



**GREEN SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF GOLD  
NANOPARTICLES SYNTHESIZED USING  
*CITRUS MEDICA* EXTRACT**

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**ABSTRACT**

This research outlines an environmentally friendly method for producing gold nanoparticles (AuNPs) that uses *Citrus medica* extract as a reducing and stabilising agent. The UV-Vis spectra showed a clear absorption peak at 540 nm, which was used to confirm the production of AuNPs. The synthesised AuNPs' spherical, 18 nm-average size was measured using transmission electron microscopy (TEM). It was feasible to confirm the existence of functional groups that were in charge of the reduction and stabilisation of AuNPs using Fourier transform infrared spectroscopy (FTIR). Four bacterial strains—*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*—were employed to test the antibacterial effectiveness of the synthesised AuNPs. Overall, the results of this study indicate that the synthesis of AuNPs with strong antibacterial activity can be achieved using *Citrus medica* extract in a practical and environmentally friendly manner. In this study gold nano particles of *Citrus medica* extract showed antibacterial activity

**Keywords:** Nano:green:organisms: AuNPs-pharma

**INTRODUCTION**

Due to its environmentally beneficial, economically advantageous, and long-lasting characteristics, green nanoparticle synthesis has attracted a lot of attention. *Citrus medica* extract has demonstrated promising results when compared to other plant extracts used for the creation of nanoparticles. The citrus fruit *Citrus medica*, usually referred to as Citron, is widely cultivated around the world. It is abundant in phytochemicals with antibacterial and antioxidant

characteristics, such as flavonoids and phenolic compounds. Due to their distinctive physical and chemical characteristics, gold nanoparticles (AuNPs) are among the nanoparticles that have been the subject of the most research. They are used extensively in many different industries, including biomedicine, electronics, and catalysis. Numerous studies have been done on the antibacterial activity of AuNPs, and the results against diverse bacterial strains have been encouraging. As a result, the purpose of this study was to synthesise gold nanoparticles using Citrus medica extract and to test their antibacterial effectiveness against various bacterial strains. The biocompatible and stable AuNPs with improved antibacterial activities are anticipated to be produced employing the green synthesis method using Citrus medica extract as a reducing and capping agent.

There are about 70 species of citrus, making it the largest genus in the Rutaceae family ( Bora, H.; 2020,Farag, M.A,Favela-Hernández,2020). These fruits have substantial antioxidant, anti-inflammatory, and anti-tumor potential because of their high quantities of bioactive substances, including flavonoids, polyphenols, and vitamins. One of the areas of modern material science research that is most active is the field of nanotechnology.(SamerBayda et al., 2019).Gold nanoparticles have a strong antibacterial capability against foodborne pathogens and can be employed in a variety of nanomedicines and nano-food packaging to shield food (items) from microbial attack. (Chen *et al.*, 2008).

In order to avoid wound contamination, which is a common problem due to bacterial contamination of wounds, prevention is key. Numerous medications are suggested in the usual wound management. An efficient antimicrobial dressing, however, might support normal wound healing. Numerous studies showed that AgNP improved antibacterial activity in vitro, and it was shown that AgNP would be an excellent choice for antibacterial wound care. In contrast, green chemistry emphasises reducing or completely eliminating the use of harmful substances. This technique is based on the use of fungi, bacteria, and plants to create silver nanoparticles. Given that plants are readily available, using them to create silver nanoparticles is thought to be useful.The procedure does not call for any culture preparation or isolation methods. As a result, the approach is regarded as economical [Bar H, Bhui DK*etal.*,2009)

## MATERIALS AND METHODS

In our research, we used several chemicals and strains to carry out the experiments and we purchased the chemicals, namely, Methanol, acetone, and distilled water, silver nitrate from the SU Chemicals Pvt. Ltd from Chennai, Tamilnadu.

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### Methodology

#### Preparation of *Citrus medica* fruit extract

A small market in Chennai is where the citrus medica fruit was obtained. The fruits were chopped into small pieces and cleaned with distilled water. Eight grammes of fruit were divided into 80 ml of distilled water and cooked for two to five minutes each. Each filtrate was collected separately and kept at 4°C for later usage after the obtained lemon extract was filtered using filter paper.

