Nano World Journal

https://doi.org/10.17756/nwj.2020-075

Research Article

Open Access

Silver Nanoparticle and Plant Molecule Combinations Synergistically Inhibit Drug Resistant Biofilms in *Candida albicans*

Ashwini K. Jadhav[1], Shivkrupa D. Halbandge[2], Archana A. Wakharde[2] and Sankunny Mohan Karuppayi[1*]

[1]*Department of Medical Biotechnology & Department of Stem Cell and Regenerative Medicine, Centre For Interdisciplinary Research, DY Patil Education Society, Maharashtra, India*

[2]*DST-FIST & UGC-SAP Sponsored School of Life Sciences, SRTM University, Maharashtra, India*

[*]Correspondence to:
Dr. Sankunny Mohan Karuppayil
Professor and Head, Department of Stem Cell and Regenerative Medicine
Centre For Interdisciplinary Research
DY Patil Education Society (Deemed to be University, NAAC Accredited with 'A' Grade)
Kolhapur, Maharashtra, 416006, India
E-mail: prof.karuppayil@gmail.com

Received: March 30, 2020
Accepted: April 16, 2020
Published: April 17, 2020

Citation: Jadhav AK, Halbandge SD, Wakharde AA, Karuppayi SM. 2020. Silver Nanoparticle and Plant Molecule Combinations Synergistically Inhibit Drug Resistant Biofilms in *Candida albicans*. *Nano World J* 6(1): 7-12.

Copyright: © 2020 Jadhav et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (http://creativecommons.org/licenses/by/4.0/) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

Published by United Scientific Group

Abstract

Anti-biofilm activity of silver nanoparticles (SNPs) in combination with four molecules i.e., Geraniol, β-Ionone, Citral and Carvacrol were analysed in a polystyrene microplate based *in vitro* biofilm model using XTT-metabolic assay. SNPs dosages required for the inhibition of anti-biofilm activity were reduced by the addition of the above molecules. MIC of SNPs against planktonic growth of *C. albicans* ATCC 90028 was reduced by eight fold in presence of geraniol. This interaction was also synergistic in case of *C. albicans* clinical isolate GMC 03. Geraniol-SNPs combination was synergistic against developing biofilms in both the strains and reduced the MIC of SNPs by 16 fold in *C. albicans* ATCC 90028. Eight fold decrease in MIC was observed in presence of 500 μg/ml of β-ionone in *C. albicans* ATCC 90028. MIC of SNPs against mature biofilm forms were brought down by sixteen fold by geraniol. Combination of SNPs-β-ionone caused eight fold decrease in the MIC of SNPs against mature biofilm forms in *C. albicans* ATCC 90028.

Keywords

Candida albicans, Synergy, Silver nanoparticles, Plant molecules, Biofilms

Abbreviations

SNPs: Silver Nanoparticles; MIC: Minimum Inhibitory Concentration; ATCC: American Type Culture Collection; FICI: Fractional Inhibitory Concentration Index; XTT: 2, 3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium- 5-carboxanilide

Introduction

Antifungal drugs such as azoles, polyenes, and echinocandins are used to treat infections caused by *Candida albicans* in humans [1]. The biofilms formed by *C. albicans* on biotic as well as abiotic surfaces like prosthetic devices are highly resistant to host immune defence's as well antifungal agents. As such there is a need to develop novel drugs to handle, the increasing number of immune-compromised patients to be treated, drug resistance, toxicity and side-effects [2-7]. Innovative strategies such as using synergistic combinations of antifungal drugs, use of virulence factor targeting drugs, drug repositioning are viable options [9, 10]. Use of synergistic drug combinations may be an advantageous strategy for antifungal drug development. It may substantially reduce the dosages of drugs, enhance with increased drug efficacy and lower toxicity. Furthermore, it is a multi target strategy that may prevent development of drug resistance [11]. Molecules

of plant origin are very well known for their anti-*Candida* activity [12-14]. Synergistic combinations of molecules such as thymol, carvacrol, allyl isothiocynate, berberine, retegeric acid, eugenol, glabridin, baicalein, and berberin with antifungal agents are reported [10, 11, 15].

