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Bactericidal efficacy of Ag and Au nanoparticles synthesized by the marine alga Laurencia catarinensis

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ABSTRACT

A laboratory experiment was conducted to evaluate the antibacterial activity of AgNPs and AuNPs prepared by the marine alga Laurencia catarinensis against six pathogens of Gram-positive and Gram-negative bacteria. All extracts proved efficient activity against these pathogenic bacterial species.

Keywords: *AgNPs; AuNPs, Laurencia catarinensis; pathogenic bacteria.*

INTRODUCTION

Marine organisms exhibit a rich chemical content that possesses unique structural features as compared to other metabolites. Marine algae are a rich source of chemically diverse compounds with the possibility of their potential use as a novel class of antimicrobial agents. In particular, many marine algae live in complex habitats exposed to extreme conditions and in adapting to new surroundings environment, they produce a wide range of secondary metabolites which cannot be found in terrestrials. However, investigations related to the search for new bioactive compounds from the marine environment can be seen as an almost unlimited field. Additionally, the biological productivity of terrestrial ecosystems has also perhaps reached what it can achieve; the marine biodiversity of the ocean can be expected to yield new therapeutic agents [1].

Increasing resistance of clinically important bacteria to existing antibiotics is a major problem throughout the world. One of the ways of preventing antibiotic resistance is by using new compounds which are not based on the existing

synthetic anti-microbial agents. So, search for novel natural sources from marine ecosystems could lead to the isolation of new antibiotics [2,3]. Among marine organisms, edible marine algae have been identified as a source of functional foods. It is believed that the physiological and genetic characteristics of marine algae are extensively used in food and medicine [4]. The ability of marine algae to produce secondary metabolites of antimicrobial value, such as volatile components phenols, terpenes, steroids, phlorotannins, and lipids has been already studied [5,6]. Among these, phlorotannins as polyphenolic secondary metabolites are found only in brown algae [7]. Red algae are considered as the most important source of many biologically active metabolites in comparison to the other algal classes. A new brominated C15 – acetogenin, namely Laurenidificin, was detected in the marine red alga *Laurencia nidifica* [8].

As mentioned before, the goal of the present work was to demonstrate the antimicrobial efficiency of the studied algal nanoparticles against tested strains of human pathogenic bacteria by disc and well diffusion methods.

MATERIALS AND METHODS

Bio-Synthesis of Au and Ag nanoparticles

Bio-Synthesis of Au and Ag nanoparticles from marine alga *Laurencia catarinensis* was carried in previously working [9].

Bactericidal potentials of Ag and Au nanoparticles

Test microorganisms

The test organisms were obtained from the Riyadh Military Hospital, Riyadh, Saudi Arabia.

Gram-positive bacteria: were *Staphylococcus aureus* (ATCC 29213), *Methicillin Resistant S. aureus* ATCC 12498 (MRSA) and *Enterococcus faecalis* (ATCC 29212).

Gram-negative bacteria: were *Klebsiella pneumoniae* ATCC 27738, *Escherchia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Application procedures.

Well diffusion method

The antibacterial activity of the AgNPs and AuNPs was assayed by following the standard Nathan's Agar Well Diffusion (NAWD) technique [10]. A single colony of each test strain was grown overnight in Blood Agar medium at 35°C. The bacterial suspensions (the inocula) were prepared by diluting the overnight cultures with 0.85% NaCl to a 0.5 McFarland standard. Six wells of 6mm diameter were made on the pre-poured Muller Hinton Agar (MHA) plates. These MHA plates were inoculated or injected by swab the bacterial suspensions to create a confluent lawn of bacterial growth. 20 µL from each of the AgNPs and AuNPs synthesized by powder, ethanolic and chloroform extract of *L. catarinensis* were loaded onto the each of the well. After 18–24 h of incubation at 37ºC, the susceptibility of the test organisms was determined by measuring the diameter of the inhibition zone around each well to the nearest mm.

Disc diffusion method

The antibacterial activity of the AgNPs and AuNPs was assayed by following the standard Kirby–Bauer disc diffusion method [11]. A single colony of each test strain was grown overnight in Blood Agar Medium at 37°C. The bacterial suspensions (the inocula) were prepared by diluting the overnight cultures with 0.85% NaCl to a 0.5 McFarland standard. Muller Hinton agar was prepared and poured on sterile Petri plates. These MHA plates were inoculated by swabbing the bacterial suspensions to create a confluent lawn of bacterial growth. Paper disc of 6mm dimension was impregnated with 20 µL from each of the AgNPs and AuNPs synthesized by powder, ethanolic and chloroform extract of *L. catarinensis*. The discs were gently pressed to have a better contact with Muller Hinton

Agar. Plates were incubated in an inverted position for 18-24 h at 37ºC and the susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition around each disc to the nearest mm.

