Biosynthesis of Silver Nanoparticles Using *Eucalyptus Bicolor* Bark and their Antimicrobial Activity

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Abstract

The current study examined the production of silver nanoparticles from silver nitrate(AgNO3) solution with the extract of *Eucalyptus bicolor* bark and the antimicrobial activity of the silver nanoparticles versus microorganisms. The silver nanoparticle was characterized by its color changes of extract, UV-Visible spectroscopy and SEM. The useful groups for alcoholic extract were recognized by using Fourier transform infrared spectroscopy analysis (FTIR). In addition to we compared between the biological effectiveness of alcoholic and nano extract of *Eucalyptus* bark against three types of pathogenic microbes: *Staphylococcus*. aureus (Gram +ve), Pseudomonas *aeruginosa* (Gram-ve), and yeast, Candida *albicans* . Alcoholic extract offered the higher effect with *Candida* by inhibition zone of 14mm , *S,aureus* of 13mm and *pseudomonas* of 10mm , While the nano extract has showed that the highest effect on growth of microorganism, *Candida* by inhibition zone of 20mm , *staph* of 19mm, and *pseudomonas* of 11mm .Furthermore,the combined effect of some antibiotics and alcoholic extract was studied against the same pathogenic microorganisms and the results showed that both :synergism and antagonism effects were seen, Carpenciline and the extract exhibited synergistic effect as evidenced by the increment of antimicrobial activity with all bacteria(synergism) while Amoxicilline and the extract exhibited antagonistic effect against *S.aureus* (antagonism), and synergistic effect against *P. aeruginosa*

Keywords: plants extract, silver nanoparticles, antimicrobial activities.

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التكوين الاحيائي لدقائق الفضة النانويه باستخدام لحاء اليوكالبتوز ونشاطهما الضد مايكروبي

رغد خويطر مايح

الجامعة التكنلوجية - قسم العلوم التطبيقية

الخلاصة

وضحت الدراسة الحالية تكوين ذرات الفضة النانوية من محلول نترات الفضة من خلال مستخلص لحاء اليوكالبتوز وحددت الفعالية الضد مايكروبية للجسيمات النانوية ضد البكتريا المرضية والخمائر . تم تمييز الذرات النانوية من خلال التغير اللوني للمستخلص ومن خلال قياس UV visibile spectroscopy وصورة المجهر الالكتروني SEM . بالاضافة الى ذلك تم تحديد المجاميع الفعالة للمستخلص الكحولي بأستخدام التحليل الطيفي للاشعة تحت الحمراء (FTIR) . كذلك تم المقارنة بين الفعالية الضد ميكروبية للمستخلص الكحولي والنانوي لنبات اليوكالبتوز من خلال تأثيره على ثلاث انواع من الاحياء المجهرية المرضية بكتريا *Staphylococcus aureus* الموجبة لصبغة كرام وبكتريا *Pseudomonas aeruginosa* السالبة لصبغة كرام و *Candida albicans*(خمائر) . اعطى المستخلص الكحولي اعلى منطقة تثبيط بلغت 14 mm في *candida* و 13mm في *S. aureus* و 10 mm في *P. aeruginosa* بينما اعطى المستخلص النانوي تأثيرا اكبر من المستخلص الكحولي على نمو الاحياء المجهرية حيث كان قطر منطقة التثبيط 20 mm في *Candida* و19 mmفي *S. aureus* و11 mm في *P. aeruginosa* . اضافة لذلك تم دراسة التأثير المشترك للمضادات الحياتية مع المستخلص الكحولي ضد نفس انواع الاحياء المجهرية المرضية واظهرت النتائج وجود فعل تازري وتضادي ،اظهر الكاربنسيلين والمستخلص وجود فعل تازري ضد كل انواع البكتريا بينما اظهر الاموكسيلين والمستخلص فعلا تضاديا ضد *S. aureus* وفعلا تازريا ضد *P. aeruginosa*

الكلمات المفتاحية: المستخلصات النباتية ،ذرات الفضة النانوية ،الفعالية الضد مايكروبية

