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Antifungal activity of silver nanoparticles synthesized from *Capparis spinosa* fruits against azole resistant *Candida* spp.

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Abstract:

This study was aimed to *In vitro* study the activity of silver nanoparticles synthesized from *Capparis spinosa* fruits against azole resistant *Candida* spp.. 120 samples were taken from 62 women with VVC and 58 samples were collected from children infected with Oral Thrush from Baghdad Hospital from February to June 2022. Microscopical examination, cultural examination to detect on *Candida* spp. The effect of *Capparies spinosa* silver nanoparticles (AgNPs) against *Candida* spp. viability was measured by MTT method. Synergistic effect of AgNPs with Fluconazole resistant *Candida* spp. was performed by MTT assay, well diffusion test .The effect of silver nanoparticles (AgNPs) synthesis from *Capparis spinosa* on viability of *Candida* spp. showed the highest inhibition percentage, the highest inhibition percentage was (88.15 and 91.42) % in *C. albicans* (V2) and *C. lusitaniae* (O38) respectively, while the lowest inhibition percentage was (77.77 and 76.31) % in *C. albicans* (V18) and *C. lusitaniae* (O36) respectively, and showed that the synergistic effect between AgNps-fruits extract with fluconazole was less than AgNps fruits extract alone and fluconazole alone. The results of statistical analysis showed significant ($p \leq 0.05$) between treatment and control of all isolates.

Keywords: *Candida* spp.; Silver Nanoparticles; *Capparis spinosa* fruits.

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Introduction

Commensal yeasts like *Candida* spp. can cause a rising number of infections, occurrence of the disease requires firstly the attachment to the host cells, followed by the invasion of the tissue, The adaptability translates into a rapid ability to respond to stress factors, to take up nutrients or to multiply under different conditions, By Through many mechanisms and virulence factors such as biofilms, *Candida* spp. become not only more resistance to antifungal but also more susceptible to cause disease , The microbial interactions can increases the virulence of a fungal strain. *In vivo*, the fungal cells face a many of challenges and, they develop more strategies serving the eventual goal is survival (Ciurea *et al*, 2020) . *Candida* spp. contains many virulence factors such as adherence to surfaces, secretion of extra hydrolytic enzymes as well as biofilm production.Moreover, when *Candida albicans* production biofilm, the resistance to traditional antifungal agentsincreases (Henriques and Silva ,2021).

Attention to nanotechnology, especially for finding treatment methods for microbial infections, has been increasing noticeable In recent years (Rai *et al* ,2016) . One of the most important applications in Medical treatments is to develop drug delivery systems that facilitate targeted delivery, increase bioavailability, and minimize the toxicity of many drugs to treated different infectious diseases (Patra *et al* ,2018) .

Capparis spinosa belonging to the Capparidaceae family is a growing in a climatic conditions, in dry deserts also cooler height of mountains(Mollica *et al* ,2019)

The components of *Capparis spinosa* plant include the alkaloids, saccharides, flavonoids and also glycosides and terpenoid moreover ,oils like volatile oils, fatty acids and steroids. The major compounds found in *C. spinosa* are flavonoids, Indoles, and Phenolic compounds (Raissi *et al*, 2016) . Plant extracts, *C. spinosa* extract ,have recently been used for nanoparticles due to it is rich in active substances that can reduce metal ions in one step and produce highly effective nanocomposites, for

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example silver nanoparticles were successfully synthesized using *C. spinosa* extract from fruit and leaves (Benakashani *et al*,2016).

The antimicrobial effect of nanoparticles produced *C. spinosa* extract was studied using different microorganisms , recently more isolates of *Candida* spp. have appeared resistant to azole antifungals, and therefore, This study aimed to investigate *In vitro* the antifungal activity of silver nanoparticles synthesized from *Capparis spinosa* fruits extract against azole resistant *Candida* spp.

Materials and Methods

Samples collection *of Candida* spp.

Two hundred twenty samples were taken from 62 women infected with Vulvovaginal Candidiasis and Fifty eight (58) samples were collected from different age of children infected with Oral Thrush from period February to June 2022. specimens were collected from IBN AL-Baladi Teaching Hospital. The samples divided in to two parts , one examined microscoply and second cultured on Sabouraud Dextrose agar.

Isolation of *Candida* spp.

All samples were streaked on chromogenic *candida* agar and Sabouraud Dextrose agar contains antibiotic (antibacterial) chloramphenicol (250 mg/L) and incubated for 48 hours at 37 °C (Al-Dabbagh *et al* ,2023) .

Identification Using Vitek 2 Compact System

According to Tan *et al* ,(2019) Vitek 2 Compact System using to confirming and identification of *Candida* spp.

Antifungals susceptibility test

According to Prakash *et al*,(2018) Kirby-Bauer method was used for Antifungals susceptibility test. Amphotrecin B, Clotrimazole, Fluconazole, Ketoconazole and Nystatin were used to test the susceptibility.

Silver nanoparticles (AgNPs) preparation

Preparation of aqueous extract of *Capparies spinosa*

The fruits of *Capparies spinosa* were obtained from Diyala city in December 2021. The fruits were washed many times using Distilled

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water To remove dust, then dried in the shade and ground finely . Twenty grams of fruit powder mixed with 100 ml of distilled water and boiled for 30 minutes Then filtered using Whatman filter papers no.1 (Benakashani *et al*, 2016). NaOH, used to change to pH 8.

