Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

Silver Nanoparticles Inhibition of SAP 7 and SAP 9 Gene Expression in Candida albicans Isiolates from Diabetic Foot Ulcers

Fatin Ali Al-Chalabi[1], Mohsen Hashim Risan[2], Rabab Majead Abed[1]

[1]Department of Biology, College of Education for Pure Science, Diyala University, Diyala, Iraq.

[2] College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.

(Received: 04 August 2023 Revised: 12 September Accepted: 06 October)

KEYWORDS

Diabetic foot ulcers, Candida albicans, SAP7, SAP9, AgNPs.

ABSTRACT:

Candida albicans is the most common yeast species in diabetic foot patients who are at risk of growing diabetic foot ulcers, this has something to do with fungal infections. This study was conducted on 203 clinical samples of diabetic patients with diabetic foot ulcers for the period from January 15 to May 15, 2022. All samples were grown on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24–48 hours under aerobic condition . Identification of Candida albicans isolates depends on morphological characteristics in culture medium, germ tube formation, chlamydospore formation, and CHROMagar and using the Vitek 2 compact system . It was found (15) isolates from C.albicans . The results indicated the presence of SAP7,SAP9 genes in all isolates (100%; n=15) of C.albicans.The treated of 4 isolates with AgNPs , our results show an increase in the gene expression values of SAP 7 for the two isolates CAA1 and CAA11 and tow isolates, CAA2 and CAA5 had reduced gene expression values .The results of our study on SAP 9 gene expression after treatment with AgNPs were that there was an increase in the gene expression values of the two isolates CAA1 and CAA11 and gene expression values decrease for two isolates, CAA2 and CAA5.

Introduction

One of the most common and serious complications of this disease is Diabetic Foot Ulcers (DFUs) in uncontrolled diabetes, the infection is predominantly polymicrobial between aerobic, Gram-positive, anaerobic, and fungal infections. Early detection of fungal infections and initiation of appropriate treatment may lead to DFU promotes wound healing and avoids amputation (Kandregula *et al*., 2022). Once the protective covering of the skin is damaged, bacterial and fungal infections can reach deeper tissues. Patients with DFUs usually require amputation of the lower extremities (Ramirez-Acuña *et al*. 2019).

Secreted aspartyl proteinase (Sap) proteins resulting from gene expression of *SAP* genes consisting of 10 genes (Sap1-10) play a major role in the virulence of *Candida albicans* during fungal infection, as they participate in the infection process by degrading various host cell proteins such as immunoglobulins, and the proteins of the complement system, allowing the fungus to escape the first line of host defense from tissue damage and resulting invasion of other microorganisms (Hube *et al*., 1994; Monika and Zbigniew, 2017).

With advances in nanotechnology, silver nanoparticles (AgNPs) have been extensively studied for their potential applications in biomedicine, such as anticancer nanodrug carriers, vaccines and drug delivery systems (Rai *et al*., 2015; Thayath *et al*., 2021). In recent studies, AgNPs have also proven effective against *C. albicans* (Selvaraj *et al*., 2014; Monteiro *et al.*, 2015) by disrupting membrane potentials and forming pores that cause leakage of ions and other substances, leading to apoptosis and causing ultrastructural changes (Kim *et al*., 2009 ؛ Hwang *et al*., 2012). Some studies found that AgNPs at low concentrations inhibit *C. albicans*, which prevents its growth and affects the formation of a mature biofilm. In addition, AgNPs combination with antifungal drug like fluconazole (FLZ) improves its inhibition potency (Monteiro *et al*., 2011; Vazquez Muñoz *et al*., 2014).

The aim: To identify the effect of AgNPs on *SAP* 7 and *SAP* 9 gene expression in *Candida albicans* isolates from diabetic foot ulcers.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

Materials and Mathods

Study Samples, Culture, Identification of *Candida albicans*

This study was conducted on 203 clinical samples of diabetics with diabetic foot ulcers for the period from January 15 to May 15, 2022. All samples were grown on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 24–48 hours under aerobic condition . Identification of *Candida albicans* isolates depends on morphological characteristics in culture medium, germ tube formation, chlamydospore formation , and CHROMagar and using the Vitek 2 compact system .