Introduction

Plants have been used in popular medicines for several thousand years and world health organization WHO expected that 80% of persons used popular medicines for their healthiness (1). Medical plants are thought to be a main source of chemical materials including beneficial effects (2). *Eucalyptus* be a part to the family mytaceae It is an active growing tree, aromatic evergreen tree and several natural substances having antagonistic activities against several microorganisms (2). The bark of the *Eucalyptus* plant was used in cure of ulcers, injuries, and other diseases (1). the ethanolic extracts from plants used for, antiviral, antimicrobial and

antitumor agents (3). Nanotechnology increases much care among the students for the properties distribution, size, and morphology of nanomaterials (4). Biological means are used to production of nanoparticales using, an ecofriendly (green synthesis) method by using silver ion (5,6).Silvernanoparticles have showed to be greatest active as it has good anti-microbial effectiveness against Viruses, bacteria, and other micro-organisms (7). Some recommendations have been advanced about the effect of silver ion against bacteria and all opinion to the heavy metal effect on protiens,DNA,and respiratory enzyme that which leads to inactivation of the proteins and DNA (7). The objective of the present study was to produce silver nanopaticles from bark extract of *Eucalyptus bicolor* and exam the antimicrobial effect of the nanoparticles against bacteria and yeast, in order to find an alternative of antimicrobial agent by joining the antimicrobial properties of *Eucalyptus bicolor* bark extract with antibiotics in order to maximize the antimicrobial effect against bacteria and yeasts.

Materials and Methods

Sample of plant: *E.bicolor* bark were taken from local garden cleaned , dried for extract.

plant extract:

Fifty g of dried material was extracted by addition 250 ml of ethanol (70 %) using soxholet apparatus for two days. Next extract solution was filtered and the solvent was detached by rotary evaporator to get the crude extract. After that extract was saved in refrigerator at 4 °C for until use.

Microorganisms:

The examined microbial strains were obtained from microbiology laboratory Applied Science department University of Technology these involved *Candida albicans pseudomonas aerugenosa* and *staph aureus*.

Preparation of silver nanoparticles

Ten milliliter of *E.bicolor* bark extract was mixed with 90 mL of 0.01 mmol/mL aqueous $AgNO_3$ and showing to sun light for 1 h. A modification from yellowish to reddish brown color was detected.

Antibmicrobial activity tests:

The silver nanoparticles produced using *E. bicolor* bark extract was tested by agar well diffusion method against *C. albicans* (yeast), *P. aeruginosa* (gram negative) and *S. aureus* (gram positive). the pure cultures of the organism were subcultured on muller–hinton agar. every strain was spreaded on the plates by spreader. wells were made on plates by gel puncture. 50 µl of extract (10,20,40,60,80 and 80mg, concentration respectively) was transferred onto each well on all plates. disc diffusion method was used for evaluating the antimicrobial activity of antibiotic with ethanolic extract. antibiotic discs were applied to the inoculated MHA plates another collection of these antibiotic discs were soaked with ethanolic extract disc then located on the inoculated plates.plates was incubation at 37 °c for overnight at the end the diameter of inhibition zone was calculated in millimeter and The tests were done in triplicate.

Descriptions of Silver Nanoparticles

Produced nanoparticles were check by measuring the UV-visible spectroscopy, UV-Vis spectral analysis was determined by using (TechcompUV2300) spectrophotometer. The morphology of the nanoparticles was investigation by Scanning Electron Microscope (SEM).

Results and discussion

UV-visible spectrum analysis:

The *E.bicolor* bark extract was yellowish in colour , after addition of silver nitrate solution and showing to sunlight for 1 h turned reddish brown (Figure 1) . Creation of nano silver was established using UV-vis spectral analysis and displayed silver surface plasmon resonance band at 410 nm (Figure 2) .

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A **B**

Figure 1: synthesis of silver nanoparticles using *E.bicolor* bark extract

A- $AgNo_3$ with *Eucalyptus* bark extract

B- Colour changed after exposing to bright sunlight

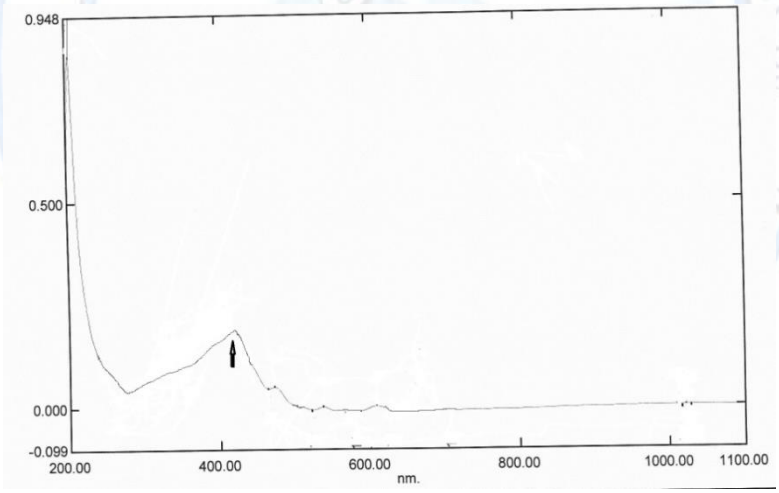

Figure 2: UV-visible spectrums of *E.bicolor* nano extract

Scanning Electron Microscopy (SEM).