Silver nanoparticles Preparation

According to Ahmad *et al*., (2004) and Ajitha *et al*., (2015) silver nanoparticles were prepared. Putting of 30 mL of 1mM (weight 0.017gm from silver nitrate and dissolve it in 100mL deionized water) aqueous solution of silver nitrate in flask; then 4.0mL of plant extract were added separately to it at room temperature, then heated with shaking water bath at 70C° for 80 min and the flask must be covered with aluminium foil. The formation of brown color indicated the synthesis of silver nanoparticles, Positive controls containing plant extract without silver nitrate and only 1mM silver nitrate as negative control were also used . The process for preparing silver nanoparticles has been repeated in order to optimize various parameters .

Silver nanoparticles Characterization

Change of Color

The color reaction mixture change was documented through visual observation. The transition from green to dark brown pointed to the synthesis of silver nanoparticles(Korbekandi *et al*,2016).

UV-vis spectroscopy

Ag+ were reduced to Ag, Double beam UV-Vis spectrophotometer (PD-303 UV) distinguished it spectrometrically at different wavelengths (300-800 nm). (Ahmad *et al*,2004)

X-ray diffractometry

This technique used primarily for the analytic determination of the crystallite size of the solution silver nanoparticles(Ali *et al*,2024). The sample was examined in the college of education Ibn- Al-Haytham/ University of Baghdad.

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Scanning electron microscope.

Scanning Electron Microscope (SEM) has also performed characterization, it is a kind of electron microscope that images a sample (AgNP) by scanning it in a raster scan pattern with a beam of electrons. The electrons interact with the atoms which make up the sample producing signals containing information about the topography and composition of the surface of the samples(Sadeghi, & Gholamhoseinpoor, 2015) .

Antifungal Activity of AgNPs Against *Candida* spp isolates

Minimum inhibitory concentration (MIC) of *Capparies spinosa* AgNPs *Against Candida* spp. Isolates

The antifungal activity of *Capparies spinosa* AgNPs against all *Candida* spp. isolates was determined by the microdilution method in 96 - well flat - bottom microplate titer on the basis of minimum inhibitory concentration (MIC) values (Elshikh *et al*,2016) .

The MIC of Fluconazole (150 mg/tablet; provided by Pfizer Pharmaceuticals, Pfizer-Roerig, Groton, CT, USA, purity, 99.7%) with concentrations (30000, 15000, 7500, 3750, 1875, 937.5) µg/ml against *Candida* spp. was determined as AgNPs .

Effect of AgNPs in the viability of *Candida* Spp

The effect of *Capparies Spinosa* AgNPs at sub MIC against *Candida* spp. viability was measured by MTT assay. The absorbance of the purple solution was measured at 590 nm by using Eliza reader . Inhibition percentage of viability was calculated as equation described by Bahuguna *et al*.,(2017).

Inhibition of viability % = O.D control- O.D treatment /O.D control × 100

Inhibitory Effect of Silver nanoparticles synthesis from *Capprais spinosa* Fruits extract, and fluconazole against *Candida* spp.

One hundred µL AgNps of fruits extract prepared with MIC concentration 31.5 and 62.5 mg/ml, and fluconazole was prepared with a concentration 15000, 7500 µg /ml. Then 0.1 ml of *Candida* spp. suspension was spread on Sabouraud dextrose agar and left 30 min to dry, then four 7mm wells were Make on the SDA, the first well is filled with

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AgNps only, the second with fluconazole only, the third with fluconazole and AgNps, and the fourth filled with distilled water, the plates were incubated in the incubator at a 37°C for a 24 hrs., after incubation the inhibition zones of for each well were measured(Birla *et al*., 2013) and (Abood, 2014).

Synergistic effect of silver nanoparticles, synthesis from *Capparis spinosa*, and Fluconazole resistant *Candida* spp. *In vitro* by MTT assay

The viability of *Candida* spp was measured by using MTT. All experiments were done in triplicate in test tubes, Added 40 µl from each isolates of *Candida* spp. Compared to 0.5 x 10^8 cell/ ml were added to 160 µl of sabouraud dextrose broth and 100 µl of sub MIC (15000,7500) µg /ml of fluconazole, and 100 µl of MIC (500, 250 and 125) mg/ml of AgNps, Moreover three control tubes for each isolate (*Candida* with AgNps, *Candida* with fluconazole and *Candida* only). All tubes incubated at 37 ºC for 24 h. 50 µl from each treatment and control were added to the wells of microtiter plates 96 wells flat bottom , then added 50 µl of MTT reagent to each well and incubate the microtiter plate for 3 h at 37 ºC. After incubation the solution was discharged and 150 µl of MTT solvent was added in to each wells that result in formation purple solution. The absorbance of the purple solution was measured at 590 nm by using Eliza reader . Inhibition percentage of viability was calculated (Bahuguna *et al*., (2017).

Statistical Analysis

SPSS software version 21/France was used for data analyzing by one way ANOVA-test to calculate the P-vale between the tests groups and control in the previous study. The P value equal or less than 0.05 was considered as the level of statistical significance. Least significant difference- LSD test was done also to compare between the groups mean in this study. The results were presented as mean ± SD.

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Results

Candida spp. Isolates

One hundred twenty specimens were taken from 62 women with VVC and 58 specimens for children with Oral Thrush. 35 cases gave positive results for *Candida* spp . Fifteen isolates of *Candida* spp . selected according to antifungal susceptibility , for study antifungal activity of AgNPs against *Candida* spp isolates in *vitro*.

