P-ISSN: 2410-8863
E-ISSN:2958-6178
https://djvs.uodiyala.edu.iq
Pages: 124-132

Diyala Journal for Veterinary Sciences
Vol. 3 No.2 June (2025)

https://doi.org/10.71375/djvs.2025.03210

Antimicrobial Effects of Capsaicin Extracted from *Capsicum annuum* on *HWP1* Gene Expression of *Candida albicans*

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Important dates: **Received :** February 29, 2025 ; **Accepted:** April 05, 2025; **Published**: June 01, 2025

Abstract

Background: Cutaneous lesions of candidiasis in dogs are characterized by erythematous moist erosions with an irregular contour and there may be alopecia, crusting, ulceration and edema. Therefore, the skin scrapings and skin biopsy are the main methods for cutaneous candidiasis diagnosis in dogs.Protein agglutinin-like sequence proteins (*HWP1*) are examples of adhesion proteins which activate with encoded many targeted genes like (*HWP1*) . *HWP1* gene which are involved in *C. albicans* on host cells in cutaneous Candidiasis. Capsaicin is a phytochemical structure is extracted from *Capsicum annuum* L., it was showed activity on adherent virulent role of different microbial skin problems such as cutaneous candidiasis. **Objective:** The aim of this study was to detect the effectiveness of capsaicin against *HWP1*gene expression for *Candida albicans* isolated from canine cutaneous candidiasis. **Material and methods:** A total of 100 skin samples from German Shepherd-K9 dogs (*Canis lupus* L.) which infected with cutaneous candidiasis. All of the isolated *C. albicans* were examined under a microscope and using biochemistry. screening and purification methods were used to look into the detection of capsaicin from *Capsicum annuum* L.. The expression of (*HWP1*) gene before and after treatment with capsaicin was compared using reverse transcription quantitative polymerase chain reaction (RT-qPCR). **Results:** The results show that the highest expression folding 1.00 before capsaicin treatment and the expression was reduced after treatment folding of this gene was reduced after capsaicin treatment which means that capsaicin inhibits the gene expression of biofilm formation of *C. albicans*. **Conclusions:** Our conclusions were the gene expression showed that the levels of gene *HWP1*were up-regulated in biofilm formation isolates.

Keywords: *Candidaalbicans*, *HWP1* gene, Capsaicin, Antifungals, *Cutaneous Candidiasis*

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Introduction:

According the size of canine organs, the skin represents the largest organ it cover the external surface of canine body , those layers have possess varying anatomical functions and process. It consists of 24% of body weight but as it were 12% of growing canine (Bonifant and Holloway,2019). *Candida albicans* is yeast like cell; it is the commonest cause of contagious fungi around the world and the biggest class of restoratively vital yeasts. This genus includes around 200 species, most of them are commensals or endosymbionts of has counting people (Talapko et al., 2021). Cutaneous lesions of candidiasis in dogs are characterized by erythematous moist erosions with an irregular contour and there may be alopecia, crusting, ulceration and edema. Therefore, the skin scrapings and skin biopsy are the main methods for cutaneous candidiasis diagnosis in dogs (Heseltine et al., 2015). In the severe immunocompromised dogs, especially newborn dogs or dogs which treated with antibiotics for a long periods, (Katiraee et al., 2022). Itraconazole, fluconazole and ketoconazole are concluded for systemic treatment and nystatin, ketoconazole, clotrimazole, miconazole are concluded for topical treatment. The drug of choice for cutaneous candidiasis treatment is recommended systemic or topical are depended on the degree of lesion involvement (Lin et al., 2025).

The adhesion proteins of cell wall for *C. albicans* act as intercellular communication between yeast and host cells, this correlation led to development of C. *albicans* pathogenicity (Poulain and Jouault, 2004). Protein agglutinin-like sequence proteins (*HWP1*) are examples of adhesion proteins which activate with encoded many targeted genes like (*HWP1*) . which are involved in *C. albicans* on host cells in cutaneous candidiasis. Also, these proteins family (*HWP1*) are characterized with antibiotics resistance of fluconazole and nystatin when they used in treatment of cutaneous Candidiasis in dogs (Bilal et al., 2022).Capsaicin is structure is extracted from *Capsicum annuum* L., it is used as a homeopathic treatment burned skin, specific reviews showed that active for treatment of different microbial skin problems such as cutaneous candidiasis(Chang et al., 2023).

