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Evaluation of Antimicrobial Activities of silver Nanoparticles Biosynthesized by *Eucalyptus Critriodora* Leaves Extra

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Abstract:

The genus Acinetobacter currently contains 34 species, the vast majority of which are not regularly implicated in causing infection. However, incidences of hospital-acquired infection with Acinetobacter species are increasing, mainly due to the rise in the number of infections caused by the species Acinetobacter baumannii in immunocompromised patients, particularly in intensive care units (ICUs). One hundred fifty clinical isolates were collected from burn units and wounds of both genders attending a burn hospital in Medical City in Baghdad, Iraq. All collected isolates were cultured on Mac-Conkey agar, blood agar, and CHROMagar orientation medium for the diagnosis of Acinetobacter baumannii isolates based on morphological and biochemical characteristics. The Vitek system was also used to identify the isolates at the species level. Silver nanoparticles (AgNPs) prepared from the hot extracts of Eucalyptus, and the crystallinity of synthesized AgNPs was investigated by an X-ray diffraction (XRD) technique and corresponding XRD patterns . In this study, we evaluated the antimicrobial and anti-biofilm activity of AgNPs against X-ray diffraction (XRD) A. baumannii. The results of the anti-biofilm activity showed AgNPs exhibited inhibition or reduction in biofilm formation at concentrations of 150, 75, and 37.5 µg/ml .Also the results of antimicrobial activity in Minimal Inhibitory Concentration (MIC) exhibited by the Eucalyptus Critriodora/ Silver (EK AG) against tested A. baumannii showed the concentration 31.5.µg/ml was inhibiting the growth of A.baumannii isolates in percent of 50% (4/8), while concentrations 18.7µg/ml were inhibitory for isolates, whilst antimicrobial activity in MIC exhibited by the EK plant against tested A.baumannii the concentration 150.µg/ml was inhibiting the growth of

A.baumannii isolates in percent of 50% (4/8), while concentrations 75 µg/ml were inhibitory for isolates. In conclusion, the silver nanoparticles (AgNPs) have antimicrobial and antibiofilm activities against the (XDR) A. baumannii isolates under study.

Keywords: *Acinetobacter baumannii, silver nanoparticles (AgNPs), Eucalyptus Critriodora, X-ray Diffraction Analysis.*

Note: Wintachai, P., Paosen, S., Yupanqui, C. T. and Voravuthikunchai, S. P. 2019. Silver nanoparticles synthesized with Eucalyptus critriodora ethanol leaf extract stimulate antibacterial activity against clinically multidrug-resistant Acinetobacter baumannii isolated from pneumonia patients. Microbial pathogenesis, 126, 245-257.

1. Introduction:

The synthesis of noble metal nanoparticles, specifically Ag, Pt, Au, and Pd, has been the subject of extensive research in the past ten years due to their unique properties for use in drug delivery, photothermal therapy, pharmaceutical formulation, electronics, optics, environmental, and biotechnology applications (Miri, et al.,2015). The use of plants and microorganisms such as bacteria, fungi, algae, and the like in green synthesis techniques has garnered a lot of interest lately. Metal nanoparticles made from plant extracts (PE) have the greatest potential for large-scale, impurity-free synthesis of nanoparticles that reduce the need for dangerous solvents and additional agents and stabilizers in the development of environmentally friendly material synthesis technologies (Sadeghi and Gholamhoseinpoor, 2015). The primary benefits of extracting plants for the synthesis of silver nanoparticles are thought to be accessibility and safety, both of which can lower the concentration of silver ions. In the last ten years, silver nanoparticles have gained recognition in the medical community for their antibacterial (Ghiassi et al., 2018), and inhibitory effects on microorganisms, such as topical ointments to prevent from protecting against open wounds and burns (Atiyeh et al., 2007). One of the most problematic nosocomial infections in recent decades has been Acinetobacter baumannii, which has spread around the world. Its remarkable ability to acquire or upregulate multiple resistance determinants has greatly increased its therapeutic value, making it one of the most effective multidrug-resistant (MDR) organisms that threaten current antibiotic therapy (Ayoub, and Hammoudi 2020). Smooth, mucoid, grayish-white Acinetobacter

colonies may be seen on solid medium at 37 °C, including sheep blood agar and tryptic soy agar, particular focus on A. baumannii was sparked by the recent epidemic linked to US military actions in Iraq (Perez et al. 2010). Isolates of Acinetobacter baumannii that have been studied in hospitals tend to be resistant to a wide range of antimicrobial drugs and may live for very lengthy lengths of time outside of hospitals (Liakopoulos et al., 2012). The present recommendations for treating infections caused by A. baumannii are greatly impacted by this tendency towards resistance, which limits the chemotherapeutic choices available (Ayoub, and Hammoudi, 2020). As far as we are aware, no study has been carried out in Iraq to investigate the efficacy of silver nanoparticles against clinical isolates of Acinetobacter baumannii that has developed widespread resistance to drugs. The goal of this study was to determine if clinical isolates of Acinetobacter baumannii, which is extremely drugs resistant (XDR), were susceptible to the silver nanoparticles as antibacterial and antibiofilm inhibitors.