Antifungal Susceptibility

The Susceptibility of the *Candida* spp. isolates were tested against five type of antifungal , results were recorded resistant , intermediate and sensitive according to the diameter of inhibition zone, The current results showed that all *Candida* spp. were resistant to each of Ketoconazole and Clotrimazole(100 %), Fluconazole (97.4%), Nystatin (73.7 %) and to Amphotericin-B (76.3 %). (**table 1and figure 1).**

Table 1: Antifungal Susceptibility of *Candida* spp. isolated from oral and vaginal infection.

Candida spp.	Amphotericin B			Fluconazole			Clotrimazole			Ketoconazole			Nystatin		
	Number of isolates (%)														
	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
C. albicans	20 (71.4)	4 (14.3)	4 (14.3)	28 (100)	0	0	28 (100)	0	0	28 (100)	0	0	18 (64.28)	9 (32.14)	1 (3.57)
C. ciferri	0	1(100)	0	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	0	0
C. kefyr	2 (100)	0	0	2 (100)	0	0	2 (100)	0	0	2 (100)	0	0	2 (100)	0	0
C. lusitaniae	7 (100)	0	0	7 (100)	0	0	7 (100)	0	0	7 (100)	0	0	7 (100)	0	0
Total	29 (76.3)	5 (13.2)	4 (10.5)	37 (97.4)	1 (2.6)	0	38 (100)	0	0	38 (100)	0	0	28 (73.7)	9 (23.7)	1 (2.6)

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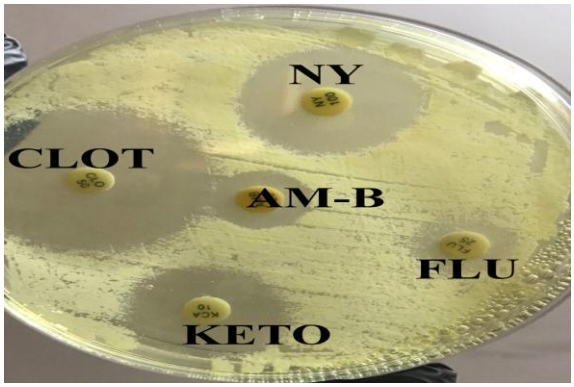

Figure 1: Antifungal drugs Susceptibility of *Candida albican*s. on mueller hinton agar at 37°C for 24 hrs.

Characterization of Silver Nanoparticles

The Color Change

Fruits and roots of *Capparis spinosa* were used for biosynthesis of silver nanoparticles. In principle, the silver nanoparticle formation was well known by changing from green to brown color while adding *C. spinosa* extract to silver nitrate solution Figure 3, first the color was changed after 10 min , and after passing 70 min the color was changed into brown this indicates to production of nanoparticles, the result is corresponding with Benakashani *et al*.(2016) in which the synthesis of silver particles by using *C. spinosa* was observed during change the color of the mixture from green to brown (Figure 2) .

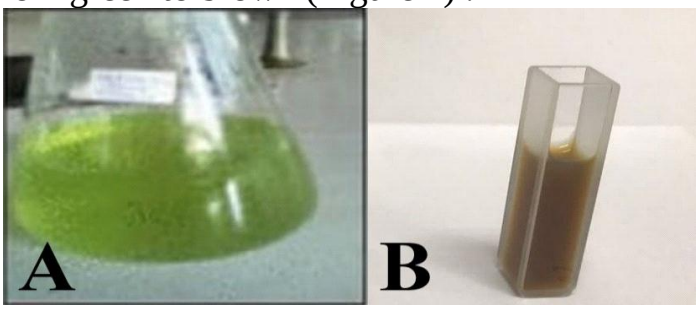

Figure 2: (A) *Capparies spinosa* extract with green color, (B) Brown color indicate the formation of silver nanoparticles.

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UV-Visible Absorbance for AgNp

Figure 3 reveals the absorbance bands of Ag NPs at a wavelength around (320-920) nm. The results observed that the absorbance spectra of biosynthesis Ag NPs as-prepared by using fruits extracts has sharply absorbance with the highest peak between 380-400 nm, and the molecules undergo electronic transitions in the UV and near the visible region that due to the small of NPs. The energy gap of the Ag NPs were calculated by plotting the square of $(\alpha h\upsilon)^2$ versus $(h\upsilon)$. Because of the small size of NPs that reduce the potential attraction between the conduction electrons and metal ions of the particle that leads to the band energy gap increases for the smaller particle, or may be the concentration increase leads to the division of the level into secondary levels that called energy gap. The values of the energy gap of Ag NPs are ranged from 3.3 to 3.4 eV as shown in the figure 3.

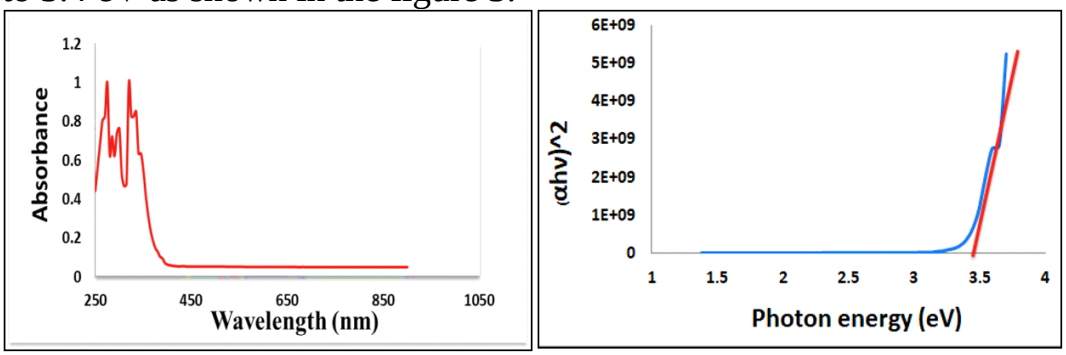

Figure 3: UV-visible spectra of *Capparis* spinosa fruits AgNPs The Absorbance bands of Ag NPs at a wavelength around (320-920) nm.

