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Inhibition growth of *Candida albicans* which isolated from renal failure and controlling by silver nanoparticles extracted from *Syzygium aromaticum*

Hussein Ali Abbood Shubbar
Department of Biology, Faculty of Science, University of Kufa, Iraq
Email: husseinshuber2020@gmail.com

R.A. Sahib
Department of Biology, Faculty of Science, University of Kufa, Iraq
Email: raghad.almusawi@uokufa.edu.iq

***Abstract*---**The samples *of C. albicans* were selected for a sensitivity test with three concentrations were selected, as follows (50 ,100 ,200) mg/L. the upper inhibition zone is 200 mg/L which increased as compared with other concentrations, the inhibition percentage used to compare between all data using SPSS V.25 computer software using a T-test. P - values of less than 0.05 was considered statistically significant. Green synthesis using to produce silver nanoparticles by *Syzygium aromaticum* after adding the plant extract obtained of a silver nanoparticles when adding 4ml extract of *S. aromaticum* and the best pH to produce (AgNps) is 8 with wavelength 435 nm and 1 molarity by UV-visible devise. Field emission-scanning electron microscope (FESEM) revealed damage of the cells and inhibition biofilm formation of *C.albicans* when treated with AgNPs produce by *S. aromaticum* as compared with control of *C.albicans* which un treated with AgNPs,the inhibition growth diameter is 19mm when using 200 mg/L caused the upper inhibition zone as compared with other concentrations.

Keywords*---Candida albicans*, Renal failure, Silver nanoparticles, *Syzygium aromaticum*

Introduction

C.albicans is responsible for an unacceptably high number of symptomatic infections each year, ranging from superficial (i.e., skin and mucous membranes)

to invasive internal organs. The majority of these infections occur in immunocompromised individuals and originate in the gastrointestinal tract. As a result, it is critical to bridge the knowledge gap regarding *candida* spp. colonization, commensal lifestyle and transition into a pathogenic state. Surprisingly, *Candida.* spp. appear to perform host-beneficial functions (Romo and Kumamoto,2020). Plants are widely used in the synthesis of nanoparticles compounds frequently have important biological functionalities that can act synergistically with the nanoparticles for various applications ranging from food to pharmaceutical and medical industries. The importance of employing sustainable industrial methods capable of proposing alternatives for the reduction of harmful chemical residues (Hongfang *etal.*, 2017). Clove (*Syzygium aromaticum*) is a plant that is grown in a number of countries, clove essential oil is widely used for its antimicrobial, antifungal, antioxidant, anesthetic, and analgesic properties these properties are strongly attributed to its main compound, eugenol, which is present in the oil in concentrations of up to 90% (Hongfang and Chuang, 2017; Radünz *etal* ., 2018). Silver nanoparticles (AgNP) are prominent in applications as antimicrobial agents that can eliminate microorganisms interacting with their cells, preventing cellular respiration and, replication (Keshari *etal.,* 2018).

Material and Methods
Vitek 2 Identification

1- Suspension Preparation: the suspension is prepared in accordance with the supplying company's instructions, as follows:
A- Preparing sterile plastic tubes.
B- Fill the sterile tube with 3 ml of saline solution at a concentration of (4.50-5.00 percent NaCl).
C- Transfer several pure colonies from the culture plate to the tube with a sterile loop and mix thoroughly until the solution becomes cloudy.
D- Using the DensiChek device, measure the turbidity of the suspension according to the Mcfarland turbidity range, depending on the type of suspension.a microscopic object is about to be examined. yeasts and semi-yeasts (YST) = 1.80-2.20
2- Inoculum of the card: the card is inoculated using the following procedure:
A- Transferred the stuck and the card to the device holder and placed them in the appropriate locations, then connected the card and the stuck via a micro-channel, and the card symbol was entered by the scanner.
B- Placing the holder in a special vacuum chamber, as the vacuuming process transfers microbes to the card as well as distributing them through the holes in it.
3- Card Sealing and Incubation: the device automatically cuts the delivery channel after 10-15 minutes, and the card is sealed, the channel port sealed to prevent leakage, then transferred to the Carousel incubator, and the cards are incubated at 35.5 + 1.0 ° C.
4- Optical System: the device's optical system directs a series of light beams at the card in order to identify the wavelengths of the interactions and translate them through color changes, turbidity, and metabolic products.
5- **Test results and analysis techniques**: he device computes the results and compares them to the results stored in the device, which includes many test measurements and for a large number of strains developing in different conditions and isolated from various locations, the device displays the results of

the tests in the form +, -, (+), (-), and a result in parentheses indicates that the test is weak.