2. Materials and methods

2.1 Plant material and preparation of the extract

The Eucalyptus Critriodora leaves used in this study were obtained from Eucalyptus Critriodora trees which are one of the evergreen trees grown in Iraq. The green nanoparticles were then synthesized using Eucalyptus Critriodora leaf extract using a conical flask. The mixture was heated to 80 °C, boiled for one hour, and then cooled. Then 10 grams of chopped eucalyptus leaves were added to 100 milliliters of distilled water. The hot aqueous extract was filtered through a piece of gauze, then spun in a centrifuge at 4000 rpm for 15 min. It was then stored at 4 degrees Celsius until needed (Rabie et al. 2023).

The leaves of Eucalyptus citriodora, commonly known as lemon-scented eucalyptus, are collected as the plant material. The selection of leaves is critical, focusing on mature and healthy foliage free from physical damage or infections. The collection site is recorded, along with geographical coordinates, to ensure traceability and repeatability. After collection, the leaves are washed thoroughly under running tap water to remove dust and other contaminants, followed by rinsing with distilled water. This step is crucial to avoid interference from external particles during the extraction process.(Harborne,1998)

Preparation of the Extract:

The washed leaves are dried in a shaded area with adequate ventilation to prevent degradation of bioactive compounds by direct sunlight. Once dried, the leaves are ground into a fine powder using a mechanical grinder. The powdered plant material is stored in an airtight container in a cool, dry place until use.(Parekh, J., & Chanda, S,2007)

For the extraction, a specified amount of leaf powder (e.g., 50 grams) is added to a solvent like distilled water, ethanol, or methanol, depending on the desired compound extraction. The mixture is then subjected to a specific extraction method such as(Singleton, V. L., & Rossi, J. A,1965):

Cold maceration: The powdered leaves are soaked in the solvent at room temperature for 24–48 hours with occasional shaking.

Hot extraction: The mixture is heated using techniques like Soxhlet extraction or boiling at controlled temperatures to release bioactive components efficiently.

After extraction, the solution is filtered using Whatman No. 1 filter paper to remove particulate matter. The filtrate, which contains the bioactive compounds, is collected and stored at 4°C for further use in synthesizing silver nanoparticles. The yield of the extract is quantified, and its phytochemical composition may be analyzed to ensure the presence of key secondary metabolites like flavonoids, terpenoids, and phenolic compounds, which play a vital role in nanoparticle synthesis.(Zheng, Y., Wang, J., & Yu, H,2015)

Maintenance of cell cultures

A549 lung cell line was maintained in RPMI (Capricorn, Germany) supplemented with 10% fetal bovine serum (Capricorn, Germany), 100 µg/mL each of penicillin and streptomycin. Cells were passaged using trypsin-ethylenediaminetetraacetic acid (EDTA; Capricorn, Germany) and reseeded at 50% confluence twice a week, then incubated at 37°C .

2.2 Biosynthesis of silver nanoparticles

Sigma-Aldrich supplied the 99.9% pure silver nitrate solution that was made with Double Deionized water. The process of creating silver nanoparticles included adding 1 millilitre of each heated leaf extract to 10 millilitres of an $AgNO_3$ solution and stirring the mixture at 40 °C and 100 rpm for 30 minutes, or until a colour change occurred. The produced AgNPs were subjected to a 5-minute microwave treatment using a 400-watt power source (Rabie *et al.* 2023). Analyses including colour shift in mixes, (XRD), and Fourier Transformation Infrared (FTIR) were

used to characterise the silver NPs that were produced from the heated eucalyptus leaf extracts.

Biosynthesis Process:

The biosynthesis of silver nanoparticles (AgNPs) involves a green and eco-friendly approach using plant extracts as reducing and stabilizing agents. In this process, the bioactive compounds present in the plant extract, such as flavonoids, alkaloids, phenolic acids, and terpenoids, reduce silver ions (Ag^{+}) to elemental silver (Ag^{0}), leading to the formation of nanoparticles. The process consists of the following steps:

Preparation of the Silver Nitrate Solution:(Saifullah, Swami,ikram,2016)

A 1 mM solution of silver nitrate ($AgNO_3$) is prepared by dissolving a specific amount of silver nitrate salt in deionized or distilled water. This solution acts as the precursor for nanoparticle synthesis.

Mixing the Extract with the Silver Nitrate Solution:

The prepared plant extract is added dropwise to the silver nitrate solution in varying ratios (e.g., 1:1 or 1:2) under constant stirring at room temperature. The reaction mixture is kept under ambient conditions or mild heating (e.g., 50–60°C) to accelerate the synthesis process.(, Chisti, Y., & Banerjee,2013)

Observation of Color Change:

The formation of silver nanoparticles is visually confirmed by a change in the color of the reaction mixture from light yellow to brown, which indicates the reduction of silver ions. The intensity of the color correlates with the concentration of nanoparticles.(Iravani,2011)

Characterization of Nanoparticles:

The synthesized nanoparticles are analyzed using techniques such as:

UV-Vis Spectroscopy: To confirm the Surface Plasmon Resonance (SPR) peak, which typically appears between 400–450 nm for silver nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR): To identify functional groups involved in the reduction and stabilization process.

Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM): To study the size, shape, and morphology of the nanoparticles.

X-ray Diffraction (XRD): To confirm the crystalline nature of the nanoparticles.

Purification of Nanoparticles:

The reaction mixture is centrifuged at high speeds (e.g., 10,000 rpm for 20 minutes) to collect the nanoparticles. The pellet is washed several times with distilled water or ethanol to remove any unreacted biomolecules or impurities.

Storage of Nanoparticles:

The purified nanoparticles are resuspended in deionized water and stored at 4°C for further applications.(Song, J. Y., & Kim, B. S,2009)

Significance:

This method is eco-friendly, cost-effective, and avoids the use of toxic chemicals. The bio-reduction process is driven by natural plant metabolites, making it sustainable and suitable for various biomedical and environmental applications.

2.3 Antibacterial activity

2.3.1 Agar well diffusion method

In order to determine the antibacterial activity of the eukalyptus extract, the current work followed the agar well diffusion test, which was established by Gonelimali *et al.* (2018). A 400 mg/ml stock solution of the extract powder was made in a tube by dissolving it in distilled water and filtering the mixture through a 0.22 Millipore filter. This whole process took around five minutes. The solvent served as the negative control.Isolate of bacteria, cultured at a young age. To make the inoculum, we transferred 3 to 5 colonies that had grown on agar plates to 5 ml of normal saline in flat tubes. After that, we kept the tubes in an incubator set at 37 ° C for 2 hours, or until a turbid growth with 1.5×10 8 CFU/ml developed, as measured by the McFarland standard tube number 0.5. A fresh, single-use swab was used to transfer the inoculum from the bacterial solution to Petri dishes. Following the manufacturer's directions, a 4 mm thick Muller-Hinton agar medium was applied to the plates. Three times at a 60° angle, the plates were turned after each application. In the end, the agar was uniformly covered with the swab. They let the plates air dry for a while after that. The next step involves aseptically punching a 6 mm diameter well in the Petri-dishes with a sterile cork borer. Afterwards, the well was filled with about 20 µl of the plant extract solution and left to incubate at 37 ° C for 18 to 24 hours. In order to measure the inhibitory zones' diameter in millimetres, the results were read using a digital vernier calliper. Numerous screening studies have been established over the past years for discovering new antibiotic or cytotoxic metabolites from different microalgae especially green algae and cyanobacteria , Many previous studies have utilized

Chlorella algae as a natural source for the reduction of various metal oxides, exploring the potential of these nanoparticles as inhibitors of different cancerous tumors. However, no previous study had addressed the reduction of bismuth oxide by the studied algae and the application of the resulting mixture on the A549 cell line. In a study presented by Hamouda et al, Chlorella was successfully used to synthesize Au/cellulose nanocomposite. It was found that this nanocombination enhanced anticancer activity on A549 cells with an ICso value of 4.67±0.17µg/µL. The suggested mechanism was signaling pathway regulation of the mitogen-activated protein kinases (MAPK). Furthermore, there was an increase in the relative expression of the tumor suppressor gene p53 compared to control cells. In another study, Chlorella algae were used to produce SnO2 nanoparticles, which demonstrated inhibitory effects on A549 cells with an LD50 value of 188 µg/mL after 24 hours of incubation .

2.3.2 Minimal ınhibitory concentration (MIC) assay

Each well had 100 µl of the diluted antimicrobial agents before 100 µl of the standardised bacterial suspension with a concentration of 1 x 108 cfu/ml was added, bringing the total volume of each well to 200 µl. Microplate column no. 11, which included broth, antimicrobial agent solvent, and bacterial inoculation, was used as a positive control to determine the sensitivity of the microbiological isolates. The negative control, which did not include inoculum, was found in column 12 of the microplate and comprised of broth and antibacterial agent solution. next this, the next day, the microtiter plates were allowed to incubate at 37 ºC for 18–24 hours.Pipetting 30 ml of Alamar blue dye into the wells of the microplates allowed for a visual evaluation of the MIC values after an hour of incubation at 37 ºC. Because the solution included resazurin, an indicator known as alamar blue dye was used. Indicative of cell health, this solution measures viability by reducing the power of living cells. Résazurin, the active ingredient in Alamar Blue Reagent, is a non-toxic, blue chemical that can pass through cells but emits very little light. The formation of resorufin, a molecule that is both very bright and red, occurs when resazurin is reduced upon entry into living cells. At least two separate measurements were taken to validate the activity of the minimum inhibitory concentration (MIC) values. These values were recorded as the lowest concentration of each fraction that did not exhibit any discernible increase.(Andrews, J. M,2001)

The Minimal Inhibitory Concentration (MIC) assay is a quantitative method used to determine the lowest concentration of an antimicrobial agent that completely inhibits the visible growth of a microorganism. This test is widely used to evaluate the antimicrobial efficacy of natural extracts, nanoparticles, and synthetic compounds.

