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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM FRUIT EXTRACTS.

Malhar Lomte, Deepak Kharat
Department of Biotechnology
K.E. T`S V.G Vaze college of Arts, Science and Commerce,
Mumbai, Maharashtra, India

Guide: Dr Amit Breed

Abstract— **This study focuses on the synthesis and applications of silver nanoparticles. The nanoparticles were synthesized using an environmentally friendly biosynthetic method where fruit extracts were used to act as reducing agents for synthesis of nanoparticles. Citrus lemon and Cocos nucifera are the extracts used. The synthesis of Ag nanoparticles was confirmed by the visible change in colour of the solution. UV-visible spectroscopy was used to monitor the synthesis of the silver nanoparticles. As it is already known that the activity of the synthesized nanoparticle will differ depending on the reducing agents used as the shape and size of nanoparticles also changes. Thus two different fruit extracts were used to determine the difference in the activity of the NPs synthesized. The Antimicrobial tests were carried out using gram positive and gram negative bacteria Staphylococcous aureus and Escherichia coli respectively were used and the inhibiting activity of the nanoparticles over these bacteria was observed. Antioxidant tests were carried out and antioxidant scavenging activity of Ag nanoparticles was calculated by colorimetric tests. The antimicrobial tests showed positive results by inhibiting the bacterial growth. The Minimal inhibitory concentration (MIC) and Minimal lethal concentration (MLC) were performed to find out the ED50 and LD50 for organisms E.coli and Staohylococcus aureus.**

Keywords— **Silver Nanoparticles, Lemon Peel extract, Coconut meat extract, antimicrobial tests.**

I. INTRODUCTION

The application of nanoscale materials structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges such as in areas of water treatment, biology, biomedical sciences, solar energy conversion etc [1]. Nanoparticles posses a very high surface area and their efficiency also depends on their sizes and shapes so the number of different reducing agents used the different Ag nanoparticles synthesized will have different sizes and shapes which will affect their antibacterial, antioxidant, anticancer activity. Phytological origin is main source of many flavonoids and corresponding flavonoid O-glycosides. It has been found that biosynthesized phytological nanoparticles are more effective than their plant extracts. Due to increase in multi drug resistant disease causing organisms there is a need of different and new medical agents. The discovery of nanoparticles has paved the way and is substituting currently used antibiotics and synthetic agents. The characteristic features of nanomaterials such as their size, shape, surface area, distribution, aggregation etc are needed to be evaluated before assessing toxicity or biocompatibility [3]. To evaluate the synthesized nanomaterials many analytical techniques including ultraviolet visible spectroscopy (UV-V spectroscopy), X-ray diffraction, Transmission electron microscopy (TEM), scanning electron microscopy (SEM) etc are used. The fruit extracts used are from Citrus Lemon peels and Cocos nucifera meat extracts as reducing agents for the synthesis of the Ag NPs. Lemon peels were used due to the antibacterial, antioxidant and antiviral properties of lemon juice. It is also know that lemon juice concentrates effectively inhibit the growth of salmonella, staphylococcus and candida infections. The flavonoids in lemon juice are known to protect the cells from damage which can lead to cancer. Coconut meat was used due to its easy availability and its antibacterial, antifungal, antioxidant and immunostimulant properties. They also contain phenolic antioxidant compounds which fight free radicals and protect the body from oxidative stress. Based on these properties the above fruit extracts were used for study.

II. MATERIAL AND METHODS

For synthesis of Ag nanoparticles fresh lemons were procured from the local market. The lemon peels were finely cut or grated, 25g of lemon peels were added to 100ml of distilled water and boiled at 100ºC and stirred continuously for 30mins. The mixture was then left to cool for 15mins and then filtered through whattman filter paper having the pore size of 110nm.The filtered mixture was then refrigerated for further use of synthesis of Ag nanoparticles. A fresh Coconut was procured from a coconut vendor and the coconut meat was separated and crushed in a mortar and pestle.25g of crushed coconut meat was added to 100ml of distilled water and

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placed in a rotary shaker for 4 hours at 110 rpm.The mixture was then filtered using a muslin cloth. For synthesis of Ag nanoparticles silver nitrate ($AgNO_3$) was procured from the college laboratory. 0.032 g of silver nitrate was weighed and added to 200ml of distilled water and mixed continuously for 15mins.The lemon extract (50ml) was taken in a beaker and (50ml) of silver nitrate stock solution was added in dark conditions and mixed for 20mins.The solution was incubated at room temperature in dark conditions for 24hrs. After 24hrs the formation of nanoparticles was characterized by change in the color from transparent and milky white to light brown. UV-V spectral analysis was also carried out to confirm the formation of nanoparticles.

