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Evaluated antifungal effect of biosynthesized silver nanoparticles from Clavispora lusitaniae on some candida spp. causing denture stomatitis

Assist. Lec. Alia Mohammed Abdulateef[1, a)]

Assist. Prof. Dr. Mohannd Khalaf M. Ameen [2, b)], and Assist. Prof. Dr.Afrodet Abdulrazaq Saleh [3, c)]

a)correspondingauther: alia7moh@gmail.com

b) mohanadkma@yahoo.com

Abstract. In our current study, the extracellular biosynthesis of silver nanoparticles was conducted with *Clavispora lusitaniae* isolated from oral cavity and evaluation of its antifungal potential against pathogens some *candida* spp. causing denture stomatitis., such as *Candida glabrata*, *Candida albicans*, *Candida tropical*, *Clavispora lusitaniae*, and *Candida dubliniensis*isolated from oral cavity of the mucosa upon contact with dentures. The production of the silver nanoparticles was confirmed by changing the color and absorption peak at (410) nm in UV-visible spectroscopy due to thesurface plasmon resonance of the silver nanoparticles. Scanning electron microscopy analysis were showed the nanoparticles were well spread and has mostly a spherical shape in the (48.73-80.49) nm size range. The antifungal capacity of Ag nanoparticles was characterized by the evaluation of the minimum inhibitory concentration (MIC), the minimum fungicide concentration (MFC), and well propagation methods. The MIC and MFC ranges are (62.5-250) µg/ml and (125-500) µg/ml, respectively.

Introduction

Candida spp. is one of the most prevalent opportunistic human pathogenic fungi, accounting for (90–100) % of mucosal infections and the fourth-leading cause of nosocomial infections (candidemia and other types of invasive candidiasis), with a mortality rate of (35-50) % in immunocompromised and critically ill patients. [1,2] Despite the innocuous coexistence of the fungi with the host, rare cases were reported in immunocompetent patients. [3]

Despite the fact that oral *candida* clings readily to the main materials of denture, many denture cleaners are only effective against bacteria, not *candida.*[4]. Denture wear is required for food intake as well as nutritional status maintenance. *Candida* thrives on denture base material, which makes it a hotspot for mycotic stomatitis [5]. Candidiasis may take several forms, one of which being denture-induced stomatitis. It is defined as erythema and inflammation of the oral mucosa underneath the denture-bearing regions, and it is clinically divided into three categories based on severity. The disease is typically treated with denture care, proper mouth rinses, and antifungal medication, but new technologies are emerging that may aid in its treatment. [6].

Antifungal drug therapy is no exception; resistance has arisen to several of the currently used antifungal drugs. Despite the fact that antifungal medication resistance does not appear to be as widespread as antibacterial drug resistance in bacteria, one long-term issue is that the number of fundamentally distinct kinds of antifungal medicines available for therapy remains relatively restricted. Because fungi are eukaryotic organisms with a structure and metabolism identical to their eukaryotic hosts. As a result, antibiotics with novel antibacterial mechanisms are unavoidably and urgently required in medicine. [7,8]

Nanotechnology is a branch of science that deals with the synthesis and development of nanoparticles. Nanoparticles offer unrivaled physicochemical, biological, and optical properties, and they're employed as antimicrobials in a variety of applications. [9]

Given that nanosilver-based materials have proven to offer intriguing, difficult, and promising properties appropriate for different biomedical applications, silver nanoparticles (AgNPs) have become one of the most researched and explored nanotechnology-derived nanostructures in recent years. The therapeutically improved customized healthcare practice is attracting a lot of curiosity among AgNPs' modern biological potential. [10]

According to recent studies, the importance of silver in the health-care industry has prompted researchers to switch from ionic or colloidal silver to silver nanoparticles (AgNPs), which have a superior safety profile. Furthermore, AgNPs exhibit a wide range of antibacterial, antifungal, and antiviral properties. [11,12] Physical, chemical, and biological techniques are currently used to synthesize AgNPs. For safety reasons, green synthesis is the best approach in biomedicine. [13]