6- Identification levels: the object's identification level is determined by mapping its tests and comparing them to the device's classification characteristics, so the object is assigned a probability ratio and a level of confidence, for example, if the probability ratio is 96-99 percent. It is excellent in terms of confidence.

Biosynthesis of Silver Nanoparticles from *Syzygium aromaticum* (Clove)

The preparation process for the synthesis of nanoparticles was carried out based on a study (Ramesh. *etal* .,2021)

A- Preparation of Plant Extract

The plant samples were cleaned with distilled water to remove dust particles followed by air drying at room temperature (25°C),Clove crushed to make powder form using a grindermixer 20 gram from sample was addedinto 80ml of distilled water and heated for 30minutes at 80°C.the plant extracts were centrifuged for 5 min at 5000 rpm followedby filtering using a whatman filter paper grade 1.These extracts were stored at refrigerator (4°C)for further use.

B- Synthesis of sliver nanoparticles(AgNPs)

1mm silver nitrate (AgNO3) solution wasprepared in distilled water. 20 ml of AgNO3(1mM) solution was separately added with 0.5,1.0, 2.0 and 4.0 ml of aqueous extracts previously prepared. thereafter, solutions were kept in sunlight for 2 minutes for reaction. After sunlight incubation the colour was changed. thesolutions were centrifuged at 10,000 rpm for 20min to collect nanoparticles and further kept in hot air oven at 60°C. Next day pellet was extracted as dried form and kept in an eppendorf tube for further experiments.

C- Characteristics of silver nanoparticles synthesized by UV-Vis spectroscopy

After exposure to sun light, the syntheses ofAgNPs in solution were characterized by using aUV-visible spectrophotometer. The synthesis of silver nanoparticles was checked by recordingthe UV-visible spectra of solutions between 300–600 nm.

D-Agar well diffusion assay for antimicrobial activity of AgNPs

Antimicrobial activity of *Syzygium. aromaticum* (Clove),water extract and their AgNPs were tested onthree risolated human *C.albicans* activity was conducted through agar well diffusion method Agar wells (6 mm diameter) were made with the help of sterilized cork borer and loaded different concentration of AgNPs, AgNO3 each concentration50mg/ml,100mg/ml and200mg/ml,size 20µl of ctuck nanomaterial were added to well and plates were incubated for 24 h at 37°C,and diameters of zone of inhibition were recorded in centimetres. Measure the percentage of inhibition according to the equation

The percentage of inhibition $(\%)\left(\frac{R1-R2}{R1}\right)X100$

R1= maximum radial growth of fungus colony (control treatment).
R2 = maximum radial growth of the colony in plates containing silver nanoparticles.

Using a scanning electron microscope to determine the effect of nanomaterial on *C.albicans* cells

Cells are selected from SDA culture medium at 24 hours of age the cells are of two types, untreated and cells that were tested for growth inhibition by silver

nanoparticles According to the steps.Prepare a solution 2.5 ml Glutaradehyed to 47.5 distilled water and mixing solution added 90 ml from Glutaradehyed diluent in to sample cells selected for examination and mixing incubation 24 h and 4°c,centrifuge 3000 for 5 min , the solution is carefully poured,added 0.5 mL from Nacl in to the precipitated cells were then re-centrifuged under the same conditions, solution is carefully poured,added and the process is repeated again added 0.5 mL from alcohol 70% in to the precipitated cells for 15 min and added 0.5 mL from alcohol 100% in to the precipitated cells for 15 min Slide is being made for examination(Bello *etal.*,2017).

Results and discussion Vitek 2 Identification of *Candida* spp.

Most of the tests for diagnosing the types of *Candida.*spp presented in the study were conducted on the Vitek 2 compact system , and the results depended on the principles of which allows species identification by comparing the biochemical profile to a large database according to the percentage shown according to *C. albicans* 92%, *C.dubliniesis* 85%, *C.krusei* 86% , *C.glabrata* 86% and *C.tropicalis* 85% figure(1)The results were approach as mentioned before(ALyassree and Alrufae 2021).

