

Comparison in Synergetic Effect for Silver Nanoparticles that Produced from *P.aeruginosa* and *P.luteola* with Oil Extract of *Cymbopogon Citraus* on Different Types of Pathogenic Bacteria

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Abstract

Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties. In addition, many reports referred to the antibacterial activity of lemongrass oil against different organisms. Two species of Gram negative bacteria *Pseudomonas* (*P.aeruginosa* and *P.luteola*) were tested for the ability of bearing silver nanoparticles by using LB medium, the two isolates of bacteria were appeared brown color when mixed the supernatant of bacterial culture with AgNO₃ solution, that referred the biosynthesis of Silver nanoparticles (Ag NPs). UV-visible spectrophotometer was utilized for estimation of (Ag NPs). The antimicrobial activity of silver nanoparticles arranged from the two isolates was tested against different types of pathogenic bacteria. Lemongrass oil was extracted and three different concentrations (v/v) of lemongrass oil with dimethyl sulfoxide (DMSO) 0.5%, 5% and 10%, were prepared to determine the MIC by using well diffusion method. The synergetic effect was demonstrated by combining the (Ag NPs) that produced from, *Pseudomonas aeruginosa* and *Pseudomonas luteola* with MIC of lemongrass oil (0.5%) against same types of clinical bacteria.

Keywords: *Pseudomonas luteola*, *Cymbopogon citraus*, LB medium

Introduction

Human beings are often infected by different microorganisms like bacteria, molds, yeasts, and viruses, present in their living environments, the development of resistant or even multi-drug resistant pathogens has become a major problem⁽¹⁾. Nanotechnology refers to an emerging field of science that includes synthesis and development of various nanomaterials⁽²⁾. Recently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver⁽³⁾. Silver ions and their derivative compounds are highly toxic to microorganisms.⁽³⁾ Silver is a safe inorganic antibacterial agent used for centuries and it has the ability of killing different types of diseases causing microorganisms⁽⁴⁾. Additionally, when compared with

other metals, silver presents the lowest toxicity for animal cells⁽⁵⁾. Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties that can promote faster wound healing.⁽⁶⁾ Biological synthesis of nanoparticles in spot light due to the fact that it is eco-friendly as compared to other methods of nanoparticle synthesis⁽⁷⁾. Nanoparticles have been successfully synthesized from bacteria, actinomycetes, algae, sugar, plants, and from many more substrates⁽⁸⁾. Lemon grass (*Cymbopogon Citraus*) is a native aromatic tall, it is a perennial herb widely cultivated in the tropics and sub-tropics, is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine⁽⁹⁾. Many reports referred to the antibacterial activity of lemongrass oil against different organisms comprising gram positive and gram negative bacteria, yeast and fungi⁽¹⁰⁾. In this work, (Ag NPs) were synthesized by two types of bacteria, *Pseudomonas aeruginosa* and *Pseudomonas luteola*. The antibacterial activity of

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synthesized silver nanoparticles with Lemon grass oil extract on different kinds of bacteria was explored.

Materials and Method

Organisms and Lemon grass oil extraction

Two species of *Pseudomonas* (*Pseudomonas aeruginosa* and *Pseudomonas luteola*) were used in this work. They were cultivated on brain heart infusion broth for 24h at 37 °C, then purified. For extraction of lemongrass oil , 25 g of lemongrass was weighted and cut it into small pieces. 300 ml of distilled water was added to the flask containing the grass and set the apparatus for distillation. The mixture was boiled and collected the distillate until no more oily drops can be seen passing over. The distillate was extracted with hexane, dried them at 45° C, yellow colored oily liquid with fresh lemon like tone was obtained ⁽⁹⁾.

Biosynthesis and characterization of silver nanoparticles

Pseudomonas aeruginosa and *Pseudomonas luteola* were cultured in LB medium. The culture flasks were incubated on shaker at 37°C and agitated at 200 rpm for 24 hours , then harvested and centrifuged at 10000 rpm for 10 minutes. The supernatant was collected for silver nanoparticles biosynthesis , supernatant (10 ml) was mixed with 5ml of silver nitrate solution (10 mM) and incubated at 30°C for 24 h in dark ⁽⁴⁾. The silver nanoparticles (AgNPs) were purified by centrifugation at 10,000 rpm for 5 min twice, and collected for further using ⁽¹¹⁾. (Ag NPs) was estimated by using UV–visible spectrophotometer (300 -900) nm , the colored nanoparticle solution shows a peak ~400 nm.