The SEM description the size, shape of silver nanoparticles, the Fig: (3) shows spherical shape nanoparticles with diameter range about ≈ 87 nm.

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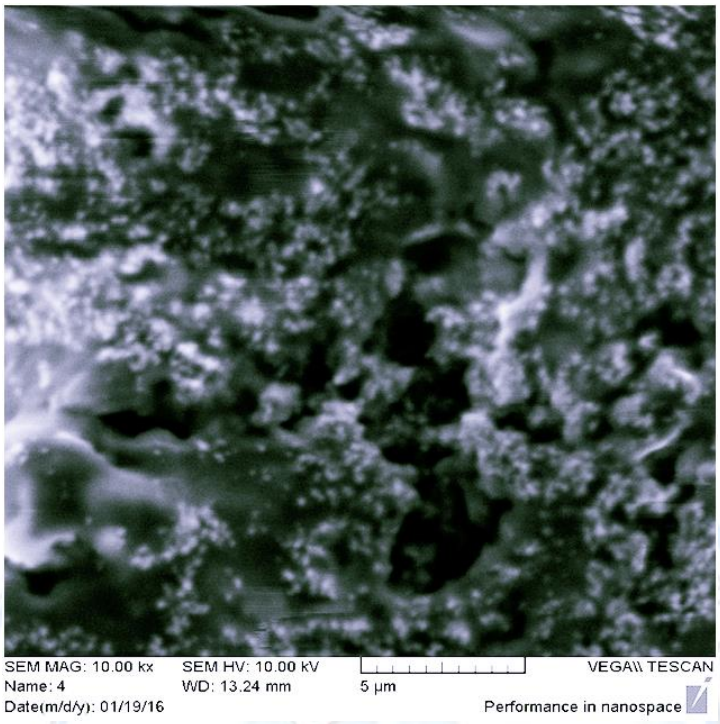

Figure 3: SEM image for synthesized silver nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR).

Fourier transform infrared spectroscopy is often used to characterize the beneficial groups. The FTIR exhibited the presence of bands at 3427.62 cm-1 as a result of phenolic OH. The band at 2360.95 cm-1 as a result of alkynes. The band at 1629.90 cm-1 as a result of C=N. The band at 1516.10 as a result of C=C. The band at 1203.62 cm-1 as a result of C-O Fig:(4) (10).

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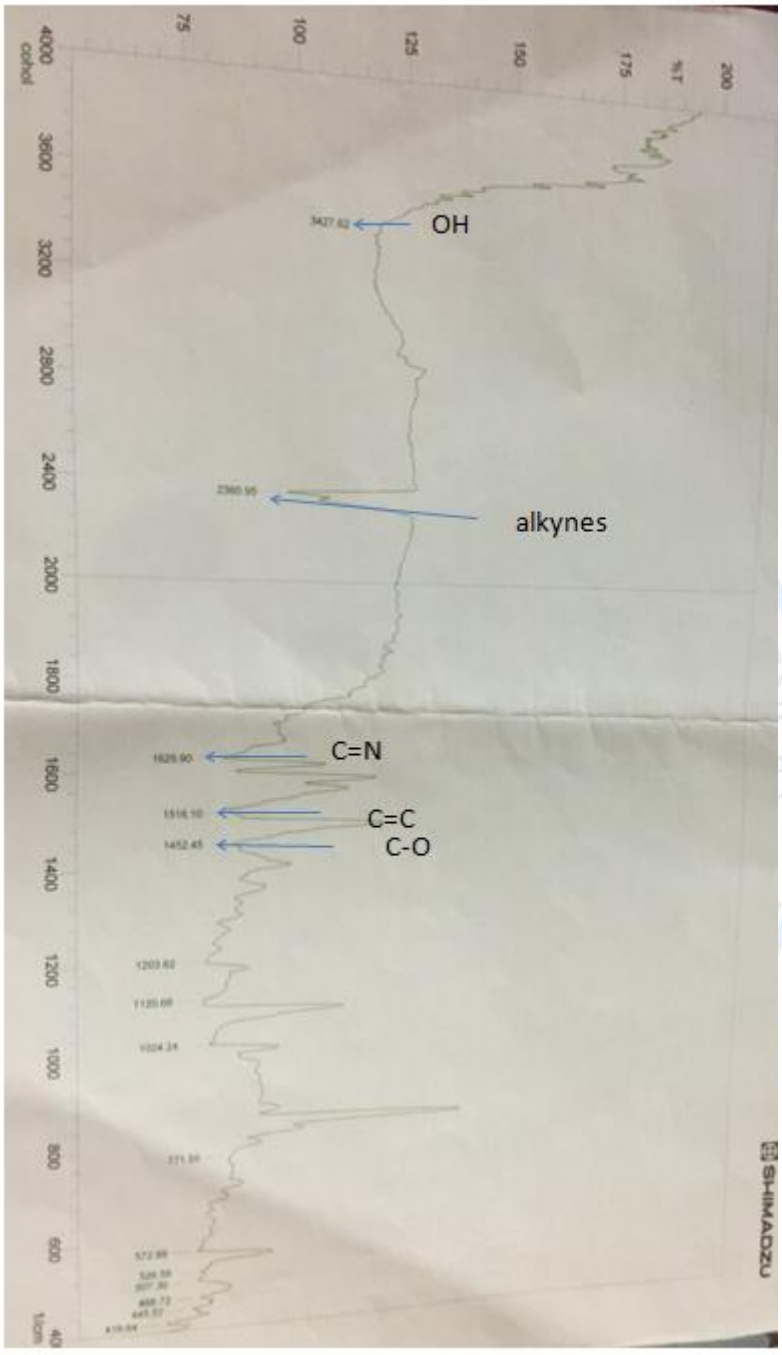