Identification Information			Analysis Time:		17.80 hours			Status:	Final						
Selected Organism			85% Probability Bionumber:		Candida dubliniensis 6112144065335370										
ID Analysis Messages															
Biochemical Details															
3	LysA	-	4	IMLTa	+	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLYLa	-
13	TyrA	+	14	BNAG	-	15	ARBa	-	18	AMYa	-	19	dGALa	+	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAdGa	-	26	dCELa	-	27	GGT	-	28	dMALa	+
29	dRAFa	-	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	ISBEa	-
39	IRHAa	-	40	XLTa	+	42	dSORa	+	44	SACa	+	45	URE	-	46	AGLU	+
47	dTURa	+	48	dTREa	+	49	NO3a	-	51	IARAa	+	52	dGATa	+	53	ESC	-
54	IGLTa	+	55	dXYLa	-	56	LATa	+	58	ACEa	+	59	CITa	+	60	GRTas	-
61	IPROa	+	62	2KGa	+	63	NAGa	+	64	dGNTa	-						

Figure (1) Types of *Candida.*spp by the Vitek 2 compact system

Synthesis of Silver Nanoparticles (AgNPs) Using *Syzygium aromaticum*

This study shows the synthesis of silver nanoparticles from a *Syzygium aromaticum* after adding the plant extract with specific concentrations and within a varying pH range between acid and base for several experiments, the study reached to obtain the synthesis of a (AgNPs) when adding 4ml extract of *S. aromaticum*, the best pH to produce silver nanoparticles in the pH 8 and wavelength 435 nm, with 1 molarity, the examination was done with a device, UV-visible spectra of solutions between (200–700) nm as shown in the figure (2). The results were conducted with what presented by (Ajitha *etal.*, 2019) explained a green chemistry route for preparing and characterizing (AgNPs) at room temperature using *Syzygium aromaticum* (cloves) extract by (UV–Vis) spectroscopy, the formation of the AgNPs was confirmed by the observation of a distinct absorption peak at a wavelength at 418 nm.

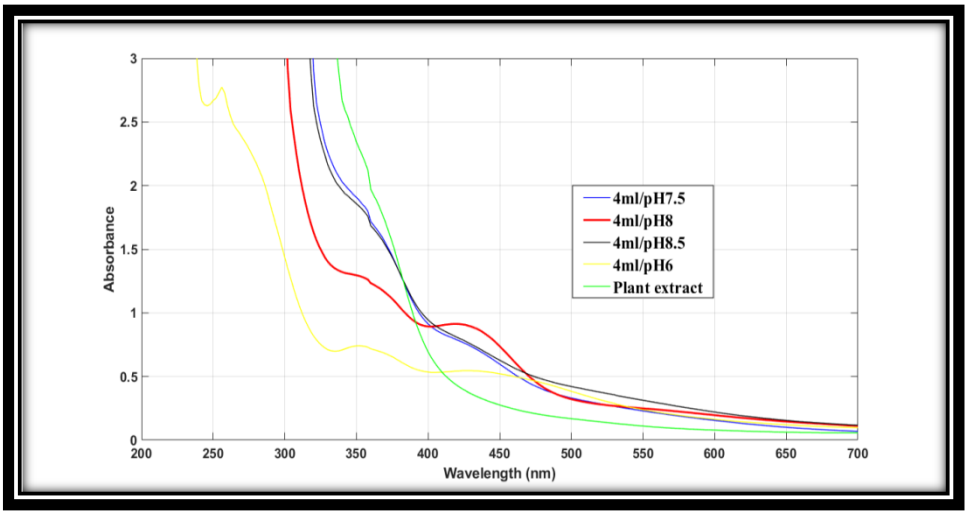

Figure (2) UV-visible curve of Silver Nanoparticles produced by the extract of *Syzygium aromaticum*

Ability of Silver Nanoparticles produced by *S.aromaticum* to inhibition growth *of C. albicans*

The samples *of C. albicans* were selected to a sensitivity test conducted for three concentrations followed (50 , 100 ,200) mg/L and three replicates for each sample, the inhibition percentage was shown in the figure (3) shows a sensitivity test of *C. albicans*.The study comes in accordance with (Fonseca *etal.*, 2022). The inhibition growth diameter is 19mm when using 200 mg/L which caused the upper inhibition zone as compared with other concentrations 50 mg/L damping diameter15mm.Statically analysis Mean ± standard deviation (SD) and less significant differences (LSD) have been used to compare between all data using SPSS V.25 computer software using a T-test. A P-value of less than 0.05 was considered statistically significant shown in the figure (4).

