patient consent were obtained according to the decision 1231 in 14\12\2023. The samples were cultured immediately after sampling on Sabouraud's dextrose agar medium (SDA), incubated at 37°C for two days for isolation of *C. albicans*. Direct microscopical examination using KOH (10%) was done to detect the causative agent for candidiasis.

Candida albicans Isolation and Identification

After the streaking of isolates onto SDA at 37°C for 2-3 days, a pure colony was transferred to culture on HiChrom Candida agar medium at 37°C for 2 days and SDA at 42°C for 2 days for *C. albicans* identification. Also, the morphological characteristics of isolates, germ tube

formation and the VITEK 2 Compact system (BioMérieux, France) using YST-ID Cards were used for yeast identification (Mahajan *et al.,* 2014).

Germ tube test

A pure tiny portion of *C. albicans* colony was transferred to a sterile tube containing 0.5 ml of human serum according to the procedure reported by Kotgire and Hatkar (2024). Then, the mixture was incubated at 37°C for 2 hours. The germ tube was examined by overspreading a loopful of the suspension onto a sterile glass slide, covering it with a cover slide, and then testing it under a microscope at a power of (40 X)

Susceptibility of Antifungals

Triplicate for each isolate were susceptibility tested toward FLU (25 µg) and NY (100 IU) using the Disc Diffusion Method on Muller-Hinton agar (Himedia, India) supplemented with 3% glucose and 0.5 µg\ mL of methylene blue. To ensure the inoculum reached a concentration of $5 \times 10^{\wedge 6}$ yeast cells/mL, the turbidity of the yeast suspensions was adjusted to match a 0.5 McFarland density standard. The diameter of inhibition zones for FLU and NY was tabulated into sensitive and resistant isolates based on the interpretation chart from the CLSI document M44 2022). At the overnight growth decreasing changeover point, the widths of the inhibition zones were measured in millimetres. One of the triplicates for each resistance isolate was treated with piperine extract (CLSI document M44, 2022). According to the research-based criteria and CLSI (2022, the diameter of the inhibition zone (mm) of *C. albicans* toward FLU is as follows: ≤ 14 (Resistant), 15-18 (Intermediate), ≥19 (Susceptible). Whereas, the diameter of the inhibition zone (mm) of *C. albicans* toward NY is: < 10 (Resistant), 10-15 (Intermediate), >15 (Susceptible).

Detection of Biofilm formation using the microtiter plate method (MTP)

A pure colony of *C. albicans* was dissolved in 4ml trypticase soya broth and incubated at 37°C for one day. Then, it was diluted 1:100 in TSB containing 1.5% glucose, and the turbidity was set to 0.5 McFarland. About 200 µl of the dilution was inoculated into sterile flat bottomed 96 well of polystyrene tissue culture plates. After that, the plate was incubated at 37°C for one day. To quantify biofilm formation, the supernatant was removed from the microplate wells, which were then washed twice with sterile phosphate-buffered saline (PBS) and inverted to air-dry. The adherent biofilms were fixed and stained by adding 200 µL of 0.1% crystal violet to each well for a 20-minute incubation. After staining, the wells were washed twice with PBS to remove excess dye and blotted dry. The bound crystal violet was subsequently solubilized by adding 200 µl of an acetone-ethanol mixture (20:80 v/v). After 10 minutes, the optical density (OD) was measured at 595 nm using an ELISA plate reader. All assays were performed in triplicate, and the mean OD of each strain (OD) was compared against the mean OD of the negative controls (ODnc), which consisted of sterile medium. Biofilm production was categorized according to the following criteria:

- Non-producer: ODs =OD nc
- Weak producer: ODnc < ODs =2 x ODnc
- Moderate producer: 2 x ODnc < ODs = 4 x ODnc
- Strong producer: ODs > 4x ODnc (Elkholy *et al.,* 2025).

Extraction and purification of piperine

Ten grams of black pepper seeds were shaken with 2.5 litters 95% ethanol in a cool place for 72 hours. Next, the extract was filtered, and the filtrate was dried at 30-40 °C by a rotary evaporator to get 1/10 (one tenth) of its original volume. The resinous substance was further dried in the oven at 60 °C. The extracts were fractionated by column chromatography using the method of Sreevidya and Mehortra (2003). The piperine was purified using an HPLC method according to Harimurti *et al.* 2023). The stock solution of extracted piperine was 200 mg\ ml.