III. RESULTS

A. Observation of colour changes of silver nanoparticles

The colour of the fruit extract was changed from light/ transparent colour to light brown colour. This colour change indicates synthesis of nanoparticles. This change is due to reductions of Ag^+ ions to Ag^0 atoms.
The reaction is as follows

Fruit extracts + $AgNO_3$ ⟶ Ag Nanoparticles

This happens due to the biomolecules in the extracts that act as reducing agents to reduce AgNO3 to Ag atoms. The colour change also varies depending on the concentrations of extracts added. More the concentration of the extract the darker will be colour.

B. UV-V spectral analysis:

For UV-V spectral analysis the wavelength was kept between 200-500nm. The highest peak observed for lemon extract synthesized nanoparticles was at 272nm with an absorbance of 4.982 and for coconut extract synthesized nanoparticles was at 208nm with an absorbance of 3.189. All the high peaks that were observed were at the lower range than the standard absorbtion peaks for the Silver Nanoparticles. The figures 1.1 and 1.2 show the graphical representation of the UV-V spectral analysis.

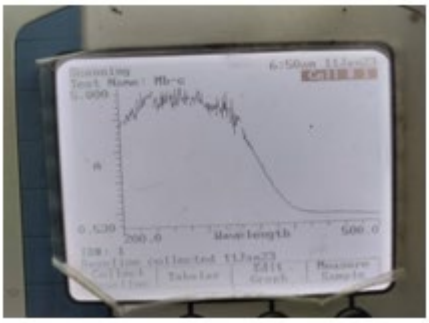

Fig 1.1

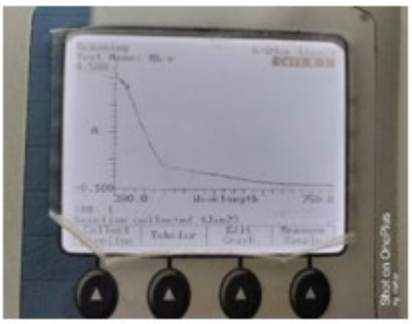

Fig 1.2

C. Antibacterial activity: Agar well method

The Antibacterial activity of synthesized nanoparticles was observed for organisms E.coli and S.aureus. After 24hrs of incubation the agar wells were observed and the nanoparticles expressed potential antibacterial effect against both gram positive and gram-negative bacteria. It was observed that zone of inhibition for S,aureus was distinct for both nanoparticles synthesized from Lemon and Coconut extracts. The zone of inhibition formed by coconut extract NPs was more as a result having high suppressing activity. The zone of inhibition for lemon was small and showed low suppressing activity. For E.coli the zones were very small and didn't show much of inhibiting activity for both coconut and lemon extract synthesized NPs. The figure 1.3 shows the results of the agar well method plates.

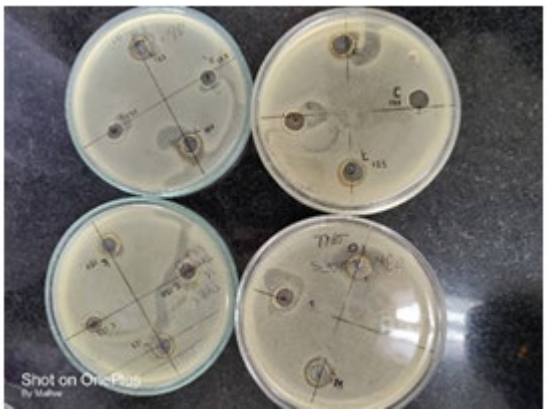

Fig 1.3

D. Antibacterial activity: Paper disc method

The experiment showed promising results for inhibiting activity of E.coli and S.aureus. The NPs synthesized from Coconut extract showed better results compared to lemon extract for inhibition of E.coli. Both the synthesized nanoparticles showed similar level of inhibition for S.aureus. From these test it is inferred that gram negative bacteria like E.coli were more sensitive to action of biologically synthesized nanoparticles. The figures 1.4 and 1.5 show the inhibition activity of the synthesized nanoparticles for both E.coli and S.aureus respectively.

