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Evaluation of Antimicrobial and Antioxidant Properties of Biosynthesized Silver Nanoparticles Using Peel Extracts of Citrus reticulata

Keshamma E.[1], Shivashankrappa L. H.[2], Geethanjali R.[3], Kavitha K.R.[4]

[1]Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[3]Assistant Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[4]Associate Professor, Department of Botany & PG Studies, Nrupathunga University, Bengaluru, Karnataka, India

Abstract: The plant extracts of Citrus reticulata well known to possesses various biological properties. Hence, the present study was designed to evaluate antimicrobial and antioxidant activities of biosynthesized silver nanoparticles from peel extract of Citrus reticulata. The multiple antibiotic-resistant bacterial isolates were isolated from clinical samples of local hospital in and around Bangalore. Antibacterial activity of the biosynthesized silver nanoparticles using peel extracts of Citrus reticulata fruit was carried out by disc diffusion method. DPPH free radical scavenging method of antioxidant assay was performed using spectrophotometric method. In our study, the results of antibacterial activity reveled that silver nanoparticles synthesised by peel extract of Citrus reticulata fruit has effective antibacterial activities on the test isolates viz. Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Pseudomonas aeruginosa as evidenced by the diameter of their zone of inhibition, and antioxidant activity of biosynthesized silver nanoparticles revealed effective free radical scavenging by silver nanoparticles. In summary, biosynthesized silver nanoparticles can be used as effective antibacterial agents even against multidrug resistant pathogenic bacteria, and possess significant free radical scavenging properties. In conclusion, our preliminary findings encourage to use eco-friendly silver nanoparticles for various medical and electronic applications.

Keyword: Citrus reticulata, Silver nano particles, Anti-microbial, Antioxidant

1. INTRODUCTION

A significant part of biosynthesis of nanoparticles is the utilization of plant concentrate to the biosynthesis reactions. Amalgamation of semi circular silver nanoparticles utilized a decontaminated apiin compound, extracted from henna leaf at ambient conditions. Natural methodologies utilizing microorganisms and plants or plant extracts for metal nanoparticle synthesis have been recommended as important options in contrast to chemical methods [1]. Plants have various reducing agents, for example, polyphenols and flavonoids etc…and are responsible for the reductions of $Ag+$ particles. These poly phenols and flavonoids are utilized as antimicrobial and antioxidant agents by the plants to shield themselves from different pathological conditions. Citrus fruits and derivatives have been notable to effectsly affect human wellbeing inferable from their high concentration of Vitamin C and bioactive mixes, for example, phenolic acid, flavonoid, limonoid, carotenoid and fiber [2], [3].

The essential oils of Citrus sp. have wide varieties of functional properties viz. attractive aroma, a repellant agent against insects and animals, and antioxidant activities. Literature study evidenced the antimicrobial properties of citrus oils [4]. In addition, the advantages of citrus oils not only limited to food industry, but citrus oil in its oil and vapour form pocessess profound antimicrobial activities against wide range microorganisms viz. Gram-positive and Gram-negative bacteria [5].

Moreover, there are a large number of studies on plant essential oils regarding their antimicrobial properties in order to develop a source of antimicrobial ingredients for the food industry [6-8]. Furthermore, literature study revealed that Citrus reticulata has been reported to pocessess anti-bacterial [9], anti-fungal [10], anti-diabetic [11], cardio-protective [12],

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anti-cancer [13], anti-arthritic [14], anti-inflammatory [15], anti-oxidant [16], anti-tubercular, and anti-anxiety medicinal properties [17].

With the rise and increment of microbial creatures impervious to numerous anti-infection agents, and the proceeding with accentuation on human services costs, various researchers have attempted to grow new, successful antimicrobial reagents liberated from resistance and cost. Such issues and needs have prompted the resurgence in the utilization of silver-based germ-killers that might be connected to broad spectrum activity and far lower penchant to initiate microbial resistance than antibiotics [18]. The antibacterial impacts of silver salts have been seen since ancient and silver is at present used to control bacterial development in an assortment of uses, including dental work, catheters and burn wounds [19-21]. With this scenario, the present study was designed to evaluate the antimicrobial and antioxidatant properties of biosynthesized silver nano particles using peel extracts of Citrus reticulata.

2. MATERIALS AND METHODS

2.1 Collection of Pathogens

The multiple antibiotic-resistant isolates viz. Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus aureus were isolated from clinical samples of local hospital in and around Bangalore and confirmed by various microscopic evaluation like Gram's staining [22]. Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne [23]. All the bacterial pathogens were further confirmed by suitable biochemical tests [24] and used for antimicrobial activity studies.

