Green Synthesis of Silver Nanoparticles Using Mixed Leaves Aqueous Extract of Wild Olive and Pistachio: Characterization, Enhancing Antioxidant, Antimicrobial Potential and Effect on Virulence Factors of Candida

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Abstract

Olea europaea subsp. *europaea* and *Pistacia lentiscus* are well known as natural sources of secondary metabolites promising in various fields. Phenolic compounds from Plant are suitable markers to differentiate varieties related to geographical area. This work aimed to enhance the antimicrobial potential of new green silver nanoparticles AgNPs using for the first time the mixed leaves extract of *Olea europaea* subsp. *europaea* var. *sylvestris* and *Pistacia lentiscus* from natural geographical association and describe their antimicrobial, antibiofilm and their effect on virulence factor of Candida. A rapid, simple environmentally approach for biosynthesis of AgNPs by using mixed plant aqueous extract acts both as reducing and capting agents without any solvent or hazardous reagents. The AgNPs were characterized by UV-Vis spectrophotometer, FTIR spectrum and the X-ray crystallography.

The AgNPs showed superior antioxidant activity by measuring DPPH, Ferric Antioxidant Reducing Power (FRAP) and the total antioxidant activity. It was most richness with flavonoids, tannins, alkaloids and total polyphenols contents compared to plant extract. The new AgNPs possess high bactericidal and fungicidal effects against clinical strains, limit spore's germination of filamentous fungi, announcing high anti-biofilm activity, synergistic effect with the conventional antibiotic's drugs and affecting virulence factors of Candida (Proteinase, Phospholipase and morphogenesis).

Introduction

Metal nanoparticles are of great importance due to their various applications principally their antibacterial potential. The silver nanoparticles are the most applied [1, 2]. Based on the toxic chemical and physical methods used for nanoparticles synthesis, there is a crucial emergency to generate an alternative non-toxic approach [3]. Green synthesis of nanoparticles has extensive regard since they are ecofriendly and rapid by single step process and relatively reproducible and more stable materials [4]. Currently, green syntheses are more compatible and simpler than the moderate Nano synthesis by microorganisms, because of the need to improve cultivation methods [5]. On the other hand, green synthesis show more advantages, by owing to antibacterial activity of silver nanoparticles because plants extracts contained chemical constituents such as phenols, reducing sugars, ascorbic acids, and others which are responsible for the bio-reduction of metal ions, the stabilization of the nanoparticles and the attachment on surface of nanomaterials. Nowadays biotechnological industries target for new natural antimicrobial drugs[3, 6].

Tunisia is rich on natural medicinal species using in traditional medicine as *Olea europaea* L. (Oleaceae) and *Pistacia lentiscus* L. (Anacardiaceae). In addition, diverse natural associations are frequently and spread in natural ecosystem as wild olive trees (*Olea europaea* subsp. *europaea* var. *sylvestris*) or oleaster forests with pistachio (*Pistacia lentiscus*). This type of natural association is isolated from all cultural practices. Therefore, the natural ecosystem reflects an extreme environment condition of this natural association. The wild olive trees or Oleaster are native in Tunisia as reported by historical reports

[7, 8]. and confirmed by nuclear and cytoplasmic molecular markers [9, 10]. *Pistacia lentiscus* L. is evergreen shrub widespread in Mediterranean forests [11].

This study described the enhancement of the antimicrobial and antioxidant activities of green synthesis AgNPs by using mixed aqueous extract from oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris*) and pistachio (*Pistacia lentiscus*) leaves. In this context, we focused on the use of oleaster and pistachio leaves as natural source of biomolecules and surface reducers of nanoparticles based on their richness on secondary metabolites as phenolic compounds and their natural association in extreme environmental showing their adaptation to environment and studying their antibacterial, antifungal actions, define the action mechanisms such as biofilm, spores germination, and virulence factor yeast reduction.

Material And Methods

Plant Material and preparation of aqueous extract

Oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris*) and Pistachio (*Pistacia lentiscus*) leaves were freshly collected form natural association from Tunisia northern forest. The leaves of plant species were surface cleaned with running tap water to remove soil and other contaminated organic contents, followed by double distilled water and air dried at room temperature. About 20 g of the leaves (10g from each specie) was cut into small pieces and then boiled with 100 mL distilled water for 20 min. The obtained extract after filtration was stored at 4°C for the further use including the analysis of its major chemical constituents.

Synthesis of nanoparticles

Aqueous solution (5 mM) of silver nitrate was prepared. The plant extract (10 mL) was added drop by drop to 20 mL of silver nitrate solution (5 mM). The reaction mixture of Ag NO_3 and leaf extract was stirred for 2 min. The color changed from yellow to reddish brown color indicating the formation of silver nanoparticles. Then, the AgNPs obtained was purified by repeated centrifugation at 10000 rpm for 10 min. The pellet was collected and dried.

Characterization of synthesized silver nanoparticles

The reduction of pure silver ions was confirmed by measuring the UV–vis spectrum of the reaction mixture against distilled water as a blank. The Spectrum analysis was done using a 2802 UV/Vis spectrophotometer (UNICO) in the 250-700 nm region. The Fourier Transform-Infrared Spectroscopy (FTIR) spectrum was recorded in the range 400-4000 cm^{-1} on a Varian FTIR 640 spectrophotometer with KBr pellets. The X-ray powder diffraction (XRD) measurements was performed on a D8 ADVANCE BRUKER diffractometer using Cu-K_{α} radiations and equipped with Lynxeye accelerator.

Antioxidant activities

DPPH radical scavenging activity

The DPPH (2,2-Diphenyl-1-picryl-hydrazyl) free radical scavenging activity was estimated by colorimetric method. One mL of sample was added to 2 mL of 1,1-diphenyl-2-picrylhydrazyl methanolic solution (1:2) mixture was incubated for 30 min in dark after shaking. The presence of an antioxidant donator of hydrogen, the DPPH. radical was reduced in 2,2-diphényl-1-picrylhydrazine (DPPH-H) reflected by the color change. The absorbance was measured at 517 nm. The scavenging activity was expressed as percentage of inhibition (PI) following this formula: PI = ((Ac – As)/As) x 100 [12].

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Total antioxidant activity (TAA)

The mixture reaction containing 0.2 mL of sulfuric acid, sodium phosphate (H_2SO_4, 0.6M $NaHPO_4$, H_2O 28 mL) and ammonium heptamolybdate ((NH□)□Mo□O□□.4H_2O4mM) at acidic pH was prepared. Then, 0.3 mL of sample was added. The reaction was placed in boiled water at 95°C for 90 min. The reduction of Mo was accompanied by green color. The absorbance was measured at 695nm. The total antioxidant activity was expressed as mg of gallic acid equivalent per g of dry matter (DM) sample MS (mg GAE/g DM).