The Structure and Properties of *Capparis spinosa* Silver nanoparticles

The crystallite structure of the synthesized Ag NPs prepared by Green Synthesis method using fruits extract as reducing agent is confirmed by XRD analysis. The obtained results illustrated that silver ions have been indeed reduced to Ag NPs using plant extracts as a reduced agent. The XRD pattern possesses very fine peaks that indicate a good crystallization. For as-grown Ag films on a glass substrate. All diffraction peaks correspond to the characteristic face-centered cubic (F.C.C) and Miller indices (h k l) values are (111), (200), and (220) planes of silver

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which are observed and compared with (JCPDS 04-0783) as shown in figure 4. The figures show several peaks have existed of Ag indicating the polycrystalline nature. The diffraction pattern shows a strong peak at the (111) plane, which confirms that a great amount of Ag NPs has aligned and grown on the glass substrate towards planes (111). The figure (4) explains that Ag is not pure and contains silver nitrate AgNO3 corresponds to (210), (122), and (231) according to Miller indices (JCPDS). It is possible to use the plant extracts with a high concentration to get pure Ag NPs. This might be due to phytochemicals responsible for the quick reduction of AgNO3 to Ag NPs in a single-step eco-friendly Figure 4.

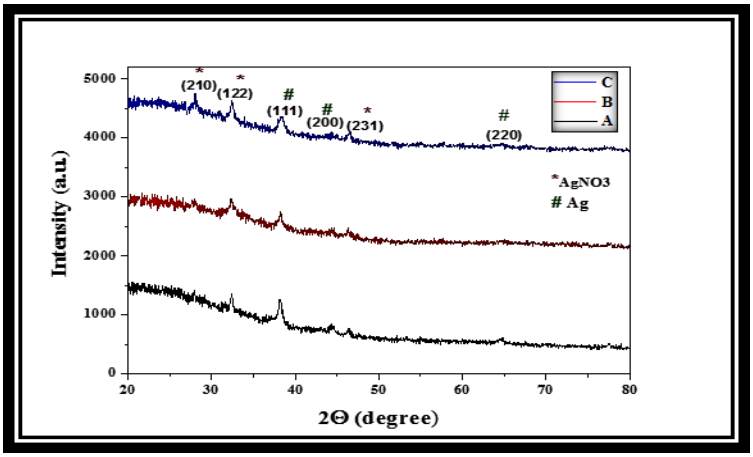

Figure 4: XRD peaks intensities of *Capparis spinosa* silver nanoparticles ; A(Fruits).

The FE-SEM analysis used to determine the morphological structure of the reaction products during biosynthesis of metal NPs. Figure 5 shows the morphological of Ag nanoparticles as prepared by *Capparis spinosa* fruits extract. The morphology explains the uniform distribution of the particles and these NPs assembled to the cluster like shape with different diameters between 20-90 nm.

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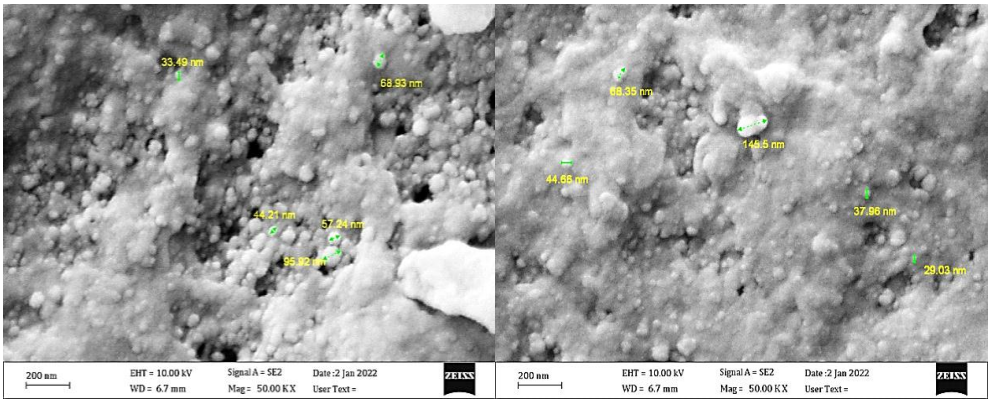

Figure 5: FE-SEM images of Ag NPs synthesized by *Capparis spinosa* fruits extract

The elemental constituents and relative abundance of the biosynthesized AgNPs were obtained from Energy Dispersive X-ray (EDX) as presented in Figure 6. The EDX spectrum reveals the purity and the complete chemical composition of AgNPs. The percentage of Ag metal found in occurrence with other chemical elements was found to be appreciable.The reduced silver nanoparticles were subjected to EDX analysis with an optical absorption characteristic peak at 3 keV. The EDX analysis showed percentage relative composition of elements such as (O) 22 %, (Si) 8.1%, (C) 21 %, Potassium (Mg) 0.7 % and Silver (Ag) 47 %. The other elements served as capping organic agents bound to the surface of the silver nanoparticles.