Biological techniques are gaining popularity because they are low-cost, employ mild reaction conditions in a range of hosts, and generate stable nanoparticles with regulated dimensions. [14,15] Plants, [16] bacteria, [17], and fungi [18] are all used in biological techniques of synthesis. Because of fungi metabolic variety, they have emerged as a leading biological option for nanoparticle production. Various species of filamentous fungi were used to biosynthesis of AgNPs, such as species of *Aspergillus,Colletotrichum, Fusarium, Neurospora, Penicillium, Rhizopus, Trichoderma, Verticillium*, [19] and endophytic fungus *Papulasporapallidula*. [20]

However, only a few yeast species have been reported on the production of AgNPs utilizing single-celled yeasts, such as *Saccharomyces boulardii* [21], *S. cerevisiae* [22], *Candida albicans* [23], *Candida utilis* [24], and *Candida lusitani*. [19] Because of yeast better tolerance and metal bioaccumulation property, large scale production, economic viability, convenient downstream processing, and most importantly, fungi produce huge amounts of proteins and enzymes that act as reducing and stabilizing agents, yeast was chosen for the extracellular biosynthesis of AgNPs. [25, 26]

Primary and secondary detection techniques were used to characterize the production of AgNPs.The appearance of silver nanoparticles was detected for the first time by observing a change in the color of the solution from pale yellow to brown, which was then confirmed by FTIR analysis. A double beam UV Spectrophotometer was used for secondary detection, which was verified by EDX and SEM analysis. [27]

2. Material and method

2.1 Biosynthesis of nanoparticles

AgNPs biosynthesis was performed by *Clavispora lusitaniae* isolated from the palatine mucosa in contact with dentures of people wearing removable dentures. by activated yeast through the inoculum of the colony on a Sabouraud dextrose ager (SDA) (HiMedia, India) plate and incubated at 37°C for (24-48) hours. *Candida* spp. were identified by morphology on corn meal agar (CMA), germ tube test, HiCrome agar, and molecular method. Then, a portion of the culture was added to Erlenmeyer flasks (250 ml volume) containing 100 ml of Sabouraud dextrose broth incubated at 37°C in a stirring incubator at 150 rpm for (24-48) hours.

2.2 AgNPs Purification and Preparation

Obtain the cell filtrate after incubation by centrifugation at 6000 rpm for10 min. In a flask (volume 250 ml), add the final concentration of 1 mM of AgNO3 into 100 ml of supernatant. Then, incubate the flask up to 24 hours in a dark room condition. With the experimental flask containing the just supernatant, the control was maintained without adding AgNO3. The presence of AgNPs indicated in the culture when the color was changed and turbidity occurred after incubation. Centrifuge the AgNPs solution formed at 6000 rpm for 25 min. Then discard the supernatant and replace it with three washes with deionized D.W. The pellet in the tube's bottom was dried at 40°C. Then carefully collected the dried powder to be kept at 4°C for other tests [28].

2.3 Biosynthesis of Silver Nanoparticles Characterization NPs

Several methods were used to characterize AgNPs synthesized by *Clavispora lusitaniae* isolate. That characterized by visual observation of the color change of the reaction mixture and then by measuring the absorbance of the colloidal suspension after the isolation NPs from the supernatant using UV-Vis spectrophotometer (Cecil (Ce-7200 – Aquarius- England) Record the absorbance spectra of nanoparticles within the range of (300–800) nm. The electron microscope utilized a scanning electron microscope SEM (Quanta450_FEI -USA) to characterize the morphology and size of AgNPs, Energy dispersive X-ray spectroscopy was used to analyze the composition of the samples AgNPs, Furthermore, the synthesized AgNPs were centrifuged to obtain pellets and then characterized by Fourier Transform Infrared Spectrometer FTIR (Jasco -Germany) for observing the functional groups.