Materials and Methods

Samples collection and isolation

One hundred skin scraps and swabs were collected from German Shepherd-K9 dogs (*Canis lupus* L.) which infected with cutaneous candidiasis for a period extended from 1st November 2023 to 30th April 2024. The skin samples were collected from dogs which attended Veterinary Teaching Hospital and private veterinary clinics in Baghdad province. The ages of infected dogs were ranged1-5 years old for both sexes males (50) and females (50). For red chili pepper fruits collections.

Isolation and identification of *Candida albicans* isolates

Skin scraps were inoculated into Sabouraud Dexyrose Agar medium (SDA) containing 0.5% chloramphenicol, penicillin at concentration of 10% and streptomycin at a concentration of 10%. The plates were incubated at 37°C for 48-72 hrs. (Hare ,2013).Germ tube formation, cultured onto

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HiCHROM Candida agar medium and VITEK 2 system were used for *C. albicans* identification in according to(Hospenthal et al., 2006; Khullar et al., 2017; Moya, 2018).

Capsaicin

Capsaicin is the bioactive component that attaches to pain receptors, resulting in a severe burning sensation. Capsaicin, a potent antioxidant, imparts the red hue to mature fruits. Chili originates from Central America and extends further southward. This crop holds significant commercial value and possesses extensive genetic variety. A comprehensive account of the crop including its origin, distribution, botanical characteristics, normal agronomic practices, and control of biotic challenges is provided (Pandit et al., 2020). Chili peppers, which belong to the genus Capsicum, are among the earliest crops to be domesticated. They contain a wide variety of naturally occurring bioactive components, which makes the plant a highly useful spice that also has positive effects on one's health.

Susceptibility to antifungal agents test

Candida albicans isolates were subjected to susceptibility test against studied antifungals Nystatin (NYS) and Chloramphenicol (CLO) and using the Disc diffusion method, following the parameters outlined in the Clinical and Laboratory Standards Institute (CLSI, 2017) M44-A document. The test was conducted on Muller Hinton agar containing glucose (2%) and 5µgs/ml of methylene blue (Michael et al., 2014). the inocula were prepared by suspending *C. albicans* colony in 5 ml of saline solution with turbidity set at 0.5 McFarland Standards. Cottoned swabs were immersed in the suspension and then applied directly onto the agar plate's surface. The plates were cultured at a temperature of 37°C. After a 24-hour period. Zones of inhibition were analyzed using CLSI interpretative method.

Extraction and purification of capsaicin

The fruits red chili pepper (*Capsicum annuum* L.) were collected from different local markets in Baghdad province. All the plant samples were similar in the storing conditions .The approval identification of *C. annuum* was depended on method described by AL-Anbari (2023). Out of 1000 grams of fresh *C. annuum***,** one hundred seeds were shaken with 2.5 litters 70% ethanol in 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, resinous substance was further dried using the oven at 60^{o}C. The extracts were fractional by column chromatography using the method of (Zhang et al., 2014). The capsaicin was purified using HPLC method according to (Collins et al., 1995), table (1).

Table (1) Conditions of HPLC method

Mobile phase	acetonitrile : D.W (80 :20)
Column	C 18 – ODS (25 cm * 4.6 mm)
Detector	UV – 280 nm
Mobile phase	Acetonitrile-water
Flow rate	1 ml / min

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Determination of Minimum inhibitory concentration (MIC) of capsaicin on *C. albicans* isolates

The MIC for all isolates had been determined according to (Moreno et al., 2006). Serial dilution method has been prepared for capsaicin by adding different ratios of their stock solution to sterile and cooled to 45°C of Muller-Hinton agar. About 5 µl was withdrawn from the above dilution (10^{-2}) with micropipette to inoculate (well method) on the antibiotic media making a Circular well of 5mm in diameter were cut by using a sterile cork borer and then low melting temperature MHA was used to seal the bottom of the wells. The results have been determined by the inhibition zone formation and measured the diameter of the zone.