Silver nanoparticles (SNPs) are studied for their antibacterial as well as antifungal activities [9, 16, 17]. Although silver nanoparticles exhibit anti-*Candida* activity, they are also known for their toxicity [18, 19]. It is advisable to invent methods that can reduce the toxicity of silver nanoparticles. Synergistic combination of drugs seems to be a good option for reducing the dosage of silver nanoparticles that may reduce its toxicity and still maintain their antimicrobial potential. In this communication, for the first time, we are reporting the efficacy of combinations of SNPs with geraniol, carvacrol, citral and β-Ionone against planktonic growth and drug resistant biofilm forms of *C. albicans.*

Materials and Methods

Cultures, culture conditions, media and chemicals

C. albicans, ATCC 90028 was purchased from IMTECH (Institute of Microbial Technology), Chandigarh, India. A clinical isolate of *C. albicans* GMC 03 was provided by Government Medical College, Nanded, Maharashtra, India. The culture was grown on YPD agar at 4 °C. YPD (1% Yeast extract, 2% Peptone, and 2% Dextrose) was prepared and used for inoculum preparation. All the ingredients of spider medium (1% Mannitol, 1% Nutrient broth, 0.2% K_2HPO_4), and 2, 3-bis (2-methoxy-4-nitro-sulfophenyl)-2H-tetrazolium- 5-carboxanilide (XTT) were obtained from Himedia Chemicals Ltd, Mumbai, India. Menadione was purchased from Sigma Aldrich Chemicals Ltd. Mumbai, India. A single colony from the YPD agar plates was inoculated in fifty ml of YPD broth and incubated at 30 °C on an orbital shaker, at 120 rpm for 24 h. Cells from the activated culture were harvested by centrifugation for 5 min at 2000 g, washed three times, and resuspended in PBS buffer (10mM phosphate buffer, 2.7 mM potassium chloride and 137 mM sodium chloride, pH 7.4). The molecules used in this work i.e. Citral, Geraniol, Carvacrol and β-ionone were purchased from Sigma-Aldrich Chemicals Ltd, Mumbai, India and Himedia Chemicals Ltd, Mumbai. Dimethyl sulphoxide (Himedia Chemicals Ltd, Mumbai) was used to dissolve phytochemicals at a final concentration of 1%. SNPs were prepared by using the leaf extract of the plant *Polyalthia longifolia* [9].

Minimum inhibitory concentration for planktonic growth

The effect of silver nanoparticles on the growth of cells of *C. albicans* was studied as per the Clinical Laboratory Standards Institute guidelines. Various concentrations of SNPs ranging from 0.75 to 100 µg ml $^{-1}$ were prepared in Spider medium in non-treated 96 well polystyrene plates (Costar, USA). Wells devoid of test compounds served as controls. 100 µl microlitres of inoculum were added to 100 µl of Spider medium in each well to obtain 1 × 10^3 cells ml^{-1}. The plates were incubated at 35 °C for 48 hours. Growth was measured by taking the absorbance at 620 nm using a microplate reader (Multiskan EX, Thermo Electron Corp., USA). The lowest concentration of the silver nanoparticles which caused a fifty percentage of reduction in the absorbance compared to the control was considered as the minimum inhibitory concentration (MIC) for growth of *C. albicans* [21].

Biofilm formation and MIC determination

Biofilms of *C. albicans* were developed on the surface of 96-well polystyrene plates. Cell suspensions of 1 × 10^7 cells ml^{-1} were prepared in PBS and 100 µl were inoculated into each well. Plates were incubated at 37 °C for 90 min at 100 rpm to allow attachment of the cells to the solid surface. Non-adhered cells were removed by washing the wells with sterile PBS. Two hundred microlitres of the spider medium were then added to each well and the plates were incubated at 37 °C for 48 h to allow biofilm formation. To analyze its effect on biofilm development, various concentrations of SNPs were prepared in spider medium and added to each well immediately after the adhesion phase and incubated for 48 h at 37 °C. To study the activity against mature *C. albicans* biofilms, SNPs were added to 24 h old mature biofilm and incubated for 48 h. After incubation, the wells were washed to remove any nonattached planktonic cells. Wells were observed for presence or absence of biofilms using an inverted light microscope (Metzer, India). Photographs were taken with a Labomed microphotography system (Labomed, India) at ×200 magnification. Biofilm growth was analyzed and confirmed with the XTT metabolic assay [21].