Procedure:A serial dilution of the test compound is prepared in a suitable growth medium, typically in 96-well microtiter plates.(Hilpert, K., & Hancock, R. E,2008)

A standardized microbial inoculum (e.g., 1×10^5 CFU/mL) is added to each well.

The plates are incubated under optimal conditions for the test organism (e.g., 24 hours at 37°C for bacteria.

The MIC is determined as the lowest concentration of the test agent where no visible turbidity (growth) is observed.(Sadiki, M., & Ibnsouda, S. K,2016)

The results can be confirmed by adding a viability indicator, such as resazurin or tetrazolium salts, which changes color in the presence of live cells.

Applications:

The MIC assay is crucial in antimicrobial research to evaluate the potency of antibiotics, nanoparticles, or plant extracts and to monitor resistance patterns in microorganisms.

3. Results and Discussion

3.1 X-ray Diffraction Analysis (XRD) of silver nanoparticles

The X-ray diffraction (XRD) method was used to examine the crystallinity of the AgNPs that were synthesised; Figure (1), displays the usual (XRD) pattern of the as-prepared silver NPs. The face-centered cubic (fcc) structure can be used to index all of the peaks in (XRD) pattern. (XRD) pattern shows the presence of diffraction peaks corresponding to (111), (200),(220), and (311) planes. When compared to bulk silver, the diffraction patterns of as-prepared silver are clearly broadened, indicating the formation of silver nanoparticles. These peaks are matched with (fcc) structure of silver (JCPDS file No. 04-0783).

Sample Preparation:

The synthesized silver nanoparticles are dried to form a powder, which is then mounted onto an XRD sample holder or glass slide.(B. D., & Stock, S. R,2001)

X-Ray Diffraction Measurement:

displays the usual XRD pattern of the as-prepared silver NPs. The face-centered cubic (fcc) structure can be used to index all of the peaks in the XRD pattern. XRD pattern shows the presence of diffraction peaks corresponding to (111), (200),(220), and (311) planes. When compared to bulk silver, the diffraction patterns of as-prepared silver are clearly broadened, indicating theformation of silver nanoparticles. These peaks are matched with (fcc) structure of silver (JCPDS file No. 04-0783).

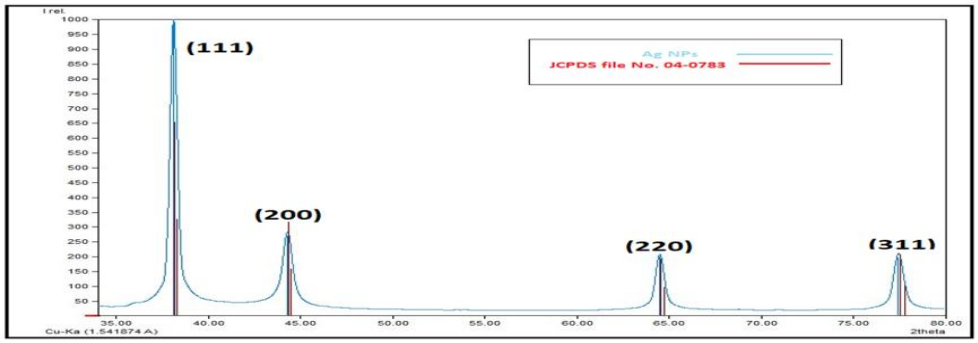

Figure (1): X-ray diffraction (XRD) analysis of Silver nanoparticles

3.2 FTIR spectroscopy of silver nanoparticles

Fourier Transformation Infrared (FTIR) examined the chemical composition and characteristics of silver nanoparticles. The sample's FT-IR spectrum is displayed in Figure 4.17. Where (N-H) stretching and bending vibrations are represented by the peaks at 3401 cm-1 and 1624 cm-1, respectively. While the peak at 2927 cm-1 due to C–H bonds, the stretching vibrations bonds of O–H appears at 2372 and 3779 cm-1.Two peaks, 1381 and 1066 cm-1correspond to C–O stretching was also observed. Finally, the FTIR spectrum of silver nanoparticles exhibited prominent peak at 802 cm-1. (Marimuthu et al.2011).