Preparation of silver nanoparticles

AgNPs solution prepared by the American company (Skyspring nanomaterials) with a purity (+99%) with a size of 50-60 nm, 0.01 g was dissolved in 10 ml of DDW (Deionizer Distal water) using an ultrasonic mixer. (Ultra sonic homogenizer) for 30 minutes at room temperature, thus obtaining a stock solution with a concentration of 1000 µg/ml The dilution equation (V1C1=V2C2) was used to prepare a series of half-dilutions.

AgNPs susceptibility test

Dilution method in nutrient medium was used to determine the minimum concentration of growth inhibiting nanoparticles (Das, *et al*., 2016). SDB medium was used to prepare a series of dilutions of silver nanoparticles with concentrations of (500, 250, 125, 62.5, 31.25, 15.62) µg / ml. 4.9 ml of the culture medium was placed in all tubes, and 5 ml of the first buffer solution was added to the second tube to obtain At a concentration of 500 µg/mL. The components of the tube were mixed well by shaking, and so on for the rest of the concentrations up to the last concentration. 5 ml of the product was withdrawn and neglected. 0.1 ml of fungal suspension with a turbidity level of 0.5 was added to all tubes. A tube was left for each nanomaterial without inoculation and it was considered a negative control, while the positive control was represented by the presence of the suspension and the medium without nanomaterials. After inoculation of the fungal isolates with the prepared concentrations of the nanomaterials, the tubes were incubated at a temperature of 37 °C for 24 hours to examine the presence of fungal growth based on the naked eye by observing the turbidity and clarity of the medium. A confirmatory culture was performed for each tube of different concentrations in order to determine the minimum inhibitory concentration (MIC) accurately, as it was planted on SDA medium and incubated at a temperature of 37 °C for 24 hours. Through the results of transplantation, the concentration at which the MIC inhibition occurred was determined, and the concentration of the MIC Sub, which is the tube from which RNA was extracted for the purpose of measuring gene expression by RT-PCR.

Molecular identification

DNA extraction

Extract genomic DNA from overnight culture according to the protocol described in the Kit from (China-Transgenbiotech). The concentreation of the DNA extract was determined by Nanodrop spectrophotometer.

PCR primers to detection of *SAP* 7 and *SAP* 9 gene

The presence of *SAP* 7 , *SAP* 9 gene was evulated using PCR as described previously. Primers sequences are listed in Table 1. PCR was performed using AccuPower ® PCR PreMix , which contains Taq polymerase, ‘dNTPs , MgCl2’ and the appropriate buffer. Each PCR tube contains 25µl reaction mixture containing 5 µl master mix, 1µl each forward and reverse primer solution, 3µl DNA and nuclease and 15 µl free water to complete the volum. PCR was performed according to the following conditions: initial denaturation at 95C° for 4sec , 1 cycles , denaturation at 95C°,20 s , 35 cycle . Annealing temperature, for, 57C° for 30s followed by a final extension 72C°,for 7sec. The amplified DNA used to be separated by 2% agarose gel electrophoresis.

Table 1: Primers sequences

Gene	Primer	Sequence(5′-3′ direction) real time PCR	(bp) Bais pair	Reference
*ACT*1	F	GACAATTTCTCTTTCAGCACTAGTAGTGA		Belmadani *etal* .2018

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

	R	GCTGGTAGAGACTTGACCAACCA	87	
SAP 7	F	GAAATGCAAAGAGTATTAGAGTTATTAC	196	Monod *etal*. 1994
	R	GAATGATTTGGTTTACATCATCTTCAACTG		
SAP 9	F	ATTTACTCCACAGTTTATATCACTGAAGGT	86	Belmadani *etal* .2018
	R	CCACCAGAACCACCCTCAGTT		
		PCR		
SAP 7 C	F	AAGAAGCAAAGAAATGCAAAGAGTA	440	Researcher
	R	TATTCAAACCGATTAAATTCCAAAA		
SAP 9 C	F	CTATGGTTCATTCAATACCGAAAAC	556	Researcher
	R	GTCGCTGAAAACATACGATAAAGTT		

RNA purification and extraction

RNA was extracted from 4 isolates (CAA1,CAA2,CAA5,CAA11) isolated from DFUs, By using the Trizol kit supplied by the Korean company Bioneer .The concentreation of the RNA extract used to be decided with the aid of via Nanodrop spectrophotometer.