Biofilm quantitation by XTT assay

To quantify biofilm growth, XTT metabolic assay was performed. The XTT solution was prepared by mixing 1 mg ml^{-1} of XTT salt in sterile distilled water. Prior to use, menadione solution was prepared in acetone and added to the XTT to a final concentration of 4 µM. The wells containing biofilm were washed with PBS to remove non-adhered cells and incubated with 100 µl of XTT-menadione solution in dark, at 37 °C for 5 h. Color product by the water soluble formazan product was measured at 450 nm using a microplate reader (Multiskan EX, Thermo Electron Corp. USA). Wells without the SNPs were referred as controls. The concentration of SNPs which caused ≥ 50 % lowering in relative metabolic activity was considered the MIC for biofilm formation [21].

Checkerboard assay for drug combination against planktonic and biofilm growth

To study the efficacy of drug combinations against planktonic and biofilm growth in *C. albicans,* checkerboard assay was performed and fractional inhibitory concentration indices (FICI) was analyzed. A two dimensional array of drug combinations were used for the preparation of drug dilutions. The efficacy of drug combinations against planktonic and biofilm growth was performed as discussed in the earlier section and the MIC value were determined. The FICI values were calculated by the formula

$$\Sigma FIC = FIC_A + FIC_B$$

FIC_A = (MIC of drug A in combination / MIC of drug A alone)

FIC_B = (MIC of drug B in combination / MIC of drug B alone)

When the value of ΣFICI ≤ 0.5, it was considered as synergism; between 0.5 and 1.0, it was additive; and when ΣFICI > 4, was considered as antagonism. A ΣFIC result of >1 but ≤ 4 was treated as indifference [22].

Results

Silver nanoparticles are synergistic with plant molecules against planktonic growth of *C. albicans*

Planktonic growth of *C. albicans* was susceptible to the molecules tested. MICs of geraniol, β-ionone, citral and carvacrol was achieved at 1000 µg/ml, 4000 µg/ml, 500 µg/ml and 250 µg/ml respectively against planktonic growth of *C. albicans* ATCC 90028 (Table 01-4). SNPs showed pronounced inhibitory effect at 1.5 µg/ml against planktonic growth of *C. albicans* ATCC 90028. There was substantial decrease in the dosage of SNPs required for inhibition of planktonic growth of *C. albicans* in presence of the above molecules. For example, MIC of SNPs against planktonic growth of *C. albicans* ATCC 90028 in combination with geraniol was achieved at 0.187 µg/ml i.e. eight times less than that of SNPs alone. Calculated FIC index for the combination was 0.249, suggesting that interaction is synergistic. Interaction was also synergistic in the case of the isolate GMC 03 (Table 1). Addition of 1000 µg/ml of β-ionone lowered the MIC of SNPs against planktonic growth of *C. albicans* ATCC 90028 from 1.5 to 0.375 µg/ml of SNPs i.e. four fold reduction in MIC of SNPs. FIC index for this interaction was 0.5 indicating that combination is synergistic. This drug combination was also tested against *C. albicans* clinical isolate GMC 03 and calculated FIC was 0.312 showing that the interaction is synergistic (Table 2). In presence of 31.25 µg/ml of citral, MIC of SNPs against planktonic growth of *C. albicans* ATCC 90028 was lowered to 0.375 µg/ml. Combination of SNPs and citral was four times more efficient than that of SNPs alone. Calculated FIC index was 0.3125. This drug combination was synergistic in both the strains of *C. albicans* (Table 3). Carvacrol-SNPs drug combination was was not efficient against planktonic growth of *C. albicans* and the calculated FIC index was 1.00 showing that the interaction is indifferent (Table 4).