2.4 Tested micro-organisms

Clinical isolates of yeasts species from mucosal membranes in contact with dentures in patients' mouths were used during this study such as *C. albicans*, *C. tropicalis, C. dubliniensis, C. glabrata*, *Clavispora lusitaniae*.

2.5 Evaluate the antifungal activity of mycosynthesized AgNPs

The antifungal efficacy of as-synthesized AgNPs from *Clavispora lusitaniae* against *Candida* spp., such as *Candida albicans*, *Candida dubliniensis*, *Candida tropical*, *Candida glabrata*, and *Clavispora lusitaniae*, was investigated using well diffusion technique [29].

Briefly, a well of 6 mm diameter was formed on Sabouraud dextrose agar plates previously infected with 100 µl of 1 × 106 fungal suspensions, then 100 µl of different concentrations of AgNPs (500, 250, 125, 62.5, 31.25 µg/ ml) were added, sterilely and disinfected in the well. After a 24 hours' incubation period at 37°C, the antifungal was assessed by measuring the diameter of the inhibition zone (mm) surrounding the well.

2.6 Determining the MIC and MFC to assess the antifungal activity of AgNPs

According to the Clinical Laboratory Standards Institute (CLSI) (M27-A2) guidelines, the minimum inhibitory concentration (MIC) was calculated by microdilution method. The MIC was calculated using the lowest concentration of each antifungal agent that inhibited yeast growth. [30, 31] After the MIC determination, 100 µl aliquots from each well that did not show any yeast growth after incubation were transferred to SDA plates and incubated at 37oC for 24 hours. The MFC is the lowest concentration that kills 100% of the original yeast population and leaves no colonies on the SDA.

2.7 Statistical analysis

The antifungal activity test of mucosynthesized AgNPs was evaluated using the statistical program (SPSS® ver. 21) software (SPSS, 2012), was performed with triplicates according to randomized complete block design (RCBD), $P \leq 0.05$.

3. Results and Discussion

3.1 Biosynthesized silver nanoparticles

In the present study, silver nanoparticles were synthesized from silver nitrate by *Clavispora lusitaniae*. After 24 hours from the start of the reaction in a dark room condition, the color of the solution changed from yellow to brown Figure (1), indicating the synthesis of silver nanoparticles by *Clavispora lusitaniae*. This occurs as a result of the reduction of Ag+ to AgNPs, as evidenced by the color of the solution. It was changed to brown due to surface plasmon resonance [32]. A UV-Visible spectrophotometer and the wavelength range set between (300–800) nm was used to make optical measurements for the detection of silver nanoparticles. The maximum absorbance peak observed at wavelength 410 nm was within the AgNPs peak range, as shown in the figure (2). This corresponds to numerous studies that found the greatest absorbance peak to be between (400–480) nm [33,34,35]. *Clavispora lusitaniae* was produced spherical SNPs varying in size from (48.73-80.49) nm, figure (3), according to nanostructure investigations conducted by SEM, which found size distribution in the range of (10-78) nm [36], spherical shaped silver nanoparticles, this is consistent with prior research [35]. At low angles (10°–80°), the XRD pattern revealed a distinct diffraction line. Bragg reflections at angles 2θ of 31.64o and 66.24o are seen in the figure (4). The face-centered cubic structure of AgNPs was confirmed by this pattern. The crystal structure of silver in the *Clavispora lusitaniae* extract was validated by XRD data, and the findings of this study are equivalent to those of numerous other studies published in the literature [37].

Figure (5) shown the main functional groups in the FTIR analysis of the biosynthesized AgNPs, the results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3470—Nh– H stretch, 1638—C=C, and 1137—C=O.

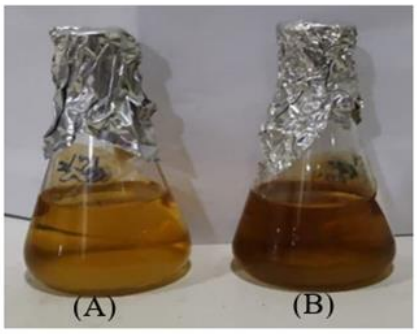

Figure (1) Biosynthesis of silver nanoparticles (A) conical flasks containing the culture supernatant of the *Clavispora lusitaniae*. (B) brown in color synthesized AgNPs solution.