Detection of gene expression by quantitative real time PCR of SAP *7*, SAP *9*

A quantitative real-time polymerase reaction assay (reverse cloning) was performed to measure the quantitative levels of mRNA, to indicate the amount of gene expression for the SPA 7 and SPA 9 genes, and the Act 1 gene was used as a standard regulator gene to calculate gene expression before and after treatment with nanomaterials. This examination was carried out for cDNA samples of the experimental groups using the Accupower 2X green star qPCR kit, which was prepared by the Korean company Bioneer and contains a cyber green dye that interacts with the amplified genes in the Real-Time PCR device. RT-PCR performed using q PCR Master mix 20µl, 1µl of each forward and reverse primer, 16µl of DEPC water and 10µl of RNA. The RT-PCR used to be performed in accordance to the following corditions : one cycle. Initial denaturation (95C°, 4 min), the 45 cycle of denaturation (95C°, 20s). Annealing/ Extension Detection scan at 55C° , for 45s, Melting 60-95 C° for 1s. Calculate gene expression using the equation

1- ΔCT =CT gene - CT House Keeping gene

2- ΔΔCT =ΔCT Treated - ΔCT Control

3- Gene expression Ratio $=2^{-\Delta\Delta CT}$

Results

The study included two hundred and three samples of diabetic foot ulcers, (105 , 51.7%) were male, (98, 48.3%) were male. These samples were collected from patients who attended Yarmouk Teaching Hospital and outpatient clinics during the period from January 15 to May 15, 2022 using sterile swabs with media and were cultured on Sabouraud Dextrose Agar (SDA). The results of phenotypic, microscopic and physiological examination . The results showed that among the 113 isolates that gave a positive result for fungal growth, 15 (13.27%) gave a positive result for *C. albicans* yeast.

AgNPs susceptibility test

After inoculation of *C. albicans* isolates with the prepared concentrations of AgNPs individually, they were incubated for 24 hours at a temperature of 37 °C. The results of the study showed that the average value of the minimum inhibitory concentration (MIC) for the used nanomaterials (125 - 250) µg/ml, while the Sub MIC value was (125- 31.25) µg/ml .

Molecular identification

Detection of (*SAP* 7 and *SAP* 9) genes by PCR

All *C. albicans* isolates (15 isolates) were subjected to detection of *SAP* 7 and *SAP* 9) genes within the polymerase chain reaction (PCR). After electrophoresis of the (PCR) product, the results showed that all the 15 studied isolates contained *SAP* 7 and *SAP* 9 with clear bands and a length of (440 bp for the *SAP* 7 gene) and

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

(556 bp for the *SAP* 9 gene), as shown in Figure (1) and Figure (2).

