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# ANITIMICROBIAL AND ANTIOXIDANT PROPERTIES OF BIOSYNTHESIZED SILVER NANOPARTICLES USING *CITRUS SP*. PEEL EXTRACTS

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**ABSTRACT:**


*The essential oil of Citrus Sp.possesses various biological properties, such as an attractive aroma, a repellant agent against insects and animals, antimicrobial and antioxidant activities. Hence, the present study was designed to evaluate antimicrobial and antioxidant activities of biosynthesized silver nanoparticles from peel extract of Citrus Sp.In our study, the results of antibacterial activity reveled that silver nanoparticles synthesised by peel extract of Citrus Sp. fruit has effective antibacterial activities on the test isolates viz.Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Proteus mirabilisand Pseudomonas aeruginosaas indicated by the diameter of their zone of inhibition.Antioxidant activity of silver nanoparticles synthesized using peel extracts of Citrus Sp. depicted effective free radical scavenging by silver nanoparticles and the antioxidant activity of silver nanoparticles was increased with increased concentration. In conclusion, the active nano compound synthesized by using peel extract of Citrus Sp. fruit can be used as effective antibacterial reagents even against multidrug resistant bacteria. Furthermore, silver nanoparticles are found to have significant DPPH free radical scavenging properties. These results encourages to use eco-friendly silver nanoparticles for electronic and medical applications.*

**KEYWORDS:** *Citrus Sp., Silver nanoparticles, Peel extract, Anti-microbial, Antioxidant.*

## INTRODUCTION :

Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods. An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Synthesis of quasi spherical silver nanoparticles used a purified apiin compound, extracted from henna leaf at ambient conditions.[1]The *Citrus Sp.*essential oils have various functional properties, such as an attractive aroma, a repellant agent against insects and animals, and antioxidant activities. A previous studies reported the antimicrobial action of citrus oils.[2] Meanwhile, citrus oils are not only available to the food industry, but are also generally recognized as safe and have been found to be inhibitory, in both oil and vapor form, against a range of both Gram-positive and Gram-negative bacteria.[3] 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.[4-6]

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of silver-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics.[7] The antibacterial effects of silver salts have been noticed since antiquityand silver is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds.[8-10]With this viewpoints, the present study was conducted to evaluate the antimicrobial and antioxidatant properties of biosynthesized silver nano particles using peel extracts of *Citrus Sp.*fruits.

## MATERIALS AND METHODS

### 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 Mysore and confirmed by various microscopic evaluation like Gram's staining.[11] Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne.[12] All the bacterial pathogens were further confirmed by suitable biochemical tests[13]and used for antimicrobial activity studies.

### Determination of Antimicrobial Activity

Antibacterial activity of the silver nanoparticles synthesized using peel extracts of *Citrus Sp.*fruit was carried out by disc diffusion method.[14] 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 Sp.*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.

### Antioxidant Assay

Antioxidant assay for silver nanoparticles was performed as previously reported with a slightmodification.[15] Different concentrations (0.025. 0.050, 0.125, 0.250, 0.5 and 1mg/ml) of silvernanoparticles were individually mixed with 0.5ml of 1mM DPPH and incubated in dark for 30minutes. After incubation the absorbance of the samples was determined at 517nm against methanol as a blank by using UV-Visible spectrophotometer (Shimadzu, Japan). DPPH methanol reagentwithout sample was used as control and Vit.C was used as standard. The percentage of inhibition wascalculated according to the following formula.