Figure 4: FTIR spectrum of *E.bicolor* extract

Antimicrobial test of an ethanolic and nano bark extract of *Eucalyptus bicolor*.

Antimicrobial activity of *E. bicolor* bark extract against three types of microorganisims was determined. Table1 displays the affect of ethanolic and nano extract of the bark of *E.bicolor* plant on bacteria G+ve , G-ve and yeast. The ethanolic extract gave the higher effect on the growth of yeast *C. albicans* by inhibition zone 14mm ,*S . aureus*.13mm and *p. aeroginosa* 10mm , While the nano extract has been exposed that the highest effect on growth of microorganism, *C. albicans* by inhibition zone 20mm, *S . aureus* 19mm and *p. aeroginosa* 11mm Fig (5) .

Table 1: Antimicrobial activity of the ethanolic and nano bark extract of *E.bicolor* Concentration mg mL-1

Microorganisims	Zone of inhibition (mm)	
	Ethanolic extract (80) mgmL-1	Nano extract (80) $mgmL^{-1}$
Candida albicans	14	20
Staphylococcus aureus	13	19
Pseudomonas aeruginosa	10	11

Table (1) showed the antimicrobial effect of both ethanolic and nano extract was more marked against bacteria G+ve than bacteria G-ve and nano extract exhibited good inhibition activity against *C. albicans*, that's may be because the differences of the cell wall structure between Gram positive and negative bacteria (11,12) . The mechanism of the antimicrobial effect of nanosilver remains unclear,but Some studies suggest that the great affinity of Ag+ to phosphorus and sulfur and affects on microorganisms (13). Because of the bacterial cell membrane containing abundance of sulfur- proteins Ag+ can react with sulfur-containing amino acids inside the cell membrane and affects bacterial cell viability (14,15). Ag^{+} can interact with phosphorus moieties in DNA and increases DNA mutation (15). as well as, nanosilver interacts with NADH dehydrogenase resulting in the separation of respiration from ATP synthesis(15).

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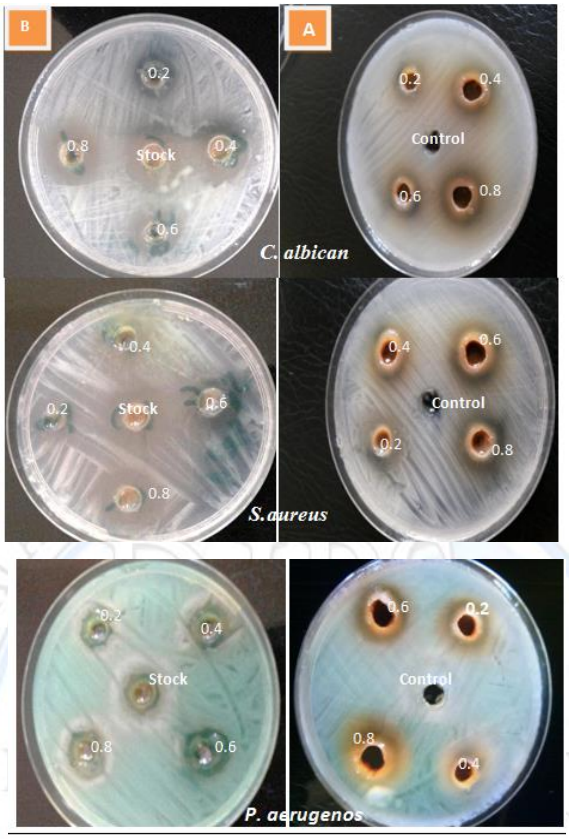

Figure 5: Inhibition zone effects of *Eucalyptus bicolor* bark of ethanolic extract and nanoextract.10,20,40,60,80 and 80mg per ml,concentration respectively, A :ethanolic extract B:nano extract.