Determination of Minimum inhibitory concentrations (MIC) of piperine on *C. albicans* isolates

To determine the MIC of piperine extract against *C. albicans*, the broth microdilution method is standardly employed using a 96-well microtiter plate. The process begins by preparing a stock solution of piperine (200 mg\ ml). The extract was dissolved in Dimethyl sulfoxide (DMSO) and subjected to two-fold serial dilutions in Mueller-Hinton broth to achieve a range of decreasing concentrations, which were (320, 160, 80, 40, 20, 10, 5 and 2.5) µg/ml. Standardized fungal suspensions were prepared from fresh colonies by adjusting them to a 0.5 McFarland standard and further diluting them to achieve a final inoculum density of approximately 1×10^{5} CFU/ml. Each well received 50 µL of the piperine dilution and 50 µL of the yeast inoculum for a total volume of 100 µL. The experimental design included positive growth controls (broth and yeast), negative sterility controls (broth only), and solvent controls (DMSO). Following incubation at 37°C for 24 to 48 hrs, the plates were visually inspected for turbidity (Thakre *et al.,* 2021).

Candida albicans DNA extraction

Genomic DNA of *C. albicans* was extracted according to the ABIOpure Extraction kit protocol: For pellet cells, a loopful of grown hypha was suspended in 200µl of Buffer CL, and twenty microliters of Proteinase K solution (20 mg/ml) were added to 200µl of Buffer CL. The tubes were vortexed and incubated at 55 °C for 60 mins. The protein digestion and cell lysis. Next, two hundred microliters of Buffer was added after incubation, and the tubes were vortexed and incubated at 70 °C for 60 min. Then, two hundred microliters of absolute ethanol were added to the sample, and the sample was vortexed to mix thoroughly. All of the contents were added to a mini-column and centrifuged for 60 secs at 9,000 rpm, and the collected tubes were replaced with another. After that, six hundred microliters of Buffer BW were added to the mini-column, then centrifuged for 1 min at 6,000 x g (>8,000 rpm), and the collection tube was replaced with a new one. Seven hundred microliters of Buffer TW were applied. Centrifuge for 1 min at >8,000 rpm, pass-through was discarded, and the mini-column was reinserted into the collection tube. The mini-column was centrifuged at full speed (>13,000 x g) for 1 min to remove residual wash buffer, then the mini-column was placed into a fresh 1.5 ml tube. Finally, one hundred microliters of Buffer AE was added and incubated for 1 min at room temperature, then centrifuged at 5,000 rpm for 5 min. The concentration and quality of the extracted DNA were evaluated using a Nanodrop spectrophotometer. This device measures the optical density (O.D.) at wavelengths of 260 nm and 280 nm to quantify the genetic material. Furthermore, the purity ratio of the DNA was calculated using the following formula: DNA\ purity = O.D.260\ O.D. 280. This specific ratio serves as a critical indicator to determine whether the sample is free from protein contamination, as established by the standards of (15). Valid DNA samples typically yield a ratio between 1.8 and 2.0. To confirm the presence and structural integrity of extracted DNA, agarose gel electrophoresis was performed. Three microliters of DNA fragments with 2 µl of loading buffer were loaded into the gel. The ethidium bromide-stained DNA bands were visualized under a UV transilluminator at a wavelength of 350 nm. The resulting fluorescent patterns were then digitally captured or photographed to document the migration and presence of the target amplicons. This step ensured that the extraction of *C. albicans* DNA was successful.