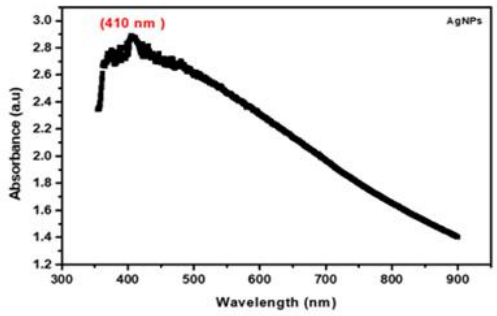

Figure (2) UV–Vis spectrum of AgNPs synthesized by supernatant of the *Clavispora lusitaniae* Abbreviation: AgNPs, silver nanoparticles

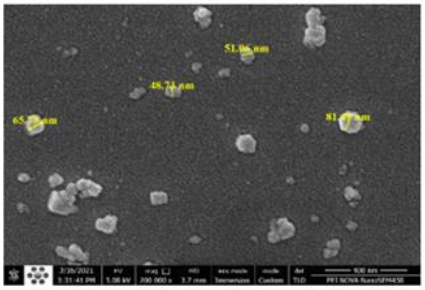

Figure (3) SEM image of synthesized AgNPs

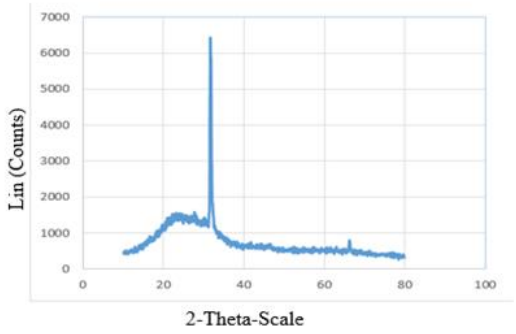

Figure (4) XRD spectrum of lyophilized cell supernatant with AgNPs

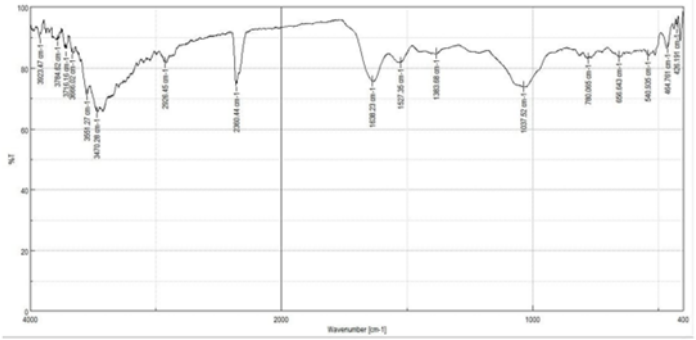

Figure (5) FTIR spectra of AgNPs synthesized by the *Clavispora lusitaniae* supernatant.

3.2 Antifungal Activity of Biosynthesized AgNPs

Antifungal activity of biosynthesis AgNPs against pathogenic yeasts associated with removable dentures, i.e. *Candida* species. It was evaluated by well diffusion agar and microdilution methods. A zone of inhibition test of mycosynthesized AgNPs against *Candida* spp. at various concentrations (500, 250, 125, 62.5, and 32.25) µg/ml. *Clavispora lusitaniae* antifungal activity was evaluated by measuring the clear zone of inhibition around wells treated with AgNPs at concentrations of (31.25–500) µg/ml. *C. albicans* was recorded the highest inhibition zone (21mm), followed by *C. dubulanesis*, *Clavispora lusitaniae* and *C. glabrata* (20mm), *C. tropicalis* (17mm), there was a significant difference between the