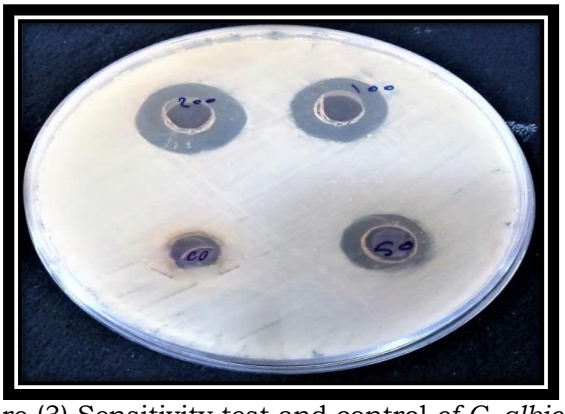

Figure (3) Sensitivity test and control *of C. albicans*.

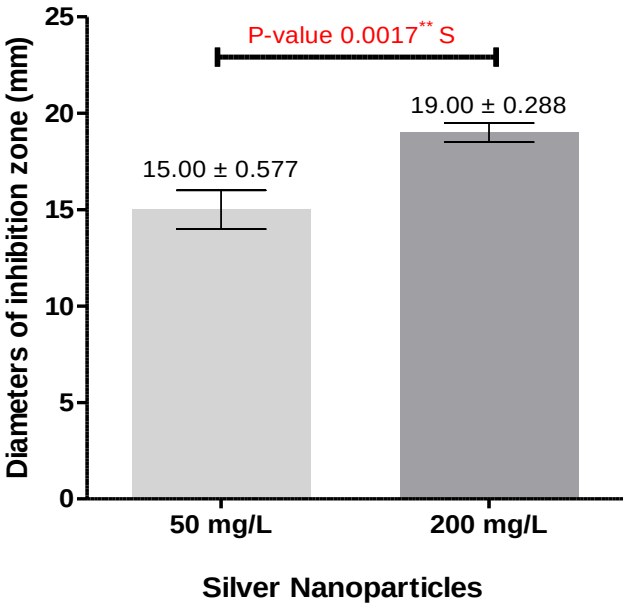

Figure (4) Compare between (50and200)mg/L,using SPSS V.25 computer software by T-test; P-value less than 0.05 .

Effect of Silver Nanoparticles on the *C. albicans* using field emission-scanning electron microscope (FESEM)

C.albicans treated with AgNPs produce by *S. aromaticum* and elevated the effect (FESEM). Figure(5 A-B) shows damage of the cells and inhibition biofilm formation as compared with control of *C.albicans* which un treated with AgNPs. These results compatible with (Fonseca *etal.*, 2022) mention that *C.albicans* were susceptible to AgNPs, caused surface damage, cell disruption, and inhibition of biofilm formation

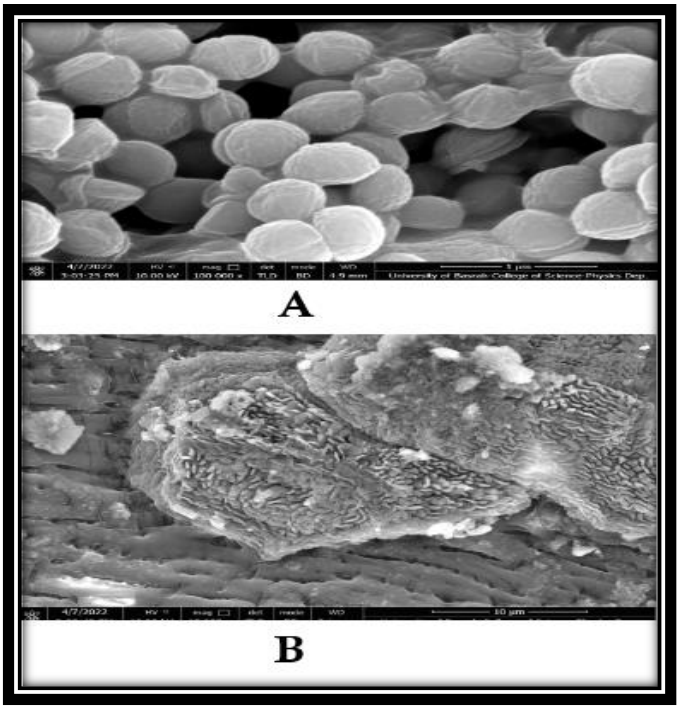

Figure (5) A- *C.albicans* untreated with AgNPs B- *C.albicans* treated with AgNPs.

Conclusion

Ability of Silver Nanoparticles produced by *S. aromaticum* to inhibition growth *of C. albicans* and Effect on the *C. albicans* using field emission- scanning electron microscope (FESEM).

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