Statistical analysis:

Statistical analyses were conducted with the SPSS 11.5 version software package on the triplicate (n = 3) test data. The mean values of each treatment were compared using one-way analysis of variance (ANOVA). The outcome results were offered as a mean with standard deviation (±SD). A p-value of less than 0.05 was considered as significant.

RESULTS

Table 1 indicated that maximum size of inhibition zones (22 mm) was observed with AgNPs of ethanol extract of *L. catarinensis* against *E. coli* and *K. pneumoniae*. Also, AgNPs of ethanol extract of *L. catarinensis* were recorded high significant inhibition zone (20 mm) against MRSA. On the other hand, AgNPs of *L. catarinensis* powder exhibited a moderate significant inhibition zone (15 mm) against *K. pneumoniae*. Chloroform extract of *L. catarinensis* AgNPs showed prominent zones (14 mm) of inhibition against *S. aureus* and MRSA. Whereas, significant small inhibition zones (9,19 and 10 mm) occurred with *L. catarinensis* ethanolic extract against E. faecalis, MRSA and *S. aureus*, respectively. But no inhibition zones (6 mm) occurred against *E. coli, K. pneumoniae* and *P. aeruginosa* by *L. catarinensis* ethanol and chloroform extracts.

Similarly, the results presented in Table 2 recommended the previous data. The maximum inhibition zones were recorded with AgNPs of *L. catarinensis* ethanol extract (21, 21, 17 and 15 mm) against *K. pneumoniae*, *S. aureus*, *E. coli* and *P. aeruginosa*, respectively followed by chloroform extract and AgNPs of *L. catarinensis* powder. While algal ethanol extract was active only against three pathogens.

Table 3 depicted that all amounts of AuNPs of *L. catarinensis* extract highly significant effective against tested pathogens followed by AuNPs algal powder. Data revealed that application of AuNPs of *L. catarinensis* ethanol extract exhibited a clear significant antibacterial activity with inhibition zones (20, 19 and 16 mm) against *K. pneumoniae*, MRSA and *E. faecalis*, respectively. In addition, AuNPs of *L. catarinensis* chloroform extract exhibited a moderate significant inhibition zones (15 and 12 mm) against most of the test pathogens. The antibacterial activity of AuNPs of *L. catarinensis* powder showed a significant activity (13 and 12 mm) against *K. pneumoniae* and *P. aeruginosa* compared to ethanol and chloroform *L. catarinensis* extracts (6 mm). However, *L. catarinensis* ethanol extract exhibited a significant antibacterial activity against *S. aureus*, MRSA and *E. faecalis*.

The antibacterial property of AuNPs of *L. catarinensis* extracts is documented in Table 4. The results clearly demonstrated that the AuNPs ethanol extract of *L. catarinensis* inhibited the growth of the tested bacteria followed by AuNPs of *L. catarinensis* chloroform extract then AuNPs of *L. catarinensis* powder compared to *L. catarinensis* extracts. Currently, *L. catarinensis* AgNPs and AuNPs were most effective against tested pathogenic bacteria. These results clearly observed in Plates 1 to 6.

DISCUSSION

Generally, the previous results recorded that the antimicrobial property of algal nanoparticles was higher than row algal extracts. That may be attributed to many reasons such as alga, metal; solvent used nanoparticles characters and tested bacteria. In general, extracts obtained using ethanol were more active than those obtained with chloroform. On the basis of the available literature, we hypothesized that *Laurencia* was inherently rich in antiseptics and halogenated compounds such as palimatic acids, bromophenols, iodine, bromine and chlorine [9]. These substances participated in Ag+ and Au+ nanoparticles which increased the inhibition effect of algal nanoparticle formation against most of the tested pathogens in relative to algal extracts effect.

Antibacterial mechanism or destruction has been extensively studied. The interaction stage between AgNPs and bacteria (*E. coli*) has been studied in detail [12] using TEM. At the initial stage of the interaction, Ag nanoparticles were found to adhere to the wall of the bacteria due to the charge of the functional group of the bacteria[13].Subsequently,thenanoparticlespenetratedthebacteriaanddestroyedthemembrane,whicheventually

killed the bacteria. The difference in sensitivity is contributed by the nature of the bacteria, in which *E. coli* is Gram-ve where MRSA is Gram+ve. It is known that Gram-ve bacteria have four layers of protective membranes consisting of a plasma membrane, a periplasmic area, a peptidoglycan layer and an outermost layer known as an external membrane made up of protein and lipopolysaccharide. Gram-Positive is only enveloped by peptidoglycan layer. Therefore, the lack of an extra layer of the membrane results in Gram-positive bacteria being more sensitive towards the presence of Ag nanoparticles [14].

