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Original Research Article

Antibacterial synergy of *Tritirachium oryzae*-produced silver nanoparticles with different antibiotics and essential oils derived from *Cupressus sempervirens* and *Asteriscus graveolens* (Forssk)

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Abstract

Purpose: *To carry out eco-friendly biosynthesis of fungi-derived silver nanoparticles (AgNPs) and investigate their antibacterial synergies with essential oils (EOs) of Asteriscus graveolens (Forssk.) Less. and Cupressus sempervirens.*

Methods: *Biosynthesis of AgNPs was carried out using a cell-free filtrate of Tritirachium oryzae. The biosynthesized AgNPs characteristics were assessed using different methods, including ultraviolet-visible spectrophotometry (UV), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) with energy-dispersive x-ray spectroscopy (EDS) and transmission electron microscopy (TEM).*

Results: *Obvious synergistic effects were observed between AgNPs and chloramphenicol, vancomycin, nitrofurantoin or tetracycline with Pseudomonas aeruginosa, through increases in fold area of inhibition (IFAs) within the range of 2.4 to 9.0. Synergistic interactions were also seen between AgNPs and the antibiotics used, depending on the strain. Increase in IFA ranged from 1- to 3-fold for S. aureus, E. coli and P. aeruginosa. Similarly, combinations of AgNPs, EO of A. graveolens and cefotaxime, nitrofurantoin or amoxicillin against P. aeruginosa led to 10-, 3- and 10-fold synergy, respectively. In contrast, the use of AgNPs and trimethoprim, tetracycline or amoxicillin against E. coli led to 1 to 6-fold synergy. The best synergistic capacity resulted from AgNPs and the EO of C. sempervirens and trimethoprim against S. epidermidis, which yielded 29-fold increase in IFA. The use of combination of AgNPs and vancomycin against P. aeruginosa led to 16.4-fold enhancement of IFA.*

Conclusion: *The findings can potentially lead to the development of a new perception of antibacterial agents (innovative medications) involving the incorporation of nanoparticles (NPs) or new materials that potentially synergize with antibiotics, NPs and the EOs of different plants.*

Keywords: *Antibacterial activity, Silver nanoparticles, T. oryzae, Synergistic effect, Essential oil, Asteriscus graveolens, Cupressus sempervirens*

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INTRODUCTION

Nanotechnology refers to the synthesis of nanoparticles and their employment in different areas such as chemistry, physics, biology, and medicine [1,2]. The use of microorganisms for nanoparticles biosynthesis is a simple and applicable tool for more complex chemical synthetic procedures [3]. There is a significant global evolution in multidrug resistance to bacteria due to increased use of antibiotics [4]. These bacteria, along with other types of microorganisms, have developed antibiotic resistance which is a global medical challenge [5]. To manage the problem of multidrug resistance, new and different antimicrobials are required [6]. In this context, the combination of silver nanoparticles (AgNPs) with various antibiotics and EOs may lead to effective synergy against bacteria, resulting in evolution of new methods of treatment.

Asteriscus graveolens (*A. graveolens*) is a Middle Eastern medicinal plant that grows in extreme desert environments. Its major EO components have been identified as *cis*-chrysanthenyl acetate, myrtenyl acetate and kessane [7]. Recent studies reported significant antifungal and anticancer effects of the EOs of *A. graveolens*. In addition, it has been reported that the EOs of *A. graveolens* exerted various degrees of antibacterial activity depending on the bacterial strain [8]. The species *C. sempervirens*, one of the rarer species of the genus *Cupressus*, grows in Jordan. Some studies have investigated the antimicrobial effects of the genus *Cupressus,* and found that α-pinene is a major bioactive component of its oils [9,10].

Combinations of NPs and antibiotics minimize the bioactive agents in the dosage, thereby lowering their noxiousness and boosting their potential antimicrobial effects [11]. This study was aimed at investigating the antibacterial effect of AgNPs when used singly and synergistically with some antibiotics or EOs extracted from leaves of *A. graveolens* and *C. sempervirens*. The study is the first to report the antibacterial effects produced by combination of AgNPs and essential oils of plants and antibiotics.

EXPERIMENTAL

Tritirachium oryzae W5H

The fungal isolate *Tritirachium oryzae* W5H employed in this study was recently identified [12]. The fungal species was identified by the use of ITS sequencing (GENWIZ, USA). The similarity of sequence with the database of NCBI was investigated and its accession no. (MK028996) was obtained and registered in NCBI database.

Preparation of silver nanoparticles

The procedure of Jaidev and Narasimha was used for AgNPs biosynthesis [13], but with some modifications in the liquid medium composition. The fungal isolate of *Tritirachium oryzae* W5H was cultured under aerobic conditions in a 100 mL of liquid media with the following chemical composition (w/v): 1.0% glucose, 1.0% yeast extract, and 0.5% NaCl. Fungal spores (2.0×10^6) were seeded in the liquid medium and incubated at 33^oC on an orbital shaker at a speed of 150 rpm. After incubation for 72 h, the fungal biomass was filtered out through Whatmann No.1 filter paper, and then washed severally with distilled water. The wet biomass (10 g) was added to 100 ml of deionized water in a glass flask and incubated for 72 h at 33°C and 150 rpm. Thereafter, the filtrate of fungal biomass was obtained by filtrating the aqueous suspension of fungal biomass through Whatmann No.1 filter paper. The AgNPs were biosynthesized by adding $AgNO_3$ to 100 ml fungal filtrate to a final concentration of 1 mM. This was incubated in the dark at 30°C and 150 rpm for 72 h. Another 100-ml fungal filtrate devoid of $AgNO_3$ was used as a control and was incubated under the same conditions. The reaction solution was sampled after 72 h incubation for AgNP characterization using UV/VIS spectroscopy.

Characterization of AgNPs

The formation of AgNPs in the reaction solution was indicated through change in the absorbance in a UV-visible spectrophotometer (SPUV-19, Sco-TECH, Germany). The image of TEM was taken using a Morgagni (Philips, Netherlands) 268 FEI electron microscope linked to Mega ViewG2 Olympus Soft Imaging Solutions, at an accelerating voltage of 40 kV. The grids of the TEM were composed by drop-casting 10 µL of the pure AgNPs distributed on Formvar coated copper TEM grids (300 mesh, Ted Pella Inc., Redding, CA) and allowing them dry aerobically. The morphology of the silver nanoparticles was studied using a SEM FEI Quanta 600 (scanning electron microscope) equipped with energy dispersive X-ray (EDX) facility for analysis of elements.

FT-IR analysis

Prior to analysis of the biosynthesized AgNPs powder with Fourier transform infrared (FTIR)

(IRAffinity-1, Shimadzu Corporation, Tokyo, Japan), the AgNPs were subjected to purification. They were centrifuged 3 times and re-distilled in sterile distilled water in order to ensure good separation of free entities from the NPs. The interaction of the biosynthesized AgNPs with proteins in dried samples was analyzed with FTIR spectroscopy using the pellet technique of potassium bromide (KBr) in diffused reflection at a resolution of 4 cm^{-1}. The AgNPs powder were mixed with KBr prior to exposing to infrared source at 400–4000 cm^{-1} [1].