Drug combinations of SNPs and selected molecules are additive and synergistic in interaction against developing biofilm of *C. albicans*

Biofilm development was susceptible to SNPs at 50 µg/ml which is 33 fold higher than the MIC for planktonic growth. Developing biofilm was sensitive to the tested molecules and MIC was achieved at 2000 µg/ml, 2000 µg/ml 1000 µg/ml and 500 µg/ml for geraniol, β-ionone, citral and carvacrol respectively. Geraniol-SNPs combination was highly synergistic against developing biofilms. Addition of 62.5 µg/ml of geraniol reduced the MIC of SNPs against developing biofilm by 16 fold in *C. albicans* ATCC 90028. FIC index for this combination was 0.0937 (Table 1). The MIC of SNPs was decreased from 50 µg/ml to 6.25 µg /ml i.e. eight fold decrease in MIC in presence of 500 µg/ml of β-ionone in *C. albicans* ATCC 90028. Calculated FIC was 0.375 indicating that the interaction is synergistic. However, this combination of drugs were additive in the clinical isolate of GMC 3 (Table 2). Combination of citral and SNPs was not efficient and interaction was indifferent in *C. albicans* ATCC 90028 while this combination was additive in the case of the clinical isolate GMC 03 (Table 3). SNPs-carvacrol drug combination was not effective against developing biofilm of both the strains of *C. albicans*. Interaction of this drug combination was indifferent and calculated FIC was 1.062 (Table 4).

Table 01: Comparison of MICs of silver nanoparticles (SNPs) and Geraniol, alone and in combination, against planktonic growth and biofilm of *C. albicans* ATCC 90028 and *C. albicans* GMC 03.

Growth	MIC (µg/ml)											
	Candida albicans ATCC 90028						*Candida albicans* GMC 03					
	Alone		In Combination				Alone		In Combination			
	SNPs	Geraniol	SNPs	Geraniol	FICI	Remarks	SNPs	Geraniol	SNPs	Geraniol	FICI	Remarks
Planktonic Growth	1.5	1000	0.187	125	0.249	Synergistic	3	1000	0.75	3.9	0.3	Synergistic
Biofilm Formation	50	2000	3.125	62.5	0.093	Synergistic	50	2000	6.25	500	0.375	Synergistic
Mature Biofilm	100	4000	6.25	1000	0.312	Synergistic	100	4000	50	125	0.531	Additive

SNPs and selected molecule combinations act synergistically against mature biofilm forms of *C. albicans*

Mature biofilm forms were susceptible to high dosages of SNPs and MIC was achieved at 100 µg/ml in both the strains of *C .albicans*. MIC against mature biofilm forms were brought down by sixteen fold i.e. from 100 µg/ml to 6.25 µg/ml, in presence of 1000 µg/ml of geraniol. Combination of SNPs with geraniol against mature biofilm of *C. albicans* ATCC 90028 was synergistic and calculated FIC was 0.3125. However, this drug interaction was additive in case of the clinical isolate GMC 03 (Table 1). Although SNPs alone were effective for the inhibition of biofilm, higher concentrations were required to irradicate the biofilms. 100 µg/ml of SNPs was required for inhibition of 50 % of biofilm formation. Combination

Table 02: Comparison of MICs of silver nanoparticles (SNPs) and β-Ionone, alone and in combination, against planktonic growth and biofilm of *C. albicans* ATCC 90028 and *C. albicans* GMC 03.

Growth	MIC (μg/ml)											
	Candida albicans ATCC 90028						*Candida albicans* GMC 03					
	Alone		In Combination				Alone		In Combination			
	SNPs	β-Ionone	SNPs	β-Ionone	FICI	Remarks	SNPs	β-Ionone	SNPs	β-Ionone	FICI	Remarks
Planktonic Growth	1.5	4000	0.375	1000	0.5	Synergistic	3	4000	0.75	250	0.312	Synergistic
Biofilm Formation	50	2000	6.25	500	0.375	Synergistic	50	8000	25	2000	0.75	Additive
Mature Biofilm	100	8000	12.5	2000	0.375	Synergistic	100	8000	50	250	0.531	Additive

Table 03: Comparison of MICs of silver nanoparticles (SNPs) and Citral, alone and in combination, against planktonic growth and biofilm of *C. albicans* ATCC 90028 and *C. albicans* GMC 03.