2.2 Determination of Antimicrobial Activity

Antibacterial activity of the biosynthesized silver nanoparticles using peel extracts of Citrus reticulata fruit was carried out by disc diffusion method [25]. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (100 µg/ml) and placed in the nutrient agar plate and kept for incubation at 37ºC for 24 hours. Zones of inhibition for (peel extracts of Citrus reticulata fruit and silver nitrate solution), silver nanoparticles and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

2.3 Antioxidant Assay:

Antioxidant assay for silver nanoparticles was carried out by modified method of Choi et al [26]. Different concentrations (0.025. 0.050, 0.125, 0.250, 0.5 and 1 mg/ml) of silver nanoparticles were individually mixed with 0.5 ml of 1 mM DPPH and incubated in dark for 30 minutes. After incubation the absorbance of the samples was determined at 517 nm against methanol as a blank by using UV-Visible spectrophotometer (Shimadzu, Japan). DPPH methanol reagent without sample was used as control and Vit.C was used as standard. The percentage of inhibition was calculated according to the following formula.

% of inhibition= [(Absorbancecontrol -Absorbancetest) / Absorbancecontrol]×100

3. RESULTS

The results of antibacterial activity portrayed that biosynthesized silver nanoparticles by using peel extract of Citrus reticulata fruit has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The zone of inhibition was 22 mm for Klebsiella pneumonia, 21 mm for Staphylococcus aureus, 20 mm for Proteus mirabilis, 18 mm for Enterobacter cloacae & Pseudomonas aeruginosa, and 17 mm for Escherichia coli. Whereas the test shows the silver nitrate solution has no effect against tested isolates (Table 1 and Figure 1). The results of antioxidant activity of biosynthesized silver nanoparticles using peel extracts of Citrus reticulata depicted effective free radical scavenging by silver nanoparticles, and the antioxidant activity of silver nanoparticles was increased with increased concentration. (Figure 2).

Table. 1. The inhibitory activity of the biosynthesized silver nanoparticles using peel extracts of Citrus reticulata fruit against the test bacteria as demonstrated by diameters of zone of inhibition.

Bacterial Pathogens	Zone of Inhibition (mm)		
	Silver Nitrate Solution	Peel extract of Citrus reticulata	Peetl extract of Citrus reticulata / Silver nanoparticles
Enterobacter cloacae	0	14	18
Escherichia coli	0	11	17
Klebsiella pneumonia	0	13	22
Proteus mirabilis	0	15	20

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Pseudomonas aeruginosa	0	13	18
Staphylococcus aureus	0	15	21

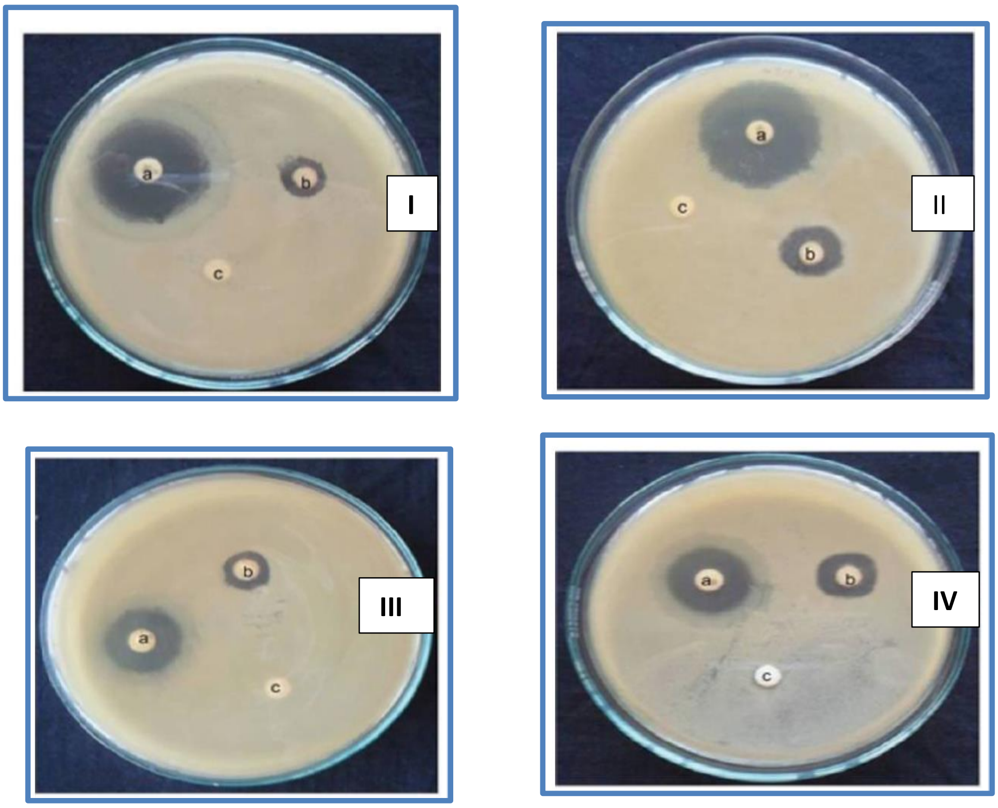

FIGURE 1: The antibactial activity of biosynthesized silver nanoparticles from peel extracts of Citrus reticulata fruit agaist the test bacterium Escherichia coli [I], Klebsiella pneumonia [II], Pseudomonas aeruginosa [III], Staphylococcus aureus [IV].