fungal species at various concentrations at 0.05 level, shown in Figures (6). The zone of inhibition values was obtained here as similar to those reported by Jalal et al., [38]. The MICs values of AgNPs against all tested *Candida* species were ranged from (62.5–250) µg/ml, whereas the MFCs were (125-500) µg/ml (Table1). In a study, [39] AgNPs synthesized by *C. glabrata* supernatant was showed MIC and MFC values (62.5–250) µg/ml and (125–500) µg/ml, respectively against *C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis, C. krusei, C. glabrata, S. aureus, E. coli, P. aeruginosa, K. pneomonaie, S. typhimurium*, and *S. flexneri* isolated from the oropharyngeal mucosa. The MIC and MFC results of the present study demonstrate that the AgNPs exhibited high anticandidal activity.

Table (1): MIC and MFC values of biosynthesized AgNPs against *Candida* strains.

No.	Fungal Isolates	MIC (µg/ml)	MFC (µg/ml)
1	*Candia albicans*	250	500
2	*Candida dubliniensis*	62	125
3	*Candida glabrata*	62	125
4	*Candida tropicalis*	250	500
5	*Clavispora lusitaniae*	125	250

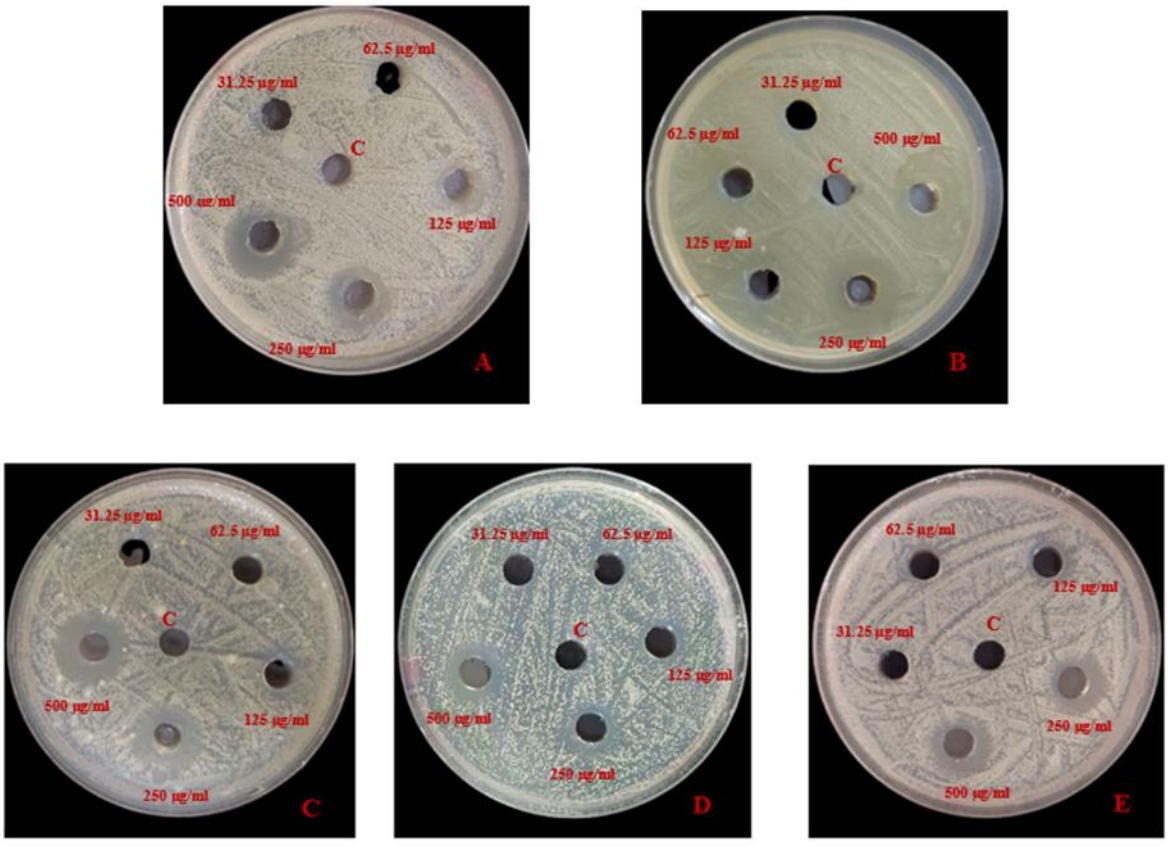

Figure 6: The anticandidal activity of mycosynthesized AgNPs at various doses was assessed using the agar well diffusion technique against several *Candida* spp.: (**A**) *C. albicans*, (**B**) *C. glabrata* (**C**) *C. dubliniensis*, (**D**) *Clavispora lusitaniae* (**E**) *C. tropicalis*

Table (2): Represents the inhibition zone (in mm) of different *candida* species at different concentrations of AgNPs (µg/ml).

No.	*Candida* spp.	Inhibition zone (mm) in Concentration (µg/ml)					Mean of *Candida* spp.
		31.25	62.5	125	250	500	
1	*C. albicans*	6	6	12	13	21	11.6
2	*C. glabrata*	6	11	14	20	20	14.2
3	*C. dubliniensis*	6	6	10	18	20	12
4	*Clavispora lusitaniae*	6	11	12	19	20	13.6
5	*C. tropicalis*	6	9	11	16	17	11.8
6	*Control*	6	6	6	6	6	6
Mean of Concentration		6	8.167	10.833	15.333	17.333	11.533

LSD Candida spp. =0.57

LSD concentration = 0.81

LSD Candida spp. * concentration = 1.38

* The means difference significant at the 0.05 level.

4. Conclusions