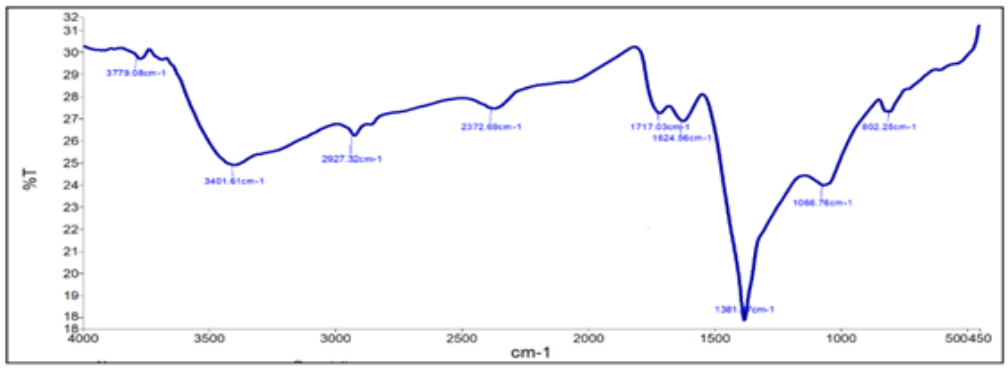

Figure (2): The Fourier Transformation Infrared (FTIR) spectrum of Agnanoparticles

3.3 Gas Chromatographymass Spectrophotometric (GC-MS) Analysis aqueous Eucalyptus extracts

The bioactive components in the aqueous Eucalyptus extracts were analysed using GC-MS. Figure (3) and Table (1) show the mass spectra of the aqueous extracts.

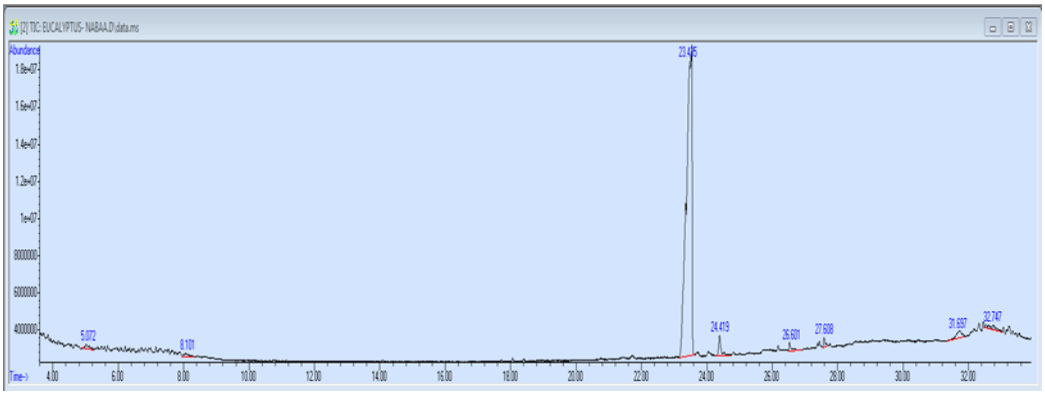

Figure (3):Total ion chromatogram of compounds in aqueous extracts from the Eucalyptus

Table (1): Analysis of bioactive components of Eucalyptus

Pk	RT(min)	Compounds	Molecular formula	M. wt (g/mol)	Area %
1	5.075	n-Hexane	C6H14	86.17	0.91
2	8.105	Pentane,3-ethyl-2,2 dimethyl	C9H20	128.25	0.81
3	23.433	Bis(2-ethylhexyl) phthalate	C24H38O4	390.5	90.23
4	24.420	Diisooctyl phthalate	C24H38O4	390.6	2.61
5	26.601	2Trifluoroacetoxypentadecane	C17H31F3O2	324.4	0.86
6	27.605	1H-Indole, 1-methyl-2-phenyl	C15H13N	207.276	0.74
7	31.699	Tetradecanoic acid, dodecyl ester	C26H52O2	396.68	2.06
8	32.746	Octane, 2,2,6-trimethyl	C11H24	56.30	1.77

Bis (2-ethylhexyl) pht-halate, this compound has a role as an apoptosis in-hibitor, an androstane receptor agonist and a plasticiser. Moreover, it has an anti-leukaemic, anti-mutagenic, antimicrobial, and cytotoxic activity (Habibet al.2009, Lee et al.2000).

Based on previous studies, thecompound Bis (2-Ethylhexyl) phthalate authenticated as potent effector on enteric gram negative bacterial infections. Herwin et al (2020).Purified this compound from leaves of Colocaciaesculenta L by TLC-Bio autography . And experimented the fraction against several bacteria, includingEscherichia coli, Pseudomonas aeruginosa, Salmonella thypi, Streptococcus mutans, and Vibriocholerae, by diffusion Agar method, the largest inhibition zone recorded on Salmonellathypi bacteria. Other researches such as(Habib and Karim,2009; Lee et al. 2010),demonstrated that the compound has a role as an apoptosis in-hibitor, anandrostane receptor agonist and a plasticiser.

Moreover, it has an anti-leukaemic, anti-mutagenic, antimicrobial, and cytotoxic activity.