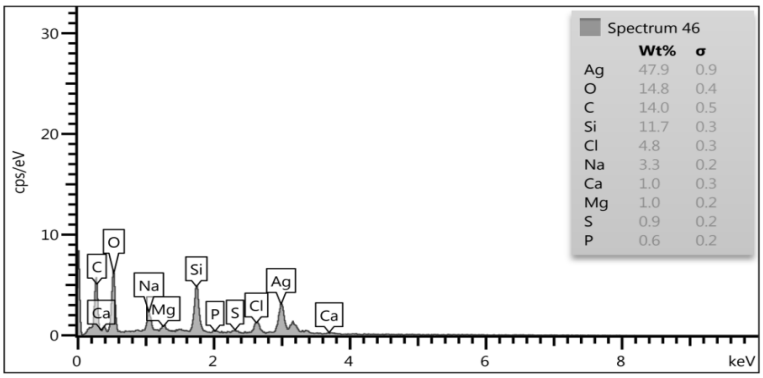

Figure 6: Energy Dispersive X-ray (EDX) fruits.

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According to the characterization of silver nanoparticles results, *C. spinosa* fruits AgNPs were selected to study their effect on *Candida* spp . isolates.

Minimum Inhibitory Concentration (MIC) of Silver Nanoparticles from *Cappris spinosa* Fruit extracts isolates of against *Candida* spp

Results in Table 2 showed that MIC values of *Cappris spinosa* Fruits extracts silver Nanoparticles was ranging 31.25-62.5 mg/ml . According to AgNPs Fruit extract, the MIC 62.5 mg/ml of AgNPs-Fruit extract were recorded against VVC *C. albicans* (V1) and *C. kefyr* (V21), while MIC 31.25 mg/ml were recorded against VVC *C. albicans* (V2, 3, 5, 18, 20), *C. kefyr* (V22) and *C. ciferri* (V23). Moreover, the MIC 31.25 mg/ml of AgNPs-fruit extract were recorded against oral *C. albicans* (O24, 25) and MIC 62.5 mg/ml of AgNPs-fruit extract were recorded against *C. lusitaniae* (O35, 36, 37, 38). On the other hand ,The results of fluconazole showed that the MIC for all isolates of *Candida* spp. were 15000 mg/ml except *C. albican* (V2) the MIC was 7500 mg/ml.

Table 2: Minimum Inhibitory Concentration (MIC) of Silver Nanoparticles from *Cappris spinosa* Fruits extract against *Candida* spp. Isolates

Candida spp.	MIC(mg/ml) AgNPs
C. albicans (V1)	62.5
C. albicans (V2)	31.25
C. albicans (V3)	31.25
C. albicans (V4)	31.25
C. albicans (V5)	31.25
C. albicans (V6)	31.25
C. kefyr (V7)	62.5
C. kefyr (V8)	31.25
C. ciferri (V9)	31.25
C. albicans (O10)	31.25
C. albicans (O11)	31.25
C. lusitaniae (O12)	62.5
C. lusitaniae (O13)	62.5
C. lusitaniae (O14)	62.5
C. lusitaniae (O15)	62.5
V:vagina, O:oral	

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Effect of AgNPs on *Candida* spp Viability

AgNps from *Cappris spinosa* fruits extracts showed high effectiveness in inhibiting the viability *of Candida* spp. AgNPs fruits extract, the highest inhibition percentage was (88.15 and 91.42) % in *C. albicans* (V2) and *C. lusitaniae* (O38) respectively, while the lowest inhibition percentage was (77.77 and 76.31) % in *C. albicans* (V18) and *C. lusitaniae* (O36) respectively.

Table 3: Inhibition of Viability of of AgNPs fruits extract against *Candida* spp.

Candida spp.	Inhibition of viability %
C. albicans (V1)	81.08
C. albicans (V2)	88.15
C. albicans (V3)	85.36
C. albicans (V4)	85.71
C. albicans (V5)	77.77
C. albicans (V6)	86.206
C. kefyr (V7)	81.81
C. kefyr (V8)	85.29
C. ciferri (V9)	82.05
C. albicans (O10)	75.609
C. albicans (O11)	81.81
C. lusitaniae (O12)	74.41
C. lusitaniae (O13)	76.31
C. lusitaniae (O14)	90.67
C. lusitaniae (O15)	91.42
V:vagina, O:oral	

Effect of silver nanoparticles, synthesis from *Capparis spinosa*, and Fluconazole against *Candida* spp. by Agar plate method

Antifungal properties of sub MIC of AgNPs and fluconazole alone or together against *Candida* spp. were tested by agar plate method. The results are shown in the Table 4. The results of statistical analysis showed significant ($p \leq 0.05$) between three treatment of all isolates. The results showed that the effectiveness of fluconazole increases significantly when

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mixed with nanoparticles, which explains the possibility of using it effectively to treat fungal infections, especially Candida infections.