In the current study, yeasts such as *Clavispora lusitaniae* were used in the biosynthesis of silver nanoparticles, the study proved that it is safe, environmentally friendly and inexpensive. Several tests were conducted to confirm the formation of silver nanoparticles such as UV, SEM, XRD and FTIR. The biological efficacy of silver nanoparticles against *Candida* spp. that causing stomatitis- was investigated.

5. References

1- Wenzel, R. P. (1995). Nosocomial candidemia: risk factors and attributable mortality. Clinical Infectious Diseases, 20(6), 1531-1534.

2- Geffers, C., &Gastmeier, P. (2011). Nosocomial infections and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). DeutschesÄrzteblatt International, 108(6), 87.

3- Al-Laaeiby, A., Ali, S., & Al-Saadoon, A. H. (2019). *Candida* species: the silent enemy. African Journal of Clinical and Experimental Microbiology, 20(4), 260-267.

4- Kamikawa, Y., Hirabayashi, D., Nagayama, T., Fujisaki, J., Hamada, T., Sakamoto, R., ... & Sugihara, K. (2014). In vitro antifungal activity against oral *Candida* species using a denture base coated with silver nanoparticles. Journal of Nanomaterials, 2014.

5- Dağistan, S., Aktas, A. E., Caglayan, F., Ayyildiz, A., & Bilge, M. (2009). Differential diagnosis of denture- induced stomatitis, *Candida*, and their variations in patients using complete denture: a clinical and mycological study. Mycoses, 52(3), 266-271.

6- Adam, R. Z., & Khan, S. B. (2021). Antimicrobial efficacy of silver nanoparticles against *Candidaalbicans*: A systematic review protocol. Plos one, 16(1), e0245811.

7- Silver, S. (2003). Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS microbiology reviews, 27 (2-3), 341-353.

8- Klasen, H. J. (2000). A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns, 26(2), 131-138.

9- Elgorban, A. M., El-Samawaty, A. E. R. M., Yassin, M. A., Sayed, S. R., Adil, S. F., Elhindi, K. M., ... & Khan, M. (2016). Antifungal silver nanoparticles: synthesis, characterization and biological evaluation. Biotechnology & Biotechnological Equipment, 30(1), 56-62.

10- Burdușel, A. C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., &Andronescu, E. (2018). Biomedical applications of silver nanoparticles: an up-to-date overview. Nanomaterials, 8(9), 681.