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

## RESULTS

The results of antibacterial activity reveled that silver nanoparticles synthesised by peel extract of *Citrus Sp.* fruit has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The zone of inhibition was 19 mm for *Enterobacter cloacae*, 16 mm for *Escherichia coli*, 21 mm for *Klebsiella pneumonia*, 19 mm for *Proteus mirabilis* and 15 mm for *Pseudomonas aeruginosa.*Whereas the test shows the silver nitrate solution has no effect against tested isolates(Table 1 and Figure 1)

Antioxidant activity of silver nanoparticles synthesized using peel extracts of *Citrus Sp.* revealed effective free radical scavenging by silver nanoparticles. The antioxidant activity of silver nanoparticles was increased with increased concentration. (Fig 2)

**Table 1: The inhibitory activity of the silver nanoparticles synthesized by peel extracts of *Citrus Sp.* 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 Sp.* fruit | Peetl extract of *Citrus Sp.*/ Silver nanoparticles |
| *Enterobacter cloacae* | 0 | 13 | 19 |
| *Escherichia coli* | 0 | 10 | 16 |
| *Klebsiella pneumonia* | 0 | 12 | 21 |
| *Proteus mirabilis* | 0 | 13 | 19 |
| *Pseudomonas aeruginosa* | 0 | 11 | 15 |
| *Staphylococcus aureus* | 0 | 16 | 20 |

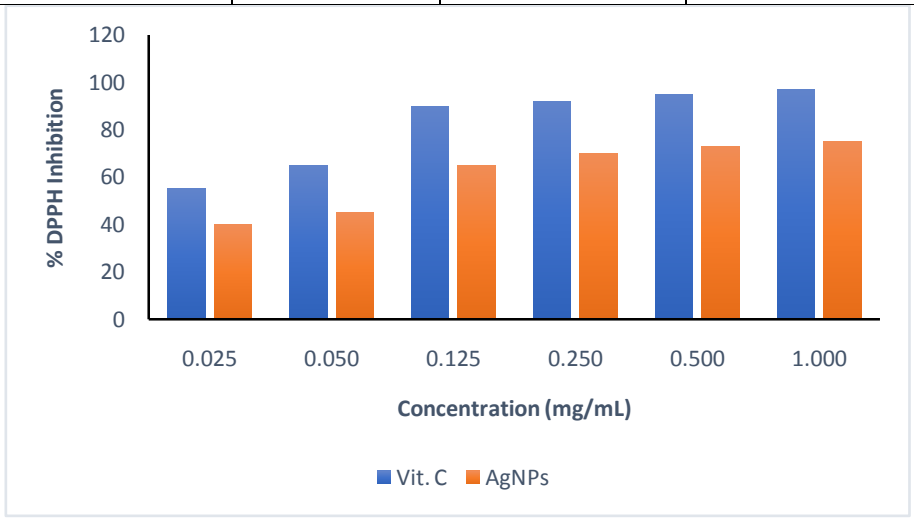
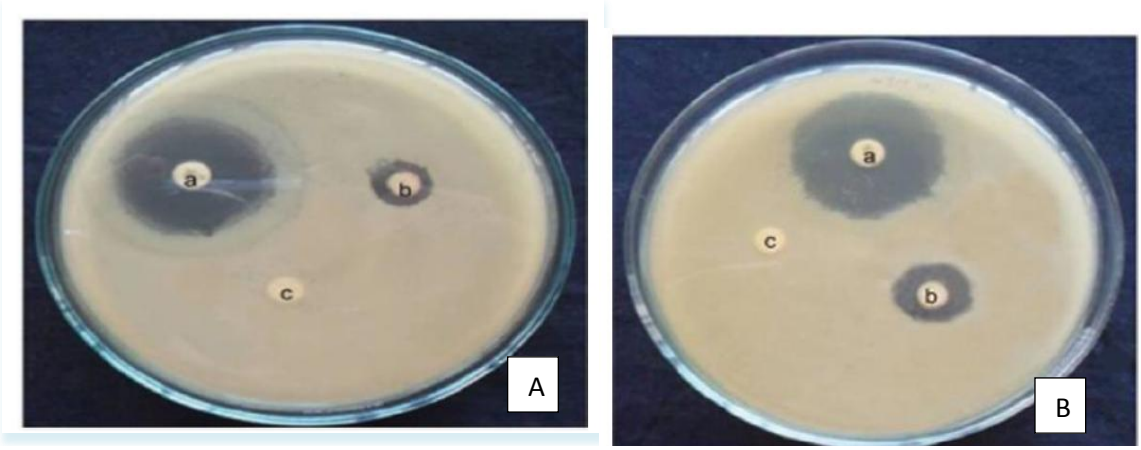