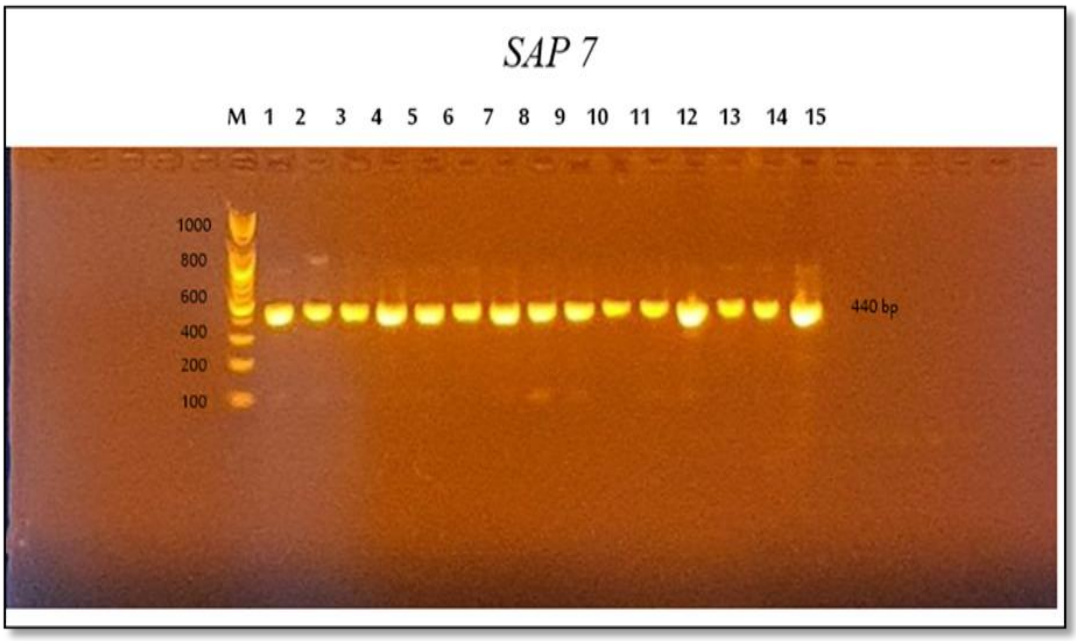

Figure .1: Electrophoresis of the product of the PCR reaction of *SAP* 7 gene (440bp) using 2% agarose gel with ethidium bromide dye (45 min, 70 v/cm2) M : marker DNA ladder 100 bp.

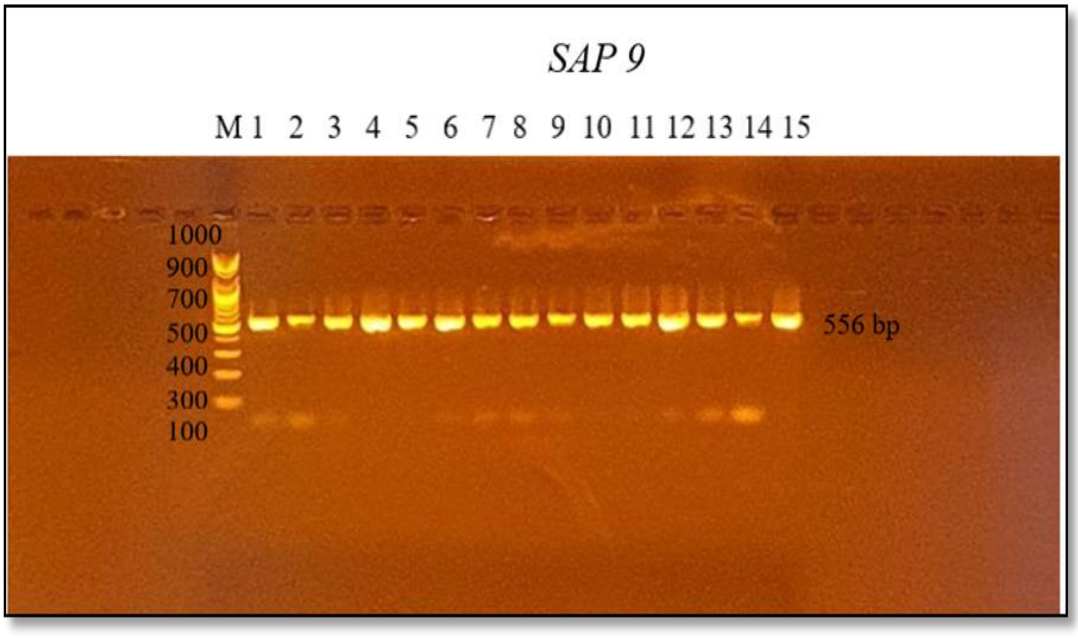

Figure .2: Electrophoresis of the product of the PCR reaction of *SAP* 9 gene (556bp) using 2% agarose gel with ethidium bromide dye (45 min, 70 v/cm2) M : marker DNA ladder 100 bp

Effect of AgNPS on the gene expression of SAP7, SAP9 genes

Four isolates of *C. albicans* (CAA1,CAA2,CAA5,CAA11) were selected to investigate the effect of silver nanoparticles on gene expression products for *SAP* 7,*SAP* 9 before and after treatment with the nanomaterial.

The results of our study showed that there was an increase in the gene expression values of *SAP*7 for the two isolates CAA1 and CAA11 and a decrease in the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

gene expression values of the two isolates CAA2 , CAA5, as shown in Table (2).