Table 4: Effect of silver nanoparticles, synthesis from *Capparis spinosa* Fruit extract, and Fluconazole against *Candida* spp. by Agar plate method

Candida spp	Inhibition zone (mm) Mean ± SD						P VALUE
	AgNps		Fluconazole		Fluconazole +AgNps		
C.albicans (V1)	10.00 ±	0.82	10.00 ±	0.82	19.00 ±	1.00	0.001
C.albicans (V2)	7.00 ±	2.16	25.67 ±	10.31	35.67 ±	3.65	0.001
C.albicans (V3)	5.00 ±	0.82	22.33 ±	1.34	54.67 ±	4.03	0.001
C.albicans (V4)	7.00 ±	0.82	11.33 ±	3.27	13.67 ±	1.53	0.001
C.albicans (V5)	6.33 ±	0.94	13.00 ±	1.70	15.00 ±	1.00	0.001
C.kefyr (V6)	10.00 ±	0.82	12.33 ±	1.34	30.00 ±	1.00	0.001
C.kefyr (V7)	10.00 ±	1.63	15.00 ±	0.82	32.33 ±	6.43	0.001
C.albicans (V8)	11.67 ±	1.25	7.67 ±	0.57	21.33 ±	3.21	0.001
C.ciferri (V9)	10.67 ±	2.62	8.67 ±	3.77	22.33 ±	2.52	0.001
C.albicans (O10)	6.00 ±	0.82	12.33 ±	1.03	13.33 ±	1.53	0.001
C.albicans (O11)	15.00 ±	0.82	15.67 ±	7.87	30.00 ±	1.00	0.001
C.lusitaniae (O12)	10.00 ±	0.82	25.67 ±	0.83	35.67 ±	4.04	0.001
C.lusitaniae (O13)	10.00 ±	0.82	27.67 ±	1.34	35.00 ±	5.00	0.001
C.lusitaniae (O14)	9.00 ±	0.82	16.00 ±	2.16	24.33 ±	9.29	0.001
C.lusitaniae (O15)	8.00 ±	0.82	9.00 ±	0.82	15.00 ±	1.00	0.001
P VALUE	NS		0.01		0.001		

Effect of silver nanoparticles, synthesis from *Capparis spinosa*, and Fluconazole against *Candida* spp. *In vitro*

Silver ions, especially Ag-NPs, are known to have strong antifungal activities. Although synthesized Ag-NPs of fruit extracts and fluconazole separately showed high antifungal activity against *Candida* spp. isolates compared with a control, the effect of both of them with fluconazole was less than AgNps and fluconazole separately , but higher than control. as illustrated in table 5 The results of statistical analysis showed significant ($p \leq 0.05$) between treatment and control of all isolates. In the VVC isolates treatment with AgNPs– Fruit extract and fluconazole, combined effect of fluconazole with AgNPs-Fruit extract, the results showed that

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higher effect was noticed on C. albicans (V2). According to oral isolates, the higher effect was noticed on C. lusitaniae (O37) respectively followed by other isolates.

Table 5: Effect of *Capparies spinosa* Fruits Extract AgNPs with Fluconazole against *Candida* spp. isolates by MTT Method.

Candida spp.	OD at 590 nm Mean ± SD									
	Control		**AgNps**		**Fluconazole**		**Fluconazole +AgNps**		P VALUE 1	P VALUE 2
***C. albicans* (V1)**	**0.37 ±**	0.01	**0.07±**	**0.05**	**0.05 ±**	**0.00**	0.21 ±	0.02	0.01	0.01
***C. albicans* (V2)**	**0.76 ±**	**0.04**	**0.09 ±**	**0.03**	**0.07 ±**	**0.00**	0.23 ±	0.00	0.01	0.01
***C. albicans* (V3)**	**0.41 ±**	**0.03**	**0.06 ±**	**0.00**	**0.06 ±**	**0.00**	0.25 ±	0.02	0.01	0.01
***C. albicans* (V4)**	**0.35 ±**	**0.01**	**0.05 ±**	**0.02**	**0.06 ±**	**0.00**	0.30 ±	0.01	0.01	0.01
***C. albicans* (V5)**	**0.45 ±**	**0.01**	**0.10 ±**	**0.02**	**0.06 ±**	**0.00**	0.24 ±	0.00	0.01	0.01
***C. albicans* (V6)**	**0.58 ±**	**0.02**	**0.08 ±**	**0.03**	**0.06 ±**	**0.00**	0.21 ±	0.00	0.01	0.01
***C. kefyr* (V7)**	**0.33 ±**	**0.01**	**0.06 ±**	**0.02**	**0.06 ±**	**0.00**	0.22 ±	0.01	0.01	0.01
***C. kefyr* (V8)**	**0.34 ±**	**0.01**	**0.05 ±**	**0.00**	**0.06 ±**	**0.00**	0.24 ±	0.05	0.01	0.01
***C. ciferri* (V9)**	**0.39 ±**	**0.03**	**0.07 ±**	**0.01**	**0.06 ±**	**0.00**	0.34 ±	0.05	0.01	0.01
***C. albicans* (O10)**	**0.41 ±**	**0.01**	**0.10 ±**	**0.03**	**0.06 ±**	**0.00**	0.26 ±	0.02	0.01	0.01
***C. albicans* (O11)**	**0.44 ±**	**0.01**	**0.08 ±**	**0.03**	**0.06 ±**	**0.00**	0.34 ±	0.04	0.01	0.01
***C. lusitaniae* (O12)**	**0.43 ±**	**0.04**	**0.11 ±**	**0.02**	**0.06 ±**	**0.00**	0.43 ±	0.07	0.01	0.01
***C. lusitaniae* (O13)**	**0.38 ±**	**0.03**	**0.09 ±**	**0.03**	**0.06 ±**	**0.00**	0.42 ±	0.02	0.01	0.01
***C. lusitaniae* (O14)**	**0.75 ±**	**0.06**	**0.07 ±**	**0.01**	**0.06 ±**	**0.00**	0.22 ±	0.03	0.01	0.01
***C. lusitaniae* (O15)**	**0.70 ±**	**0.26**	**0.06 ±**	**0.02**	**0.06 ±**	**0.00**	0.29 ±	0.06	0.01	0.01
P VALUE	**NS**		**NS**		**NS**		NS			
P value 1=Between tested groups and control /p value 2=between tested groups only										