Plant material

The aerial parts of *C. sempervirens* and *A. graveolens* (Forssk) were collected between May and June in 2017 from Dhana Natural Reserve (DNR), Jordan. The plants were kindly identified by Prof. Dr. Salih Al-Quraan, Biology Department, College of Science, University of Mutah, Jordan. Voucher specimens of *C. sempervirens* (NO MU2018-5620) and of *A. graveolens* (Forssk) (NO MU2018-5621) were deposited in the Department of Biology, Faculty of Science, Mutah University, Jordan. The fresh plant materials were dried at room temperature in the shade and then crushed into fine powder.

Extraction of essential oils

Fifty grams each, of the dried and powdered aerial parts of *C. sempervirens* and *A. graveolens* (Forssk) was hydro-distilled for 3 h, employing simple Clevenger apparatus. Diethyl ether was used to extract oil from the aqueous phase, and the oil was dried over anhydrous sodium sulfate (Na_2SO_4). The oils obtained was kept at 4˚C prior to use.

Evaluation of antibacterial activity

Bacterial strains

In this study, five bacterial species were used: Gram-positive, beta-lactamase (BL)-producing clinical isolates of *S. aureus* and *S. epidermidis*, and 3 genera of gram-negative bacterial strains i.e. clinical isolate *P. aeruginosa,* an Extended Spectrum Beta-Lactamase producer (ESBL), *E. aerogenes* ATCC 13048, and *E. coli* ATCC 25922. The clinical bacterial isolates were obtained from Al Bashir Hospital (Amman - Jordan). *Staphylococcus aureus* (BL) and *S. epidermidis* (opportunistic dermatitis) were isolated from dermatitis patients, while *P. aeruginosa* (ESBL) was obtained from a patient with burn inflammation. The identities of all isolates were confirmed using BIOMÉRIEUX VITEK® 2.

Preparation of bacterial suspension

Bacterial culture media were produced according to the procedure of Laboratory Standards Institute (CLSI M7-A7, 2012). Single bacterial colony was cultured overnight in sterilized 5-ml nutrient broth (NB) at 37°C. The bacterial growth was adjusted to 0.5 McFarland Standard (final absorbance of 0.1 at 620 nm) using sterile NB broth.

***Antibacterial effect of EOs of C. sempervirens and A. graveolens* Forssk**

A stock solution of 100 mg/mL of each EO was prepared in DMSO. In the well diffusion method, 100 µl of each EO-DMSO solution was put in a well (10 mg per well), while in the disc diffusion procedure, 5 µl of original EO was applied to each disc (5 µg/disc). In MIC assays, serial dilutions were prepared from a stock solution of 100 mg/mL to obtain 3300, 1100, 370, 123, 40.7, 13.6, 4.53 and 1.5 µg/mL. After 24 h, the zone of inhibition of each sample was compared to that of each EO tested separately, so as to determine any synergistic antibacterial action between the EOs and the tested antibiotics.

Assessment of antibacterial effect of AgNPs

In the well diffusion method, 100 µl of each solution (EO-DMSO mixture) was put in each well (10 mg per well). For disc diffusion procedure, 5 µl of original EO was loaded to each disc (5 µg/disc). In MIC assay, serial three-fold dilutions of 100-µL AgNPs solution (172.6 µg) was made using 200-µL sterilized broth (NB) to get solutions containing 57.46, 19.15, 6.38, 2.13, 0.71, 0.24, 0.08 and 0.03 µg/mL.

Determination of synergy between AgNPs and EO

In the synergy experiments using well diffusion method, 50 µl of AgNPs and EO-DMSO solutions were mixed together to obtain 86.28 µg/mL and 5 mg/mL, respectively. For synergy experiments using disc diffusion, the ready-made antibiotics discs used were: ampicillin (10 mcg), amikacin (30 mcg), cefotaxime (30 mcg), vancomycin (30 mcg), nitrofurantoin (300 mcg), gentamicin (10 mcg), ciprofloxacin (5 mcg), trimethoprim/ sulphamethoxazole (1.25 mcg/23.75 mcg), tetracycline (30 mcg), imipenem (10 mcg), chloramphenicol (30 mcg) and amoxicillin (20/10 mcg). In experiments on coupled synergistic effect, 10 µl of either EO or AgNPs was used, while for studies on synergy of combination of three substances, each tested antibiotic disc, 5 µl of undiluted EO (5 µg per disc) and/or 5 µl of

AgNPs solution (8.63µg/disc) were used jointly [14]. The synergistic effect was estimated in terms of increase in fold area (IFA) as in Eq 1.

$IFA = B^2\text{-}A^2/A^2$ ..... (1)

where *A* and *B* are the zones of inhibition (mm) produced by only antibiotic or combination of antibiotic with AgNPs or antibiotics with either EO or combination of antibiotic, AgNPs and EO. When the inhibition value was zero, then the diameter of the disc was considered 6 mm for calculation purposes [15].

Determination of minimum inhibitory concentration (MIC)

Minimum inhibition concentration (MIC) values were determined using 96-well microtiter plates. Three-fold consecutive dilution was carried out. Suspensions of standard microorganisms adjusted to 0.5 McFarland standard (approximately 10^8 CFU/mL for bacteria), were inoculated on the microplates. The growth of the microorganisms was monitored through sub-culturing of each well content on nutrient agar. The MIC values are the minimal concentrations of the essential oil required to inhibit the growth of microorganisms [16].

Statistical analysis

All experiments were carried out in triplicate except the antibiotics disc assays which were done in duplicate. Student's *t*-test was used for statistical analysis. All analyses were done with Microsoft Excel 2010 software. Values of $p \leq 0.05$ were taken as indicative of statistical significance of differences.

RESULTS

Silver nanoparticles

In this study, *Tritirachium oryzae* (accession no. MK028996) was used for the preparation of an aqueous extract and for the bio-reduction of silver ions into AgNPs. Silver nanoparticle formation from *Tritirachium oryzae* was assessed by monitoring the peak absorbance at 425 nm in the ultraviolet-visible spectrum (Figure 1 A). The colour of the mixture of culture filtrate and silver nitrate changed to intense brown after 72 h of incubation (Figure 1B), while silver nitrate-free solution (control) did not display any change in colour. The infrared spectra of the AgNPs biosynthesized from *Tritirachium oryzae* contained bands at 3292.6 and 2994.23 cm^{-1} which were due to the stretching vibrations of primary and secondary amines, respectively (Figure 2). Their corresponding bending vibrations were observed at 1649.19 and 1535 $cm^{-}1$, respectively. The two bands observed at 1388.79 and 1060.88 $cm^{-}1$ could be due to the extended vibrations of the C-N bonds in aromatic and aliphatic amines, respectively.