Table .2: Gene expression of the SAP7 gene before and after treatment with AgNPs

Fold Change2^-ΔCt	ΔΔCt	ΔCTC	ΔCTE	CT *SAP7* Untreated	CT *SAP7* Treatemnt	Sample
1	0	0	0	27.65	27.15	Control
58.08123	-5.86	4.62	-1.24	27.3	27.17	CAA1
0.297302	1.75	-1.1	0.65	28.7	29.99	CAA2
0.116629	3.1	-2.56	0.54	26.5	28.77	CAA5
9.917662	-3.31	0.26	-3.05	27.65	27.15	CAA11

The results of our study on SAP 9 gene expression after treatment with AgNPs were that there was an increase in the gene expression values of the two isolates CAA1 and CAA11 and a decrease in the gene expression values of the two isolates CAA2 , CAA5, as shown in Table (3).

Table .3: Gene expression of the SAP7 gene before and after treatment with AgNPs

Fold Change2^-ΔCt	ΔΔCt	ΔCTC	ΔCTE	CT *SAP7* Untreated	CT *SAP7* Treatemnt	Sample
1	0	0	0	27.65	27.15	Control
58.08123	-5.86	4.62	-1.24	27.3	27.17	CAA1
0.297302	1.75	-1.1	0.65	28.7	29.99	CAA2
0.116629	3.1	-2.56	0.54	26.5	28.77	CAA5
9.917662	-3.31	0.26	-3.05	27.65	27.15	CAA11

Discussion

Fifteen of *C. albicans* isolates were obtained from clinical samples of DFU. The results of our study are consistent with the findings of Bansal *et al*. (2008), among 103 patients with DFU *C. albicans* make up 14% of clinical cases. AL-Ameri (2022) concluded that the percentage of *Candida albicans* isolated from DFU was 11.5%, and Helal *et al*. (2023) the percentage was 12.5% . *C. albicans* is the most common type of yeast in diabetic foot patients who are at danger of growing diabetic foot ulcers, which are related with fungal infections, and it has been confirmed that unbalanced blood sugar is associated with weak immune defenses, and specific types of yeast can reason serious infections

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

in diabetic patients with from immunodeficiency (khanoo *et al*., 2023). The *Candida* sp. It represents the most common strains isolated from DFU among a wide range of fungal strains, and *C. albicans* infection is more common than other *Candida* in DFU patients (Shubha *et al*., 2013; Tariq and Abbas, 2022) .

The results of our study showed that the minimum inhibitory concentration 'MIC' of AgNPs towards *C. albicans* isolates (125 - 250) µg/ml, while the Sub MIC value was (125- 31.25) µg/ml . The study did not agree with other studies, as Arsène *et al*. (2023) found that MIC of AgNPs ranged from (16 µg/ml) to (32 µg/ml) against clinical *C. albicans* isolates. In another similar study, AgNPs showed significant antifungal activity against *C. albicans* at a concentration of 15.6 µg/mL (Ravi and Kannabiran, 2021). The study by Abed-Alwahed and Al-baqi (2020) confirmed that AgNPs have been fungicidal in opposition to all fluconazole-resistant isolates at concentrations as low as 12.5 µg/mL . AgNPs function by way of producing reactive oxygen species and free radicals, which reason protein denaturation, DNA damage, lipid peroxidation, and cell wall damage. Therefore, they alter the permeability of the cell membrane, causing cell death (Mansoor *et al*., 2021). In a study by Jia and Sun (2021) it was indicated that the synergistic action of AgNPs and the antifungal fluconazole could be for the treatment of fluconazole-resistant fungal infections. AgNPs showed antifungal activity against clinical strains of Candida, including *C. albicans*, they are common in nosocomial infections. The results indicated that the physicochemical parameters of AgNPs such as functional groups on their surface interfere with their antifungal activity (Ribeiro *et al*., 2023).