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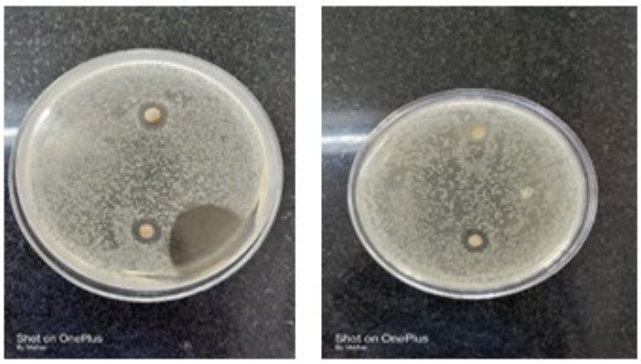

Fig 1.4 and 1.5

E. MIC (Minimal inhibitory concentration) test:

Nanoparticles synthesized from different plant extracts and also have different size, shape inhibit the microorganisms at different concentrations. To find the minimum concentration of the synthesized NPs that inhibits the growth of micro-organism MIC (minimal inhibitor concentration) was carried out. From the tests carried out the results showed that both the nanoparticles inhibit the growth of the E.coli and S.aureus. For S.aureus NPs synthesized from lemon extract showed best results as no growth was observed in any of the tubes whereas for NPs synthesized from coconut extract growth was observed in tube having NP concentration of 0.1ml. For E.coli NP synthesized from lemon extract showed no growth in any of the tubes as for NPs synthesized from coconut extract showed growth in all tubes except for the tube with NP concentration of 0.5ml. The figures 1.6 and 1.7 show the addition tables for the MIC.

Addition table (for lemon extract synthesized NPs)

SR No	Volume of sample (NPs)	Nutrient Broth (ml)	Culture(ml)
1	0.5	4.5	0.1
2	0.4	4.6	01
3	0.3	4.7	0.1
4	0.2	4.8	0.1
5	0.1	4.9	0.1

Addition table(for coconut extract synthesized NPs)

SR No	Volume of sample (NPs)	Nutrient broth (ml)	Culture(ml)
1	0.5	4.5	0.1
2	0.4	4.6	0.1
3	0.3	4.7	0.1
4	0.2	4.8	0.1
5	0.1	4.9	0.1

Fig 1.6 MIC of *S.aureus* for coconut extract synthesized NPs

Fig 1.7 MIC of *S.aureus* for lemon extract synthesized NPs

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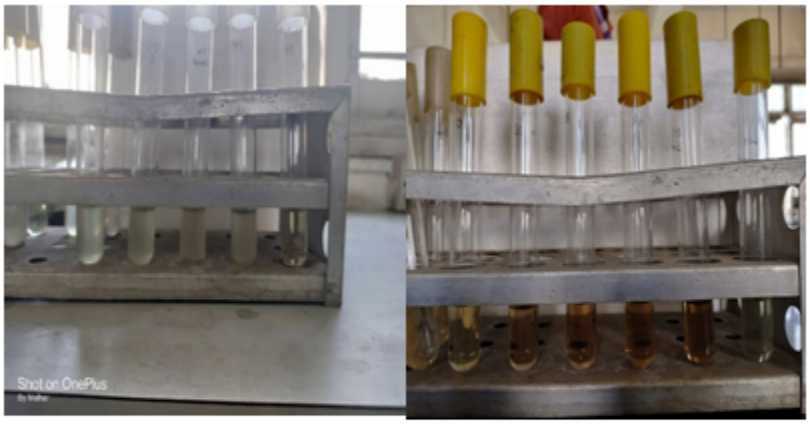

Fig 1.8 MIC of *E.coli* for coconut extract Synthesized NPs

Fig 1.9 MIC of *E.coli* for lemon extract synthesized NPs

F. Antioxidant DPPH assay:

To find the antioxidant scavenging activity of fruit extract synthesized nanoparticles antioxidant assay was carried out. The nanoparticles synthesized from the coconut extract showed gradual decrease in absorbance at 520nm. The % scavenging activity of for Coconut extract NP was from 4.91% to 32.78% in a gradual increasing order. The % scavenging activity for Lemon extract NPs was from 11.9% to 38.09%. These results showed that the NP synthesized from lemon extract have showed more DPPH scavenging activity than coconut extract NPs. The figures 1.10 and 1.12 show the addition table for the assay and figure 1.11 and 1.14 show the graphical results.