The highest inhibition percentage of treatment with AgNPs was 88.15 and 91.42 in C. albicans (V2) and C. lusitaniae (O38) respectively, however the lowest inhibition percentage was 77.77 and 74.41 in C. albicans (V18) and C. lusitaniae (O35) respectively.In fluconazole group the highest inhibition percentage was 90.78 and 92 in C. albicans (V2) and C. lusitaniae (O37) respectively, however the lowest inhibition

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percentage was 81.81 and 84.21 in C. kefyr (V21) and C. lusitaniae (O36) respectively. The highest inhibition percentage in combined of fluconazole with AgNPs-Fruit extract was 69.73 and 70.66 in C. albicans (V2) and C. lusitaniae (O37) respectively, however the lowest inhibition percentage was 12.82 and 0.0 in C. ciferri (V23) and C. lusitaniae (O35) respectively(table 6).

Table 6: Inhibition perecentage of *Cappa ries spinosa* Fruits Extract AgNPs with Fluconazole against *Candida* spp. isolates Viability by MTT Method.

Candida spp.	Inhibition perecentage (%)		
	Agnps	Fluconazole	Fluconazole+Agnps
C. albicans (V1)	81.08	86.48	43.24
C. albicans (V2)	88.15	90.78	69.73
C. albicans (V3)	85.36	85.36	39.02
C. albicans (V4)	85.71	82.85	14.28
C. albicans (V5)	77.77	86.66	46.66
C. albicans (V6)	86.206	89.65	63.79
C. kefyr (V7)	81.81	81.81	33.33
C. kefyr (V8)	85.29	82.35	29.41
C. ciferri (V9)	82.05	84.61	12.82
C.albicans (O10)	75.609	85.36	36.58
C.albicans (O11)	81.81	86.36	22.72
C.lusitaniae (O12)	74.41	86.04	0
C.lusitaniae (O13)	76.31	84.21	10.52
C.lusitaniae (O14)	90.67	92	70.66
C.lusitaniae (O15)	91.42	91.42	58.57

Discussion

About 80% of healthy people harbor *Candida* spp. as a normal flora on The urinary tract, surfaces of the mouth, intestines and vagina In addition to soft and moisturized skin. When the mucosal defenses of the host are weakened, this opportunistic fungus can invade the circulation

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and spread to other organs. This *Candida albicans* is responsible for an impressive 60% of mucosal infections and 40% of cases of candidemia (Gonçalves *et al*, 2016; Pappas *et al* ,2018) .Susceptibility test for the isolates was performed by using the Kirby– Bauer disc diffusion method(Khatami *et al*,2020 ; Muhyaddin *et al*,2023), Ketoconazole, Clotrimazole, Fluconazole, Amphotericin-B, and Nystatin antifungal discs were used to detect on the susceptibility of Cadidal isolates for these antifungal (Maryoush *et al* ,2024).

The current results showed (100 %) *of Candida* spp were resistant to each of Ketoconazole and Clotrimazole, 97.4% Fluconazole, 73.7 % of *Candida* spp were resistant to Nystatin and 76.3 % to Amphotericin-B.

The antifungal resistance is very clearly observed in non- *albicans Candida* (NAC) spp compared to *C. albicans* (Mendling, 2015; Al-Kareemi *et al*,2023).. The non- *albicans Candida* (NAC) isolated from patients showed higher rate of resistance to studied antifungal than *C. albican.*

Many membrane proteins are found in pathogenic yeast, and over-expression of membrane transporters is the mechanism by which *Candida* resists azole medications (Fluconazole, Clotrimazole, and Ketoconazole). The cell membrane, vacuole membrane, and mitochondrial membrane all contain these proteins. Environmental sensing, modification of drug, detoxification of drug, nutrient transported, signal transduction, moreover drug efflux, are just a few of the many physiological functions they serve (Lamping *et al* ,2007;Khandelwal *etal*,2019).These drugs, (Fluconazole, Clotrimazole, and Ketoconazole), are more effective for the treatment 80% of Non-severe Vulvovaginal Candidiasis and needs more duration for treatment of severe kind (Sobel, 1997; Surain and Aggarwal, 2020) . On the other hand, the use of azole in the treatment of Vulvovaginal Candidiasis is more therapeutically effective than Nystatin cream and suppositories. In addition to the high effectiveness of azoles, it is considered an antifungal with few side effects and is medically safe for treatment of Vulvovaginal Candidiasis (VVC) and good bioavailability, (Willems *et al*,2020) .

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Moreover , the resistance of fungi, including *Candida* spp, to antifungals drugs has increased causing a major problem in the treatment of fungal infections, Approximately 10% of patients with candida vaginitis fail to respond to conventional treatment (Shokohi *et al*,2016) . The antifungal activity of silver Nanoparticles from *Cappris spinosa* fruits extracts was determined on the basis of MIC values. The results showed that the MIC was 31.25-62.5 mg/ml in AgNPs- Fruit extract.