Ferric Reducing Antioxidant Power (FRAP)

The mixture reaction containing 100 µL of the sample solution (silver nanoparticles or plant extract) with 3 mL of FRAP reagent and were incubated at room temperature in dark for 10 min. The FRAP method relies on the reduction by the antioxidants, of the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)1,3,5-triazine) accompagnied by the apparition of bleu color. The absorbance was measured at 593 nm (Analytik Jena) spectrophotometer. The FRAP value was expressed as mg Ascorbic acid Equivalent Antioxidant Capacity (AEAC) per g of sample [13].

Phytochemical characterization of EA and AgNPs

Total polyphenols Content Total polyphenols content in the sample solution was estimated using the method of Folin Ciocalteu method as described by [14] and the results were expressed as mg of gallic acid equivalent (GAE) per mL (mg GAE/mL of extract).

Total Flavonoids content The flavonoids content was estimated by using aluminum chloride colorimetric technique at 765 nm. Results expressed as mg quercetin equivalents per mL of extract based on a quercetin calibration curve [15].

Tannins content

The tannins content was determined by the vanillin method in acid medium. Tannic acid was served as a standard and Tannin content was expressed as mg tannic acid equivalent per mL of extract. The absorbance was measured at 760nm [16].

Total Alkaloids content

Total alkaloids content was estimated after extraction with glacial acetic acid and ethanol and precipitated with Draggendroff's reagent. The residue treated with sodium sulfite and thiourea solution. Atropine standard solution was prepared (Sigma chemical, USA) and optical density was measured at 435 nm [17].

Antibacterial and antifungal potentialities of the silver nanoparticles

Well Agar diffusion method for antimicrobial detection

A clinical bacteria and fungi strains from a Tunisian clinical laboratory were used here as fellow: gram-negative bacteria species (*Klebsiella pneumoniae; Escherchia coli; Enterobacter cloacae)* and gram-positive bacteria *(Staphylococcus aureus; Micrococcus luteus)*. Fungi species are belonging to *Candida albicans, Candida parapsilosis, Penicillium spp*, and *Aspergillus spp.* Before use, the AgNPs was diluted in distilled water and adjusted to the appropriate concentration. The cell suspension (0.1 mL) adjusted to 10^7CFU/mL for bacteria and 10^5spores /mL for fungi were transferred separately into the surface of agar plates and 40 µL of the tested AgNPs were aseptically pipetted into wells (6mm). The plates were incubated at 37°C. The observation of inhibition zone around the wells indicates the antimicrobial activity and the diameter of inhibition zone was measured in mm. Ceftazidime CAZ30 was used as positive control for gram negative bacteria and Vancomycin for gram positive bacteria, Amphotericin B and Fluconazole 25 were used as fungicide standards. All tests were performed in triplicate [18].

Minimum Inhibitory Concentration (MIC) determination

The MIC was determined by broth dilution method by conducting broth culture of pathogen strains in the presence of different concentrations of silver nanoparticles. The incubation was performed in Eppendorf tube containing 1mL of Nutrient broth (NB) and 10CFU/mL of the pathogen strains. The negative control tube contained the NB without AgNPs. High rotational speed of 200 rpm was maintained to avoid the aggregation of the nanoparticles. The absorption was measured at 600nm, to depict bacterial and fungal growth, no increase in absorbance indicates the MIC [2].

Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MCF) determinations
The MBC and MFC were determined by transferring an aliquot of 10µL from the tube corresponding to MIC values on the surface of the appropriate agar plates and the inoculated plates were incubated at 37°C for 24h. The lowest concentration of AgNPs at which no visible colony was observed on the surface of the agar plate was reported as the MBC or MFC according to the modified method [19]. All assays were performed in quadruplicate.

Based on the method previously used [20] showing that values of report of MBC/MIC reflects the bactericide and bacteriostatic effects. The values of MBC/MIC ≤ 4 showed that the tested compound

was considered as bactericide agent, and the values of MBC/MIC> 4 showed that the compound has bacteriostatic effect.

Anti-biofilm activity

The probably effect on biofilm formation of the bacteria species have been tested on Eppendorf tubes by using the method used for *Candida* species [21, 22].

Spores germination inhibition

To evaluate the effect of the silver nanoparticles on spore germination of fungi species, the mixed reaction consisting of v/v of the AgNPs and the conidial suspensions (10^5spores/mL) mixed in Eppendorf tube containing1mL of 5% glucose as previously described [23].

Synergistic antibiotic effect of AgNPs with conventional antibiotics

In order to enhance the antibacterial effect of the synthesized AgNPs, we have tested their synergistic effect with conventional antibiotics by using the method of agar diffusion of [24].

Evaluation of AgNPs on the virulence factors of Candida species

Phospholipase detection

The phospholipase activity (Pz) was determined based on the method of [25]. Briefly, the Egg yolk agar medium was used and a volume of 10 µL of yeast suspension adjusted at 10^7CFU/mL, was deposit into the wells. Then the plates were incubated for 48h at 37°C. The diameter of colonies and the diameter of zone opacity were measured and the phospholipase activity (Pz) was calculated as follow:

$$Pz = \frac{Colony\ diameter\ (in\ mm)}{\text{Zone opacity+colony diameter (in mm)}}$$

Proteinase detection

The bovine serum albumin medium was used and the wells punched on the surface were inoculated by 10µl of Candida suspension (at 10^7CFU/mL), after incubation of 48h at 37°C, based on the modified method of Staib as detailed by [26]. The Proteinase activity (Pz) was calculated as detailed above in phospholipase activity assay.

Morphogenesis change

The effect of the addition of AgNPs on Candida albicans morphogenesis, was examined by direct microscopic observation of 100µL of *Candida albicans* suspension from 1mL culture in Sabouraud broth

for 48h at 37°C in the presence of AgNPs (10µL of 250µg/mL) and coloration with bleu cotton and compared to control tube culture without the addition of AgNPs.

Results

UV-vis spectroscopy study

The formation of AgNPs can be visualized by the color change of the mixture which is turned from pale yellow to brown color (Figure 1A). This color change in the reaction mixture strongly indicates the reduction of Ag^{+} ions to Ag^{0}. UV-Vis spectroscopic analysis elucidates a single absorbance peak at 422 nm (Figure 1B) indicating the formation of AgNPs. Ancient studies suggested that peak located between 410 and 450 nm has been observed for AgNPs and might be attributed to spherical nanoparticles[27].