Observation Table (For lemon extract synthesized nanoparticles)

Volume of stock (ml)	Methanol (ml)	DPPH (ml)	Absorbance 520nm	%Scavenging activity
0.2	0.8	3	0.37	11.9%
0.4	0.6	3	0.34	19.04%
0.6	0.4	3	0.30	28.57%
0.8	0.2	3	0.28	33.33%
1	-	3	0.26	38.09%

Fig 1.10

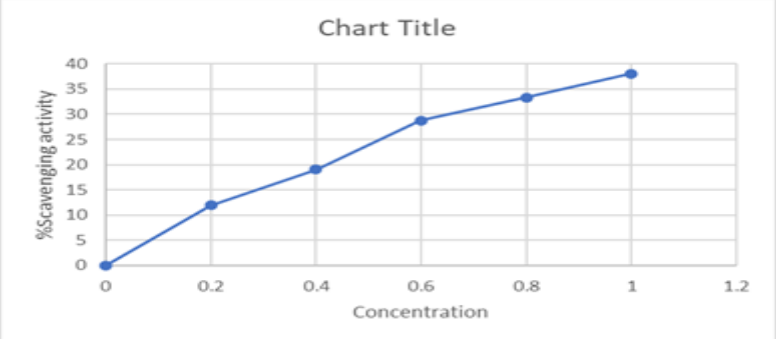

Fig 1.11

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Observation Table (for coconut extract synthesized nanoparticles)

Volume of stock (ml)	Methanol (ml)	DPPH (ml)	Abs 520nm	scavenging activity (%)
0.2	0.8	3	0.58	4.91%
0.4	0.6	3	0.52	14.75%
0.6	0.4	3	0.50	18.03%
0.8	0.2	3	0.48	21.31%
1	-	3	0.41	32.78%

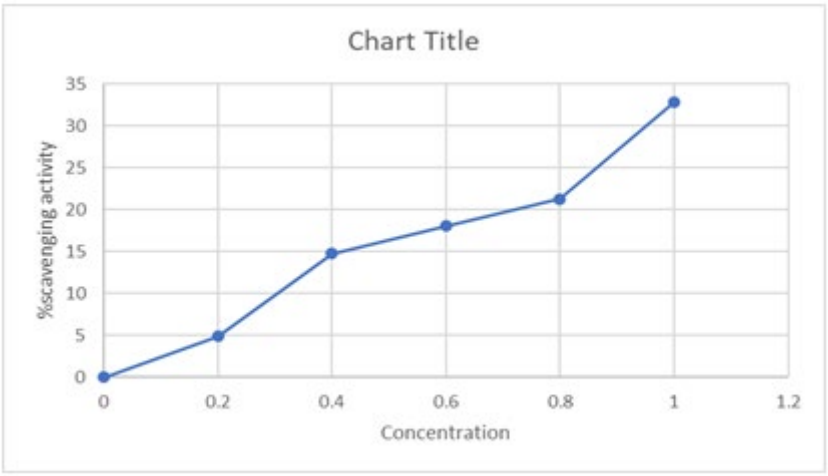

Fig 1.12 and 1.13

Fig 1.16 DPPH antioxidant assay for lemon extract synthesized NPs

Fig 1.17 16 DPPH antioxidant assay for coconut extract synthesized NPs

IV. CONCLUSION

The results of the following tests were compared and it was observed that both the nanoparticles synthesized from lemon extract and coconut extract showed almost same results. For all the antibacterial the results seen were similar and neither of the synthesized nanoparticles were more potent. For MIC the lemon extract synthesized nanoparticles showed the best inhibitory effect as no growth was observed in any of the tubes. For DPPH assay the lemon extract synthesized nanoparticles showed slightly better results than coconut extract synthesized nanoparticles.

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