MIC Determination of lemongrass oil and antibacterial action of (Ag NPs)

Lemongrass oil MIC and the antibacterial activity of (Ag NPs) prepared from *P.aeruginosa* and *P.luteola* were calculated as procedure of well diffusion method^(12, 13) .Three different concentrations (v/v) of lemongrass oil with dimethyl sulfoxide (DMSO) 0.5%, 5% and 10%, were prepared⁽¹⁰⁾ , different types of bacteria including

Staph. aureus, *Enterococcus faecalis*, *Strep.agalactiae*, *Klebsiella pneumoniae*, *E. coli*, *Acineto.bomannii* (clinical isolates) were used in this work. The plates were incubated for 24 h , the diameters of inhibition zones for the Lemongrass concentrations and (Ag NPs) extract when used alone and together were measured.

Results and Discussion

Producing organisms

Among the noble metals, silver is the best choice metal in the field of biological system ⁽⁴⁾. Selection of eco-friendly nontoxic agents are the most important issues which must be considered in green synthesis of nanoparticles⁽⁷⁾. Two species of genus *Pseudomonas* (*P.aeruginosa* and *P.luteola*) were obtained from Department of Biology\ College of Science\ Unversity of Baghdad used in this work after purification on MacCkonkey agar and Nutrient agar. Bioproduction of (Ag NPs) was investigated by mixing the bacterial supernatant with (AgNo3) solution. The two bacterial isolates showed the brown 3reported in another organisms like *Bacillus methylotrophicus* ⁽²⁾ and in fungi as *Fusarium semitectum*, *Aspergillus fumigatus* ⁽¹⁴⁾ .

Estimation of silver nanoparticles

The biosynthesis of silver nanoparticles for the two bacterial isolates was done by UV.Spectrophotometer at a wavelength range of 200-900 nm. This method has been appeared to be appropriat sensitive way to check the intense surface plasmon resonances of (Ag NPs) in the range of waves 300–600 nm⁽²⁾. The results appeared that high absorption peak at 371 nm was observed from *P. aeruginosa* while *P.luteola* perceived the high absorption peak at 369 nm. Wavelengths 300 – 800 nm are generally used for characterizing metallic nanoparticles ranging in size from 2 nm up to around 100 nm ⁽⁶⁾. The exposed to the AgNo3 solution exhibit the wide spectrum range 390 to 410 nm. The presence of the broad resonance refers the aggregation of the silver nanoparticles in the solution , 370 nm indicated to the transverse plasmon vibration in silver nanoparticles ⁽¹⁵⁾.

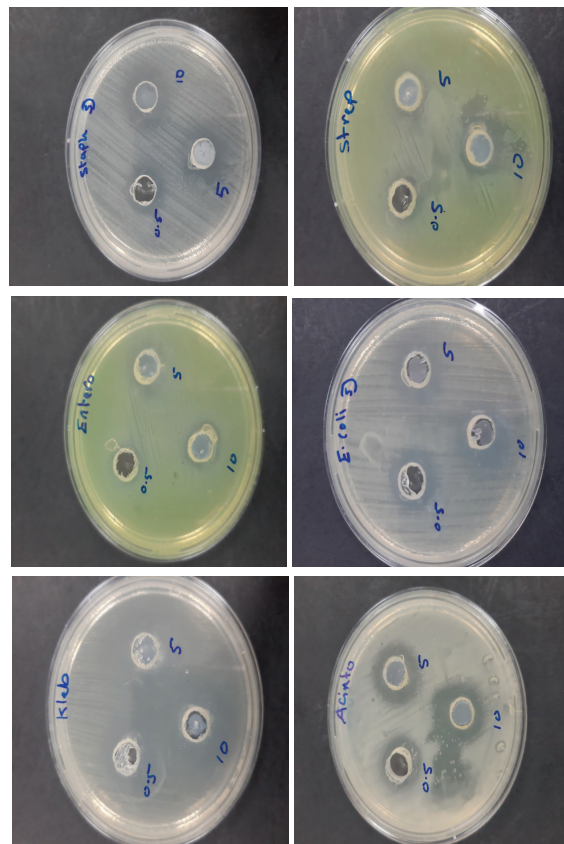
MIC of Lemongrass oil

Treatment using plant-based medicine appears to be an alternative approach because of the adverse effects associated with the use of synthetic drugs ⁽⁹⁾. The MIC of lemongrass oil was determined against different types of pathogenic bacteria. The MIC for all bacterial

isolates was 0.5% (Figure 1 , Table 1) . lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, scabies, flatulence, headaches, blood circulation problems and excessive perspiration due to its antimicrobial and antibacterial activities. It has also been used as carminative, stimulant, emmenagogue, diuretic and antiseptic ⁽¹⁶⁾