Table 1. Comparative antimicrobial efficiency of the AgNPs of *Laurencia catarinensis* against tested strains of human pathogenic bacteria by disc diffusion method. (Data are expressed as mean of three replicates ±SD).

Bacterial organism	Mean of inhibition zone diameter (mm)					LSD at 5 %	LSD at 1%
	L. catarinensis ethanol extract	*L. catarinensis* chloroform extract	AgNPs from *L. catarinensis* powder	AgNPs from *L. catarinensis* ethanol extract	AgNPs from *L. catarinensis* chloroform extract		
E.coli	6.00 ± 0.05*	6.00 ± 0.35 *	12.0 ± 1.21c	22.0 ± 1.32h	13.0 ± 0.43d	2.8	3.83
K. pneumoniae	6.00 ± 0.31*	6.00 ± 0.35 *	15.0 ± 1.31e	22.0 ± 1.34h	11.0 ± 0.51c		
P. aeruginosa	6.00 ± 0.24*	6.00 ± 0.35 *	11.0 ± 1.22	15.0 ± 1.43e	12.0 ± 0.26c		
S. aureus	10.0 ± 0.58b	8.00 ±	9.00 ± 0.22b	17.0 ± 0.93f	14.0 ± 0.62d		
MRSA	10.0 ± 1.15b	11.0 ±	6.00 ± 0.53*	20.0 ± 0.41g	14.0 ± 0.95d		
E. faecalis	9.00 ± 1.70b	7.00 ±	11.0 ± 0.31c	17.0 ± 1.71f	10.0 ± 0.66b		

* = Not effected.

Values followed by the letter (a) are not significantly different at p<0.05 level.

Values followed by different letters (b,c,d,…etc) indicate significant difference at p<0.05 level.

Table 2. Comparative antimicrobial efficiency of the AgNPs of *Laurencia catarinensis* against tested strains of human pathogenic bacteria by well diffusion method. (Data are expressed as mean of three replicates ±SD).

Bacterial organism	Mean of inhibition zone diameter (mm)					LSD at 5 %	LSD at 1%
	L. catarinensis ethanol extract	*L. catarinensis* chloroform extract	AgNPs from *L. catarinensis* powder	AgNPs from *L. catarinensis* ethanol extract	AgNPs from *L. catarinensis* chloroform extract		
E.coli	6.00 ± 0.05*	6.00 ± 0.35 *	9.00 ± 0.39	17.0 ± 1.02	14.0 ± 0.73	2.81	3.80
K. pneumoniae	6.00 ± 0.31*	6.00 ± 0.35 *	9.00 ± 0.15	21.0 ± 1.05	11.0 ± 0.53		
P. aeruginosa	6.00 ± 0.24*	6.00 ± 0.35 *	11.0 ± 0.43	15.0 ± 0.87	8.00 ± 0.68		
S. aureus	10.0 ± 0.58b	8.00 ± 0.57a	11.0 ± 0.52	21.0 ± 1.33	10.0 ± 0.19		
MRSA	10.0 ± 1.15b	11.0 ± 0.43c	8.00 ± 0.43	11.0 ± 0.17	12.0 ± 0.44		
E. faecalis	9.00 ± 1.70b	7.00 ± 0.27a	10.0 ± 0.65	17.0 ± 0.41	11.0 ± 0.61		

* = Not effected.

Values followed by the letter (a) are not significantly different at p<0.05 level.

Values followed by different letters (b,c,d,…etc) indicate significant difference at p<0.05 level.

Table 3. Comparative antimicrobial efficiency of the AuNPs of *Laurencia catarinensis* against tested strains of human pathogenic bacteria by disc diffusion method. (Data are expressed as mean of three replicates ±SD).

Bacterial organism	Mean of inhibition zone diameter (mm)					LSD at 5 %	LSD at 1%
	L. catarinensis ethanol extract	*L. catarinensis* chloroform extract	AgNPs from *L. catarinensis* powder	AgNPs from *L. catarinensis* ethanol extract	AgNPs from *L. catarinensis* chloroform extract		
E.coli	6.00 ± 0.05*	6.00 ± 0.35 *	8.00 ± 0.51a	10.0 ± 1.00b	15.0 ± 1.53e	3.03	4.10
K. pneumoniae	6.00 ± 0.31*	6.00 ± 0.35 *	13.0 ± 1.00d	20.0 ± 2.59g	15.0 ± 1.00e		
P. aeruginosa	6.00 ± 0.24*	6.00 ± 0.35 *	12.0 ± 1.03c	14.0 ± 0.35d	12.0 ± 0.43c		
S. aureus	10.0 ± 0.58b	8.00 ± 0.57a	9.00 ± 0.78b	14.0 ± 0.92d	12.0 ± 0.61c		
MRSA	10.0 ± 1.15b	11.0 ± 0.43c	6.00 ± 0.01 *	19.0 ± 2.05g	10.0 ± 0.04b		
E. faecalis	9.00 ± 1.70b	7.00 ± 0.27a	8.00 ± 0.89a	16.0 ± 1.16e	12.0 ± 1.00c		

* = Not effected.