FT-IR analysis

FT-IR analysis was performed to identify the bond linkages and functional groups of the active components in the obtained AgNPs based on the peaks and the values in the IR region. All the observed intense bands were compared with standard values to identify the functional groups. As shown in figure 3, the intense band of AgNPs at 3226 cm^{-1} denotes the presence of C-H of aromatic compounds[28]. The absorption peak at 1633 cm^{-1} correspond to the C=C bending[29]. Further, the peak emerged at 1322 cm^{-1} corresponds to O-H bending vibrations. Finally, the band at 1012 cm^{-1} can be assigned C-O stretching vibration indicated the presence of phenols and aliphatic amines[30]. (Figure 2)

Structural studies

The crystalline nature of nanoparticles was confirmed by X-ray crystallography. The XRD pattern of the synthesized AgNPs is illustrated in Figure 3. The observed 2θ values at 37.01, 45.38, 64.43 and 78.62° corresponding to the (111), (200), (220) and (311) reflections, respectively, which indicated that spherical silver nanoparticles are crystalline in nature with face-centered cubic structure (fcc)[29,30]. The crystallite size was calculated using the Scherer's formula that depends on the peak position and FWHM of the dominant reflection. Scherer's equation is $d = k \lambda / \beta \cos \theta$ where, d is the average crystallite size of the nanoparticles, k is the geometric factor equal to 0.9, λ is the wavelength of X-ray radiation source equal to 1.54Å,, β is the angular FWHM (full-width at half maximum) of the XRD peak at the diffraction angle θ[31]. The calculated average crystallite of the AgNPs is 23 nm.

Antioxidant activities

Based on the obtained results, mixed leaves extract showed high total antioxidant activity (Figure 4B), this may be due to their richness by various phytoconstituents from both studied plant species. The antioxidant activities using free radical scavenging the DPPH radical, the total antioxidant activity and the Ferric Antioxidant Reducing Power mehtods showed that the AgNPs preserve an important amount of

antioxidant activity (Figure 4) which may be due to the stability of the antioxidant activity in nanomaterials.

Phytochemical characterization of plant extract AE and AgNPs

Results showed that the synthesized nanoparticles were more richer in tannins, total polyphenols and flavonoids compared to the mixed leaves aqueous extracts, (Table 1), the observed data can explain the enhancement of the antimicrobial properties of the AgNPs compared to the plant extract one.

Table 1
Phytochemical constituent of mixed aqueous extract of *Olea europaea* var. *Sylvestris* and *Pistacia lentiscus* leaves and synthesized AgNPs

Phytoconstituents	**Screening**	
	Plant extract	AgNPs
Tannins content (mg TAE/mL)	5b	11.6a
Total Polyphenols content (mg GAE/mL)	1.35c	1.48c
Flavonoids content (mg QE/ml)	0.54d	0.55d
Alkaloids content (mg/ml)	0.93cd	1.58c
TAE: tannic acid equivalent; GAE: gallic acid equivalent; QE: quercetin equivalents		

Antibacterial and anti-candida screening

The synthesized silver nanoparticles (NP) showed an antibacterial activity against all tested gram positive and gram negative clinical bacteria strains, unlike the aqueous extract which was unable to limit the growth of used bacteria strains. The observed results indicate that the increase of zone inhibition expressed in mm given by the silver nanoparticles compared to $AgNO_3$ and the development of the sensibility of strain *Escherichia coli* which was resistant to Ag NO_3 (Figure 5A)

The present results mentioned that the synthesized AgNPs (NP) gave more inhibitory activity against *Candida* species compared to Ag NO_3. The aqueous plant extract was unable to limit any Candida species. Our synthesized nanoparticles were more effective than the fungicide amphotericin B with 19.66 and 17.33mm compared to 19mm and 10mm against Candida albicans and *Candida parapsilosis*, respectively by the AgNPs and Amphotericin B (Figure 5B.) MIC values ranged from 31 to 500 µg/mL related to the tested pathogens strains. Based on calculated value of MBC/MIC ≤ 4, AgNPs showed bactericide and fungicide effects against all tested pathogens strains. In the present work, we have investigated the silver nanoparticles effect on filamentous fungi genus *Penicillium* and *Aspergillus*, and the finding show that only the synthesized silver nanoparticles can affect mycelium growth compared to

the plant extract or Ag NO3. Superior inhibitory activity was given against spores germination of *Penicillium* with 98.4%.

Anti-biofilm activity

The investigation of the synthesized AgNPs effect on biofilm formation elucidate that AgNPs used at the corresponding MIC value were qualified to limit biofilm formation of all tested gram positive and gram negative bacteria with value ranging from 32.74 to 83%, but they were more efficient on biofilm of *Candida* albicans strains with (87%) and *Candida parapsilosis* with 63% (Table 2).

Table 2
Biofilm inhibition and eradication properties of silver nanoparticles

Microorganisms pathogens	Biofilm inhibition (%)
Klebsiella pneumoniae	56.75±0.29b
Enterobacter cloacae	76.64±1.33a
Escherchia coli	73.64±6.39a
Staphylococcus aureus	83.00±1.41a
Micrococcus luteus	32.74±1.75c
Candida albicans	87d
Candida parapsilosis	63d

Antibiotic synergistic effect of AgNPs

According to the observed results, the synthesized nanoparticles booster the effect of the tested conventional antibiotic currently used or make bacteria strains more sensitive to used antibiotic. For example, both bacteria strains were sensible to NA but they were sensitive when it was associated with AgNP, and TM against *Micrococcus* (Figure 6.)

Silver nanoparticles on virulence factor of Candida strains

In order to illustrate the ability of the silver nanoparticles on factor virulence of yeast species, here we reported the effect of AgNPs on hydrolytic enzymes production and yeast morphogenesis. As a result Table 3, mentioned that, the enzymes hydrolase proteinase and phospholipase were higly reduced in Candida species growth additioned with silver nanoparticles. Morover, Figure 7, illustrated that in vitro co-culture model of *Candida* strains, confirmed the superior inhibitory effect of the AgNPs used at MIC of 250µg/mL, on *Candida albicans* morphogenesis compared to untreated *Candida albicans* culture, the observation indicate the absence of any morphogenesis key virulence change for biofilm resistance such as: germ tube, chlamydospore and filamentous hyphae, as well as the alteration of the scare Blastospore

in the presence of AgNPs. For both *Candida* species, the results indicate that the addition of AgNPs in co-cultural model, affect hardly the number of Blastospore observed (so affect the cell multiplication phenomena).