#### Synthesis of Gold nanoparticles (AuNPs)

The resultant colour shift was seen after combining 10 mL of aqueous lemon extract with 90 mL of a 1 mM AuCl<sub>3</sub> solution to make Au NPs. The AuNPs solution was then spun at 10,000 rpm for 15 min at 4 °C to purify it. Then, after floating in deionized water, the resultant pellet was then dried and kept at room temperature for further use

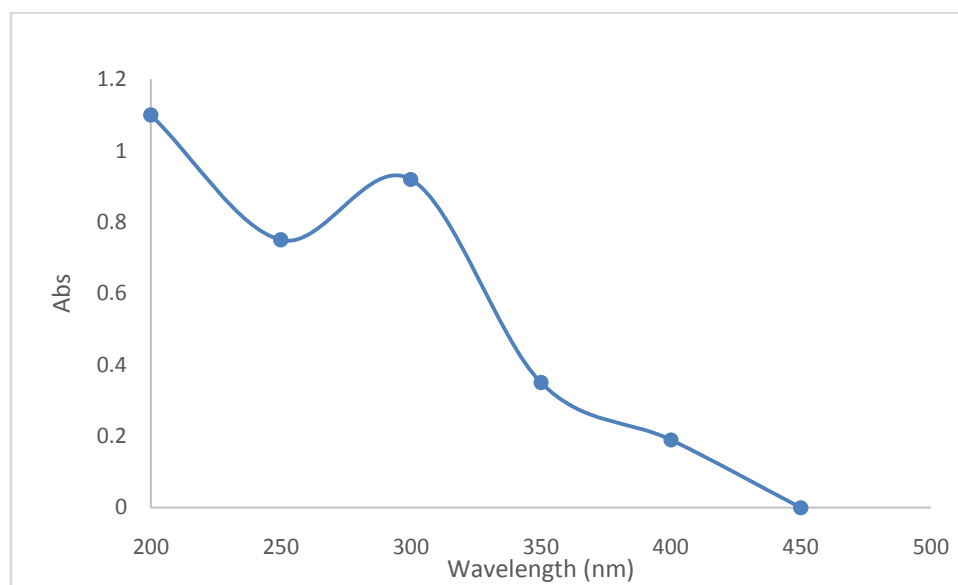


**Citrus medica**fruit extract Synthesised Au-NPs

## Characterization of AuNPs

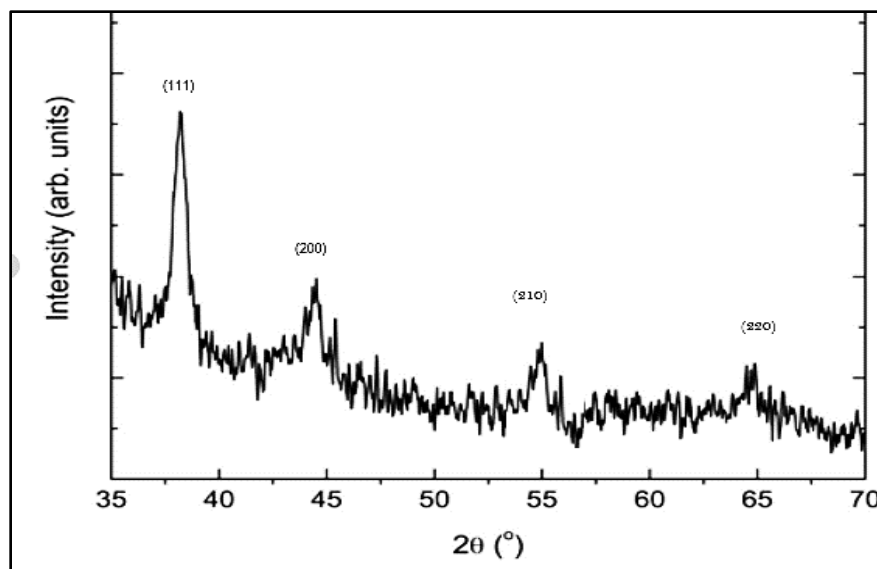
### UV-Vis analysis

A Labomed double-beam UV-visible spectrophotometer with a 1.0 nm spectral bandwidth was used to record the UV-Vis absorption spectra of synthetic Citrus medica AuNPs (CL-AuNPs). AuNPs samples' absorption spectra were also captured to assess their stability. Three millilitres of the reaction mixture were placed for spectrum analysis. A quartz cuvette for spectral investigation of the reaction mixture. Between 300 and 700 nm in wavelength, spectral analysis was done.