Molecular methods

The DNA of *C. albicans* isolates was extracted according to (Aysha et al., 2015**)**

Polymerase Chain Reaction technique:

Table (2) shows the primers of *HWP1* gene for *C. albicans* isolates were selected according to (Aysha *et. al.*, 2015). with molecular weight (318 base pairs)

Table (2): The sequences of primers are: *HWP1*

.

HWP1-F	ATGACTCCAGCTGGTTC
HWP1-R	TAGATCAAGAATGCAGC

The PCR reactions for detection of studied gene for *C. albicans* isolates were performed in 20 µl volumes containing 5 µl of nuclease free water, 10 µl of GoTaq Green Master Mix 2X containing, 1 µl each of 10 pmol forward and reverse primers and 3 µl of genomic DNA. PCR was applied according to the PCR program described by (Aysha *et al.*, 2015). Amplification of *HWP1* gene of *C. albicans* was carried out with initial denaturation 95C for 5 min. was followed by 30 cycles of denaturation at 95C for 30Sec, annealing at 58 C for 30sec, and extension at 72C for 30 sec and final extension at 72°C for 7 minutes. PCR product of *HWP1* gene was analyzed on 2% agarose gel. The 100 bp DNA ladder (Promega, USA) was used and the PCR products were stained using ethidium bromide and visualized by an image analyzer.

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. All procedures include data analysis, qPCR amplification, and whole RNA purification. Comparative Computed tomography (Ct) technique was used to quantify the degree of gene transcription and determine the level of gene expression for the *HWP1* and ITS genes (mRNA level). As an endogenous control, the Ct of the ITS gene was utilized to calibrate the Ct values of other genes. The calculations for folding gene expression are as follows: * Ct= cycle of threshold (number of cycles required for florescent signal to cross threshold). * HK= housekeeping gene.

Results and Discussion

Microscope examination

Direct Microscopic using Potassium Hydroxide (KOH 10%) and indirect Microscopic Lactophenol Cotton Blue (LPCB stain and germ tube formation) examinations were done for *C.*

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albicans identification. the direct microscopic of *C .albicans* appeared as smoothed and whitish . Lacto phenol cotton blue smears showed dark blue budding yeasts without pseudohyphae inoculated with human serum from *Candida* spp. The germ tubes formed by *C. albicans* isolates was appeared a long tubes appendages extend of yeasts as shown in figures (1).