Values followed by the letter (a) are not significantly different at $p<0.05$ level.

Values followed by different letters (b,c,d,…etc) indicate significant difference at $p<0.05$ level.

Table 4. Comparative antimicrobial efficiency of the AuNPs of *Laurencia catarinensis* against tested strains of human pathogenic bacteria by well diffusion method. (Data are expressed as mean of three replicates ±SD).

Bacterial organism	Mean of inhibition zone diameter (mm)					LSD at 5 %	LSD at 1%
	L. catarinensis ethanol extract	*L. catarinensis* chloroform extract	AgNPs from *L. catarinensis* powder	AgNPs from *L. catarinensis* ethanol extract	AgNPs from *L. catarinensis* chloroform extract		
E.coli	6.00 ± 0.05*	6.00 ± 0.35 *	10.0 ± 1.11b	12.0 ± 0.91c	15.0 ± 0.56e	2.3	3.11
K. pneumoniae	6.00 ± 0.31*	6.00 ± 0.35 *	10.0 ± 1.04b	18.0 ± 1.08f	15.0 ± 0.35e		
P. aeruginosa	6.00 ± 0.24*	6.00 ± 0.35 *	11.0 ± 0.56c	14.0 ± 1.32d	10.0 ± 1.21b		
S. aureus	10.0 ± 0.58b	8.00 ± 0.57a	9.00 ± 0.71b	13.0 ± 0.66d	11.0 ± 0.63c		
MRSA	10.0 ± 1.15b	11.0 ± 0.43c	9.00 ± 0.82b	13.0 ± 1.80d	11.0 ± 0.59c		
E. faecalis	9.00 ± 1.70b	7.00 ± 0.27a	8.00 ± 0.18a	15.0 ± 1.13e	11.0 ± 0.33c		

* = Not effected.

Values followed by the letter (a) are not significantly different at $p<0.05$ level.

Values followed by different letters (b,c,d,…etc) indicate significant difference at $p<0.05$ level.

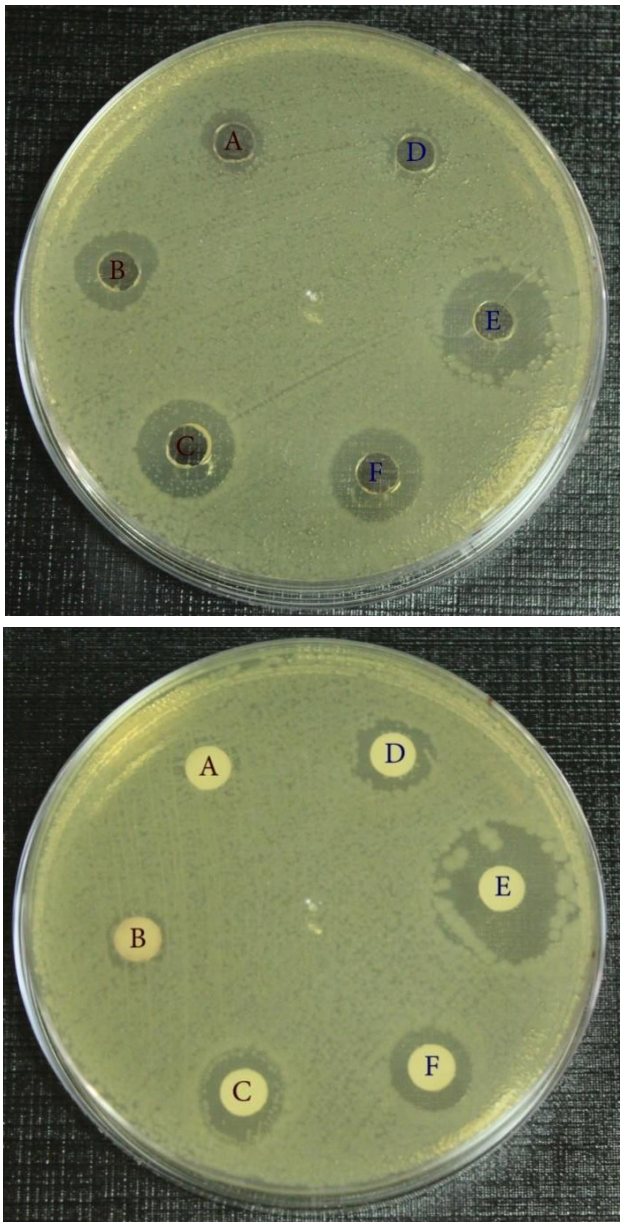

Plate 1. Antibacterial activity of AgNPs and AuNPs of *L. catarinensis* powder, ethanol and chloroform extracts against *E. coli* by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis* chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