11- Shuaib, U., Hussain, T., Ahmad, R., Zakaullah, M., Mubarik, F. E., Muntaha, S. T., & Ashraf, S. (2020). Plasma-liquid synthesis of silver nanoparticles and their antibacterial and antifungal applications. Materials Research Express, 7(3), 035015.

12- Dung, T. T. N., Nam, V. N., Nhan, T. T., Ngoc, T. T. B., Minh, L. Q., Nga, B. T. T., &Quang, D. V. (2020). Silver nanoparticles as potential antiviral agents against African swine fever virus. Materials Research Express, 6(12), 1250g9.

13- Abdelghany, T. M., Al-Rajhi, A. M., Al Abboud, M. A., Alawlaqi, M. M., Magdah, A. G., Helmy, E. A., &Mabrouk, A. S. (2018). Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review. BioNanoScience, 8 (1), 5-16.

14- Kuppusamy, P., Ichwan, S. J., Parine, N. R., Yusoff, M. M., Maniam, G. P., &Govindan, N. (2015). Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of Brassica oleracea L. and studies on their physicochemical and biological properties. Journal of Environmental Sciences, 29, 151-157.

15- Guo, M., Li, W., Yang, F., & Liu, H. (2015). Controllable biosynthesis of gold nanoparticles from a Eucommiaulmoides bark aqueous extract. Spectrochimicaacta part A: molecular and biomolecular spectroscopy, 142, 73-79.

16- Ajitha, B., Reddy, Y. A. K., & Reddy, P. S. (2015). Biosynthesis of silver nanoparticles using Momordicacharantia leaf broth: evaluation of their innate antimicrobial and catalytic activities. Journal of Photochemistry and Photobiology B: Biology, 146, 1-9.

17- Srivastava, P., Braganca, J. M., &Kowshik, M. (2014). In vivo synthesis of selenium nanoparticles by Halococcussalifodinae BK18 and their anti- proliferative properties against HeLa cell line. Biotechnology progress, 30(6), 1480-1487.

18- Soni, N., & Prakash, S. (2012). Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitology research, 111(5), 2091-2098.

19- Eugenio, M., Müller, N., Frasés, S., Almeida-Paes, R., Lima, L. M. T., Lemgruber, L., ... &Sant'Anna, C. (2016). Yeast-derived biosynthesis of silver/silver chloride nanoparticles

20- Muhsin, T., &Hachim, A. (2016). Antitumor and antibacterial efficacy of mycofabricated silver nanoparticles by the endophytic fungus Papulasporapallidula. American J. Bioeng. Biotech, 2(1), 24-4and their antiproliferative activity against bacteria. Rsc Advances, 6(12), 9893-9904.

21- Kaler, A., Jain, S., & Banerjee, U. C. (2013). Green and rapid synthesis of anticancerous silver nanoparticles by Saccharomyces boulardii and insight into mechanism of nanoparticle synthesis. BioMed research international, 2013.

22- Niknejad, F., Nabili, M., Ghazvini, R. D., &Moazeni, M. (2015). Green synthesis of silver nanoparticles: advantages of the yeast Saccharomyces cerevisiae model. Current medical mycology, 1(3), 17.

23- Bhat, M. A., Nayak, B. K., & Nanda, A. (2015). Evaluation of bactericidal activity of biologically synthesised silver nanoparticles from *Candidaalbicans* in combination with ciprofloxacin. Materials Today: Proceedings, 2(9), 4395-4401.

24- Waghmare, S. R., Mulla, M. N., Marathe, S. R., &Sonawane, K. D. (2015). Ecofriendly production of silver nanoparticles using *Candida*utilis and its mechanistic action against pathogenic microorganisms. 3 Biotech, 5(1), 33-38.

25- Azizi, S., Ahmad, M. B., Namvar, F., & Mohamad, R. (2014). Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalgaSargassummuticum aqueous extract. Materials Letters, 116, 275-277.