After electrophoresis of the PCR product, the results showed that all 15 studied isolates contain *SAP* 7 and *SAP* 9 with clear bands and a length of (440 bp for the *SAP* 7 gene) and (556 bp for the *SAP* 9 gene). Secreted aspartate proteases (Saps), encoded through a family of 10 *SAP* genes (*SAP1–SAP*10) have long been recognized as a virulence-related trait of *C. albicans*, the important function of Saps is proteolysis, however they also play a role in Cell-cell adhesion (Naglik *et al*., 2003; Naglik *et al*., 2008). Researcher Ilkhanizadeh-Qomi and co (2020) found that clinical strains of *C. albicans* have strong protein activity and high expression levels of related genes. *SAP*1-8 gene products have been shown to be released into the extracellular vacuoles while *SAP*9 and *SAP*10 gene products are bound to the cell membrane (Modrzewska *et al*., 2016). People with diabetes are more susceptible to fungal infections due to their high blood sugar environment and lowered immunity. Because of the higher blood glucose concentration, *Candida* isolates isolated from diabetic sufferers present significantly higher hemolytic enzyme activity, which might also contribute to the improved enzyme activity among diabetics (Manfredi *etal*., 2002; Fatahinia *etal*., 2015). Several studies have demonstrated an association between the activity of hydrolytic enzymes and increased pathogenic ability of *C. albicans* (Bramono *etal*., 2006; Ingham *etal*., 2012). On the other hand, researcher Sacristan and his group (2011) demonstrated that there is a relationship between Sap protein production and biofilm formation .

Conclusion:

According to our research result, *Candida albicans* was isolated from diabetic foot ulcers. All *C. albicans* isolates contain *SAP*7,*SAP*9 gene, and AgNPs has an effect on the gene expression of *SAP*7,*SAP*9.

References

1. **Kandregula**, S., Behura, A., Behera, C.R., Pattnaik, D., Mishra, A., Panda, B. and Mohanty, S., 2022. A Clinical Significance of Fungal Infections in Diabetic Foot Ulcers. *Cureus*, *14*(7).
2. **Ramirez-Acuña**, J.M., Cardenas-Cadena, S.A., Marquez-Salas, P.A., Garza-Veloz, I., Perez-Favila, A., Cid-Baez, M.A., Flores-Morales, V. and Martinez-Fierro, M.L., 2019. Diabetic foot ulcers: current advances in antimicrobial therapies and emerging treatments. Antibiotics, 8(4), p.193.
3. **Hube**, B., Monod, M., Schofield, D.A., Brown, A.J.P. and Gow, N.A.R., 1994. Expression of seven members of the gene family encoding secretory aspartyl proteinases in Candida albicans. *Molecular microbiology*, *14*(1), pp.87-99.
4. **Monika**, S. and Zbigniew, O., 2017. Contribution of aspartic proteases in Candida virulence. Protease inhibitors against Candida infections. *Current Protein and Peptide Science*, *18*(10), pp.1050-1062.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

