STUDY ON ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES FROM MANGROVE (*RHIZOPHORA MUCRONATA*) BARK EXTRACT

AJI JOVITHA A.T [1], DR. B. DEIVASIGAMANI [2]

1. Research Scholar, Centre for Advanced Studies in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India,
2. Associate Professor, Centre for Advanced Studies in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India.

ABSTRACT

Mangroves fill in saline water front natural surroundings in the tropical and subtropical areas. Mangrove environment is of an incredible natural and financial noteworthiness. The AgNPs exhibit higher antibacterial activity on marine ecosystem. In this study, we investigated the antibacterial effects of silver nanoparticles (AgNps) were tested against, two gram-positive and gram-negative pathogenic bacteria viz., *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* at 20 µg concentration. They were highly sensitive to AgNPs, whereas less sensitive to $AgNO_3$.Furthermore, the green synthesized AgNPs from *Rhizophora mucronata* screened for antibacterial activity. In the antibacterial activity of optimized AgNPs was found remarkably sensitive to the AgNPs with 23mm of inhibition zone. The standard antibiotics of streptomycin at 10 µg, when tested against the bacteria, which revealed high antibacterial activity against all the pathogenic bacteria. The MIC concentration evaluated against the bacteria showed were 18.10 ± 0.65 µg/mL for *Bacillus subtilis and Staphylococcus aureus showed 26.05 ± 0.92* µg/mL, respectively. Our research outcome opens up new improved antimicrobial composition in pharmaceutical and medical sectors for the treatment of bacterial disease for marine and aquaculture.

Keywords: *Rhizophora mucronata*, Antimicrobial, Minimum Inhibitory Concentration (MIC)

I.INTRODUCTION:

Nanotechnology is a creating interdisciplinary field of research scattering physical, synthetic, organic and designing sciences. Nanotechnology is logical innovation that manages nanometer estimated things. The activity of nanomaterials in biotechnology joins the fields of science and material science. Nanoparticles set forward a basically helpful stage, showing exceptional properties with possibly wide-running application. for example, synthetics, material industry, clinical diagnostic (future nanobots), medication and quality conveyance, hardware conclusion, counterfeit inserts, tissue engineering (Santosh Kumar *et al*., 2011) Along, the biogenic element of AgNPs evokes an ideal decision for drug and pharmaceutical fields. Because of the flare-up of pathogenic contaminations around the world, the requirements for cutting edge nanomaterial have been proposed to the network to create opposition against the diseases (Tran *et al*., 2013). In spite of the fact that they have the authority over the size and state of the nanoparticles, they represent a few threats, when they are being utilized in the clinical applications. Besides, they are costly, need high pressure, energy, and temperature, and need utilization of some toxic synthetic compounds for the adjustment of nanoparticles, prompting unfavorable impacts when they are being applied in the clinical and pharmaceutical applications (Pankaj Kumar *et al*.,2012) Microorganisms are much of the time infect human what's more, causes diseases in the current living conditions. Use of silver-based antimicrobial agent might be utilized against antibiotic resistance microbes (Al-Dhabi *et al*.,2019) The AgNPs can cause cell lysis, inhibit cell transduction or may likewise prompt cell phosphorylation (Sondi and Salopek-Sondi 2004). Recently, we were synthesized AgNPs with *Rhizophora mucronata* bark extract and it was shown more stability (Aji Jovitha and Deivasigamani 2020)

II.MATERIALS AND METHODS

The analytical grade chemicals of Streptomycin and Muller Hinton Agar (MHA) medium were procured from the Hi-media Lab Pvt. Ltd Chennai, India. The pathogenic bacteria such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* obtained from Division of Microbiology and Immunology, Annamalai University, Chidambaram, TamilNadu.

2.1Preparation of bark extract of *Rhizophora mucronata*

Mangrove Plant bark from *Rhizophora mucronata* was Collected. The collected bark materials were washed in tap water and rinsed in distilled water to remove dust and dirt particles. The freshly cleaned barks were left to dry for 3-4 days. Dried plant barks were powdered using electrical mixture and the powdered were protected from sunlight for further use.2gm of bark powder was taken and mixed with beaker containing 50ml of distilled water and it was boiled at 50-60°C for 15 minutes. Then the extract was filtered through Whatman no1 filter paper then the extract was stored.

2.2Antibacterial Activity

The antibacterial potential of AgNPs was examined by agar well diffusion method described by (Perez *et al.*, 1990). The bacterial microorganisms were cultivated consistently with a cotton swab on Muller Hinton Agar (MHA) medium. The agar wells (5 mm) were made with sterile cork borer and then the wells were loaded with AgNPs (50 µg/mL), plant extract (50 µg/mL), $AgNO_3$ (1mM; 20µL) and streptomycin (positive control; 20µg/mL). The plates were hatched for 24 h at 37 ºC. The zone of inhibition (mm) was recorded after incubation periods.