Exploration of some active phytochemicals in the algal extract

The presence or absence of the active phyto-components that has been displayed in Table 1 have many active phyto-chemicals in the extract of C. vulgaris; these phytochemi-cals support the creation process of NPs. The enhancement activity discussed by , by which these phytochemicals act as reducing and capping factors for metal oxide as well as stabilize the created nanoparticles by avoiding agglomeration. Within the cell components of C. vulgaris, lipids are made up of different cellular in-gredients, mainly glycolipids, and hydrocarbons. Besides lipids, proteins occupy half of the dry weight within the fully grown algal cells. Furthermore, carbohydrates such as cellulose and starch exist as crucial structural compo-nents in C. vulgaris. All these components are suggested to play an essential role in both reduction and stabilization along with AgNPs preparation .

Extraction Methods:Hydrodistillation: Used for essential oil extraction, yielding compounds like citronellal, eucalyptol, and limonene.(H. J. D., & Deans, S. G,2000)

Solvent Extraction: Ethanol, methanol, or water is used to extract polar and non-polar phytochemicals.(B., & Singh, S,2010)

Analytical Techniques:Gas Chromatography-Mass Spectrometry (GC-MS): Identifies volatile compounds in essential oils.(Aslam, F., & Javed, A,2015)

High-Performance Liquid Chromatography (HPLC): Quantifies phenolic and flavonoid content.

Fourier Transform Infrared Spectroscopy (FTIR): Determines functional groups in bioactive molecules.

Total Phenolic and Flavonoid Content Assays: Use colorimetric methods to measure overall levels of these compounds.(Harborne, J. B,1998)

Significance:The bioactive components of Eucalyptus are known for their antibacterial, antifungal, anti-inflammatory, and antioxidant properties, making them valuable for pharmaceutical and environmental applications.

3.3. Antimicrobial activities of AgNPs and extract

3.3.1 Antibacterial Activity by Agar Well Diffusion Method

Antibacterial activity of *Eucalyptus Critriodora* revealed a maximum zone of inhibition, 22.15mm at 2.4 mg/mL, 17.45mm at 1.2 mg/mL, 14.02 mm at 0.6 mg/mL and12.49 mm at 0.3 mg/mL by agar well diffusion method as shown in Figure (4).

Figure (4): Agar well diffusion method of Eucalyptus Critriodora agiants. A. Baumannii

Table (2) Agar-well diffusion test results of Eucalyptus Critriodora agiants A. Baumannii

Isolate No.	Eucalyptus Critriodora extract concentration mg/ml	maximum zone (mm)
1	2.4 1.2 0.6 0.3	22.15 17.45 14.02 12.49
2	2.4 1.2 0.6 0.3	23.44 17.35 13.53 11.67
3	2.4 1.2 0.6 0.3	25.12 18.85 13.12 12.17

4	2.4 1.2 0.6 0.3	24.33 19.10 15.77 13.42
5	2.4 1.2 0.6 0.3	23.65 17.73 14.29 11.87
6	2.4 1.2 0.6 0.3	22.18 20.01 17.01 13.86
7	2.4 1.2 0.6 0.3	24.22 21.18 16.01 12.13
8	2.4 1.2 0.6 0.3	23.12 19.65 17.56 14.44

3.3.2 Anti- Biofilm Activity of AgNPs

The Anti-biofilm effect of AgNPs against (XDR) *A. baumannii* isolates (4 isolates were strong biofilm producer and 4 were moderate biofilm producer) as seen in Table (3), and Figure (5). Overall, AgNPs exhibited inhibition/reduction in biofilm formation at the concentration of 150, 75, 37.5 µg/ml. One study found that AgNPs have a capacity to prevent the formation of biofilms of *Pseudomonas aeruginosa* and *Escherichia coli* (Fernandes *et al*. 2023).