Table 3
Comparison between the virulence factors expressed in *Candida* species in the absence and the presenc of AgNPs after 48h of incubation with or without AgNPs.

Key virulence	**Candida albicans**		**Candida parapsilosis**	
	Untreated strains	AgNPs	Untreated	AgNPs
Hydrolytic enzymes Proteinase (Pz in mm)	0.569	0.78	0.38	0.68
Phospholipase (Pz in mm)	0.68	0.88	0.63	0.887
Biofilm Morphogensis	+++	-	++	-
Pz= 1(negative); Pz (0.9-0.99:+); Pz: (0.8-0.89 (++); Pz: (<0.7:++++)				
Biofilm morphogenis change (+++) ; absence of any morphogensis change (-)				

Discussion

It is well known that the green synthesis of nanoparticles materials has attracted the attention of many studies due to their advancement over other methods as single step process, cost effective and emerged as ecofriendly environment [4].This report demonstrated for the first time the biosynthesis of AgNPs using aqueous extract from the equal mixture of *Olea europaea* subsp. *europaea* var. *sylvestris* and *Pistacia lentiscus* leaves. Here we proved that firstly, two *Olea europaea* subsp. *europaea* var. *sylvestris* and *Pistacia lentiscus* natural associated species can be used as natural sources to synthesize silver nanoparticles by enhancing their antimicrobial potential compared to other AgNPs synthesized from individually plant as reported previously in literature. For example as reported by the *P lentiscus* leaf extracts which possess moderate antimicrobial activities and scare works focused on the biosynthesis of silver nanomaterial from this specie [32].

Secondly, here the AgNPs synthesis were completely free from any chemical solvents and hazardous reagents similar to the green synthesis described previously [33], based on the use of two cultivars (Leccino and Carolea) of *Olea europaea* growing in the same pedoclimatic conditions for green biosynthesis of silver nanoparticles with new properties as antibacterial potential against, and were able to induce toxicity in breast cancer cell lines. In adition, here the mixed leaves extract enhance the

biosynthesis of AgNPs, on one hand, the size of silver nanoparticles of 23nm, were smaller than those obtained only by Pistachio leaves extract as reported by [34]. show that the Ag NPs from Pistachia ethanol extract ranged from 24 to 26nm. On the other hand, the mixed leaves from both plant species enhance the antibacterial potential as compared to those obtained by silver nanoparticles from Pistachio which not exceed 13mm of zone inhibition agaisnt *Escherchia coli* and *Staphylococcus aureus* [34].

Face to the emergence of multidrug resistant bacteria and biofilms producer's strains to currently used antibiotics, AgNPs may play a crucial role compared to the conventional antibiotics by action of multiple antagonism mechanism. In this work, we revealed a significant antibacterial and antifungal efficacy of AgNPs against all tested clinical strains. This finding may be attributed to its richness with secondary metabolites from both natural associated plant species. On the other hand, Olive leaves are polyphenol rich compounds that are known to have antioxidant, antimicrobial, and anti-inflammatory activities. In literature the anti-inflammatory and antibacterial effect against gram positive bacteria is due to the Olive component Oleuropein [35]. Furthermore, here the enhancement of AgNPs from the mixed leaves extract observed by the superior antibacterial activity by exhibiting maximum antibacterial effect against both gram negative and gram positive bacteria.

In addition, the results mentioned that the susceptibility of Gram positive and Gram negative bacteria to biosynthesized AgNPs was found to vary from study to other, related to the pathogen strains tested [36] and the concentration of the inoculum or solvent used [37]. For example, here the zone inhibition given by the AgNPs against *Staphylococcus aureus* and *Escherchia coli* were 18.66mm and15.33 mm respectively, these diameters were superior to those obtained by the AgNPs from *Pistachia lenticus* with 13mm and 13mm respectively against the same bacteria strains, these data could be due to the maximum richness of the mixed metabolites from both plant leaves [38]. Few works aimed the antifungal potential of AgNPs, here we successfully describe the high antifungal potential of our AgNPs against *Candida* species and *Penicillium*. The observed results on filamentous fungi confirmed that the silver nanoparticles enhanced the antifungal behavior against mycelium and spores [39]. Numerous works, described the antifungal potential of Olive plant against filamentous fungi like *Rhizopus*, *Fusarium* and *Alternaria* as reported by [40]. These funding mentioned that the antifungal behavior of the biosynthesized silver nanoparticles from mixed extracts could be due to the olive composition, which was able to affect the spores and the mycelium fungi growth. In literature, the major active components in Olive leaf are known to be Oleuropein and its derivatives as owing superior antifungal potential against fungi [41]. It is essential to note that, the new AgNPs exhibited novel bactericidal and fungicidal potential which may be highly relevant in infections caused by filamentous fungi and MDR bacteria strains. Consequently, the broad spectrum killing caused by AgNPs, have encouraged their use as antimicrobial drugs including multidrug strains MDR [2, 42].

Numerous studies have shown that nanoparticles generally improved the pharmaceutical characteristics of antifungals, as lower toxicity and enhancing antifungal potential, and the possibility of prolonged action [40]. The reported data show that the presence of AgNPs in the Candida growth can limit the virulence factor as enzyme production and biofilm formation.

In literature, several mechanisms have been reported for antimicrobial activity of AgNPs such as disruption of the bacterial cell membrane, interference in the respiratory electron transport chain formation of reactive oxygen species (ROS) [43, 44]. Nanoparticles exhibited new or improved properties depending upon their size and morphology. The pathogenicity of *Candida* species was attributed to the factor virulence such as enzyme hydrolases to invade host cells and biofilm formation to adhere to solid surface. The present work was the first to highlight the effect of silver nanoparticles from mixed leaves from Olive and Pistachia on Candida key virulence factors by means of enzyme production (proteinase, phospholipase) and morphogenesis reduction. Our results illustrated that the addition of silver nanoparticles can reduce the enzyme production and the germ tube and filamentous hypheae. Recently scare works, reported the reduction of enzyme and biofilm by Candida albicans by the addition of green silver nanoparticles. However, the exact mechanism of action on biofilm by silver nanoparticles is not known. In addition, the inhibition of yeast morphogenesis; like germ tube and filamentous hyphae lead to the suppression of biofilm formation in *Candida* strains [45, 46].