To check for the presence of silver ions in the solution, SEM-EDS analysis was performed. The micrograph and the peak of optical absorption for EDS (Figures 3 A and 3B), which were seen at approximately 3 keV, indicated the presence of AgNPs in the reaction medium of *T. oryzae* W5H. The AgNPs were consistent in their native shapes. Further characterization of the AgNPs was performed with TEM micrographs of the nanoparticles created using 1 mM AgNO3 (Figure 4). The NPs were polydisperse, and showed approximately spherical shape. The size of the AgNPs was determined by counting an average of 50 cells on a sample grid. The results showed that the sizes of the NPs were in the range of 7–75 nm.

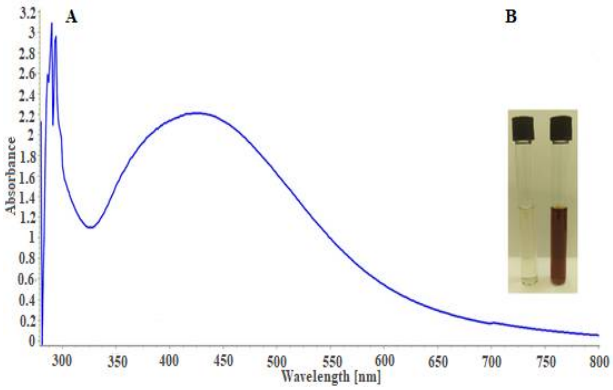

Figure 1: Solution of 1 mM $AgNO_3$ before and after bioreduction by *Tritirachium oryzae* W5H at 33 °C (a) Ultraviolet-visible spectra and (b) an inset represent color change

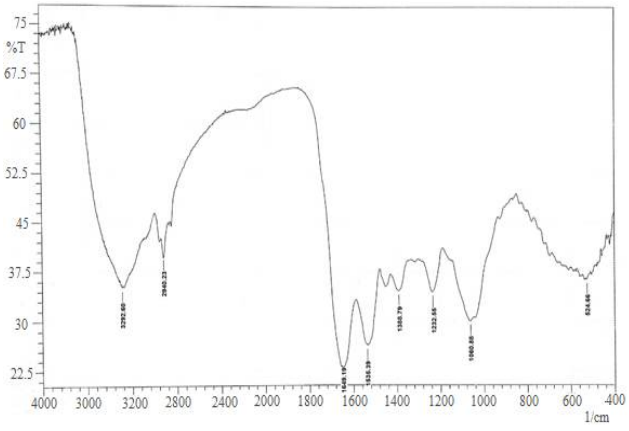

Figure 2: FT-IR spectra of biologically synthesized silver nanoparticles using *Tritirachium oryzae* W5H

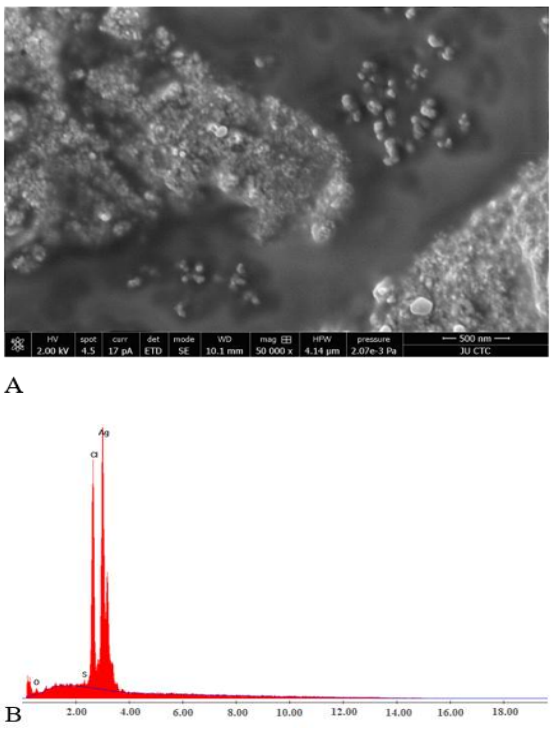

Figure 3: Representative scanning electron microscopy (SEM) micrograph of (A) Biosynthesized AgNPs (B) Energy dispersive spectrometer (EDS) spectrum of AgNPs synthesized by *Tritirachium oryzae* W5H

Antibacterial effect of AgNPs and of EOs

Initially, the antibacterial effects of the AgNPs were determined using the well diffusion method which is a more reliable procedure than the disc diffusion method. The diameters of the inhibition zones produced by AgNPs against various pathogenic bacteria were determined using well diffusion method (Table 1). The AgNPs inhibited *S. epidermidis*, *E. aerogenes, E. coli* and *S. aureus,* with zones of inhibition ranging from 16 to 20.3 mm, while the inhibition of *Pseudomonas aeruginosa* was intermediate (12 mm). Combination of AgNPs and *A. graveolens* EO produced reciprocal synergy (increase in the inhibition zone) against *E. aerogenes* and *S. aureus* only. When the EO of *A. graveolens* was replaced with the EO of *C. sempervirens*, a strong synergistic effect was observed against *Pseudomonas aeruginosa*, and a substantial increase in the inhibition zone against *S. aureus* was also observed. However, the antibacterial effects of AgNPs, EOs and AgNPs:EOs against the gram-positive and gram-negative bacteria tested (Table 1) varied between members of both groups.

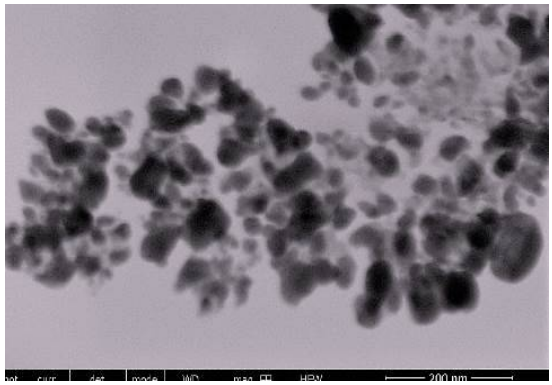

Figure 4: TEM micrograph of silver particles synthesized by *Tritirachium oryzae* (scale bar: 200 nm)

Synergistic effects against *E. coli*

The synergistic effects of combination of AgNPs and antibiotics showed a wide variation not only among groups but also between members of the same group (Table 2 and 3). When NPs were combined with aminoglycosides i.e., gentamicin and amikacin, the AgNPs produced appreciable synergy depicted by increases in IFA (1.6 and 0.4, respectively). The inhibition zones of gentamicin and amikacin significantly increased from 10 and 17 mm, to 16 and 20 mm, respectively.