The first work of synthesis Ag-NPs from aqueous extract of *C. spinosa* fruit was studied by Ebrahimi *et al*.(2019). their results showed that synthesized Ag-NPs from fruit aqueous extract showed relatively acceptable antifungal activity against all fungi (*C. albican*, *C. kefyr*, *C. lusitaniae*, *C. ciferri*). These results agreed with the current results that showed high effect of Ag-NPs from fruit aqueous extract.

The current study showed the effectiveness of silver nanoparticles prepared from *Cappris spinosa* fruits against *Candida* spp , and this effect appears clearly through reducing the percentage of Candida's viability in different AgNPs concentration of AgNPs MIC.

However, to the best of our knowledge, there are hardly any reports of the effect of AgNPs on fungal cells at the ultrastructural level. It was reported that when *C. albicans* was exposed to AgNPs, the membrane changed significantly, "pits" appeared on the cell surface, pores formed, and eventually the cells died (Kim *et al* ,2019).

The synergistic action of AgNPs and plant extracts against *Candida* spp. may be a reflection of the antibacterial, antioxidant, anticancer, and hepatoprotective characteristics of *Capparis spinosa* (Gull *et al*,2015) .

Moreover, AgNPs, are known to have High antifungal activities. AgNPs fruit extracts and fluconazole separately showed high antifungal activity against *Candida* spp. isolates compared with a control, the effect of both of them with fluconazole was less than AgNPs and fluconazole separately , but higher than control

The effectiveness of AgNPs appears through its effect on the budding process, as well as its effect to the disruption of cell membrane integrity,

also, defects in green synthesis pathway and efflux bump functions were also detected, as the expressions of some related genes *ERG5*, *ERG1*, *ERG25*, *ERG11*, *MDR1*, and *CDR2* genes were disregulated and membrane ergosterol level was reduced (Sun *et al*,2016; Maryoush *et al* ,2024). but the underlying mechanisms were not much elucidated. In wild-type *C. albicans* strain SC5314, AgNPs exerted antifungal activity by altering membrane microenvironment, predominantly ergosterol content and fatty acid composition (Radhakrishnan *et al* ,2018) . AgNPs could also induce intracellular Reactive oxygen spp production . These factors are essential and Important for resistance of drug in the fungal cells (Mukhopadhyay *etal*,2004; Neto *etal* ,2014) . Another study indicated that the synergistic action of AgNPs and fluconazole antifungal drugs could be a potential way to treat fluconazole-resistant fungal infections (Jia *et al* ,2021) .

Conclusions

AgNPs from *Cappris spinosa* fruits extracts had effective on the Candida spp. viability ,All isolates showed decrease in viability . Moreover , Combined effect of silver nanoparticles and fluconazole on *Candida* spp. showed antagonistic effect of the interaction of AgNPs-fruits extract and Fluconazole**.**

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Ethical clearance: The study protocol was approved by the Ethics Committee of Biology Department , Science College, Mustansiriyah University(BCSMU/11021 /00075M)

Conflict of interest: None

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النشاط المضاد للفطريات لجسيمات الفضه النانوية المصنعة من ثمار *Capparis spinosa* ضد *Candida* spp. المقاومة للأزول.

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مستخلص البحث: تُعدّ المبيضات جزءًا شائعًا من الميكروبات البشرية، لا سيما في الجهاز الهضمي والجهاز البولي التناسلي. هدفت هذه الدراسة إلى دراسة النشاط المضاد للفطريات لجسيمات الفضة النانوية المُصنّعة من ثمار نبات الكبر (*Capparis spinosa*) ضد أنواع المبيضات المقاومة للأزول في المختبر. في هذه الدراسة، جُمعت 120 عينة من 62 امرأة مصابة بالتهاب المهبل الفطري، و58 عينة من أطفال مصابين بداء المبيضات الفموي من مستشفى بغداد للفتره من شباط الى تموز 2022. تم إجراء فحص مجهري وفحص زراعي للكشف عن أنواع المبيضات. وقُيس تأثير جسيمات الفضة النانوية المستخلصة من نبات الكبر (*Capparis spinosa*) على حيوية أنواع المبيضات باستخدام طريقة MTT. كما تم تقييم التأثير التآزري لجسيمات الفضة النانوية مع أنواع المبيضات المقاومة للفلوكونازول باستخدام اختبار MTT واختبار انتشار الحفر في أطباق الآجار. وأظهر تأثير جسيمات الفضة النانوية المُصنّعة من نبات الكبر على حيوية أنواع المبيضات أعلى نسبة تثبيط،وكانت أعلى نسبة تثبيط (88.15 و91.42)% في *C. albicans* (V2) و*C. lusitaniae* (O38) على التوالي، في حين كانت أقل نسبة تثبيط (77.77 و76.31)% في *C. albicans* (V18) و *C. lusitaniae* (O36) على التوالي، كما أظهر أن التأثير التآزري بين مستخلص ثمار نبات الكبر وجسيمات الفضة النانوية مع الفلوكونازول كان أقل من تأثير مستخلص ثمار نبات الكبر وجسيمات الفضة النانوية وحده، وتأثير الفلوكونازول وحده. أظهرت نتائج التحليل الإحصائي تاثيرا معنوية ($p \geq 0.05$) بين المعاملة والسيطرة لجميع العزلات.

الكلمات المفتاحية: أنواع الكانديدا؛ جزيئات الفضة النانوية؛ ثمار نبات الكبر الشوكي.