5. **Rai**, M., Ingle, A.P., Gupta, I. and Brandelli, A., 2015. Bioactivity of noble metal nanoparticles decorated with biopolymers and their application in drug delivery. International journal of pharmaceutics, 496(2), pp.159-172.
6. **Thayath**, J., Pavithran, K., Nair, S.V. and Koyakutty, M., 2021. Cancer nanomedicine developed from total human serum: A novel approach for making personalized nanomedicine. Nanomedicine, 16(12), pp.997-1015.
7. **Selvaraj**, M., Pandurangan, P., Ramasami, N., Rajendran, S.B., Sangilimuthu, S.N. and Perumal, P., 2014. Highly potential antifungal activity of quantum-sized silver nanoparticles against Candida albicans. Applied biochemistry and biotechnology, 173(1), pp.55-66.
8. **Monteiro**, D.R., Takamiya, A.S., Feresin, L.P., Gorup, L.F., de Camargo, E.R., Delbem, A.C.B., Henriques, M. and Barbosa, D.B., 2015. Susceptibility of Candida albicans and Candida glabrata biofilms to silver nanoparticles in intermediate and mature development phases. Journal of Prosthodontic Research, 59(1), pp.42-48.
9. **Kim**, K.J., Sung, W.S., Suh, B.K., Moon, S.K., Choi, J.S., Kim, J.G. and Lee, D.G., 2009. Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals, 22(2), pp.235-242.
10. **Hwang**, I.S., Lee, J., Hwang, J.H., Kim, K.J. and Lee, D.G., 2012. Silver nanoparticles induce apoptotic cell death in Candida albicans through the increase of hydroxyl radicals. The FEBS journal, 279(7), pp.1327-1338.
11. **Monteiro**, D.R., Gorup, L.F., Silva, S., Negri, M., De Camargo, E.R., Oliveira, R., Barbosa, D.D.B. and Henriques, M., 2011. Silver colloidal nanoparticles: antifungal effect against adhered cells and biofilms of Candida albicans and Candida glabrata. Biofouling, 27(7), pp.711-719.
12. **Vazquez-Muñoz**, R., Avalos-Borja, M. and Castro-Longoria, E., 2014. Ultrastructural analysis of Candida albicans when exposed to silver nanoparticles. PloS one, 9(10), p.e108876.
13. **Byadarahally** Raju, S. and Rajappa, S., 2011. Isolation and identification of Candida from the oral cavity. *International Scholarly Research Notices*, *2011*.
14. **Kumar**, R., 2010. *Speciation of Candida isolates in significant count from urine samples* (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India).
15. **Kangogo**, M.C., Wanyoike, M.W., Revathi, G. and Bii, C.C., 2011. Phenotypic characterization of Candida albicans from clinical sources in Nairobi, Kenya. African Journal of Health Sciences, 19(3-4), pp.19-23.
16. **Kaup**, S., Sankarankutty, J., Balasubrahmanya, H.V., Kulkarni, S. and Nirmala, M., 2016. Speciation of Candida using HiCrome candida differential agar. *Int J Curr Microbiol Appl Sci*, *5*(7), pp.267-74.
17. **Das**, B., Khan, M.I., Jayabalan, R., Behera, S.K., Yun, S.I., Tripathy, S.K. and Mishra, A., 2016. Understanding the antifungal mechanism of Ag@ ZnO core-shell nanocomposites against Candida krusei. Scientific reports, 6(1), p.36403.
18. **Belmadani**, A., Semlali, A. and Rouabhia, M., 2018. Dermaseptin-S1 decreases Candida albicans growth, biofilm formation and the expression of hyphal wall protein 1 and aspartic protease genes. Journal of applied microbiology, 125(1), pp.72-83.
19. **Monod**, M., Togni, G., Hube, B. and Sanglard, D., 1994. Multiplicity of genes encoding secreted aspartic proteinases in Candida species. Molecular microbiology, 13(2), pp.357-368.
20. **Bansal**, E., Garg, A., Bhatia, S., Attri, A.K. and Chander, J., 2008. Spectrum of microbial flora in diabetic foot ulcers. Indian journal of pathology and microbiology, 51(2), p.204.
21. **AL-Ameri**,A.Y.K.,2022.Molecular study of fungal isolates associated with diabetic foot ulcer patients in Al_Najaf province and evaluate inhibition activity of some natural products. PhD thesis. Faculty of Science, University of Kufa.
22. **Helal**, S.K., Ali, W.A. and Ghazzay, H.I., 2023. The study of fungal infections and diabetic foot ulcer in Iraqi patients. Journal of Survey in Fisheries Sciences, 10(3S), pp.3410-3418.
23. **khanoo**, N.M., Al-laaeiby,A.I., Jafer,F.N., Marzoq,A.K., 2023. The association of yeast infections with diabetic foot ulcer. Journal of Survey in Fisheries Sciences, 10(4S), pp.371-378.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