Table 1: Antibacterial effect of the AgNPs, EOs of the *A. graveolens*, *C. sempervirens* and their synergistic effect on the test bacterial strains using well diffusion method

Bacterium	NP	EO	NP+EO
	A. graveolens		
E.aerogenes	20 ± 1.0	10.7 ± 0.6	22.3 ± 0.6
S. epidermidis	20.3 ± 0.6	13.3 ± 0.6	20 ± 1.0
E. coli	18 ± 1.0	-	13.7 ± 0.6
S. aureus	16 ± 0.6	16.3 ± 0.6	17 ± 1.0
P. aeroginsa	12 ± 1.0	-	-
	C. sempervirens		
E. aerogenes	20 ± 1.0	12.6 ± 0.6	12.6 ± 0.6
S. epidermidis	20.3 ± 0.6	13.3 ± 0.6	15.3 ± 1.2
E. coli	18 ± 1.0	11 ± 1.0	14 ± 0.0
S. aureus	16.3 ± 0.6	13.6 ± 0.6	19 ± 1.0
P. aeroginosa	12 ± 1.0	10.6 ± 0.63	19 ± 1.0

Imipenem produced a slight increase in IFA (0.07) when combined with nanoparticles (NPs). Silver nanoparticles alone produced an 18 mm inhibition zone against *E. coli*. The synergistic effects of amikacin and NPs, and synergistic effects of NPs and the EO of *A. graveolens* (Table 2) resulted in IFAs of 0.4 and 0.7, respectively, although *E. coli* was completely resistant to the EO of *A. graveolens* when used alone (Table 1). Trimethoprim and amoxicillin showed significant effects (IFAs of 6 and 3, respectively) in the presence of EO of *A. graveolens* when used against *E. coli*. However, *E. coli* was completely resistant to trimethoprim, amoxicillin and EO of *A. graveolens* when applied individually (Table 2). This phenomenon was not observed with the EO of *C. sempervirens* (Table 3). The effect of tetracycline and amoxicillin against *E. coli* was remarkably enhanced up to 1- and 3-fold in the presence of the EO of *A. graveolens*, but no synergy was observed when these antibiotics were combined with AgNPs.

Synergistic effects against *E. aerogenes*

Out of the 12 antibiotics tested, only ampicillin, imipenem and cefotaxime showed negligible synergistic effect in combination with AgNPs against *E. aerogenes* (Table 4 and 5). In contrast, the use of combination of nitrofurantoin, ampicillin and amoxicillin with the EO of *A. graveolens* (Table 4) against *E. aerogenes* resulted in IFAs of 0.3, 2.3 and 1.04, respectively. Similarly, for tetracycline, trimethoprim, ampicillin and amoxicillin, significantly slight-to-moderate synergies were observed when combined with NPs and the EO of *A. graveolens* against *E. aerogenes*. The increases in synergy (expressed as IFA) were 0.26, 0.4, 0.2 and 1.0, respectively. It was observed that the best synergistic effects of antibiotics against *E. aerogenes* were produced with the combinations with *C. sempervirens* EO (Table 5), where cefotaxime had an IFA of approximately 1.7, while the combination of EO, AgNPs and cefotaxime resulted in complete bacterial resistance (0 mm inhibition zone) or even negative synergy. The latter results may indicate that the combination produced ineffective materials (Table 5). The explanation based on ineffectiveness is most probably correct since the EO and the NPs independently showed antibacterial effects against *E. aerogenes*.

Table 2: Antibacterial and synergistic effects of *A. graveolens* essential oil, antibiotics and silver nanoparticles against *E. coli*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	12.0 ± 1.1	6.0 ± 0.1*	-	20.0 ± 0.8*	1.8	17.0 ±0.3*	1.0
Gentamicin	10.0 ± 1.2	16.0 0.3*	1.6	-	-	16.0 ± 0.5*	1.6
Chloramphenicol	33.0 ± 0.6	24.0 ± 1.2*	-	24.0 ± 0.2*	-	20.0 ± 0.1*	-
Amikacin	17.0 ± 0.3	20.0 ± 0.6*	0.4	17.0 ± 0.2*	-	22.0 ± 1.0*	0.7
Trimethoprim	6.0 ± 0.1	6.0 ± 0.1	-	6.0 ± 0.1	-	16.0 ± 0.5*	6.0
Vancomycin	10.0 ± 0.5	6.0 ± 0.1*	-	-	-	6.0 ± 0.1*	-
Nitrofurantoin	15.0 ± 0.3	16.0 ± 0.8	0.13	14.0 ± 0.5	-	9.0 ± 0.3*	-
Ampicillin	6.0 ± 0.1	6.0 ± 0.2	-	12.0 ± 0.2*	3.0	12.0 ± 0.2*	3.0
Ciprofloxacin	30.0 ± 0.8	10.0 ± 0.1*	-	-	-	6.0 ± 0.1*	-
Imipenem	26.0 ± 0.1	31.0 ± 0.5*	0.42	10.0 ± 0.7*	-	13.0 ± 0.1*	-
cefotaxime	25.0 ± 1.4	11.0 ± 1.0*	-	6.0 ± 0.1*	-	9.0 ± 0.3*	-
Amoxicillin	6.0 ± 0.1	6.0 ± 0.1	-	6.0 ± 0.1	-	12.0 ± 0.4*	3.0

Table 3: Antibacterial and synergistic effects of *C. sempervirens* essential oil, antibiotics and silver nanoparticles against *E. coli*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	12.0 ± 0.7	6.0 ± 0.7*	-	6.0 ± 0.1*	-	12.0 ± 0.5	-
Gentamicin	10.0 ± 0.6	16.0 ± 0.5*	1.6	18.0 ± 0.3*	2.2	18.0 ± 0.6*	2.2
Chloramphenicol	33.0 ± 0.5	24.0 ± 0.6*	-	23.0 ± 0.2*	-	16 ± 0.1*	-
Amikacin	17.0 ± 0.3	20.0 ± 0.3*	0.38	20.0 ± 0.5*	0.4	20.0 ± 0.3*	0.4
Trimethoprim	6.0 ± 0.2	6.0 ± 0.1	-	6.0 ± 0.3	-	6.0 ± 0.9	-
Vancomycin	10.0 ± 0.6	6.0 ± 0.6*	-	6.0 ± 0.1*	-	6.0 ± 0.9*	-
Nitrofurantoin	15.0 ± 0.1	6.0 ± 0.2*	-	10.0 ± 0.2*	-	9.0 ± 0.1*	-
Ampicillin	6.0 ± 0.1	6.0 ± 0.1	-	12.0 ± 0.4*	3.0	6.0 ± 0.2	-
Ciprofloxacin	30.0 ± 0.6	10.0 ±* 0.6	-	6.0 ± 0.3*	-	6.0 ± 0.6*	-
Imipenem	29.0 ± 0.2	30.0 ± 0.2	0.07	22.0 ± 0.6*	-	20.0 ± 0.4*	-
Cefotaxime	25.0 ± 0.3	11.0 ± 0.3*	-	6.0 ± 0.3*	-	9.0 ± 0.3*	-
Amoxicillin	6.0 ± 0.1	6.0 ± 0.1	-	12.0 ± 0.7*	3.0	13.0 ± 0.5*	3.7

The combination of AgNps with different antibiotics and either EO of *A. graveolens* or EO of *C. sempervirens* showed different selective synergistic efficacies against *E. aerogenes*.