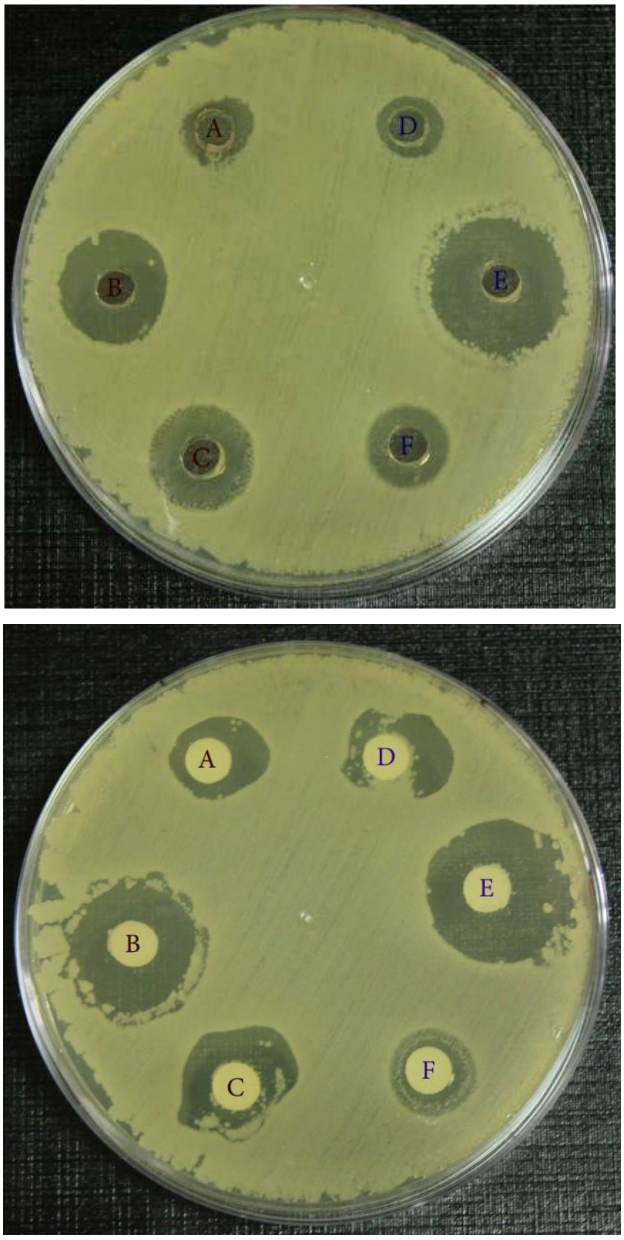

Plate 2. Antibacterial activity of AgNPs and AuNPs of *L. catarinensis* powder, ethanol and chloroform extracts against *K. pneumonia* by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis* chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