Table (1) : The MIC of lemongrass oil on different clinical bacteria

Bacteria	0.5%	5%	10%
<i>Staph.aureus</i>	14 mm	16 mm	18 mm
<i>Strep.agalactiae</i>	12 mm	14 mm	18 mm
<i>Entero.faecalis</i>	11 mm	13 mm	14 mm
<i>E.coli</i>	14 mm	17 mm	19 mm
<i>Kleb.pneumoniae</i>	16 mm	30 mm	40 mm
<i>Acineto.baumannii</i>	15 mm	20 mm	30 mm



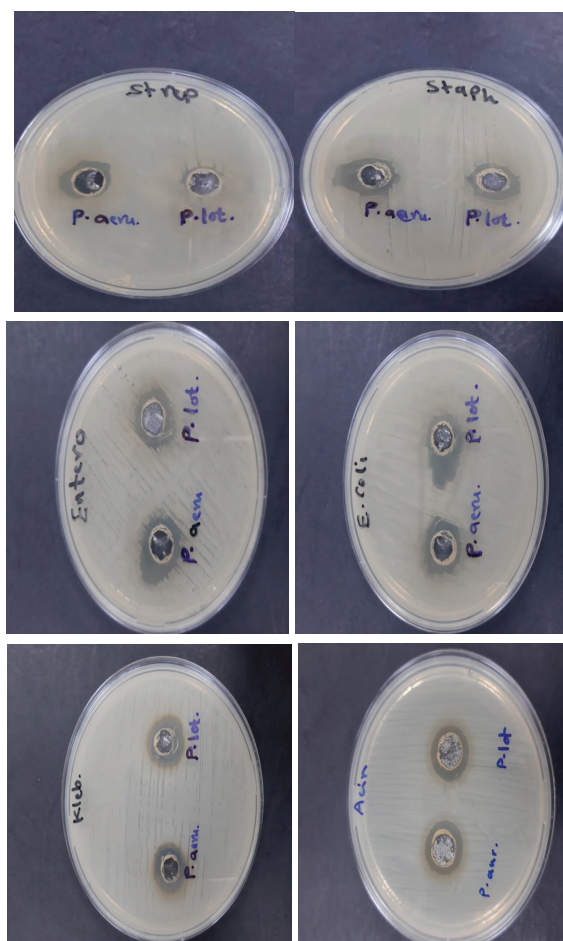
Figure(1): MIC of Lemongrass oil extract on Mullar Hinton Agar

Antimicrobial activity of silver nanoparticles

Silver is a nontoxic, safe inorganic antimicrobial agent adopted for centuries and it has the inclination of killing different type of diseases motive microorganisms. Silver has been published to be a sturdy antibacterial, antifungal and antiviral agent .The antimicrobial action of biosynthesized (Ag NPs) from two bacterial isolates were investigated facing a span of pathogenic microorganisms by applying well diffusion method. The diameter of inhibition growth zone is monitored in (Fig 2 and Table 2).The results appeared that the antibacterial activity of (Ag NPs) from *P. aeruginosa* was higher than (Ag NPs) from *P.luteola* against *Staph.aureus*, *Strep. agalactiae* and *Entero.faecalis*, while the antibacterial activity of (Ag NPs) from *P.luteola* was higher against *E.coli* . The antibacterial action of (Ag NPs) from the two species was equal against *Kleb.pneumoniae* and *Acineto.baumannii*. Most of the antibiotic resistance mechanisms are irrelevant for nanoparticles (NPs) because the mode of action of NPs is direct contact with the bacterial cell wall, without the need to penetrate the cell; this raises the hope that NPs would be less prone to promoting resistance in bacteria than antibiotics. Therefore, attention has been focused on new and exciting NP-based materials with antibacterial activity (17).