Polymerase Chain Reaction technique

The primers of the *PLB1* gene and Internal Transcribed Spacer (ITS) region for *C. albicans* isolates were selected according to Naglik *et al. (*2003). The sequences of primers are: *PLB1-F*(CCTATTGCCAAACAAGCATTGTC), *BLP1-R (*CCAAGCTACTGATTTCACCTGCTCC*)* and ITS-F (5′-TCCGTAGGTGAACCTGCGG-3), ITS-R (5′-

TCCTCCGCTTATTGATATGC-3′), with molecular weights (179 bp and 650 bp, respectively). The PCR reactions for the detection of the *PLB1* gene and ITS region for *C. albicans* isolates were done in a total volume of 20 µls contain 5 µl of nfw, 10 µls from Go. Taq Master Mix, 1 µl each of 10 pmol forward and reverse primers and 3 µl of genomic DNA. PCR was applied as described by Naglik *et al.,* 2003). Amplifications of *PLB1* of *C. albicans* with initial denaturation at 95 °C for 6 mins. follow by 35 cycles of denaturation at 95C for 30Sec, annealing at 58°C for 30sec (For *PLB1* gene and ITS region), extensions at 72C for 45 secs and final extension at 72°C for 6 minutes. PCR product of the *PLB1* gene was analyzed on a 2% agarose gel. 100 bp. DNA ladder (Promega, U.S.A.) was utilized with staining of the PCR product using ethidium bromide and captured by an image capturer.

Gene Expression method

The extraction of RNA was done from the sample using the TRIzolTM Reagent's instructions. After being converted to cDNA, RNA/miRNA concentration is a factor in the analysis and calculation of gene expression levels. To evaluate the suitability of the samples for downstream applications, the concentration of the extracted RNA was quantified using a Quantus Fluorometer. The assay was prepared by mixing 1 µL of the RNA sample with 199 µL of diluted QuantiFluor Dye. Following a 5-minute incubation at room temperature in the dark, the fluorescence was measured to determine the precise RNA concentration. This fluorometric method ensures higher sensitivity and specificity for single-stranded RNA compared to traditional spectrophotometry. To quantify the expression levels of the *PLB1* gene, a one-step qRT-PCR SYBR Green assay was utilized. This method allowed for the simultaneous reverse transcription of RNA and quantitative amplification of the target cDNA in a single reaction vessel. Specific primer sequences were designed for each gene of interest to ensure precise amplification. To account for variations in sample loading and RNA quality, the mRNA levels of the ITS region were amplified as an endogenous control, serving as a housekeeping reference to normalize the expression data of the target genes. The RT-qPCR reaction volume and components for the detection of the *PLB1* gene and ITS region for *C. albicans* isolates were done in a total volume of 20 µls containing 1.5 µl of RNase-free water, 1.5 µls of RT mix, and 10 µls of Go. Taq qPCR Master Mix, 2 µl each of 10 pmol forward and reverse primers, and 4 µls of RNA. PCR was applied as described by Naglik *et al. (*2003). Amplifications of *PLB1* of *C. albicans* with cDNA synthesis at 37°C for 15 mins., initial denaturation 95°C for 6 mins. follow by 35 cycles of denaturation at 95°C for 30Sec, annealing at 58°C for 30sec (For *PLB1* gene and ITS region), extensions at 72°C for 45 secs and final extension at 72°C for 5 minutes. All procedures include data analysis, qPCR amplification, and whole RNA purification. Comparative Ct technique was used to quantify the degree of gene transcription and determine the level of gene expression for the *PLB1* gene and ITS region (mRNA level). As an endogenous control, the Ct of the ITS region was utilized to calibrate Cts for another gene. Calculations for folded gene expression are as follows:

ΔCt= Ct (*PLB1* gene) – Ct (ITS)

ΔΔCt= ΔCt (sample) – ΔCt (control)

Folding = $2^{-\Delta\Delta Ct}$

3. Results

Identification of Yeast Isolates

The identification of *C. albicans* isolates depended on morphological and physiological features, microscope and microscopic examinations, and the Vitek 2 system. The macroscopic appearance of *C. albicans* isolates that were cultured on SDA medium incubated at 37°C for 2-3 days was white to cream colored, smooth, glabrous and yeast-like colonies. Whereas, *C. albicans* isolates that were cultured on HiChrom Candida agar medium incubated at 37°C for

2 days appeared as light green smooth colonies. The Microscopic examination of cells appeared as pseudohyphae and budded, as shown in figures 1 and 2. *Candida albicans* was isolated more frequently from vaginal swabs than from oral swabs (with the percentages 19.2% and 13.2%, respectively). Positive percentages of identified *C. albicans* among males and females were 25.0% and 75.0%, respectively. A statistically significant difference was observed between study groups of genders concerning the causes. (Tables 1 and 2).