Synergistic effects against *P. aeruginosa*

The combination of tetracycline, chloramphenicol, vancomycin and trimethoprim with AgNPs produced varied degrees of enhancement of antimicrobial effect against *P. aeruginosa* i.e. 2.4, 6.0, 9.0 and 6.0 folds, respectively (Table 6 and 7). However, with vancomycin and nitrofurantoin, *P. aeruginosa* was transformed completely from a resistant to a sensitive strain, with significant inhibition zones of 19 and 16 mm, respectively. However, the other antibiotics showed negative or no synergy against *P. aeruginosa* when combined with AgNPs. The combination of AgNPs with the EO of *C. sempervirens* (Table 7) and vancomycin produced a selective synergistic efficacy against *P. aeruginosa* (up to 16.4-fold). However, when the EO of *C. sempervirens* was replaced with EO of *A. graveolens*, the resulting IFA was zero (Table 6). A mixture of the EO of *A. graveolens* with nitrofurantoin, ampicillin, cefotaxime or amoxicillin produced significant synergistic effects against *P. aeruginosa,* with large IFA increase of 3, 5.3, 6.1 or 10. Moreover, *P. aeruginosa* changed from being resistant to these antibiotics to a susceptible status (Table 6).

Synergistic effects against *S. epidermidis*

When combined with AgNPs, the antibacterial effect of trimethoprim, ciprofloxacin, or cefotaxime showed significant synergism (0.7 – 10-fold increase). The effect of trimethoprim (10-fold increase) against *S. epidermidis* was superior to those of the other antibiotics (Table 8 and 9). The combination of cefotaxime, NPs and EO of *A. graveolens* (Table 8) resulted in a significant fold increase in area of inhibition (10-fold). Interestingly, ampicillin showed significant synergistic effect only when combined with AgNPs and EO of *A. graveolens*, which was accompanied by transformation of the organism from being completely-resistant to reasonably-sensitive. The EO of *A. graveolens* when combined with either tetracycline, chloramphenicol or cefotaxime resulted in 0.36-, 0.62- or 3.0-fold increases in antibacterial effects, respectively (Table 8).

Table 4: Antibacterial and synergistic effects of *A. graveolens* essential oil, antibiotics and silver nanoparticles against *E. aerogenes*

Antibiotic (A)	A	NPs+A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	16.0 ± 0.5	13.0 ± 0.3*	-	13.0 ± 0.1*	-	18.0 ± 0.7*	0.26
Gentamicin	30.0 ± 0.3	25.0 ± 0.7*	-	26.0 ± 0.3*	-	14.0 ± 0.3*	-
Chloramphenicol	29.0 ± 0.1	26.0 ± 0.8*	-	25.0 ± 0.2*	-	20.0 ± 0.5*	-
Amikacin	27.0 ± 0.7	24.0 ± 0.5*	-	25.0 ± 0.7*	-	26.0 ± 0.8	-
Trimethoprim	16.0 ± 0.3	10.0 ± 0.5*	-	6.0 ± 0.1*	-	19.0 ± 0.1*	0.4
Vancomycin	21.0 ± 0.5	18.0 ± 0.4*	-	18.0 ± 0.1*	-	19.0 ± 0.3*	-
Nitrofurantoin	21.0 ± 1.2	20.0 ± 0.2*	-	24.0 ± 0.5*	0.3	21.0 ± 0.6	0.0
Ampicillin	11.0 ± 0.7	12.0 ± 0.3	0.2	20.0 ± 0.3*	2.3	12.0 ± 0.2*	0.2
Ciprofloxacin	26.0 ± 0.3	25.0 ± 0.5*	-	19.0 ± 1.1*	-	15.0 ± 0.6*	-
Imipenem	28.0 ± 0.8	30.0 ± 0.6*	0.15	22.0 ± 0.7*	-	23.0 ± 0.4*	-
cefotaxime	20.0 ± 0.5	22.0 ± 0.7*	0.2	18.0 ± 0.4*	-	6.0 ± 0.3*	-
Amoxicillin	14.0 ± 0.2	12.0 ± 0.2*	-	20.0 ± 0.3*	1.04	20.0 ± 0.5*	1.0

Table 5: Antibacterial and synergistic effects of *C. sempervirens* essential oil, antibiotics and silver nanoparticles against *E. aerogenes.*

Antibiotic (A)	A	NPs+A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	16.0 ± 0.3	13.0 ± 0.4*	-	13.0 ± 0.1*	-	6.0 ± 0.7*	-
Gentamicin	30.0 ± 0.4	25.0 ± 0.3*	-	26.0 ± 0.3*	-	29.0 ± 0.3*	-
Chloramphenicol	29.0 ± 0.5	26.0 ± 0.7*	-	25.0 ± 0.2*	-	6.0 ± 0.2*	-
Amikacin	27.0 ± 0.1	24.0 ± 0.3*	-	25.0 ± 0.5*	-	12.0 ± 0.2*	-
Trimethoprim	16.0 ± 1.1	10.0 ± 0.1*	-	6.0 ± 0.3*	-	12.0 ± 0.1*	-
Vancomycin	21.0 ± 0.7	18.0 ± 0.2*	-	18.0 ± 0.2*	-	6.0 ± 0.2*	-
Nitrofurantoin	21.0 ± 0.3	20.0 ± 0.5	-	24.0 ± 0.5*	0.3	20.0 ± 0.6*	-
Ampicillin	11.0 ± 0.8	12.0 ± 0.1	0.2	13.0 ± 0.8*	0.4	15.0 ± 0.5*	0.86
Ciprofloxacin	26.0 ± 0.5	25.0 ± 0.7	-	20.0 ± 0.9*	-	19.0 ± 0.1*	-
Imipenem	28.0 ± 0.3	30.0 ± 0.4*	0.15	33.0 ± 0.3*	0.4	19.0 ± 0.4*	-
cefotaxime	20.0 ± 0.2	22.0 ± 0.1*	0.2	33.0 ± 0.6*	1.7	6.0 ± 0.3*	-
Amoxicillin	14.0 ± 0.4	12.0 ± 0.2*	-	20.0 ± 0.3*	1.0	16.0 ± 0.1*	0.3