Growth	MIC (μg/ml)											
	Candida albicans ATCC 90028						*Candida albicans* GMC 03					
	Alone		In Combination				Alone		In Combination			
	SNPs	Citral	SNPs	Citral	FICI	Remarks	SNPs	Citral	SNPs	Citral	FICI	Remarks
Planktonic Growth	1.5	500	0.375	31.25	0.312	Synergistic	3	500	0.375	62.5	0.25	Synergistic
Biofilm Formation	50	1000	50	7.81	1.007	Indifferent	50	1000	25	62.5	0.562	Additive
Mature Biofilm	100	2000	6.25	1000	0.562	Additive	100	2000	6.25	1000	0.562	Additive

Table 04: Comparison of MICs of silver nanoparticles (SNPs) and Carvacrol, alone and in combination, against planktonic growth and biofilm of *C. albicans* ATCC 90028 and *C. albicans* GMC 03.

Growth	MIC (μg/ml)											
	Candida albicans ATCC 90028						*Candida albicans* GMC 03					
	Alone		In Combination				Alone		In Combination			
	SNPs	Carvacrol	SNPs	Carvacrol	FICI	Remarks	SNPs	Carvacrol	SNPs	Carvacrol	FICI	Remarks
Planktonic Growth	1.5	250	0.75	125	1.000	Indifferent	3	250	0.187	125	0.562	Additive
Biofilm Formation	50	500	3.125	500	1.0625	Indifferent	50	500	50	7.81	1.015	Indifferent
Mature Biofilm	100	1000	6.25	1000	1.0625	Indifferent	100	1000	100	7.81	1.007	Indifferent

of SNPs-β-ionone caused 8 fold decrease in MIC of SNPs. The calculated FIC index for this drug interaction was 0.375 indicating synergistic interaction. This drug interaction was additive in the clinical isolate, GMC 03 (Table 2). SNPs-citral interaction was additive and FIC index was 0.562 in both the strains of *C. albicans* (Table 3). Carvacrol combination with SNPs showed indifferent interaction (Table 4).

Discussion

In this study, biosynthesised SNPs were tested against growth and biofilm formation of *C. albicans*. However, the dosage required to irradicate biofilm was high that may lead to toxicity. Our main thrust was to minimise the dosage of SNPs required for inhibition that can reduce the risk of toxicity. Studies on combinations of silver nanoparticles and molecules of natural origin and their use against *C. albicans* are lacking. Dosages of SNPs are reduced by the addition of plant molecules and MIC was reduced by several folds (Tables 1-4). This lowered dosage of SNPs may be physiologically relevant as higher dosage may lead to toxicity.

Out of the four molecules tested, geraniol and β-ionone acted synergistically with SNPs against mature and pre-formed biofilm of *C. albicans*. Addition of geraniol reduced the MIC by sixteen fold while addition of β-ionone reduced the MIC by eight fold against mature biofilms of *C. albicans*

(Tables 1 and 2). Use of these drug combinations may be beneficial to treat infections related to biofilms of *C. albicans*. Physically synthesized SNPs are reported to be cytotoxic against human hepatocellular carcinoma (HepG2) cells above 7.03 ppm of concentration [23]. Silver is eliminated from the human body through the liver and kidneys. Silver is not toxic at low dosages [24]. High concentrations of silver can exert potential toxic side-effects to human cells by preventing DNA and protein synthesis [25]. Studies have shown that silver nanoparticles may be safe at low concentrations [26, 27]. The use of SNPs in combination with plant molecules can be effective to combat *Candida* infection due its high efficacy and to the fact that dosages of the SNPs can be substantially reduced. There are some reports addressing the synergistic and additive interactions of silver nanoparticles with antifungal agents and antibiotics [9, 13, 28-31]. Silver is well-known for its antimicrobial efficacy even at low concentrations [32]. Currently, silver nanoparticles have gained remarkable attention as antimicrobial agents [33].