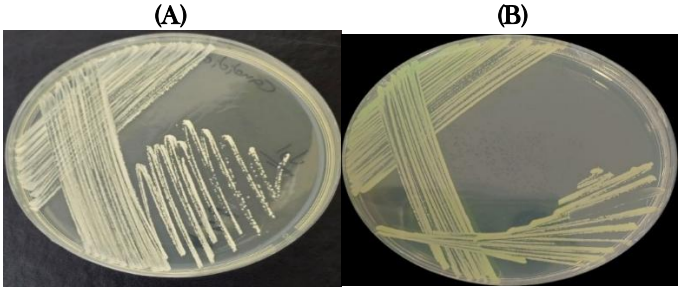

Figure 1. Macroscopic examination of *C. albicans* isolates cultured on: (A) SDA incubated at 37°C for 2-3 days and (B) HiChrom Candida agar medium incubated at 37°C for 2 days

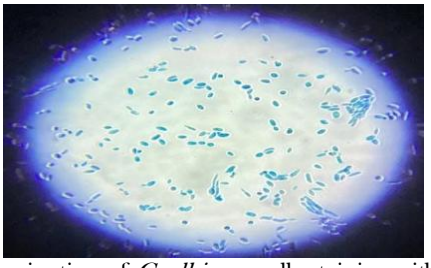

Figure 2. Microscopic examination of *C. albicans* cells staining with lactophenol cotton blue (40x)

Table 1. Percentage of *C. albicans* isolates concerning the site of lesions

Results	Oral swabs (%)	Vaginal swabs (%)	Total (%)
C. albicans	7 (13.2%)	9 (19.2%)	16 (16.0%)
Non albicans Candida	9 (16.9%)	17 (36.1%)	26 (26.0%)
Negative	37 (69.8%)	21 (44.7%)	58 (58.0%)
Total	53 (100.0%)	47 (100.0%)	100 (100.0%)
P value	0.0335*		

Table 2. Percentage of *C. albicans* isolates concerning lesion sites and patients' gender

Results	Oral swabs (%)	Vaginal swabs (%)	Total (%)
Males	4 (57.14%)	0 (0.0%)	4 (25.0%)
Females	3 (42.86%)	9 (100.0%)	12 (75.0%)
Total	7 (100.0%)	9 (100.0%)	16 (100.0%)
P value	0.019*		

Antifungal sensitivity test for *C. albicans*

According to the susceptibility test of 16 isolates of *C. albicans* against nystatin and fluconazole antifungals using the disc diffusion method (Kirby-Bauer). The results showed that only five isolates of *C. albicans* (31.25%) (2 oral isolates and 3 vaginal isolates) were resistant to both

studied antifungals. According to the biofilm formation, the moderate former isolates of biofilm were the most prevalent among other isolates (7 isolates out of 16), as shown in Table 3. A statistically significant difference was observed between the susceptibility test of antifungals against *C. albicans* concerning the sample sources.

Effect of piperine on resistant isolates of *Candida albicans*

The MIC of piperine (40 µg\ ml) showed the effect against isolates of *C. albicans* with ascending inhibition zones (5, 8, 9, 14 and 16) mm as shown in Figure 3.

Table 3. The antifungal susceptibility test of *C. albicans* concerning sample sources and biofilm formation

Isolate No. of *C. albicans*	Source of isolates	Inhibition zone toward FLU (mm)	Inhibition zone toward NY (mm)	Biofilm strength ODc=0.07
Ca1	Oral swabs	17	14	Moderate
Ca2		22	13	Weak
Ca3		7	8	Strong
Ca4		15	22	weak
Ca5		23	10	Moderate
Ca6		15	10	Moderate
Ca7		14	9	Moderate
Ca8	Vaginal swabs	20	11	Weak
Ca9		11	5	Strong
Ca10		19	21	Non former
Ca11		13	8	Moderate
Ca12		16	19	Moderate
Ca13		15	10	Moderate
Ca14		8	2	Strong
Ca15		24	15	Non former
Ca16		21	18	Weak
P value		0.021*		