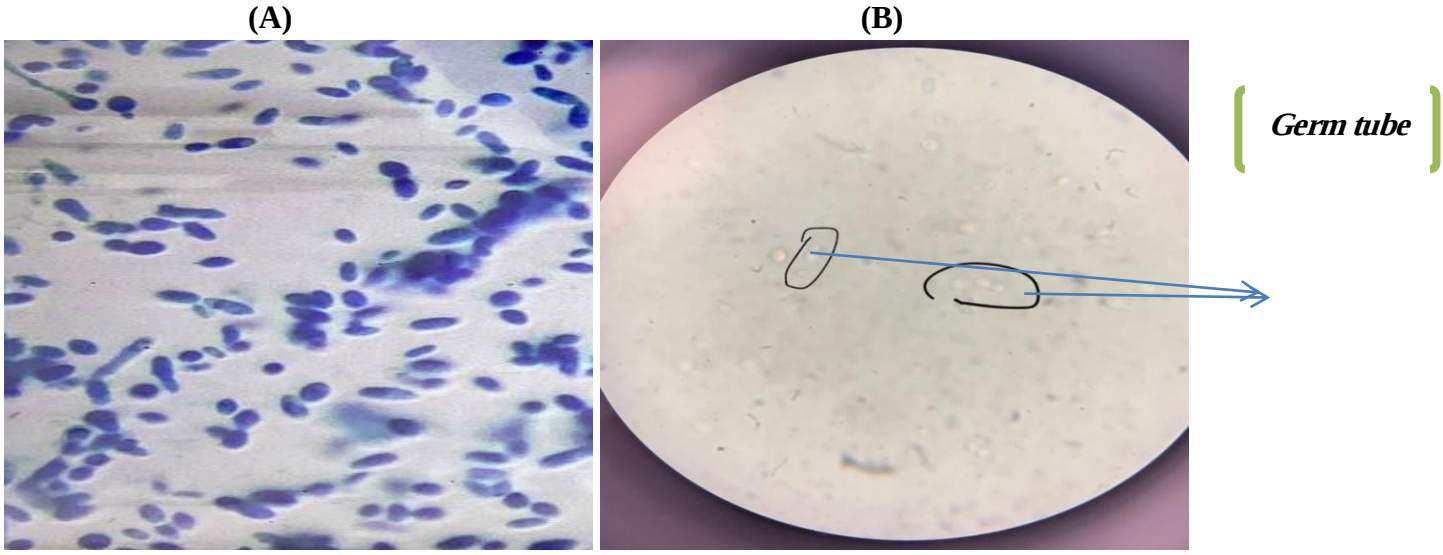

Figure (1): Microscopic examination of *C. albicans* isolates (40x): (A) stained with LPCB stain and (B) germ tube formation.

Antibiotics sensitivity test for *C. albicans*:

According to the susceptibility test of 14 isolates of *C. albicans* against nystatin and clotrimazol antifungals using Disc diffusion method (Kirby-Bauer). The results showed that only four isolates of *C. albicans* (28.6%) were resistant to both studied antibiotics. (CLSI, 2017 and CLSI 2023). This results agreed with (Abeer et al., 2019) and disagreed with (Nguyen et al., 2024) who found that 66.6% and 45.8% of *candida albicans* isolates were resistant for nystatin ,clotrimazole, repectively. Number of collected samples, virulent role of identified strain, hygiene state of dogs and presence of normal canine surface microbiota all these may be play important roles in resistant of *C. albicans* isolates to studied antifungals.

Effect of purified capsaicin on resistant isolates of *Candida albicans*:

only three isolates of *C. albicans* were sensitive to purified capsaicin (50 mg\ ml) using MIC method with ascending inhibition zones (9, 13 and 16) mm. (Menezes et al., 2022) and (Moghadam et al., 2023) found that the MIC of capsaicin extracts from *Capsicum chinense* was 15 mg/ml and 20 mg\ml for *C. albicans*, respectively. Capsaicin exerts its effects acts preventing ergosterol biosynthesis of *C. albicans* cells as well as reducing biofilm formation (Behbehani et al., 2023).

Molecular identification of *Candida albicans* using PCR technique

The detection of genes (*HWP1* and *ITS*) by PCR of four isolates of *C. albicans*. The amplified virulence genes were identified depended onto annealings with m. wt for product. Present of uni

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Vol. 3 No.2 June (2025)
https://doi.org/10.71375/djvs.2025.03210

band from extract DNA. indicate effictive of this methods use in DNA. Extractions. results showed that the four isolates have the virulence genes (*HWP1*) and housekeeping ITS gene, figure (2).

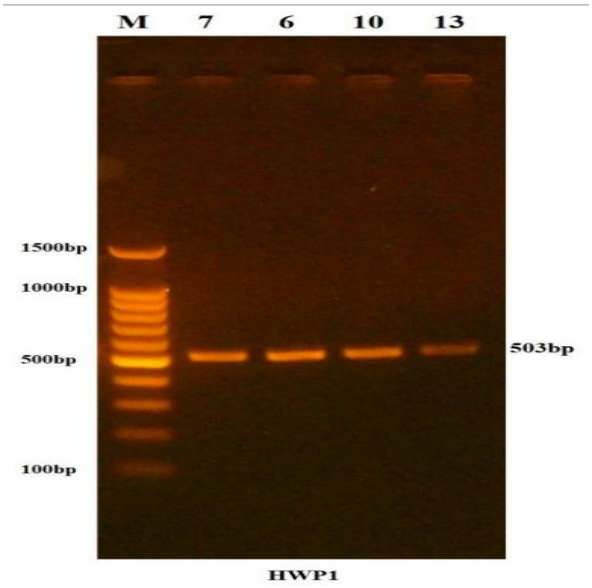

Figure (2) Electrophoreses of HWP1 uniplex PCR products for *C. albicans* using 2% agarose gel at 7volts/ cm. for 60 mins. M: 100 DNA ladder, lane 1-4: uniplex genes PCR products.