Anti-*Candid*a activity of SNPs are reported to be due to the production of ROS, nuclear fragmentation, formation of pores on cell membrane, effect on cell wall [34-38] and their effect on genes involved in signal transduction [39]. The inhibitory activity of plant molecules on *C. albicans* may be due to membrane damage, inhibition of oxidative phosphorylation and respiration [40]. The mechanism of synergistic interaction of silver nanoparticles with plant molecules is not clear. It seems to be complex and multi-factorial. The mechanism behind the synergistic and additive effect of SNPs-plant molecules may involve attacking of different cellular targets by SNPs and plant molecules in combination. Further *in vivo* and cytotoxicity studies has to be done in future.

Conclusions

Efficacy of biofabricated SNPs-plant molecule combinations against growth and biofilm formation in *C. albicans* is presented. Combinations of SNPs –geraniol and SNPs- β-ionone exhibited synergisitic inhibitory activity against growth and biofilm forms of *C. albicans*. These combinations considerably brought down the dosages of SNPs as well as plant molecules and SNPs required for inhibitory activity against biofilms and growth of *C. albicans* may reduce the risk of toxicities. Further, studies are necessary to test its inhibitory activity *in vivo*. Detailed toxicity studies are required to be done.

Conflict of interest

No conflict of interest.

Authors contribution

Ashwini Jadhav and Shivkrupa Halbandge contributed equally in this publication.

Acknowledgments

SMK and SDH are thankful to UGC-Innovative program, New Delhi for providing financial assistance to SMK.

Funding

This work was supported by the University Grant Commission –XII plan Innovative Programme, New Delhi, India.

References

1. Mikolajewska A, Schwartz S, Ruhnke M. 2012. Antifungal treatment strategies in patients with haematological diseases or cancer: from prophylaxis to empirical, pre-emptive and targeted therapy. *Mycoses* 55(1): 2-16. https://doi.org/10.1111/j.1439-0507.2010.01961.x
2. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, et al. 2012. Hidden killers: human fungal infections. *Sci Transl Med* 4(165): 165rv13. https://doi.org/10.1126/scitranslmed.3004404
3. Cowen LE. 2008. The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. *Nat Rev Microbiol* 6(3):187-198. https://doi.org/10.1038/nrmicro1835
4. Fuentefria AM, Pippi B, Lana DFD, Donato KK, de Andrade SF. 2018. Antifungals discovery: an insight into new strategies to combat antifungal resistance. *Letters in Applied Microbiology* 66(1): 2-13. https://doi.org/10.1111/lam.12820
5. Wang D, Meiser B, Eisen HJ, Eifert S. 2019. Immunosuppression, Including Drug Toxicity, Interactions, New Immunosuppressants in the Pipeline. In: Feldman D, Mohacsi P (eds) Cardiovascular Medicine. Heart Failure, Springer, pp 241-253.
6. Sanglard D, Coste A, Ferrari S. 2009. Antifungal drug resistance mechanisms in fungal pathogens from the perspective of transcriptional gene regulation. *FEMS Yeast Res* 9(7): 1029-1050. https://doi.org/10.1111/j.1567-1364.2009.00578.x
7. McCarthy MW, Baker T, Satlin MJ, Walsh TJ. 2018. Antibacterial and Antifungal Agents: The Challenges of Antimicrobial-Resistant Infections in Immunocompromised Hosts. In: Segal B (eds) Management of Infections in the Immunocompromised Host. Springer, pp 297-315.
8. Halbandge SD, Mortale SP, Karuppayil SM. 2017. Biofabricated silver nanoparticles synergistically activate amphotericin b against mature biofilm forms of *Candida albicans*. *Open Nanomed J* 4(1): 1-16. http://doi.org/10.2174/1875933501704010001
9. Raut JS, Bansode BS, Jadhav AK, Karuppayil SM. 2017. Activity of allyl isothiocyanate and its synergy with fluconazole against *Candida albicans* biofilms. *J Microbiol Biotechnol* 27(4): 685-693. https://doi.org/10.4014/jmb.1607.07072
10. Cui J, Ren B, Tong Y, Dai H, Zhang L. 2015. Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*. *Virulence* 6(4): 362-371. https://doi.org/10.1080/21505594.2015.1039885
11. Perugini Biasi-Garbin R, Saori Otaguiri E, Morey AT, Fernandes da Silva M, Belotto Morguette AE, et al. 2015. Effect of eugenol against *Streptococcus agalactiae* and synergistic interaction with biologically produced silver nanoparticles. *Evid Based Complement Alternat Med* 2015: 861497. https://doi.org/10.1155/2015/861497
12. Leite MC, de Brito Bezerra AP, de Sousa JP, de Oliveira Lima E. 2015. Investigating the antifungal activity and mechanism(s) of geraniol against *Candida albicans* strains. *Med Mycol* 53(3): 275-284. https://doi.org/10.1093/mmy/myu078
13. Chaillot J, Tebbji F, Remmal A, Boone C, Brown GW, et al. 2015. The monoterpene carvacrol generates endoplasmic reticulum stress in the pathogenic fungus *Candida albicans*. *Antimicrob Agents Chemother* 59(8): 4584-4592. https://doi.org/10.1128/AAC.00551-15
14. Doke SK, Raut JS, Dhawale S, Karuppayil SM. 2014. Sensitization