2.3Minimum Inhibitory Concentration (MIC) of AgNPs

The minimum inhibitory concentration of AgNPs was examined as described by (Balashanmugam *et al*., 2020). The dynamics of bacterial susceptibilities were recorded on the solid medium. The optimized AgNPs were tested against four bacterial pathogens. 5 mL of the optimally grown bacterial suspension was treated with AgNPs at different concentrations such as 10, 20, 30, 40, 50 µg/mL. The culture suspensions were incubated for 24 h at 37 ºC. A loop full of culture was streaked into MHA medium plates and incubated at 37 ºC for 24 h. The MIC values of AgNPs were documented.

III.RESULTS AND DISCUSSION:

Synthesized silver nano particle showed excellent anti-microbial activity against the *Bacillus subtilis*,followed by *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* Antibacterial impact was size and dose dependent and was more effective against gram negative microbes than gram positive microscopic organisms.(Fig 1 & Table 1) It was seen that negative charge on cell surface of gram-negative microorganisms was higher than gram positive microscopic organisms. So, the association between gram positive and silver nanoparticle was stronger than gram negative (Mallikarjuna, *et al*.,2011, Anima Nanda, and Shahnaz Majeed ,2014) The optimized AgNPs of respective minimum inhibitory concentration against the four different bacteria pathogens were investigated. Among, *Bacillus subtilis* was found extremely sensitive to AgNPs by showing 18.10 ± 0.65 mm of zone of inhibition followed by *S. aureus* of 26.05 ± 0.92 mm, *Klebsiella pneumoniae* of 31.21 ± 0.79 mm and *Escherichia coli* mm was recorded. (Fig. 2& Table 2)

Antimicrobial study revealed that the synthesis nanoparticles from *Rhizophora mucronata* bark extract was found to be very efficient against test pathogens such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli , Klebsiella pneumonia* a maximum zone of inhibition was seen in the concentration around 40 µl when compared to other concentration 10 - 30 µl against both gram positive and gram negative pathogens which was co-relating (Thukkaram Sudhakar *et al*., 2014 and Manimaran *et al* .,2016) In comparative studies 40 µl of AgNPs AgNO3, plant extaract of *Rhizophora mucronata* bark extract and streptomycin was added against several test pathogens by well diffused method.(Fig.3) A maximum zone of inhibition was seen in standard antibiotic streptomycin and biologically synthesized silver nanoparticles.

IV.CONCLUSION:

The AgNPs shows highest antibacterial activity was observed in (gram-positive and gram-negative bacteria) *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*. We have confidence in that the silver nanoparticle has greatest potential for applications in catalysis, biomedical, and pharmaceutical industries. We conclude that the effective antibacterial activity of biologically synthesized AgNPs from mangrove plant *Rhizopora mucronata* bark extract reported in this study can be exploited as an antibacterial agent with marine environment and therapeutic treatments for aquatic animals.