26- Agarwal, H., Kumar, S. V., &Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach. Resource-Efficient Technologies, 3(4), 406-413.

27- Dhabalia, D., Ukkund, S. J., Syed, U. T., Uddin, W., &Kabir, M. A. (2020). Antifungal activity of biosynthesized silver nanoparticles from *Candidaalbicans* on the strain lacking the CNP41 gene. Materials Research Express, 7(12), 125401.

28- Sarvamangala, D., Kondala, K., Murthy, U. S. N., Rao, B. N., Sharma, G. V. R., &Satyanarayana, R. (2013). Biogenic synthesis of AGNP's using Pomelo fruit—characterization and antimicrobial activity against Gram+ Ve and Gram− Ve bacteria. International Journal of Pharmaceutical Sciences Review and Research, 19(2), 30-35.

29- Jalal, M., Ansari, M. A., Shukla, A. K., Ali, S. G., Khan, H. M., Pal, R., ... &Cameotra, S. S. (2016). Green synthesis and antifungal activity of Al 2 O 3 NPs against fluconazole-resistant *Candida* spp. isolated from a tertiary care hospital. RSC advances, 6(109), 107577-107590.

30- Pereira, L., Dias, N., Carvalho, J., Fernandes, S., Santos, C., & Lima, N. (2014). Synthesis, characterization and antifungal activity of chemically and fungal- produced silver nanoparticles against T richophytonrubrum. Journal of applied microbiology, 117(6), 1601-1613.

31- Abed-Alwahed, F. R., & Al-Baqi, N. J. A. (2020). Effect of the silver nanoparticles on erg11expression in fluconazole-resistant *Candidaalbicans* isolates. Plant Arch, 20, 616-620.

32- Muhsin, T. M., &Hachim, A. K. (2014). Mycosynthesis and characterization of silver nanoparticles and their activity against some human pathogenic bacteria. World Journal of Microbiology and Biotechnology, 30(7), 2081-2090

33- Niknejad, F., Nabili, M., Ghazvini, R. D., &Moazeni, M. (2015). Green synthesis of silver nanoparticles: advantages of the yeast Saccharomyces cerevisiae model. Current medical mycology, 1(3), 17.

34- Dhabalia, D., Ukkund, S. J., Syed, U. T., Uddin, W., &Kabir, M. A. (2020). Antifungal activity of biosynthesized silver nanoparticles from *Candidaalbicans* on the strain lacking the CNP41 gene. Materials Research Express, 7(12), 125401.

35- Al-Abdullah, Z. T., & Al-Shuhaib, Z. (2017). AS abdulridah and MN Aboud,"Synthesis and Characterisation of Antibacterial Silver Nanoparticles,". Journal of Chemical, Biological and Physical Sciences, 7(2), 377-383.

36- Ibraheem, S. Q., & AL-Abdullah, Z. T. (2019). Analytical studies of synthesized silver nanoparticles and their applications in degradation of different pollutants. Journal of Chemical, Biological and Physical Sciences.

37- Kumar, D., Karthik, L., Kumar, G., &Roa, K. B. (2011). Biosynthesis of silver nanoparticles from marine yeast and their antimicrobial activity against multidrug resistant pathogens. Pharmacologyonline, 3, 1100-1111.

38- Jalal, M., Ansari, M. A., Alzohairy, M. A., Ali, S. G., Khan, H. M., Almatroudi, A., & Siddiqui, M. I. (2019). Anticandidal activity of biosynthesized silver nanoparticles: effect on growth, cell morphology, and key virulence attributes of *Candida* species. International journal of nanomedicine, 14, 4667.

39- Jalal, M., Ansari, M. A., Alzohairy, M. A., Ali, S. G., Khan, H. M., Almatroudi, A., &Raees, K. (2018). Biosynthesis of silver nanoparticles from oropharyngeal *Candidaglabrata* isolates and their antimicrobial activity against clinical strains of bacteria and fungi. Nanomaterials, 8(8), 586.