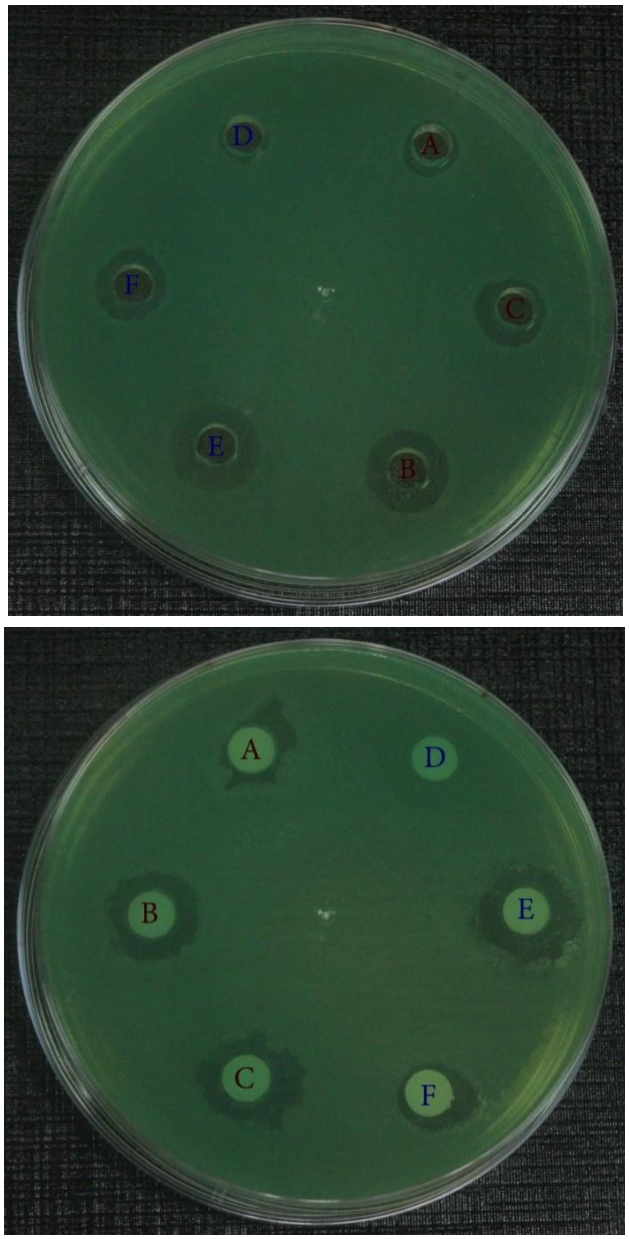

Plate 3. Antibacterial activity of AgNPs and AuNPs of *L.catarinensis* powder, ethanol and chloroform extracts against *P. aeruginosa* by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis*chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

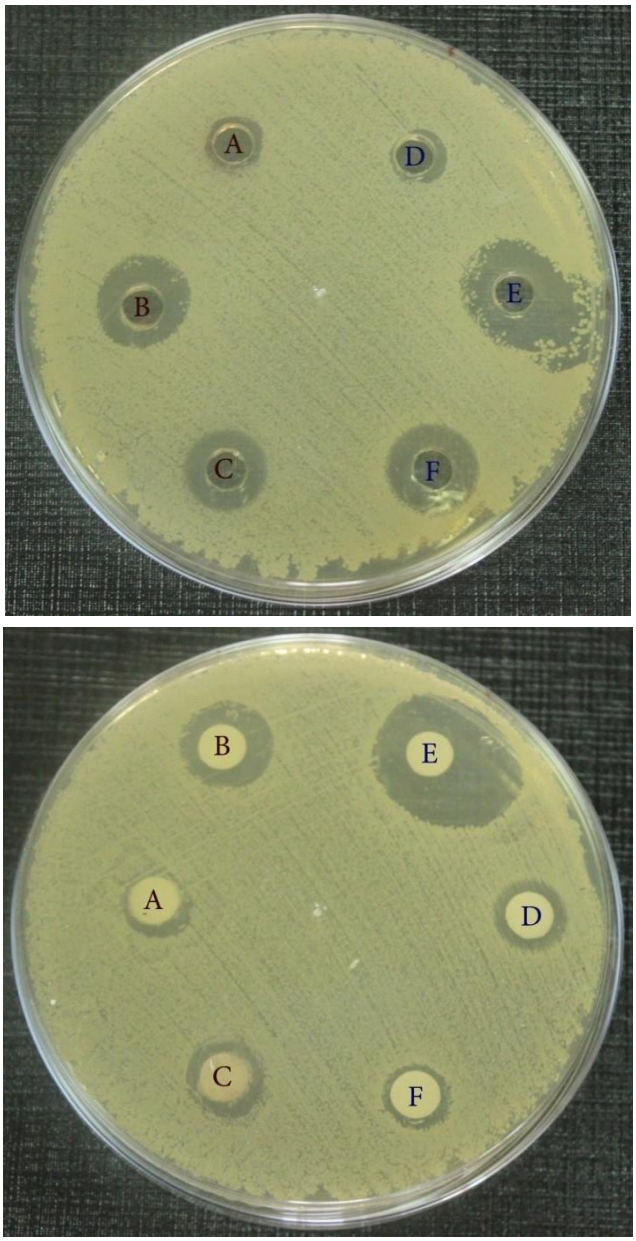

Plate 4. Antibacterial activity of AgNPs and AuNPs of *L. catarinensis* powder, ethanol and chloroform extracts against *S. aureus* by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis* chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