4.1 Figures and Tables:

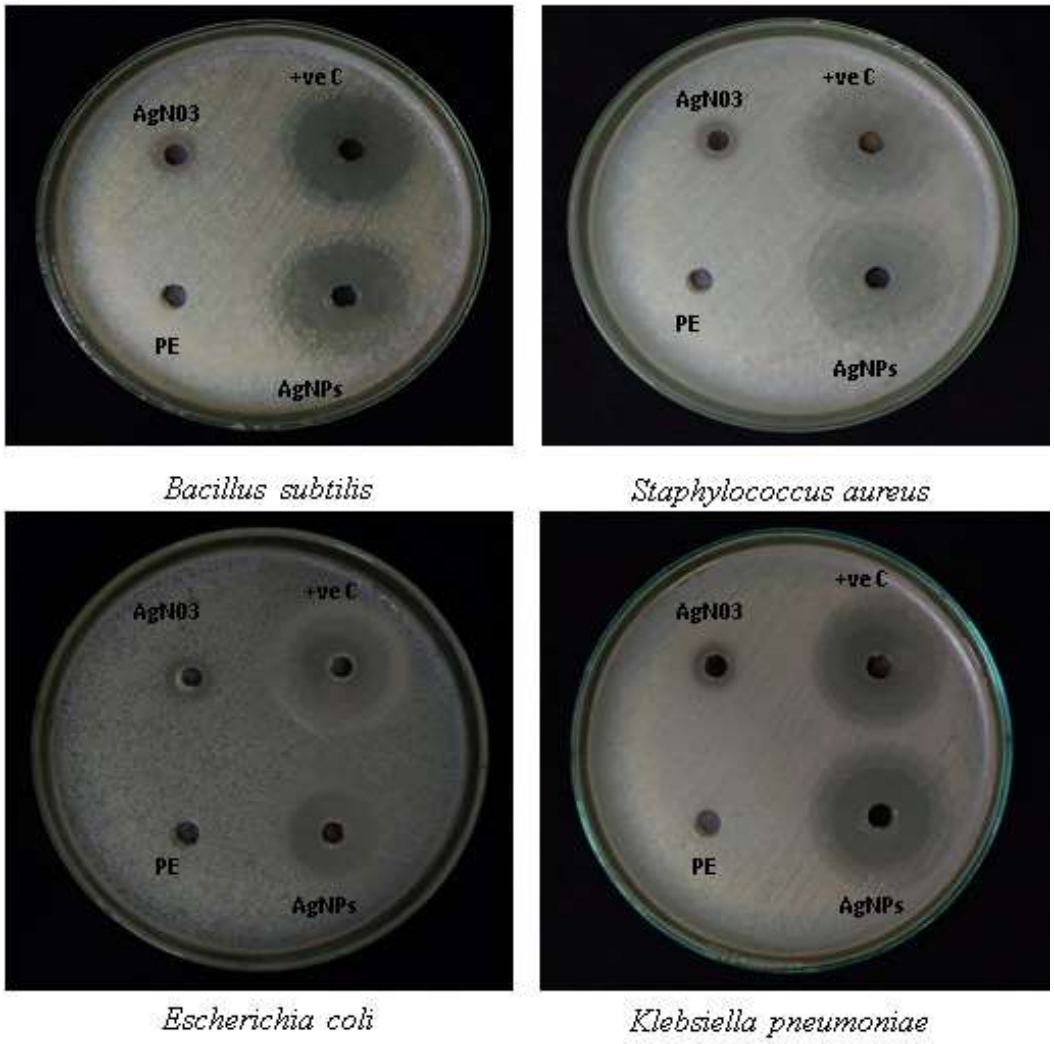

Figure 1 Antibacterail activity of AgNPs synthesized from *R.mucronata* bark extract

Abbreviations: $AgNO_3$ – Siver nitrate, AgNPs – Biologically synthesized silver nanoparticles from *R.mucronata* bark, $+^{ve}$ control – streptomycin, PE – Plant extract,

Table 1 Antibacterial Activity of AgNPs

S. No.	Microorganism	Zone of inhibition (mm in diameter)			
		Positive control (+ve C) (20 µg/mL)	Silver nanoparticles (AgNPs) (50 µg/mL)	Silver nitrate (AgN03) (1mM)	Plant extract (PE) (50 µg/mL)
1	*Bacillus subtilis*	27	25	8	0
2	*Staphylococcus aureus*	25	22	9	0
3	*Escherichia coli*	23	20	7	0
4	*Klebsiella pneumoniae*	26	24	9	0