Table 6: Antibacterial and synergistic effects of *A. graveolens* essential oil, antibiotics and silver nanoparticles against *P. aeruginosa*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	12.0 ± 0.1	22.0 ± 0.3*	2.4	10.0 ± 0.2*	-	6.0 ± 0.7*	-
Gentamicin	22.0 ± 0.3	19.0 ± 0.1*	-	6.0 ± 0.2*	-	11.0 ± 0.3*	-
Chloramphenicol	11.0 ± 0.2	29.0 ± 0.5*	6.0	6.0 ± 0.1*	-	6.0 ± 0.1*	-
Amikacin	25.0 ± 0.5	21.0 ± 0.7*	-	20.0 ± 0.5*	-	25.0 ± 0.5	-
Trimethoprim	6.0 ± 0.2	6.0 ± 0.9	-	6.0 ± 0.7	-	6.0 ± 0.7	-
Vancomycin	6.0 ± 0.1	19.0 ± 0.3*	9.0	6.0 ± 0.3	-	6.0 ± 0.1	-
Nitrofurantoin	6.0 ± 0.2	16.0 ± 0.6*	6.0	12.0 ± 0.1*	3.0	6.0 ± 0.3	-
Ampicillin	6.0 ± 0.3	6.0 ± 0.1	-	15.0 ± 0.5*	5.3	6.0 ± 0.4	-
Ciprofloxacin	25.0 ± 0.1	23.0 ± 0.3*	-	20.0 ± 0.4*	-	20.0 ± 0.5*	-
Imipenem	30.0 ± 0.5	28.0 ± 0.2*	-	19.0 ± 0.1*	-	20.0 ± 0.1*	-
cefotaxime	6.0 ± 0.3	6.0 ± 0.3	-	16.0 ± 0.3*	6.1	6.0 ± 0.2	-
Amoxicillin	6.0 ± 0.1	6.0 ± 0.4	-	20.0 ± 0.1*	10.0	15.0 ± 0.6*	5.3

Table 7: Antibacterial and synergistic effects of *C. sempervirens* essential oil, antibiotics and silver nanoparticles against *P. aeruginosa*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs +EO	IFA
Tetracycline	12.0 ± 0.2	22.0 ± 0.2*	2.4	11.0 ± 0.6	-	13.0 ± 0.7	0.15
Gentamicin	22.0 ± 0.5	19.0 ± 0.3*	-	18.0 ± 0.3*	-	6.0 ± 0.3*	-
Chloramphenicol	11.0 ± 0.8	29.0 ± 0.1*	6.0	10.0 ± 0.6	-	6.0 ± 0.4*	-
Amikacin	25.0 ± 0.4	21.0 ± 0.4*	-	20.0 ± 0.4*	-	19.0 ± 0.3*	-
Trimethoprim	6.0 ± 0.1	6.0 ± 0.7	-	6.0 ± 0.7	-	6.0 ± 0.8	-
Vancomycin	6.0 ± 0.4	19.0 ± 0.3*	9.0	6.0 ± 0.6	-	25.0 ± 0.1*	16.4
Nitrofurantoin	6.0 ± 0.3	16.0 ± 0.1*	6.0	6.0 ± 0.1	-	6.0 ± 0.4	-
Ampicillin	6.0 ± 0.1	6.0 ± 0.1	-	6.0 ± 0.5	-	6.0 ± 0.3	-
Ciprofloxacin	25.0 ± 0.5	23.0 ± 0.1*	-	6.0 ± 0.4*	-	6.0 ± 0.5*	-
Imipenem	30.0 ± 0.4	28.0 ± 0.2*	-	19.0 ± 0.1*	-	20.0 ± 0.7*	-
cefotaxime	6.0 ± 0.5	6.0 ± 0.6	-	6.0 ± 0.2	-	6.0 ± 0.9	-
Amoxicillin	6.0 ± 0.1	6.0 ± 0.1	-	10.0 ± 0.2	1.8	6.0 ± 0.2	-

The combination of cefotaxime with AgNPs and the EO of *A. graveolens* resulted in a significant fold increase in inhibition (10-fold). There were similar responses in bacteria using the EO of *C. sempervirens* (Table 9) in addition to AgNPs in different combinations. The IFAs of tetracycline, amikacin, trimethoprim, vancomycin, nitrofurantoin, cefotaxime and amoxicillin were enhanced against *S. epidermidis* by 1.8-, 0.8-, 29.0-, 0.12-, 0.16-, 2.4- and 2.4-fold, respectively. The effect of trimethoprim against *S. epidermidis* was significantly superior, with an IFA of 29-fold when used in combination with AgNPs and the EO of *C. sempervirens* (Table 9). The efficacy of the trimethoprim and cefotaxime combination with AgNPs when used against *S. epidermidis* was selectively enhanced, as reflected in complete change in inhibition diameters from 0 to 20 and 0 to 10 mm, respectively. On the other hand, the effects of cefotaxime and amoxicillin against *S. epidermidis* were substantially enhanced (0 to 11, and 0 to 11 mm, respectively) when synergized with the EO of *C. sempervirens*.

Table 8: Antibacterial and synergistic effects of *A. graveolens* essential oil, antibiotics and silver nanoparticles against *S. epidermidis*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs+EO	IFA
Tetracycline	18.0 ± 0.2	11.0 ± 0.3*	-	21.0 ± 0.2*	0.36	16.0 ± 0.4*	-
Gentamicin	19.0 ± 0.3	16.0 ± 0.3*	-	20.0 ± 0.5	0.11	12.0 ± 0.1*	-
Chloramphenicol	22.0 ± 0.5	11.0 ± 0.5*	-	28.0 ± 0.9*	0.62	17.0 ± 0.7*	-
Amikacin	16.0 ± 0.6	14.0 ± 0.1*	-	16.0 ± 0.1	-	12.0 ± 0.3*	-
Trimethoprim	6.0 ± 0.1	20.0 ± 0.5*	10.0	6.0 ± 0.3	-	14.0 ± 0.5*	4.4
Vancomycin	19.0 ± 0.3	13.0 ± 0.6*	-	6.0 ± 0.1*	-	17.0 ± 0.2*	-
Nitrofurantoin	13.0 ± 0.2	11.0 ± 0.3*	-	6.0 ± 0.6*	-	6.0 ± 0.2*	-
Ampicillin	6.0 ± 0.1	6.0 ± 0.1	-	6.0 ± 0.2	-	12.0 ± 0.3*	3.0
Ciprofloxacin	20.0 ± 0.5	26.0 ± 0.6*	0.7	6.0 ± 0.5*	-	6.0 ± 0.5*	-
Imipenem	30.0 ± 0.3	30.0 ± 0.2	-	30.0 ± 0.4	-	25.0 ± 0.1*	-
Cefotaxime	6.0 ± 0.2	10.0 ± 1.0*	1.8	12.0 0.3*	3.0	20.0 ± 0.2*	10.1
Amoxicillin	6.0 ± 0.1	6.0 ± 0.1	-	6.0 ± 0.1	-	6.0 ± 0.1	-