Furthermore, a combination of conventional antifungals with natural compounds can also minimize the toxicity of these drugs by reducing the dose request. Therefore, the focus of this study was to explore the new AgNPs by enhancing the antibacterial potential of conventional antibiotic drugs tested on clinical strains. Here the association of silver nanoparticles ameliorate the action of conventional antibiotic or make resistant bacteria more sensible against it. After demonstrating the simple method of synthesis, the antioxidant potential of AgNPs by testing DPPH radical scavenging, Ferric Antioxidant Reducing Power (FRAP) as well as the total antioxidant activity was determined, thus the combination of the antioxidant effects and the antibacterial and antifungal activities encourage the use of the green biosynthesized AgNPs in pharmaceutical field.

In conclusion to our knowledge, this is the first study evaluating the antioxidant and antimicrobial effects of silver nanoparticles biosynthesized from *Olea europaea* subsp. *europaea* var. *sylvestris* and *Pistacia lentiscus* leaves. The synthetized AgNPs is rich in secondary metabolites and has an antioxidant activity. The reported AgNPs exhibited markedly bactericidal and fungicidal effects against clinical pathogen strains. The synergistic interaction with the conventional antibiotic as well as the effect on bacteria biofilm and the spores of filamentous encouraged their formulation in pharmaceutical and medical purposes.

Declarations

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Competing interests

The authors declare no conflict of interests

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Authors' contributions

Essghaier Badiaa: Microbial Methodology, Data curation, writing original draft , **Rihab Dridi , Hannachi Hedia**, **Ben khedher Ghada**: methodology, validation, **Med Faouzi Zid, Chaffei Chiraz**: Supervision.

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Figures

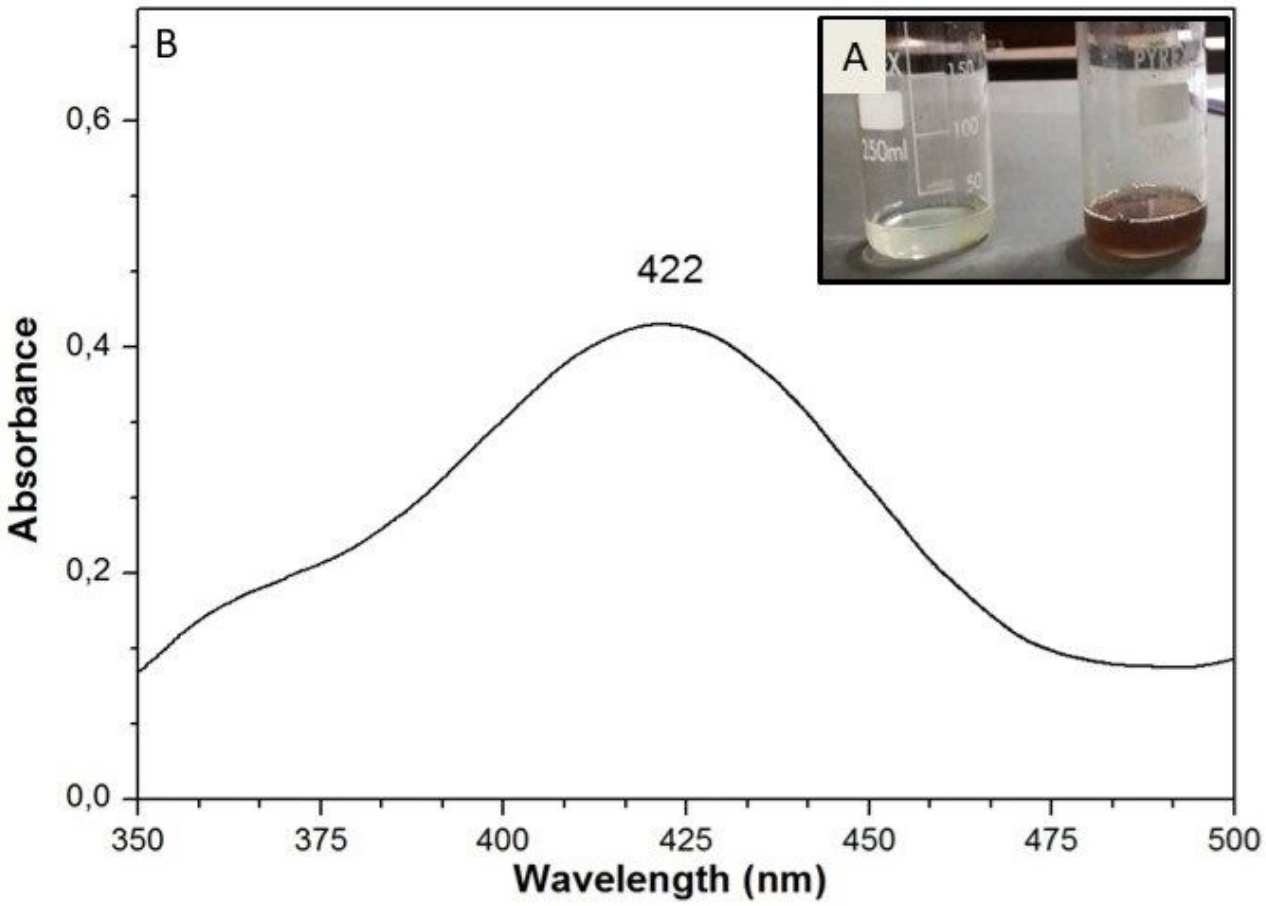

Figure 1

A Plant extract and synthesized AgNPs, B: UV–vis spectrum of synthesized AgNPs.