Table (3): The anti-biofilm effect of Ag against (XRD) A. Baumannii

Bacterial isolate	MICµg/ml
1	75
2	75
3	75
4	150
5	150
6	300
7	37.5
8	37.5

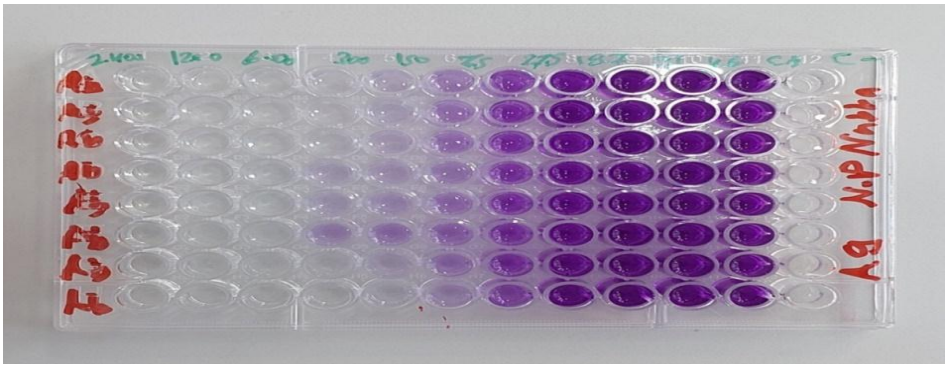

Figure (5): The Anti-biofilm effect of Ag against (XDR) A. Baumannii

4. Determination of Minimum Inhibitory Concentrations (MIC) of EK/Ag (silver), and Eucalyptus (EK Plant) Extract

The antimicrobial activity of EK/Agwere testedby broth micro-dilution method to determination minimum inhibitory concentrations (MIC) against eight (XDR) *A.baumannii*isolates and the results were explained in Figure (6), MICs of plant extract ranged at (31.5- 18.7 µg/ml). The concentration 31.5 µg/ml was inhibiting the growth of (XDR) *A. baumannii*isolates in percentage of 50% (4/8), while concentrations18.7µg/ml were inhibitory for isolates.

MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. MBC determinations are undertaken less frequently and their major use has been reserved for isolates from the blood of patients with endocarditis. Standardized

methods for determining MICs and MBCs are described in this paper. Like all standardized procedures, the method must be adhered to and may not be adapted by the user. The method gives information on the storage of standard antibiotic powder, preparation of stock antibiotic solutions, media, preparation of inocula, incubation conditions, and reading and interpretation of results.(Andrews, J. M. ,2001)

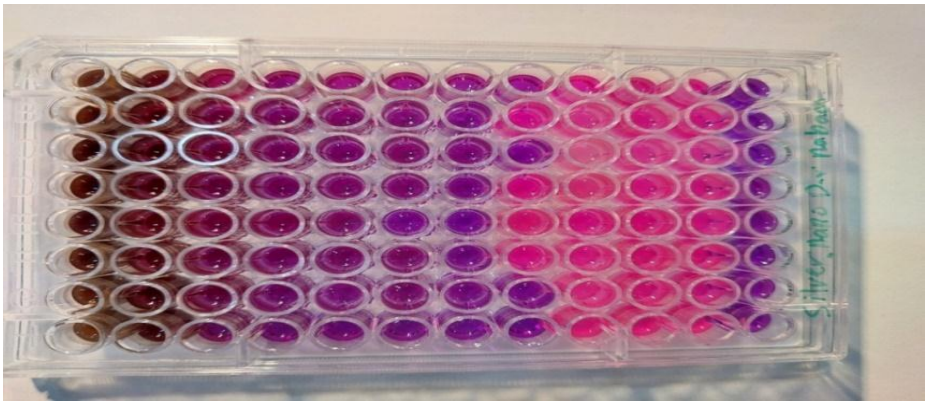

Figure (6): The results of broth microdilution method to MIC values of EK/Ag against XDR A. baumannii. (C-)" Negative control (only broth & solvent), (C+)" Positive control (only bacteria, broth & solvent), Wells with Blue color had no or inhibited growth, but, Wells with red color with growth.

The antimicrobial activity of EK were testedby broth micro-dilution method to determination minimum inhibitory concentrations (MIC) against eight (XDR) *A.baumannii* isolates and the results were explained in Figure (7), MICs of plant extract ranged at (150- 75 µg/ml). The concentration 150 µg/ml was inhibiting the growth of *A. Baumannii* isolates in percentage of 50% (4/8), while concentrations75 µg/ml were inhibitory for isolates .

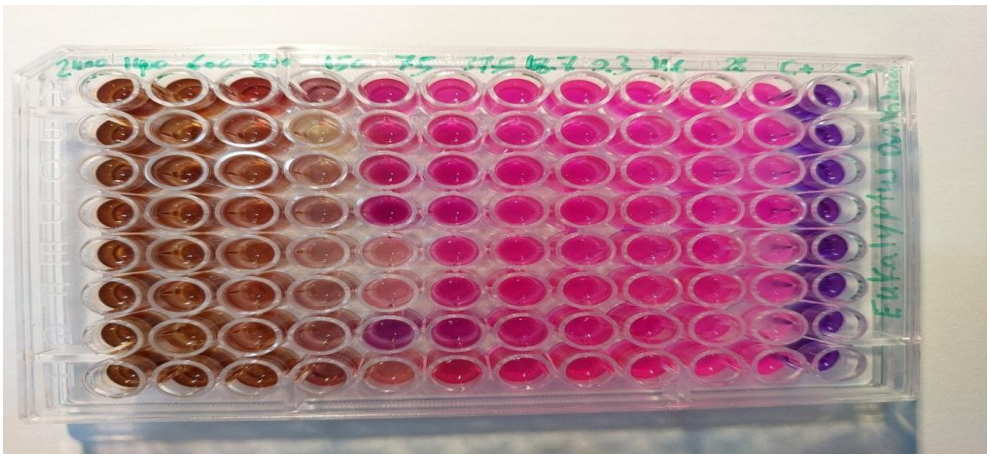

Figure (7): The results of broth microdilution method to determination minimum inhibitory concentrations (MIC) values of *EK plant extract* against XDR *A.baumannii*. (C-)" Negative control (only broth & solvent), (C+)" Positive control (only bacteria, broth & solvent), Wells with Blue color had no or inhibited growth, but, Wells with red color with growth