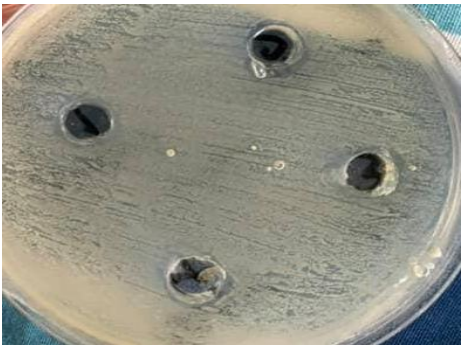

Figure 3. Anticandidal effects of piperine on resistant isolates of *C. albicans*

Molecular detection of the *PLB1* gene of *Candida albicans* isolates

The DNA was extracted from five resistant *C. albicans* isolates (Ca3, Ca7, Ca9, Ca11 and Ca14). PCR technique showed that all isolates (100%) carried the *PLB1* gene and ITS region with molecular weights of 179 bp and 650 bp, respectively (Figure 4). The present results show that the *PLB1* gene was expressed in all tested isolates. The expression level shows that this gene was regulated in biofilm formed isolate with high expression, folding 1.00 before piperine treatment, and the expression was reduced after piperine treatment for resistance isolates of *C. albicans*. The most significant impact of piperine extract on *PLB1* gene expression was observed in the C. alP11 isolate, with a mean ± SD of (25.47 ± 1.2). This isolate exhibited a fold change of 0.19 relative to the control isolate (C. al11) with a mean ± SD of (26.73 ± 0.82), as detailed in Table 4. A highly statistically significant difference was observed between *PLB1* gene expression for resistance *C. albicans* isolates (before and after treatment with piperine) (Figure 5).

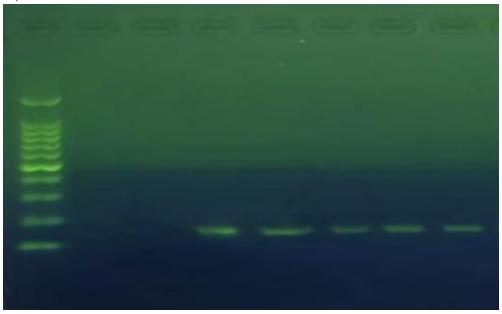

Figure 4. PCR product of *PLB1* gene (179 bp), which was electrophoresed on 1.5% agarose at 5 volts/cm2. 1x TBE buffer for 1:00 hours. N: DNA ladder (100)

Table 4. The expression of *PLB1* gene values for resistance *C. albicans* isolates (before and after treatment with piperine)

Isolate's No.	ITS region	*PLB1* gene	Δct	ΔΔct	Folding
C.al3*	27.61	27.22	-0.39	0.00	1.00
C.al7	27.2	26.81	-0.39	0.00	1.00
C.al9	26.01	25.77	-0.24	0.00	1.00
C.al11	25.74	25.35	-0.39	0.00	1.00
C.al14	27.09	26.91	-0.18	0.00	1.00
mean ± SD	26.73 ± 0.82				
C.alP3**	25.81	27.01	1.20	1.54	0.45
C.alP7	25.95	27.84	1.89	2.28	0.27
C.alP9	24.58	24.62	0.04	0.43	0.97
C.alP11	23.16	25.56	2.40	2.79	0.19
C.alP14	25.77	27.33	1.56	1.95	0.34
mean ± SD	25.47 ± 1.2				
P value	0.0015*				

*C.al1= Candida albicans isolate No. 1 before being treated with piperine extract, **C.alP1= Candida albicans isolate No. 1 after being treated with piperine extract.