Table (2) : The antibacterial activity of (AgNPs) synthesized from two species *P. aeruginosa* and *P. luteola* measured by mm

Bacteria	AgNPs of <i>P.aeruginosa</i>	AgNPs of <i>P.luteola</i>
Staph.aureus	19 mm	15 mm
Strep.agalactiae	15 mm	7 mm
Entero.faecalis	20 mm	16 mm
E.coli	15 mm	17 mm
Kleb.pneumoniae	16 mm	16 mm
Acineto.baumannii	14 mm	14 mm



Figure(2): Antibacterial activity of (AgNPs) from two isolates on Mullar Hinton Agar

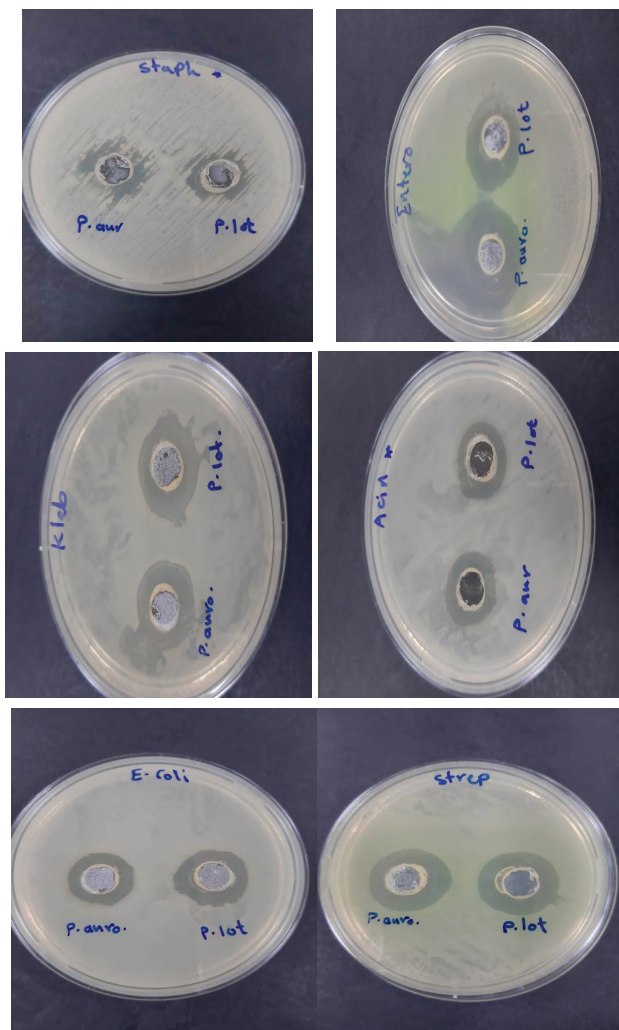
Synergetic effect of combined (AgNPs) from *P. aeruginosa* and *P. luteola* with MIC of Lemongrass oil extract

Several studies regarding the synergistic activity of nano-Ags in combination with other compounds have been reported (18). The result showed increasing in antibacterial activity when combined AgNPs that produced from two species of *Pseudomonas* (*P. aeruginosa* and *P. luteola*) with Lemongrass oil extract against all clinical types of bacteria that used in this study, but the synergetic effect from *P. aeruginosa* was more than *P. luteola* against all types of clinical bacteria except in bacteria *E.coli* the synergetic effect of *P. luteola* was the highest (Figure 3 and Table 3). Synergistic activity was done by using lemongrass (*Cymbopogon citratus*) essential oil associated with silver nanoparticles (AgNP) synthesized by green synthesis against *Candida albicans*(19) .Other combinational activities of AgNPs

with antibiotics and detergents were also seen ⁽¹⁸⁾.

Table (3) : The synergetic effect of combined (AgNPs) with MIC of Lemongrass oil extract

Bacteria	P.auroginosa	P.luteola
Staph.aureus	18 mm	15 mm
Strep.agalactiae	22 mm	20 mm
Entero.faecalis	38 mm	22 mm
E.coli	15 mm	17 mm
Kleb.pneumoniae	23 mm	20 mm
Acineto.baumannii	18 mm	17 mm



Figure(3): Synergetic effect of (AgNPs) from two isolates with Lemongrass oil extract against different clinical bacterial types on Mullar Hinton Agar

Conclusion

Silver nanoparticles which are zerovalent, can be a valuable alternative to ionic silver. Nano-Ags are a non-toxic and safe antibacterial agent for the human body. In addition, nano-Ags are also reported to possess antifungal activity, anti-inflammatory properties, antiviral activity and anti-angiogenic activity⁽¹⁸⁾. AgNPs that produced from two species of *Pseudomonas* (*P. aeruginosa* and *P. luteola*) showed increasing in antibacterial activity when combined with MIC of Lemongrass oil extract against different clinical types of bacteria. The synergetic effect from *P. aeruginosa* was more than *P. luteola* against most types of clinical bacteria.

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Conflict of Interest : Nil

Ethical Clearance- Taken from Department of Biology/ College of Science/ University of Baghdad committee

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