24. **Shubha**, K.S., Hudeda, S. and Lakshmidevi, N., 2013. Isolation and identification of pathogens from diabetic foot infections from kr hospital, mysore. International Journal of Development Research, 3(5), pp.023-026.
25. **Tariq**, M.H.M. and Abbas, U.K., 2022. Molecular detection of agglutinin-like sequence 1 gene in Candida albicans that is isolated from diabetic foot patients. Mustansiriya Medical Journal, 21(1), p.73.
26. **Arsène**, M.M.J., Viktorovna, P.I., Alla, M., Mariya, M., Nikolaevitch, S.A., Davares, A.K.L., Yurievna, M.E., Rehailia, M., Gabin, A.A., Alekseevna, K.A. and Vyacheslavovna, Y.N., 2023. Antifungal activity of silver nanoparticles prepared using Aloe vera extract against Candida albicans. Veterinary World, 16(1), p.18.
27. **Ravi**, L. and Kannabiran, K., 2021. Antifungal potential of green synthesized silver nanoparticles (AgNPS) from the stem bark extract of Kigelia pinnata. Research Journal of Pharmacy and Technology, 14(4), pp.1842-1846.
28. **Abed-Alwahed**, F.R. and Al-baqi, N.J.A., 2020. EFFECT OF THE SILVER NANOPARTICLES ON ERG11 EXPRESSION IN FLUCONAZOLE-RESISTANT CANDIDA ALBICANS ISOLATES. Plant Archives (09725210), 20(2).
29. **Mansoor**, S., Zahoor, I., Baba, T.R., Padder, S.A., Bhat, Z.A., Koul, A.M. and Jiang, L., 2021. Fabrication of silver nanoparticles against fungal pathogens. Frontiers in Nanotechnology, 3, p.679358.
30. **Jia**, D. and Sun, W., 2021. Silver nanoparticles offer a synergistic effect with fluconazole against fluconazole-resistant Candida albicans by abrogating drug efflux pumps and increasing endogenous ROS. Infection, Genetics and Evolution, 93, p.104937.
31. **Ribeiro**, L.G., Roque, G.S.C., Conrado, R. and De Souza, A.O., 2023. Antifungal activity of mycogenic silver nanoparticles on clinical yeasts and phytopathogens. Antibiotics, 12(1), p.91.
32. **Naglik**, J.R., Challacombe, S.J. and Hube, B., 2003. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. *Microbiology and molecular biology reviews*, *67*(3), pp.400-428.
33. **Naglik**, J.R., Moyes, D., Makwana, J., Kanzaria, P., Tsichlaki, E., Weindl, G., Tappuni, A.R., Rodgers, C.A., Woodman, A.J., Challacombe, S.J. and Schaller, M., 2008. Quantitative expression of the Candida albicans secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. *Microbiology (Reading, England)*, *154*(Pt 11), p.3266.
34. **Ilkhanizadeh-Qomi**, M., Nejatbakhsh, S., Jahanshiri, Z. and Razzaghi-Abyaneh, M., 2020. Aspartyl proteinase and phospholipase activities of Candida albicans isolated from oropharyngeal candidiasis in head and neck cancer patients. Jundishapur Journal of Microbiology, 13(9).
35. **Modrzewska**, B., Kurnatowski, P. and Khalid, K., 2016. Comparison of proteolytic activity of Candida sp. strains depending on their origin. *Journal de Mycologie Médicale*, *26*(2), pp.138-147.
36. **Manfredi**, M., McCullough, M.J., Al-Karaawi, Z.M., Hurel, S.J. and Porter, S.R., 2002. The isolation, identification and molecular analysis of Candida spp. isolated from the oral cavities of patients with diabetes mellitus. Oral microbiology and immunology, 17(3), pp.181-185.
37. **Fatahinia**, M., Poormohamadi, F. and Mahmoudabadi, A.Z., 2015. Comparative study of esterase and hemolytic activities in clinically important Candida species, isolated from oral cavity of diabetic and non-diabetic individuals. Jundishapur Journal of Microbiology, 8(3).
38. **Bramono**, K., Yamazaki, M., Tsuboi, R. and Ogawa, H., 2006. Comparison of proteinase, lipase and alpha-glucosidase activities from the clinical isolates of Candida species. Japanese journal of infectious diseases, 59(2), p.73.
39. **Ingham**, C.J., Boonstra, S., Levels, S., de Lange, M., Meis, J.F. and Schneeberger, P.M., 2012. Rapid susceptibility testing and microcolony analysis of Candida spp. cultured and imaged on porous aluminum oxide. PLoS One, 7(3), p.e33818.
40. **Sacristan**, B., Blanco, M.T., Galan-Ladero, M.A., Blanco, J., Perez-Giraldo, C. and Gomez-Garcia, A.C., 2011. Aspartyl proteinase, phospholipase, hemolytic activities and biofilm production of Candida albicans isolated from bronchial aspirates

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 547-555 | ISSN:2251-6727

of ICU patients. Medical mycology, 49(1), pp.94-97.

1-