Antimicrobial activity of antibiotics:

Eucalyptus bicolor ethanolic extract had antimicrobial activity on the bacteria and yeast when combined with different antibiotics. Disc diffusion method was used for evaluating the antimicrobial activity. Table (2) shows the inhibition zones diameter of antibiotics with and without *Eucalyptus bicolor* extract against bacteria (G ve+ ,G ve-)and yeast . The mixture of antibiotics with *Eucalyptus bicolor* extract had synergism and antagonism effect against microorganisim. Carpencilin with *Eucalyptus bicolor* extract exhibited an increase in its antimicrobial activity : *S.aureu*s from (7mm) to (15mm), *P.aerugenosa* from (nil) to (12). Amoxicillin when joined with *Eucalyptus bicolor* extract showed decrease in its antimicrobial against *S.aureu*s from (15) to (14), but it exhibited an increase against *P.aerugenosa* from (nil) to (10) Fig(6).

Table 2: Antimicrobial activity of antibiotics and combination of ethanolic extract of *Eucalyptu bicolor* with antibiotics

Microorganisms	Inhibition Zone (mm)			
	AM	AM+E	CAR	+E CAR
Staphylococcus aureus	15	14	7	15
Pseudomonas aeruginosa	nil	10	nil	12

E: extract AM: Amoxicillin, CAR: Carpencilin

Table 3: Percentage of synergistic/antagonistic effect of combination of *Eucalyptus bicolor* ethanolic extract with antibiotics

Microorganism	synergistic/antagonistic effect %	
	CAR	AMO
Staphylococcus aureus	+114.2	-6.6
Pseudomonas aeruginosa	+66.66	+100

+synergism, - antagonism AM: Amoxicillin, CAR: Carpencilin

Table (2) exhibited antimicrobial effect of antibiotics was more marked against gram-positive bacteria than gram-negative that's may be because the differences of the cell wall structure between Gram positive and negative bacteria (11,12). Mixture of *Eucalyptus bicolor* extract with antibiotics exhibited synergistic affect in most tests, table (2,3) The mechanism of the synergistic effect unclear, but, studies suggest that Plant antimicrobials not have a strong antimicrobial activity alone, but when they were taken with antibiotics they increase the effect of that antibiotics (16,17). *Eucalyptus bicolor* extract has the phenols, flavonoids, tannins and alkaloids. Alkaloids have ability association with bacterial DNA leading to destroy it, while phenol effect on microbial enzyme leading to missing their function (1,2). An antagonistic effect was seen in some of the tests, that's may be because of several compounds that inhibited the antimicrobial activity of the effective compounds as well as some compounds interfered with crude extract that may have affected on the act of one another therefore purification and separation, of the crude extract will increase in bioactivity than the crude extract (18,19,20).

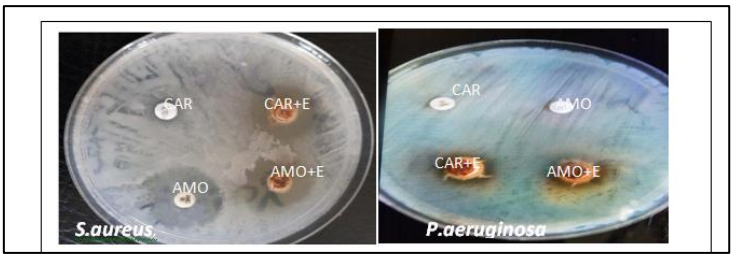

Figure 6: Combined effect of antibiotics and ethanolic extract of Eucalyptus *bicolor* selective pathogens. against some

E: extract AM: Amoxicillin, CAR: Carpencilin

Conclusion

The current study examined antimicrobial activities of silver nanoparticles with the *Eucalyptus bicolor* bark extract. Silver nano material showed antimicrobial activity against bacteria and yeast. The silver nanoparticle was established by its color changes of extract, UV-Visible spectroscopy and SEM. The FTIR was used to recognize active groups. the results got displayed the role of Eucalyptus *bicolor* extract in treatment of infectious diseases when combined with antibiotics.

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