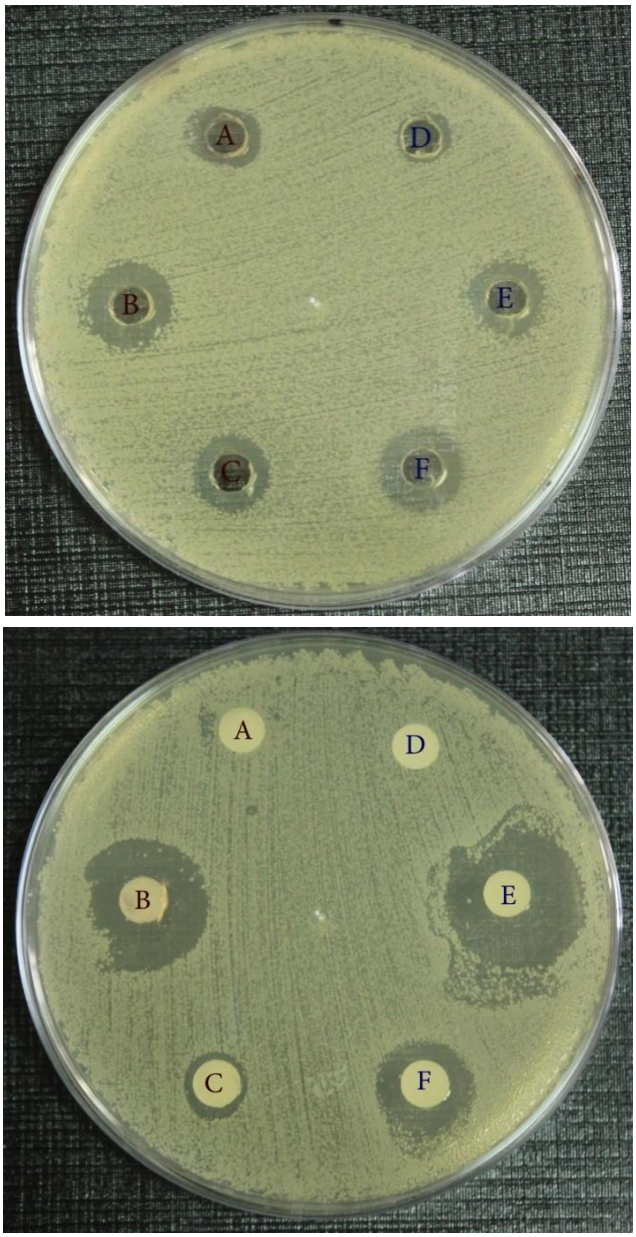

Plate 5. Antibacterial activity of 1mM of AgNPs and AuNPs of *L. catarinensis* powder, ethanol and chloroform extracts against MRSA by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis* chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

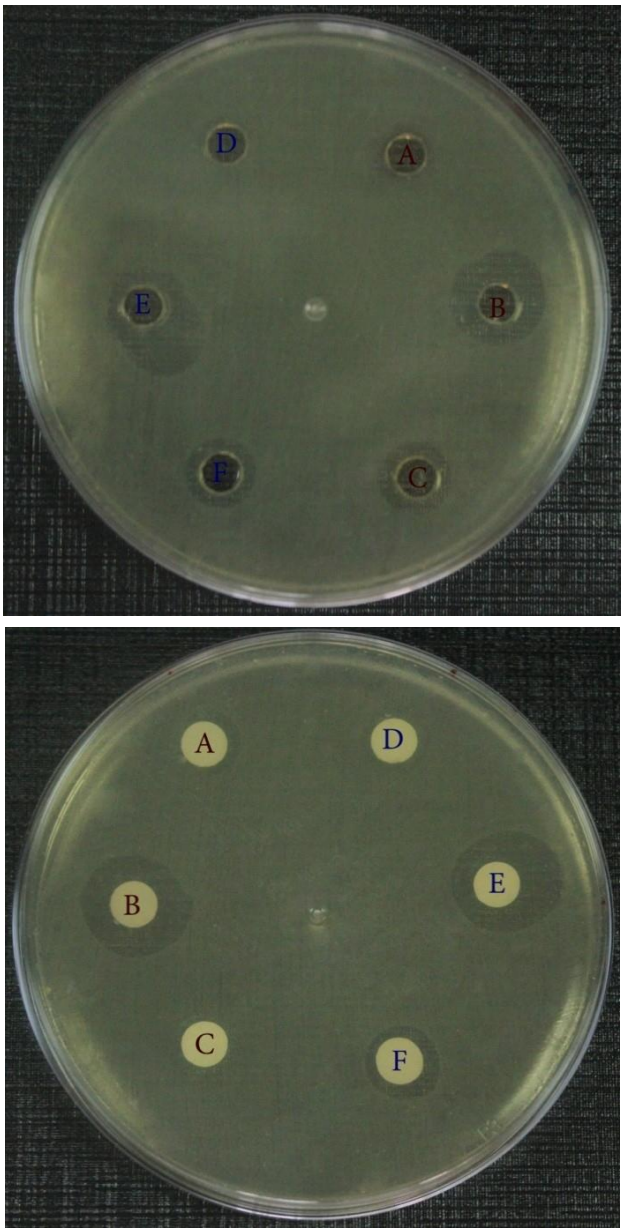

Plate 6. Antibacterial activity of AgNPs and AuNPs of *L. catarinensis* powder, ethanol and chloroform extracts against *E. faecalis* by well and disc diffusion methods.