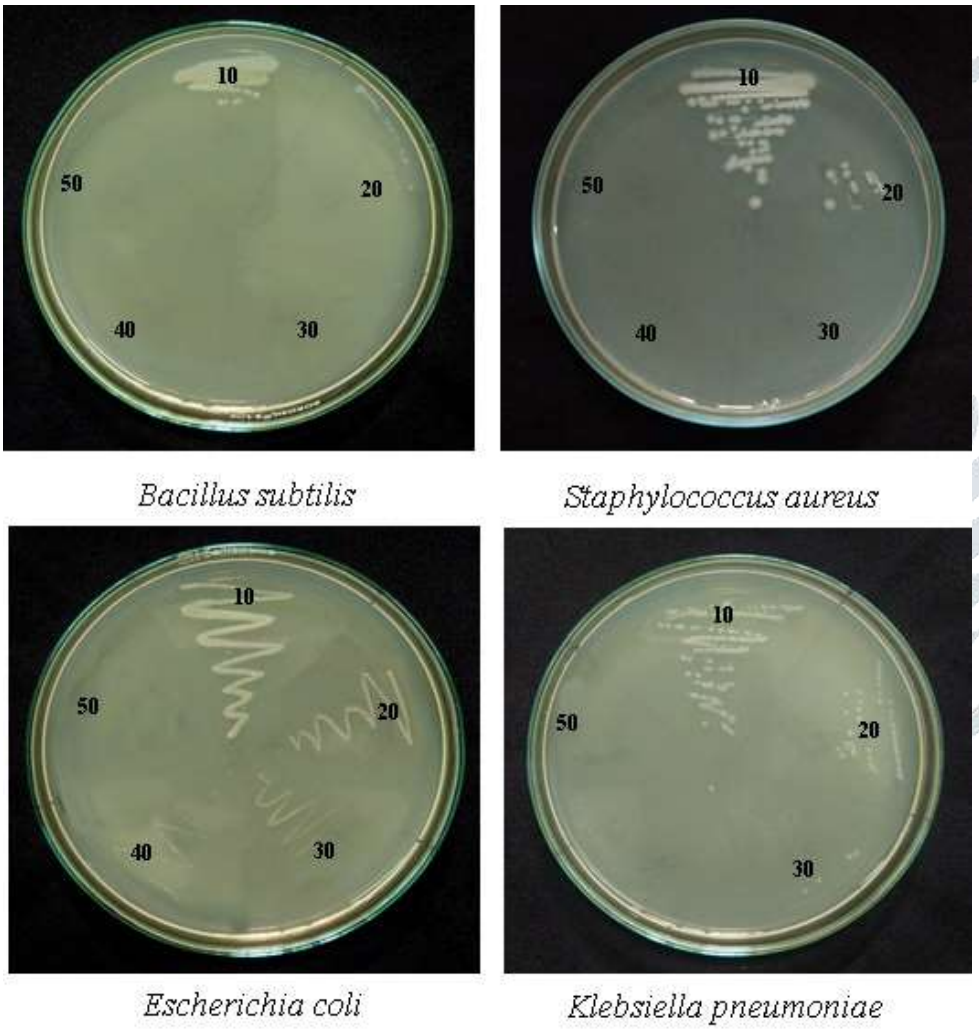

Figure 2 Minimum inhibitory concentration of AgNPs from Rhizophora mucronata bark extract

Table 2 Minimum inhibition concentration of fAgNPs

S. No.	Name of the microorganism	MIC (µg/mL)
1	***Bacillus subtilis***	18.10 ± 0.65
2	***Staphylococcus aureus***	26.05 ± 0.92
3	***Escherichia coli***	40.30 ± 0.56
4	***Klebsiella pneumoniae***	31.21 ± 0.79

*Different concentrations of AgNPs are significantly different at P<0.05 level

(Least significant difference) Mean followed by ±S.D

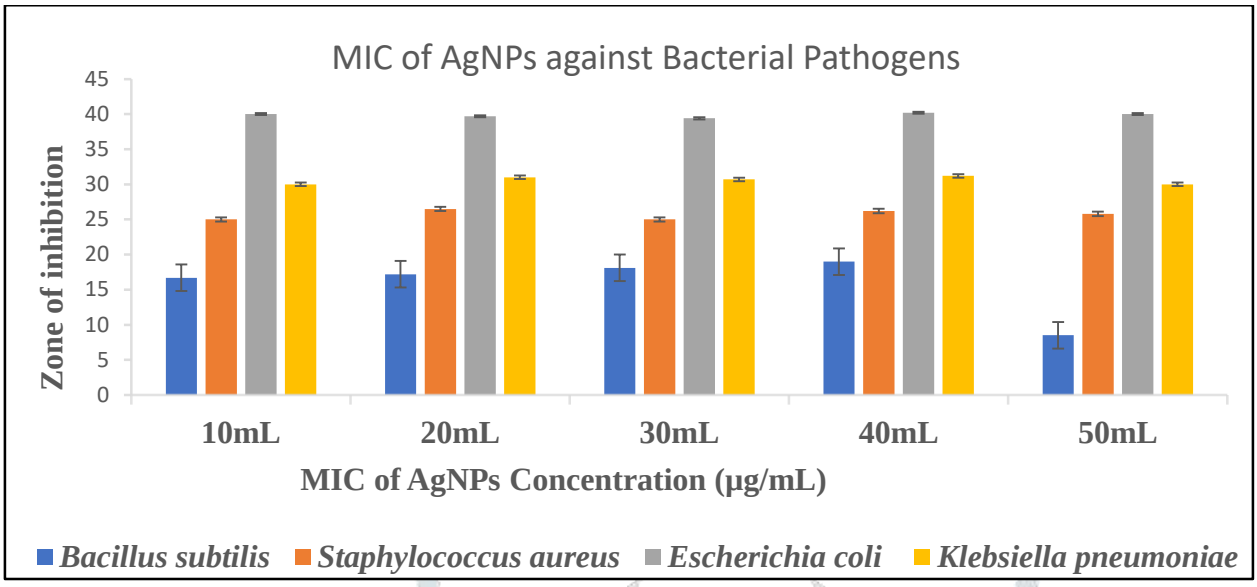

Figure 3 MIC of AgNPs against various Bacterial pathogens

V.ACKNOWLEDGMENT

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