Note: a-Citrus reticulata peel extract; b-silver nitrate solution; c-using test bacterium

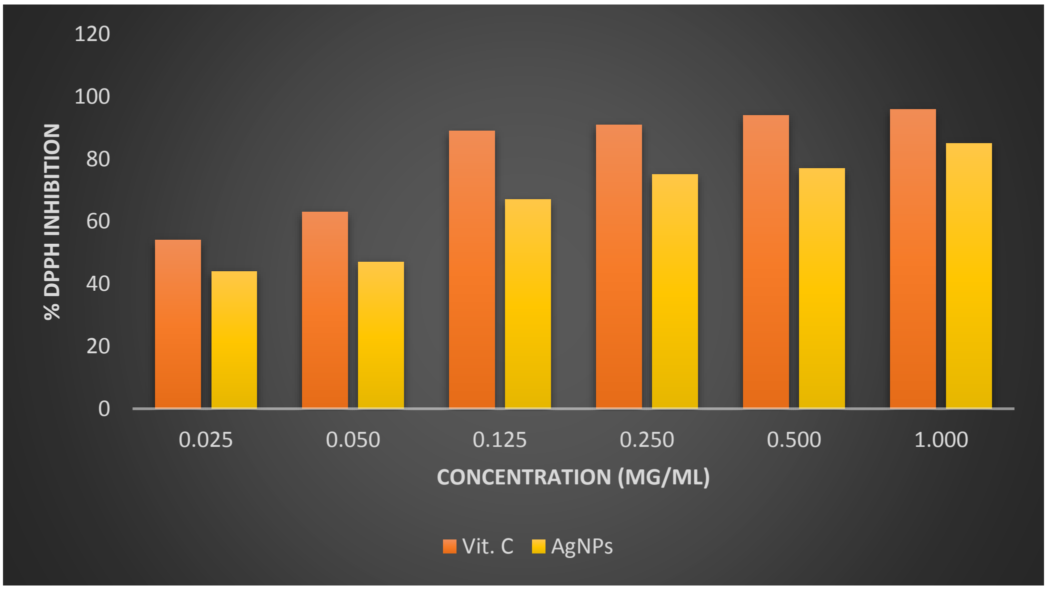

FIGURE 2: Antioxidant Activity of Biosynthesized Silver Nanoparticles

4. DISCUSSION

The results of antimicrobial activity delineated that biosynthesized silver nanoparticles utilizing peel extracts of Citrus reticulata fruit displayed viable antibacterial activities on the test isolates as demonstrated by the diameter of their zone of inhibition. Our outcomes showed that the biosynthesized silver nanoparticles using peel extracts of Citrus reticulata has another system to eliminate microorganisms not found in peel extracts of Citrus reticulata fruit alone. The results of the present investigation are in accordance with discoveries of Hindi et al [27].

The mechanism of the inhibitory effects of silver ions on microorganisms was partially known. previous studies demonstrated that the positive charge on the silver ions play a pivotal role for it's antimicrobial activity through the electrostatic attraction between negatively charged cell membrane of microorganism and positive charged nanoparticles [28-30]. In contrast, Sondi and Salopek-Sondi reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of silver nanoparticle, and was closely associated with the formation of 'pits' in the cell wall of bacteria. Then, silver nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. However, because those studies included both positively charged silver ions and negatively charged silver nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged silver nanoparticles[31]. Amro et al suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins [32]. Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of E. coli during treatment with silver nanoparticles [31]. Recently, Danilczuk et al reported silver-generated free radicals through the ESR study of silver nanoparticles that the antimicrobial mechanism of silver nanoparticles could be due to the formation of free radicals and subsequent free radical–induced membrane damage [33].

Antioxidant activity of biosynthesized silver nanoparticles from peel extracts of Citrus reticulata revealed effective free radical scavenging by silver nanoparticles. Our results are in accordance with previous findings[34-36] .Previous studies demonstrated that the antioxidant property could be due to development of reducing power. Reductones, which have strong reducing power, are generally believed not only to react directly with peroxides but also to prevent peroxide formation by reacting with certain precursors [37]. Silver nanoparticles are suggested to act as electron donors, reacting with free radicals to convert them to more stable products, which can terminate radical chain reaction. Furthermore, the reducing power of silver nanoparticles correlated well with the radical scavenging activity.

5. CONCLUSION

This study findings delineated that biosynthesized silver nanoparticles can be used as effective antibacterial agents even against multidrug resistant pathogenic bacteria, and possess significant free radical scavenging properties. Hence, our preliminary findings encourages to use eco-friendly silver nanoparticles for various medical and electronic applications.

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