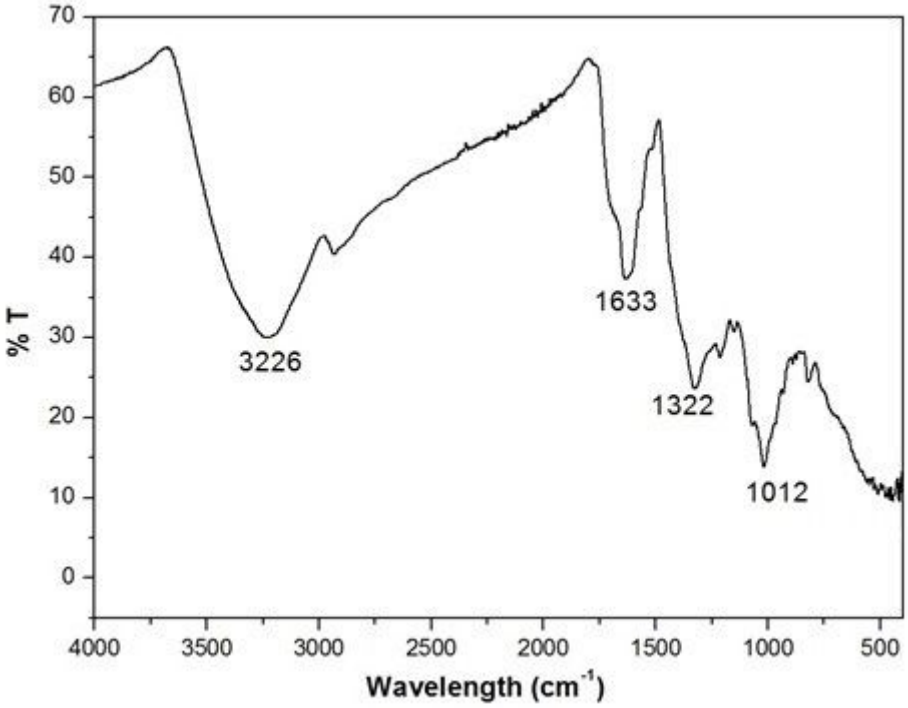

Figure 2

FTIR spectrum of AgNPs

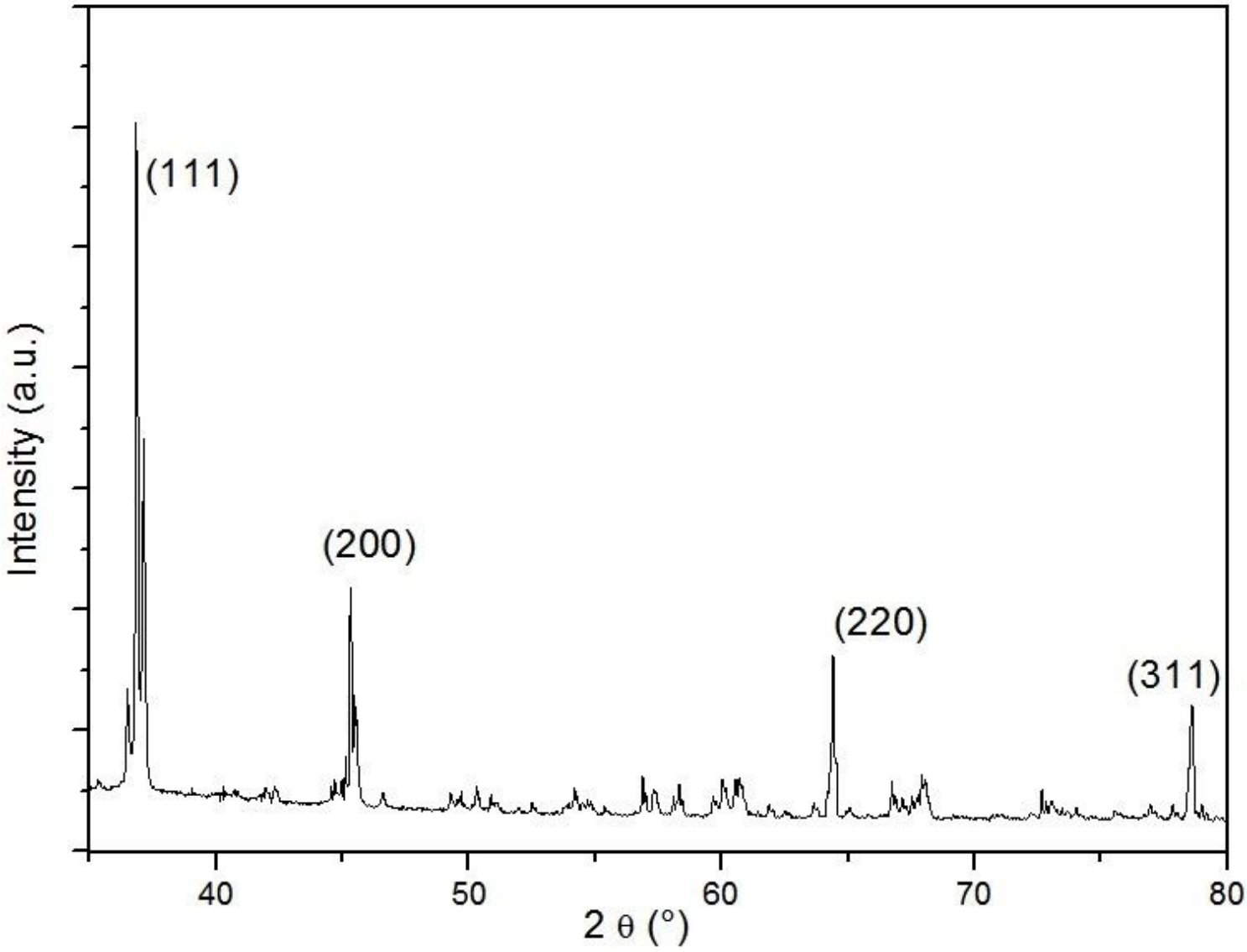

Figure 3

X-ray diffraction (XRD) pattern of synthetized AgNPs

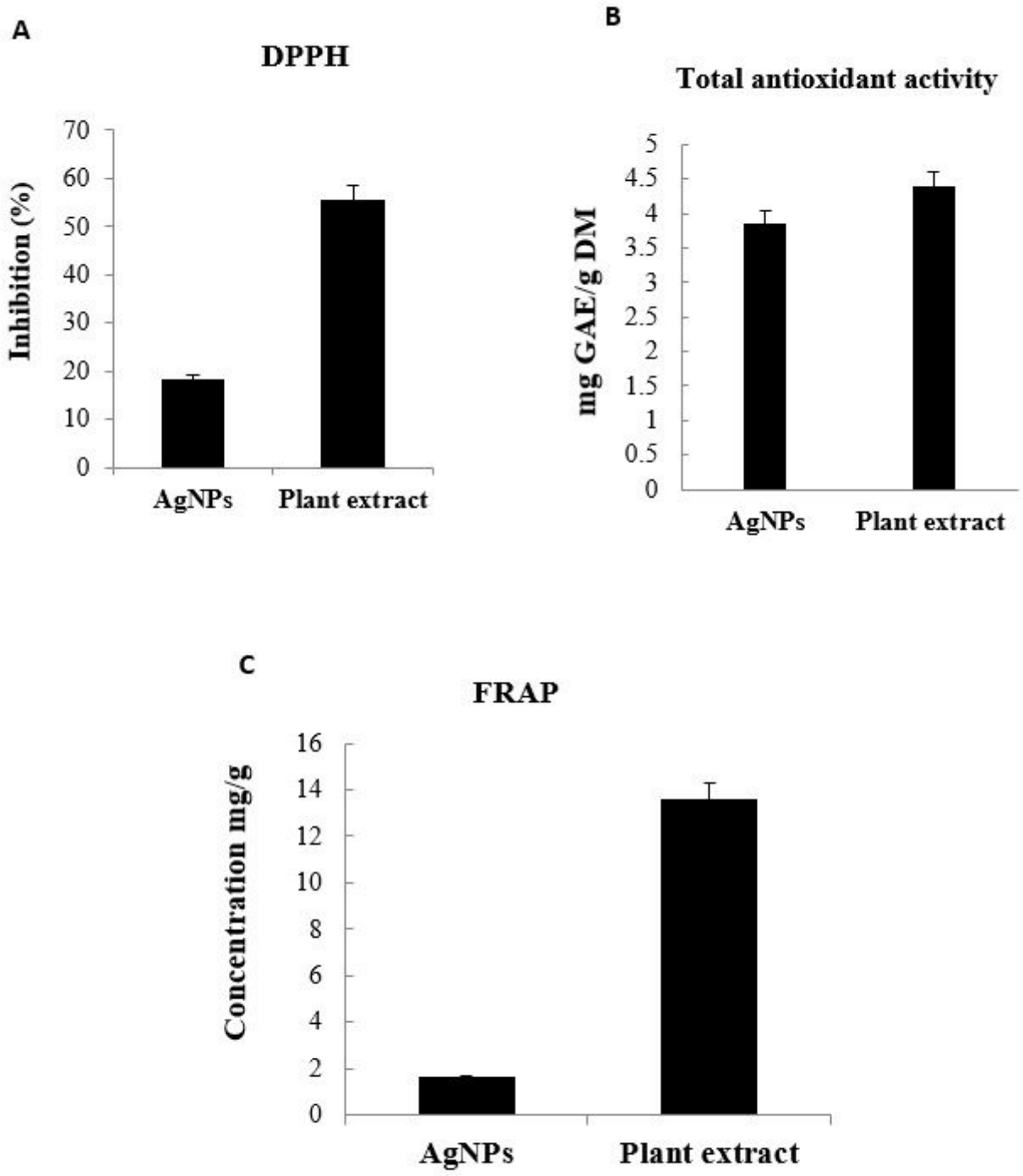

Figure 4

Antioxidant activity of plant extract and silver nanoparticles by Free radical scavenging activity (A), the total antioxidant activity (B) and the Ferric Antioxidant Reducing Power (FRAP) (C).