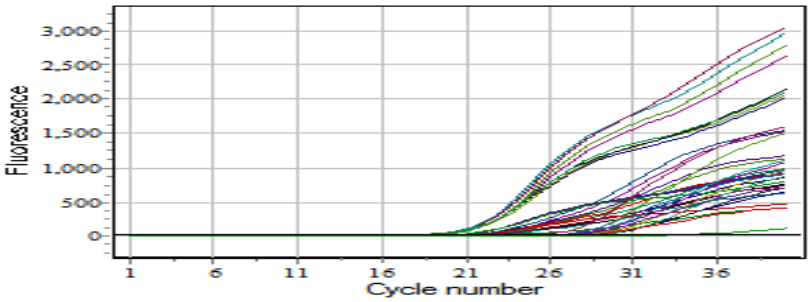

Figure 5. A curve shows the expression levels of the *PLB1* gene in *C. albicans* isolates treated with piperine extract

4. Discussion

Among patients with candidiasis, *C. albicans* was the most isolated species of *Candida* genus; this result agreed with (Mohammed *et al.,* 2019; Mohammed *et al.,* 2022; Bahi and Mustafa, 2023). who found that *C. albicans* was the most identified *Candida* spp. isolated from patients with candidiasis. *Candida albicans* is the only phospholipase-producing species, and it is worldwide the most frequently isolated from both humans and animals. Also, *C. albicans* is more virulent in comparison with other species of Candida because it has a broad expression of virulence factors (Chiang *et al.,* 2024). The number of collected samples may be related to these results, which did not show a predominance of non-albicans Candida. The results showed that only five isolates of *C. albicans* (31.25%) were resistant to both studied antibiotics. These results disagreed with those of Mohammed *et al.* 2029), who found that all isolates were (100) % susceptible to Nystatin (NY). Whereas, the highest resistance was for Fluconazole (FLU), which was (45.45) % for *C. albicans* isolates. Number of collected samples, virulent role of identified strain, hygiene state of persons and presence of normal human surface microbiota all may play important roles in resistance of *C. albicans* isolates to studied antifungals. Some studies (Min *et al.,* 2017; Noguira *et al.,* 2017) found that the MIC of piperine extracts from *P. nigrum* was 32 mg/mL for *C. albicans*. Piperine acts to prevent ergosterol biosynthesis of *C. albicans* cells as well as reducing biofilm formation (Manoharan *et al.,* 2017).

The DNA was extracted from five resistant *C. albicans* isolates (C3, C7, C9, C11 and C14). PCR technique showed that all isolates (100%) carried. Its region and *PLB1* gene have molecular weights of 650 bp and 179 bp, respectively. This result agrees with (Mohmoodi *et al.,* 2025), who found that (57.8%) of *C. albicans* isolates carried this gene. The discrimination of results may be due to the highest virulence of *C. albicans* and the variation in the source of isolated sites or hosts. A study done by (Gharaghani, 2022) evaluated the expression patterns of genes in the *PLB1* gene family in clinical oral, vaginal and urine specimens by reverse transcriptase-PCR (RT-PCR). They showed that the *PLB1* gene was expressed at all time points and inoculation densities. The most significant impact of piperine extract on *PLB1* gene expression was observed in the C. alP11 isolate, which exhibited a fold change of 0.19 relative to the control isolate (C. al11). The variation in the source of collected isolates, nutrient competition of identified yeast and some transcription factors such as EFG1, BCR1 and CPH1 play important roles in the morphogenesis and biofilm formation of *C. albicans,* as results these effects on the expression of virulence genes concern the *PLB1* gene. All these factors may be explained by the downregulation of the studied virulence gene.

5. Conclusion

This study concluded that the percentage of *C. albicans* identification using cultural and biochemical methods was the same (100%). PCR results showed that all isolates carried the *PLB1* gene. The gene expression showed that the levels of the *PLB1* gene were regulated in biofilm formed isolate with high expression, increasing 1.00-fold before piperine treatment, and the expression was reduced after treatment. The fungi were interpreted as either resistant, intermediate, or susceptible to an antibiotic drug. The result was compared with the standard inhibition zone.

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Novelty statement

The novelty of the study is focused on the physiologically active compounds of piperine extract that can be employed as novel anti-fungal pharmaceuticals due to the lack of an approved vaccination for any fungal disease and the lack of readily accessible, secure, and efficient medicines for some diseases or fungi that are resistant to synthetic treatments. It is imperative to seek into alternative sources of anti-fungal medications.

Author contributions

These authors each contributed equally.

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Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis, or presentation of the research.

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