**Fig. 1: Antioxidant Activity of Silver Nanoparticles**



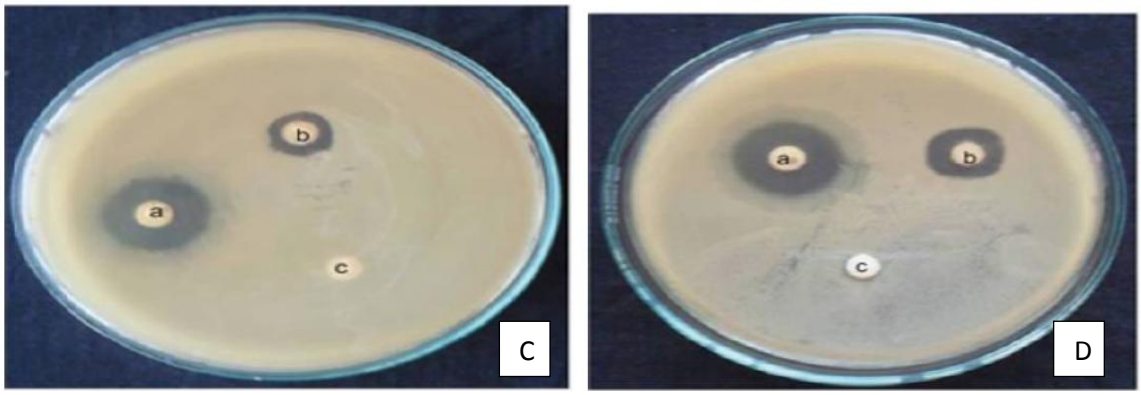



**Fig. 1: The antibactial activity of silver nanoparticles synthesized from peel extracts of *Citrus Sp.* fruit agaist the test bacterium *Escherichia coli* [A], *Klebsiella pneumonia* [B], *Pseudomonas aeruginosa* [C], *Staphylococcus aureus* [D]**

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

## DISCUSSION

The results of antimicrobial activitydepicted that silver nanoparticles synthesisedusing peel extracts of *Citrus Sp.* fruit exhibited effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. Our results demonstrated that the silver nanoparticles synthesised by peel extract of *Citrus Sp.* fruit has another mechanism to kill bacteria not found in peel extract of *Citrus Sp.* fruitalone. The results of the present study are in concurrence with findings ofHindi et al.[16]

The mechanism of the inhibitory effects of silver ions on microorganisms was partially known. previous studies demonstrated that the positive charge on the silver ionsplay a pivotal role for it's antimicrobial activity through the electrostatic attraction between negatively charged cell membrane of microorganism and positive charged nanoparticles.[17-19] In contrast, Sondi and Salopek-Sondireported 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 thepermeability, 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.[20]Amro et alsuggested 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.[21] 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.[20]Recently, Danilczuket 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.[22]

Antioxidant activity of silver nanoparticles synthesized using peel extracts of *Citrus Sp.* revealed effective free radical scavenging by silver nanoparticles. Our results are in agreement with previous findings.[23-25]Previous studies revelaed 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.[26] 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.

## CONCLUSION

In conclusion, the active nano compound synthesized by using peel extract of *Citrus Sp.* fruit can be used as effective antibacterial reagents even against multidrug resistant bacteria. Furthermore, silver nanoparticles are found to have significant DPPH free radical scavenging properties. These results encourages to use eco-friendly silver nanoparticles for electronic and medical applications.

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