(A) AuNPs synthesized by *L. catarinensis* powder	(D) AgNPs synthesized by *L. catarinensis* powder
(B) AuNPs synthesized by *L. catarinensis* ethanol extract	(E) AgNPs synthesized by *L. catarinensis* ethanol extract
(C) AuNPs synthesized by *L. catarinensis* chloroform extract	(F) AgNPs synthesized by *L. catarinensis* chloroform extract

In our study, the obtained data indicated that the studied Ag and Au algal nanoparticles were highly affected against both Gram+ve and -ve tested bacteria. These results may be due to the efficiency of metal-algal nanoparticles which have a double inhibition effect on tested organisms i.e metal effect and algal chemical constituents. Different natural antimicrobial compounds have been described in the studied alga belonging to a wide range of chemical compounds including terpenes, acetogenins, indoles, phenols, fatty acids, flavonoids, amino acids and volatile halogenated hydrocarbons. In this respect, some workers reported that the red alga *L. catarinensis* extracts were characterized by HPLC and being to contain natural antimicrobial compounds such as volatiles, aliphatic, aromatic compounds,

amino acids and fatty acids [9]. She reported that several volatile compounds were identified in ethanol and chloroform extracts from *L. catarinensis* mainly fatty acids, alkenes, phenols, and compounds such as Phytol (2-hexadacen -1-01, 3, 7, 11, 15- tetramethyl) and neophytadiene.

L. marianensis afforded a number of new metabolites: the brominated diterpene, 10-hydroxykahukuene, two sesquiterpenes, 9-deoxyelatol and isoda-ctyloxene, one brominated C15- acetogenin, laurenmariallene and two new naturally occurring halogenated sesquiterpenes [9]. All these algal bioactive compounds which synthesized in the form of Ag+/Au+ nanoparticles showed better activity against tested bacteria compared to algal extracts in our investigation. These results is in a harmony with other workers who explained that the accumulation of positively charged (Au^{+}/Ag^{+}) gold and silver nanoparticles on the negatively charged cell membrane of microorganisms leads to conformational changes in the membrane which loses permeability control which in turn causes the cell death [15].

The mechanism of action of silver is linked with its interaction with thiol group compounds found in the respiratory enzymes of microbial cells. Silver particles bind to the bacterial cell wall, cell membrane and inhibit the respiration process. In the case of E. coli, silver acts by inhibiting the uptake of phosphate and releasing mannitol, succinate, phosphate, proline, and glutamine from E. coli cells [16]. Furthermore, the silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area which provides better contact with microbes. The NPs get to attack the cell membrane and also penetrate inside the microbes. The bacterial plasma membrane contains sulfur-containing proteins and the silver nanoparticles interact with the proteins in the cell as well as with the phosphorus-containing compounds such as DNA. When AgNPs enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria protect the DNA from the Ag ions. The NPs attack the respiratory chain, so cell division leading to cell death. The NPs released Ag ions in the bacterial cells (enhance their bactericidal activity) [16]. Moreover, the surface Plasmon resonance plays a major role in the determination of optical absorption spectra of metal nanoparticles which shift to a longer wavelength with an increase in particles size. The size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells, so it will have a higher percentage of interaction than bigger particles. The nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects which enhance the reactivity of NPS to corrupt the bacterial cell. Additionally, the antimicrobial efficacy of the nanoparticle depends on the shapes of the NPs also, this can be confirmed by investigating the suppression of bacterial growth by differentially shaped nanoparticles [15].

Gold nanoparticles exploit their unique chemical and physical properties for transporting and unloading the pharmaceuticals. First, the Au core is essentially inert and their ease of synthesis, monodisperse NPs can be formed with core sizes ranging from 1 to 150 nm. The second advantage is imparted by their ready functionalization, generally through thiol linkages [17]. In addition, their photophysical properties could trigger drug release at a remote place. Furthermore, the delivery of small AuNPs make them a useful scaffold for efficient recognition and delivery of biomolecules such as proteins or nucleic acids like DNA or RNA [18]. Absorbed light by gold nanoparticles leads to heating of these particles and upon transport subsequently to heating of the particle environments. The resulting localized heating causes irreversible thermal cellular destruction. The plasmonicphotothermal therapy is a minimally-invasive oncological treatment strategy. Some scientists reported about the microbiological intelligence of gold nanoparticles when conjugated with polyparaphenylene ethylene to identify three different strains of E. coli in minutes [15]. In relation to taxonomic groups, our results are in accordance with previously workers who reported that the members of the red algae exhibited high antibacterial activity [19-21].

CONCLUSIONS

Synthesis of AgNPs using biological resources like marine algae is a challenging alternative to chemical synthesis since this novel biogenic method were eco-friendly methods. The obtained data clearly indicate the algal extracts can be used as an effective capping as well as the reducing agent for the synthesis of AgNPs. Silver and gold nanoparticles synthesized by *Laurencia catarinensis* are quite stable and no visible changes in a long time. All used analysis showed that there is a major distribution of particle size with many different shapes such as pyramidal, spherical, polygonal, rod and hexagonal with highly smooth edges. Their size ranged from 49.58 – 86.37nm. This can be supportive for medicaluses.

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