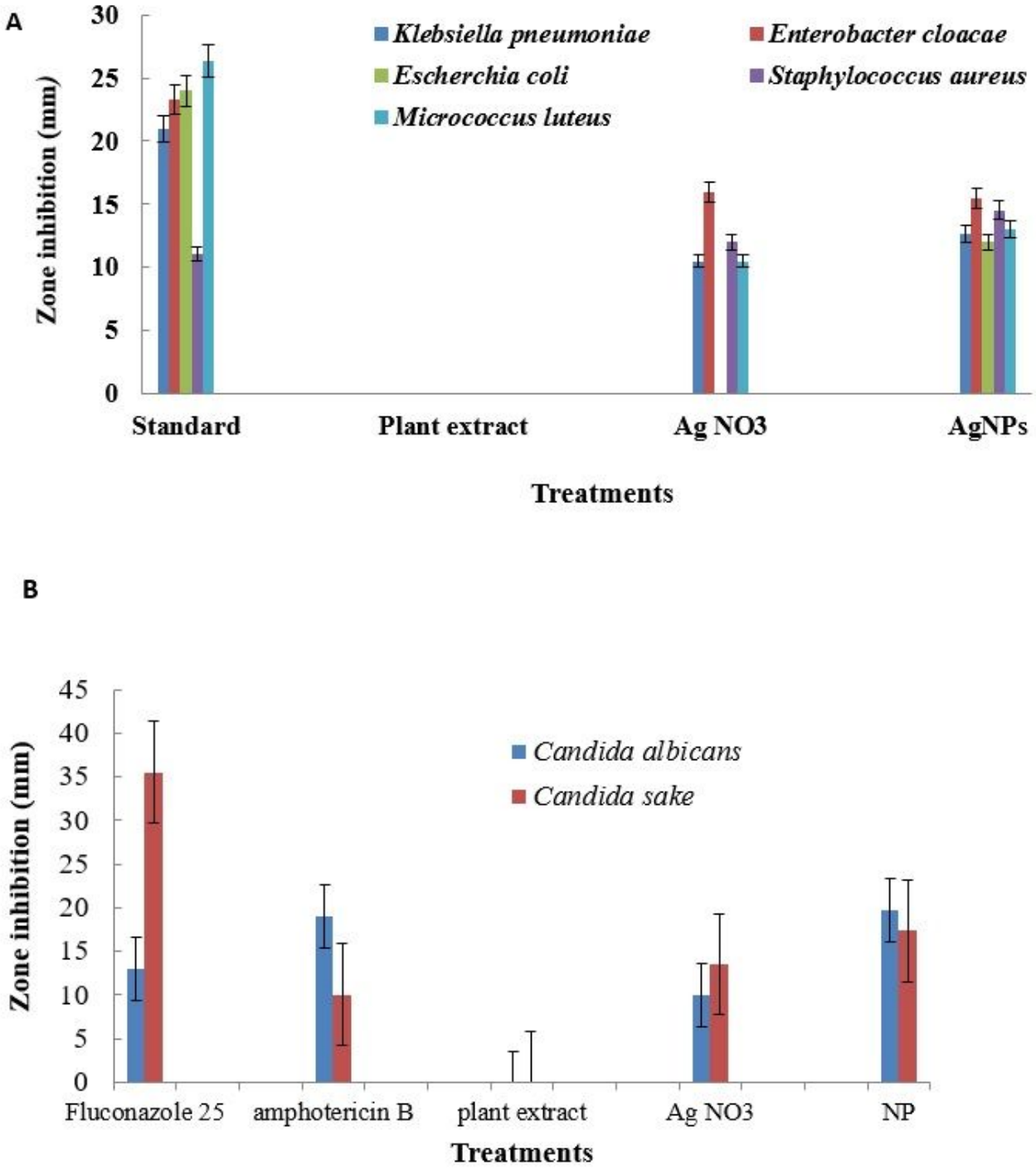

Figure 5

A. Antibacterial activity of silver nanoparticles (NP) against clinical bacteria strains compared to antibiotic standard, AgNO3 and Olea europaea var. Sylvestris and Pistacia lentiscus leaves aqueous extract. B: Silver nanoparticles effects, on Candida species compared to fungicides (Fluconazole 25 and amphotericin B) and plant extracts. Values expressed diameters in mm. Bars represent Standard errors.