Table 9: Antibacterial and synergistic effects of *C. sempervirens* essential oil, antibiotics and silver nanoparticles against *S. epidermidis*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A +NPs+EO	IFA
Tetracycline	18.0 ± 0.5	11.0 ± 0.1*	-	13.0 ± 0.3*	-	30.0 ± 0.1*	1.8
Gentamicin	19.0 ± 0.3	16.0 ± 0.6*	-	19.0 ± 0.2	-	14.0 ± 0.3*	-
Chloramphenicol	22.0 ± 0.3	11.0 ± 0.5*	-	26.0 ± 0.2*	0.4	20.0 ± 0.7*	-
Amikacin	16.0 ± 0.2	14.0 ± 0.3*	-	17.0 ± 0.1*	-	20.0 ± 0.4*	0.8
Trimethoprim	6.0 ± 0.1	20.0 ± 0.4*	10.1	6.0 ± 0.4	-	33.0 ± 0.5*	29.0
Vancomycin	19.0 ± 0.5	13.0 ± 0.5*	-	22.0 ± 0.6*	0.34	20.0 ± 0.3*	0.12
Nitrofurantoin	13.0 ± 1.0	11.0 ± 0.1*	-	17.0 ± 0.3*	0.71	14.0 ± 0.1	0.16
Ampicillin	6.0 ± 0.1	6.0 ± 0.3	-	11.0 ± 1.0*	2.4	10.0 ± 0.1*	-
Ciprofloxacin	20.0 ± 0.8	26.0 ± 0.1*	0.7	20.0 ± 0.3	-	11.0 ± 0.6*	-
Imipenem	30.0 ± 0.6	30.0 ± 0.6*	-	16.0 ± 0.3*	-	13.0 ± 0.3*	-
Cefotaxime	6.0 ± 0.1	10.0 ± 0.8*	1.8	13.0 ± 0.8*	3.7	11.0 ± 0.2*	2.4
Amoxicillin	6.0 ± 0.1	6.0 ± 0.7	-	13.0 ± 0.2*	3.7	11.0 ± 0.2*	2.4

Synergistic effects against *S. aureus*

Staphylococcus aureus was susceptible to all antibiotic discs utilized in this study except ampicillin and cefotaxime in which the inhibition zones were increased 3 and 2.7 folds, respectively (Table 10 and 11). The combination of AgNPs and the EO of *A. graveolens* (Table 10) increased the efficacies of nitrofurantoin and amoxicillin against *S. aureus* by 0.84 and 2.3 folds, respectively, whereas the effect with trimethoprim was negligible (0.01-fold increase). Amoxicillin showed identical effect with AgNPs against *S. aureus* when used either with the EO of *C. sempervirens* (Table 11) or the EO of *A. graveolens* (Table 10) (2.3-fold increase).

Minimal inhibitory concentrations

The MICs of the NPs ranged from 6.4 – 19.2 µg/mL (Table 12). The MIC values for the different bacterial species were as follows: 6.4 µg/mL for *S. aureus, S. epidermidis and P. aeruginosa;* and 19.2 µg/mL for *E. coli* and *E. aerogenes*.

Table 10: Antibacterial and synergistic effects of *A. graveolens* essential oil, antibiotics and silver nanoparticles against *S. aureus*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A+NPs+EO	IFA
Tetracycline	25.0 ± 0.3	21.0 ± 0.3*	-	20.0 ± 0.1*	-	20.0 ± 0.6*	-
Gentamicin	21.0 ± 0.6	20.0 ± 0.3	-	20.0 ± 0.5	-	18.0 ± 0.6*	-
Chloramphenicol	33.0 ± 0.2	16.0 ± 0.1*	-	20.0 ± 0.3*	-	19.0 ± 1.1*	-
Amikacin	20.0 ± 0.5	16.0 ± 0.6*	-	19.0 ± 0.3	-	12.0 ± 0.3*	-
Trimethoprim	21.0 ± 0.5	21.0 ± 0.6	-	21.0 ± 0.6	-	22.0 ± 0.1*	0.01
Vancomycin	20.0 ± 1.1	15.0 ± 0.2*	-	17.0 ± 0.2*	-	14.0 ± 0.5*	-
Nitrofurantoin	14.0 ± 0.6	13.0 ± 0.5	-	20.0 ± 0.6*	1.0	19.0 ± 0.6*	0.84
Ampicillin	10.0 ± 1.0	20.0 ± 0.2*	3.0	6.0 ± 0.1*	-	7.0 ± 0.1*	-
Ciprofloxacin	24.0 ± 0.6	17.0 ± 0.5*	-	6.0 ± 0.1*	-	10.0 ±0.8*	-
Imipenem	28.0 ± 0.3	19.0 ± 0.1*	-	12.0 ± 0.2*	-	14.0 ± 1.0*	-
Cefotaxime	13.0 ± 0.3	25.0 ± 0.3*	2.7	12.0 ± 0.1*	-	12.0 ± 0.3*	-
Amoxicillin	11.0 ± 0.3	10.0 ± 0.3	-	8.0 ± 0.8*	-	20.0 ± 0.3*	2.3

Table 11: Antibacterial and synergistic effects of *C. sempervirens* essential oil, antibiotics and silver nanoparticles against *S. aureus*

Antibiotic (A)	A	NPs+ A	IFA	EO+A	IFA	A+NPs+EO	IFA
Tetracycline	25.0 ± 0.2	21.0 ± 0.8*	-	22.0 ± 0.3*	-	20.0 ± 0.5*	-
Gentamicin	21.0 ± 0.3	20.0 ± 0.6	-	19.0 ± 0.3*	-	6.0 ± 0.1*	-
Chloramphenicol	33.0 ± 0.3	16.0 ± 0.3*	-	19.0 ± 0.6*	-	16.0 ± 0.6*	-
Amikacin	20.0 ± 0.1	16.0 ± 0.3*	-	19.0 ± 0.1*	-	18.0 ± 0.3*	-
Trimethoprim	21.0 ± 0.8	21.0 ± 0.5	-	22.0 ± 0.1*	0.1	19.0 ± 0.3*	-
Vancomycin	20.0 ± 1.3	15.0 ± 0.3*	-	17.0 ± 0.3*	-	11.0 ± 0.8*	-
Nitrofurantoin	14.0 ± 0.2	13.0 ± 0.6	-	19.0 ± 0.3*	0.84	18.0 ± 0.1*	0.65
Ampicillin	10.0 ± 0.2	20.0 ± 0.2*	3.0	10.0 ± 0.6	-	16.0 ± 0.3*	1.6
Ciprofloxacin	24.0 ± 0.3	17.0 ± 0.2*	-	12.0 ± 0.3*	-	16.0 ± 0.6*	-
Imipenem	28.0 ± 0.1	19.0 ± 0.1*	-	18.0 ± 0.8*	-	20.0 ± 0.8*	-
Cefotaxime	13.0 ± 0.2	25.0 ± 0.3*	2.7	11.0 ± 0.1*	-	8.0 ± 0.1*	-
Amoxicillin	11.0 ± 0.2	10.0 ± 0.6	-	13.0 ± 0.3*	0.4	20.0 ± 0.6*	2.3