The high minimum inhibitory concentrations (MICs) required to prevent the development of *Acinetobacter baumanniii* isolates suggest that these bacteria may have been very resistant and caused infections in the individuals included in this investigation. Our findings are in agreement with those of a study by Nowak *et al.*, (2021) that found nano-silver to be significantly more effective against microbes than ionic silver. This is due, in part, to nano-silver's capacity to release silver ions, which can bind to various components of bacterial cells, including their DNA, ribosomes, proteins, and plasma membranes. Size, shape, and surface-to-volume ratio are factors in nano-antimicrobial metals' actions. The mechanics of diffusion in the growth medium are also important considerations. The ion release is one manner in which nano-metals affect microbes, as previously mentioned.

According to Wintachai *et al.*, (2019), silver nanoparticles made with *Eucalyptus Critriodora* leaf extract inhibited (XDR) *A. baumannii* DNA condensation, caused bacterial cell death, and significantly affected biofilm formation, biofilm-grown cells, bacterial attachment, and invasion of human lung cells in a concentration-dependent manner. Our study confirms that silver nanoparticles with *Eucalyptus Critriodora* leaf extract could be a promising anti-

(XDR) *A. baumannii* agent for treatment, and they open the door to the development of numerous other effective biomedical applications**.**

Conclusion

The results of this study showed that A. baumannii poses a significant challenge in clinical settings, as it is resistant to multiple antibiotics. The high proportion of extensively drug-resistant (XDR) isolates further complicates treatment options and highlights the urgent need for alternative strategies to combat this pathogen. The findings indicate that A. baumannii exhibits extremely high resistance to β-lactam antibiotics, including those in the penicillin class and all generations of cephalosporins studied. The lower resistance rate observed with carbenicillin suggests that this antibiotic may still be effective in some cases. In our study demonstrates that EK/Ag (silver) with Eucalyptus Critriodora leaf extract shows promise as an effective agent against multi-drug resistant (MDR) A. baumannii. This suggests that it may have potential as a treatment option for A. baumannii infections. Additionally, the findings indicate that EK/Ag with Eucalyptus Critriodora leaf extract may have broader applications in biomedical settings.

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تقييم الانشطة المضادة للميكروبات لجزيئات الفضة النانوية المصنعة بيولوجيا من اوراق نبات الكالبتوس Eucalyptus Critriodora

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مستخلص البحث:
حظيت جسيمات الفضة النانوية (AgNPs) باهتمام كبير نظرا لخصائصها المضادة للميكروبات وتطبيقاتها المحتملة في المجالات الطبية الحيوية والبيئية. تستكشف هذه الدراسة التخليق الحيوي لجسيمات الفضة النانوية باستخدام مستخلص أوراق اليوكالبتوس كعامل طبيعي مختزل ومثبط . يوفر نهج التوليف الأخضر بديلا صديقا للبيئة وفعالا من حيث التكلفة وغير سام للطرق الكيميائية التقليدية. تم تمييز الجسيمات عدة تحاليل لتأكيد تكوينها واستقرارها ومورفولوجيتها وطبيعتها البلورية. تم تقييم النشاط المضاد للميكروبات للمادة مقابل مجموعة من السلالات البكتيرية إيجابية الجرام (المكورات العنقودية ذهبية ، العصية الرقيقة) وسالبة الجرام (الإشريكية القولونية ، الزائفة الزنجارية) ، وكذلك مسببات الأمراض الفطرية (المبيضات البيضاء وفطر اسبرجيلس نيجر). أظهرت النتائج تأثيرات مثبطة كبيرة للعينات الراكدة البومانية التي تم جمعها من مستشفى مدينة الطب وعلى الاوساط الزرعية مثل الكروم والمكاونكي اكار. تعزى الآلية المضادة للميكروبات إلى الإجهاد التأكسدي الناجم عن الجسيمات النانوية ، وتعطيل الأغشية الميكروبية ، والتداخل مع العمليات الخلوية. كشف التحليل المقارن مع المضادات الحيوية التقليدية عن كفاءة معززة في ابادة الجراثيم ، مما يسلط الضوء على إمكانية جسيمات الفضة النانوية المركبة بيولوجيا كعوامل جديدة مضادة للميكروبات. تشير النتائج إلى أن الجسيمات النانوية المخلقة طبيعيا تدخل في تطبيقات المستحضرات الصيدلانية وضمادات الجروح وأنظمة تنقية المياه. يوصى بإجراء مزيد من الدراسات حول السمية الخلوية والتوافق الحيوي لضمان استخدامها العلاجي الآمن.

الكلمات المفتاحية :البكتريا الراكدة البومانية ,جسيمات الفضة النانوية ,مستخلص اوراق الكالبتوس ,فحص حيود الاشعة السيني

ملاحظة : هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه ؟ نعم : √