Gene expression of *HWP1* virulence genes in *C. albicans*:

The folding rate (the gene expression) of ITS as housekeeping gene, *ALS5* and *HWP1* as virulent genes were variant among four *C. albicans* isolates before and after capsaicin treatment as shown in figures (3) and tables (3)**.**

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https://doi.org/10.71375/djvs.2025.03210

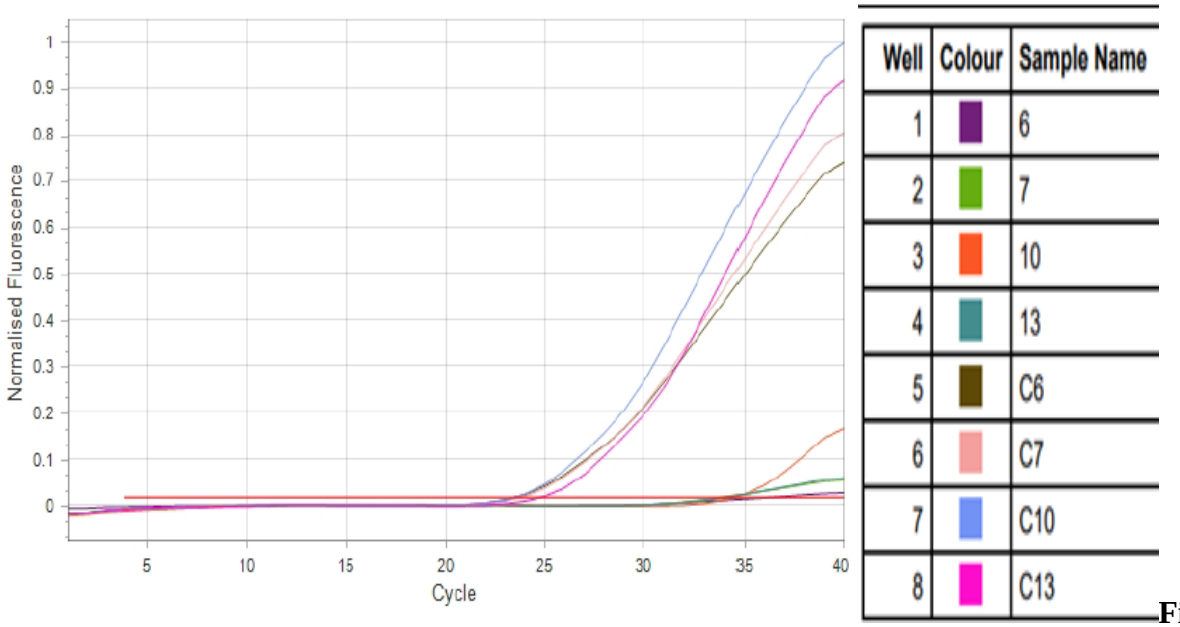

Figure (3): The amplified curve and Ct of the biofilm gene (*HWP1*) of the treated and untreated *C. albicans* isolates

Table (3): Gene expression values for *HWP1* gene in *C. albicans* isolates

Isolate No.	*ITS* CT	*HWP1* CT	Δ CT	ΔΔ CT	Folding 2-ΔΔ CT
C6	23.03	25.60	2.57	0.00	1.000
C7	23.22	25.05	1.84	0.00	1.000
C10	22.56	26.37	3.82	0.00	1.000
C13	23.04	25.76	2.72	0.00	1.000
CC6	23.31	34.45	11.13	8.56	0.0026
CC7	24.16	36.01	11.85	10.02	0.0010
CC10	21.59	34.50	12.92	9.10	0.0018
CC13	23.10	30.31	7.21	4.48	0.0448

CONCLUSION

This study was concluded that the percentage of *C. albicans* identification using cultural and biochemical was the same (100%). The gene expression showed that the levels of gene *HWP1*were up-regulated in biofilm formation isolates.

Acknowledgment: The author gratefully acknowledges the Microbiology department, College of Veterinary medicine, Diyala University and Biotechnology department, college of science, Diyala university for providing the facilities and necessary resources.

Conflict of Interest: Author declares that there is no conflict of interest.

P-ISSN: 2410-8863
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Diyala Journal for Veterinary Sciences
Vol. 3 No.2 June (2025)

https://doi.org/10.71375/djvs.2025.03210

Funding Sources: No fund was received by Author

Authors Contributions: The author of this manuscript has designed, conducted the experiments, drafted and amended all versions of the manuscript and submitted it to this journal for publication.

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