Table 12: Minimum inhibitory concentration (MIC) of EOs of *A. graveolens* and *C. sempervirens*, AgNPs and EOs plus NPs against tested bacterial strains

Bacterial strain	NPs (µg/mL)	EOs (µg/mL)	NPs+EOs (µg/mL: µg/mL)
		A. graveolens	
S. aureus	6.384	370	3.192:183.5
S. epidermidis	6.384	370	3.192:183.5
E. coli	19.154	1100	3.192:183.5
E. aerogenes	19.154	1100	9.577:550
P. aeruginosa	6.384	370	1.064:61.0
		C. sempervirens	
S. aureus	6.384	370	3.192:183.5
S. epidermidis	6.384	370	3.192:183.5
E. coli	19.154	1100	9.577:550
E. aerogenes	19.154	1100	9.577:550
P. aeruginosa	6.384	1100	28.731:1650

When the AgNPs solution was combined with the EO of *A. graveolens*, there were synergistic responses against *S. aureus, S. epidermidis, E. coli*, *E. aerogenes* and *P. aeruginosa* (Table 12), and the MIC values decreased from 6.4 – 19.2 µg/mL to 1.0 - 9.5 µg/mL. However, when the EO of *A. graveolens* was replaced with the EO of *C. sempervirens*, there were synergistic effects against most of the bacteria except *P. aeruginosa.*

DISCUSSION

Fungi are major sources of secondary metabolites and have been shown to be cost-effective and eco-friendly tools for biosynthesis of nanoparticles [17]. Silver nanoparticle formation by the fungi *Tritirachium oryzae* W5H was assessed by monitoring the absorbance peak at 425 nm in the ultraviolet-visible spectrum [18]. It has been reported that silver ions are extracellularly reduced to generate stable AgNPs in water [3]. The biosynthesis of AgNPs from different fungi has been studied widely [19].

Two possible mechanisms for the formation of silver nanoparticles by *Fusarium oxysporum* and *Bacillus licheniformis* have been reported [20]. One is through NADH-dependent nitrate reductase, and the other is through the quinine shuttle process. To confirm the presence of silver ions in the solution, SEM-EDS analysis was carried out. The EDS optical absorption peak was observed at approximately 3 keV, indicating the presence of AgNPs in the reaction solution of *Tritirachium oryzae* W5H. In addition, FT-IR analysis revealed the presence of the signature amino acid peaks (3292.6 cm^{-1}, 2994.23 cm^{-1}, 1388.79 cm^{-1} and 1060.88 cm^{-1}), which in turn, supports the existence of proteins in fungal cell-free filtrates, as observed in the UV/VIS spectra [1]. Studies have shown that the binding of proteins to nanoparticles occurs either through the free amine groups or through the cysteine residues in the proteins [21]. It is well known that the size and shape of the AgNPs affect the absorbance peak [13,22].

The AgNPs exhibited clear cytotoxicity against *S. epidermidis*, *E. aerogenes*, *E. coli*, *S. aureus* and *P. aeruginosa,* with inhibition zones in the range of 12 - 20.3 mm. It has been reported that variations in the responses to nanoparticles could be due to differences in cell surface components between gram-negative and gram-positive bacteria [2]. The catalytic reactivity and other related properties, such as antimicrobial effect of AgNPs depend on their specific surface areas. As the specific surface area of nanoparticles increases, their biological efficacy increases due to increase in surface energy [23]. Silver nanoparticles are highly toxic to microorganisms because of their highly reactive component species and their large surface areas [15]. The antimicrobial activities of naturally occurring AgNPs from other fungal sources have been studied.

The increase in the resistance to different antibiotics can be controlled using new methodologies, such as synergy with either AgNPs or the EOs of plants. The best synergistic effects of the antibiotics were obtained in the presence of AgNPs and either the EO of *A. graveolens* or the EO of *C. sempervirens*. The antibiotics with the most synergistic activity included trimethoprim, amoxicillin, vancomycin and cefotaxime. For example, trimethoprim and amoxicillin showed substantial activity against *E. coli,* with IFAs of 6 and 3, respectively, in the presence of the EO of *A. graveolens* but not in the presence of the EO of *C. sempervirens*, although *E. coli* was completely resistant to each of them individually. The effect of the EO of *C. sempervirens* against *S. epidermidis* and *S. aureus* were superior to those of the other materials, but much less effect was shown against *E. coli, E. aerogenes* and *P. aeruginosa*.

The EO of *C. sempervirens* is free of steroids and alkaloids [24]**.** This may explain why gram-negative bacteria were less susceptible to this plant oil than gram-positive bacteria. Steroids are well recognized for their antibacterial effects and their capacity to cause leakages from liposomes [25]. It is also possible that other components were involved in some type of synergism with other active compounds [26].

However, no data have been previously reported on the potential antibacterial effects of such cooperative interaction between antibiotic discs, biosynthesized AgNPs from *Tritirachium oryzae,* and the EOs of either *A. graveolens* or of *C. sempervirens* against the tested bacteria. The antibacterial synergistic effect of AgNPs, EOs and antibiotics, especially trimethoprim, amoxicillin, vancomycin and cefotaxime was much greater than the antibacterial effect of AgNPs only, which indicates the synergistic effect of these components. It has been reported that the absorption of a drug increases several times in the presence of nanoparticles, suggesting that AgNPs could be used as potential drug delivery system [3]. On the other hand, nanoparticle-antibiotic or nanoparticle-antibiotic-EO combinations most likely minimized the amounts of combined agents needed, thereby lowering noxiousness and raising antimicrobial potential [11].

These observations show the potential of AgNPs against *S. aureus, S. epidermidis* and *P. aeruginosa* when used in combination with trimethoprim, amoxicillin, vancomycin and cefotaxime and an EO. In this study, the tested bacteria which included those that cause atopic dermatitis, urinary tract infection and burn infection, as well as *E. coli,* were predominantly antibiotic-resistant [27-29]. Therefore, the increases in the level of inhibition of some of these antibiotic-resistant bacteria or even antibiotic-susceptible bacteria is considered an important finding and a successful therapeutic strategy.

CONCLUSION

The potent synergistic effect of essential oils (EOs) of *A. graveolens* (Forssk.) Less. and *C. sempervirens* and/or NPs with the used antibiotics is a new approach to controlling antibiotic-resistant bacteria. Thus, synergism between biosynthesized AgNPs, EOs of different plants and tested antibiotics can potentially be exploited when applied in a cream or ointment application to affected body surfaces to achieve appropriate healing.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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