of *Candida albicans* biofilms to fluconazole by terpenoids of plant origin. *J Gen Appl Microbiol* 60(5): 163-168. https://doi.org/10.2323/jgam.60.163

15. El-adly A, Shabana I. 2018. Antimicrobial activity of green silver nanoparticles against fluconazole-resistant *Candida albicans* in animal model. *Egypt J Bot* 58(1): 119-132.
16. Monteiro DR, Gorup LF, Silva S, Negri M, de Camargo ER, et al. 2011. Silver colloidal nanoparticles: antifungal effect against adhered cells and biofilms of *Candida albicans* and *Candida glabrata*. *Biofouling* 27(7): 711-719. https://doi.org/10.1080/08927014.2011.599101
17. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S, et al. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3(2): 279-290.
18. Stensberg MC, Wei Q, McLamore ES, Porterfield DM, Wei A, et al. 2011. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. *Nanomedicine (Lond)* 6(5): 879-898.
19. Jadhav A, Mortale S, Halbandge S, Jangid P, Patil R, et al. 2017. The dietary food components capric acid and caprylic acid inhibit virulence factors in *Candida albicans* through multitargeting. *J Med Food* 20(11): 1083-1090. https://doi.org/10.1089/jmf.2017.3971
20. Liu W, Li LP, Zhang JD, Li Q, Shen H, et al. 2014. Synergistic antifungal effect of glabridin and fluconazole. *PLoS One* 9(7): e103442. https://doi.org/10.1371/journal.pone.0103442
21. Ali A, Jadhav A, Jangid P, Patil R, Shelar A, et al. 2018. The human muscarinic acetylcholine receptor antagonist, Dicyclomine targets signal transduction genes and inhibits the virulence factors in the human pathogen, *Candida albicans*. *J Antibiot (Tokyo)* 71(4): 456-466. https://doi.org/10.1038/s41429-017-0013-z
22. Shinde RB, Raut JS, Chauhan NM, Karuppayil SM. 2013. Chloroquine sensitizes biofilms of *Candida albicans* to antifungal azoles. *Braz J Infect Dis* 17(4): 395-400. https://doi.org/10.1016/j.bjid.2012.11.002
23. Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jiménez MJ, et al. 2015. Effect of silver nanoparticles on *Candida albicans* biofilms: an ultrastructural study. *J Nanobiotechnology* 13: 91. https://doi.org/10.1186/s12951-015-0147-8
24. Lansdown AB. 2006. Silver in health care: antimicrobial effects and safety in use. In: Hipler UC, Elsner P (eds) Biofunctional Textiles and the Skin. Curr Probl Dermatol, Basel, Karger Publishers, pp 17-34.
25. Hidalgo E, Domínguez C. 1998. Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts. *Toxicol Lett* 98(3): 169-179. https://doi.org/10.1016/s0378-4274(98)00114-3
26. Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 27(1): 76-83. https://doi.org/10.1016/j.biotechadv.2008.09.002
27. Monteiro DR, Silva S, Negri M, Gorup LF, de Camargo ER, et al. 2013. Antifungal activity of silver nanoparticles in combination with nystatin and chlorhexidine digluconate against *Candida albicans* and *Candida glabrata* biofilms. *Mycoses* 56(6): 672-680. https://doi.org/10.1111/myc.12093