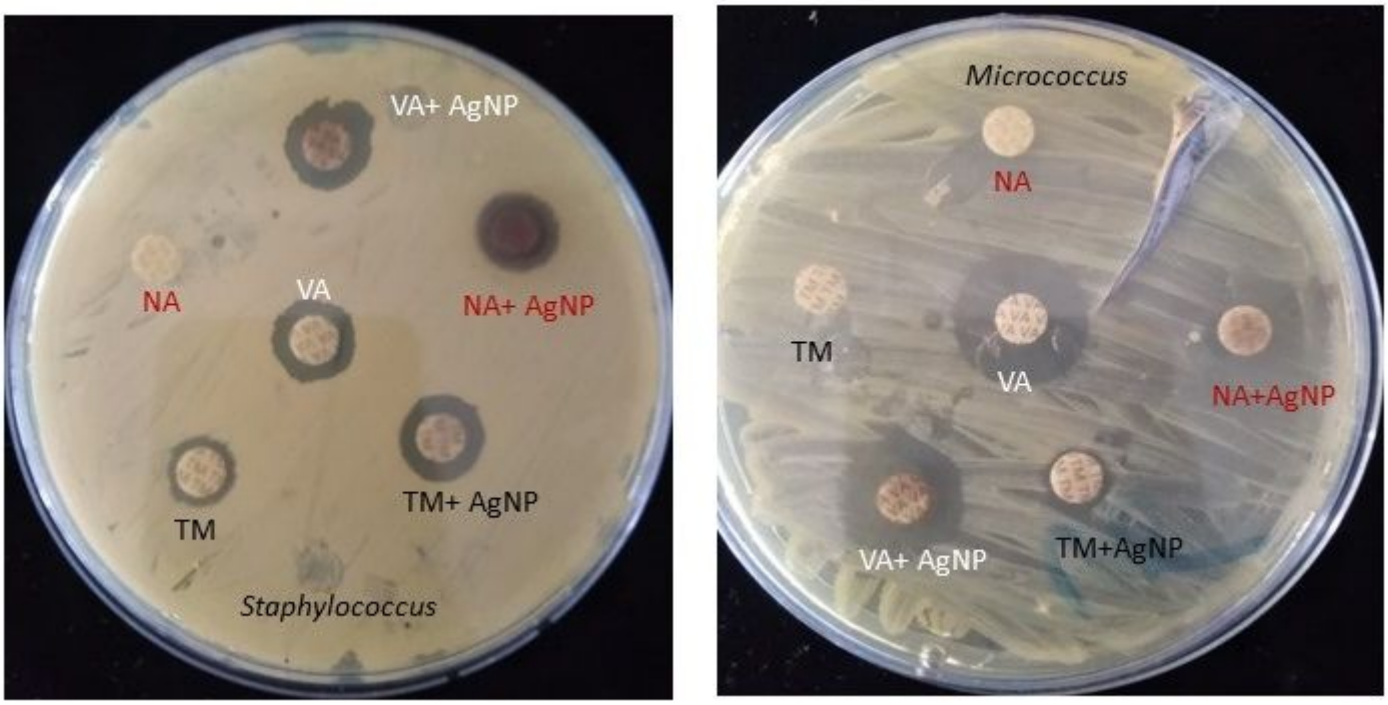

Figure 6

Assessment of the individual and synergistic effects of the synthesized silver nanoparticles (AgNPs) and the conventional antibiotics: acide nalidixique NA, tobramycine ™ and vancomycine VA

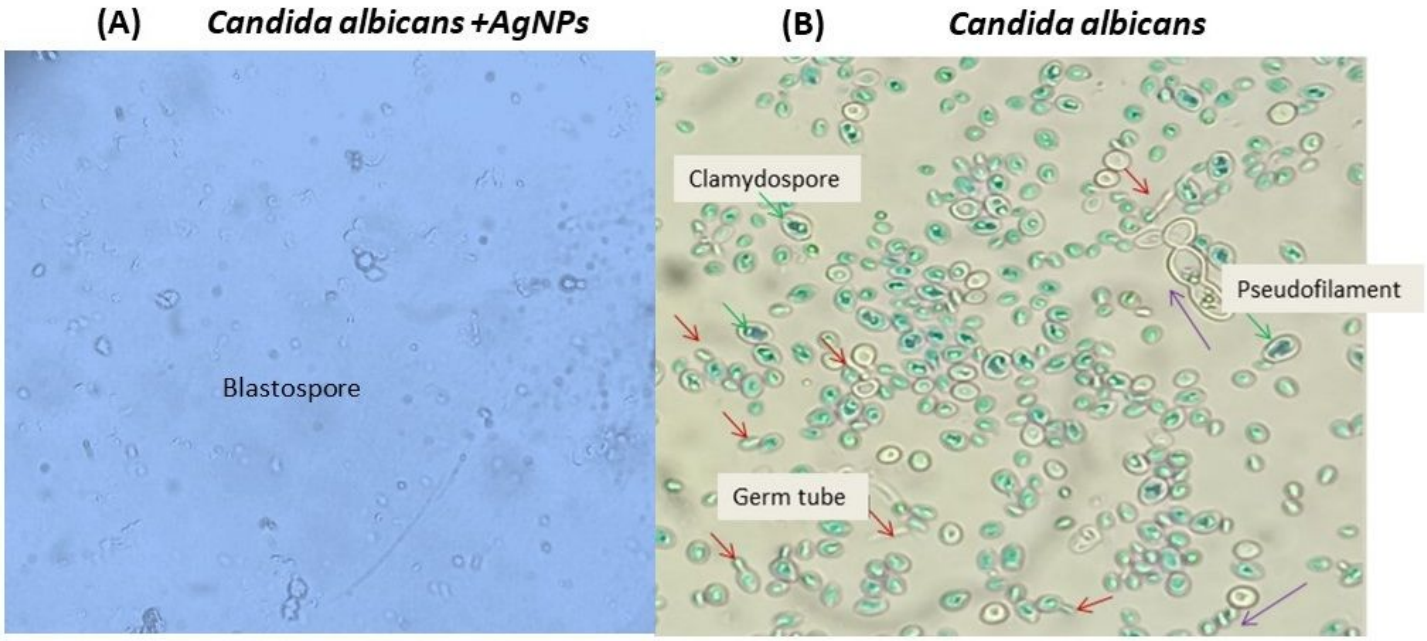

Figure 7

Microscopic observation of Candida albicans growth culture of 48h, after coloration with bleu cotton of (A): Silver nanoparticles effect's on Candida albicans morphogenesis compared to untreated Candida albicans growth without the addition of AgNPs (B). (gr X100).