28. Ghosh IN, Patil SD, Sharma TK, Srivastava SK, Pathania R, et al. 2013. Synergistic action of cinnamaldehyde with silver nanoparticles against spore-forming bacteria: a case for judicious use of silver nanoparticles for antibacterial applications. *Int J Nanomedicine* 8(1): 4721-4731. https://doi.org/10.2147/IJN.S49649
29. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, et al. 2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine: NBM* 6(1): 103-109. https://doi.org/10.1016/j.nano.2009.04.006
30. Longhi C, Santos JP, Morey AT, Marcato PD, Durán N, et al. 2016. Combination of fluconazole with silver nanoparticles produced by Fusarium oxysporum improves antifungal effect against planktonic cells and biofilm of drug-resistant *Candida albicans*. *Med Mycol* 54(4): 428-432. https://doi.org/10.1093/mmy/myv036
31. Tutaj K, Szlazak R, Szalapata K, Starzyk J, Luchowski R et al. 2016. Amphotericin B-silver hybrid nanoparticles: synthesis, properties and antifungal activity. *Nanomedicine: NBM* 12(4): 1095-1103. https://doi.org/10.1016/j.nano.2015.12.378
32. Dastjerdi R, Montazer M. 2010. A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties. *Colloids Surf B Biointerfaces* 79(1): 5-18. https://doi.org/10.1016/j.colsurfb.2010.03.029
33. Le AT, Le TT, Nguyen VQ, Tran HH, Dang DA, et al. 2012. Powerful colloidal silver nanoparticles for the prevention of gastrointestinal bacterial infections. *Adv Nat Sci: Nanosci Nanotechnol* 3(4): 045007. http://doi.org/10.1088/2043-6262/3/4/045007
34. Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, et al. Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. *Biometals* 22(2): 235-242. https://doi.org/10.1007/s10534-008-9159-2
35. Radhakrishnan VS, Reddy Mudiam MK, Kumar M, Dwivedi SP, Singh SP, et al. 2018. Silver nanoparticles induced alterations in multiple cellular targets, which are critical for drug susceptibilities and pathogenicity in fungal pathogen (*Candida albicans*). *Int J Nanomedicine* 13: 2647-2663. https://doi.org/10.2147/IJN.S150648
36. Huh AJ, Kwon YJ. 2011. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Control Release* 156(2): 128-145. https://doi.org/10.1016/j.jconrel.2011.07.002
37. Hwang IS, Lee J, Hwang JH, Kim KJ, Lee DG. 2012. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals. *FEBS J* 279(7): 1327-1338. https://doi.org/10.1111/j.1742-4658.2012.08527.x
38. Vazquez-Muñoz R, Avalos-Borja M, Castro-Longoria E. 2014. Ultrastructural analysis of *Candida albicans* when exposed to silver nanoparticles. *PLoS One* 9(10): e108876. https://doi.org/10.1371/journal.pone.0108876
39. Halbandge SD, Jadhav AK, Jangid PM, Shelar AV, Patil RH, et al. 2019. Molecular targets of biofabricated silver nanoparticles in *Candida albicans*. *J Antibiot* 72: 640-644. https://doi.org/10.1038/s41429-019-0185-9
40. Pauli A. 2006. Anticandidal low molecular compounds from higher plants with special reference to compounds from essential oils. *Med Res Rev* 26(2): 223-268. https://doi.org/10.1002/med.20050