### X-Ray diffraction analysis

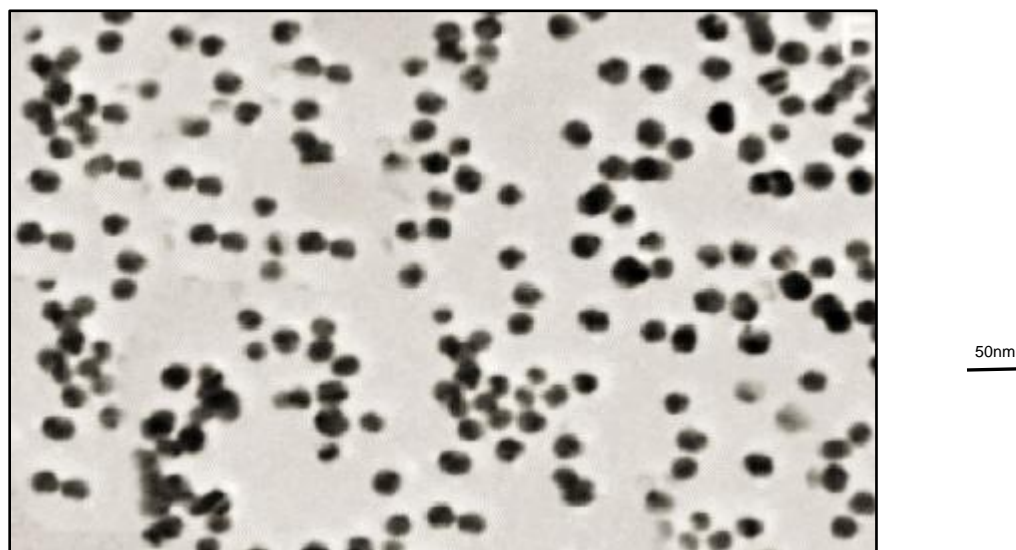
Using a JEOL JDX Powder X-Ray Diffract Metre and Cu-K radiation with a wavelength of  $\lambda = 1.5406$  Å, data on the synthesised Au nanoparticles were collected between the ranges of  $2\theta$  of 10 and 70 with a step scan of 0.04.



X-ray diffraction pattern of silver nanoparticles of *Citrus medica* extract. Peaks are assigned to diffraction from the (111), (200), (210) and (220) planes of Gold indexed. The diffraction peaks were of X-ray diffraction pattern face-centered cubic structure that is indexed to the gold metal. In good agreement with the typical diffraction pattern of cubic gold metal, the lattice constant derived from the XRD pattern is  $a = 3.073$ .

### TEM study

Morphological studies of shape, and size of nanoparticles Gold nanoparticles were viewed using an (JEM2100) HR TEM JEOL the TEM method of transmission electron microscopy. The size of AuNPs was measured using Image J programme.



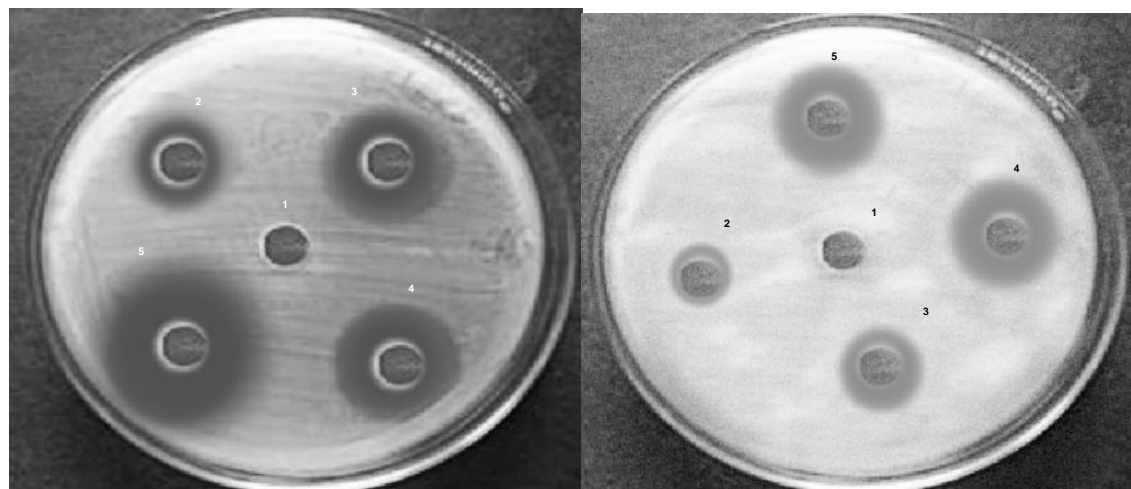
TEM photos proved the presence of small particles with diameter ranged between 20 to 40 nm.

### ANTIBACTERIAL ACTIVITY

The diffusion method using agar wells was employed to assess the antibacterial activity towards *B. subtilis* and *S. aureus*. On (MH) agar medium–containing petri plates, the freshly developed chosen organisms were subcultured. After creating the 6 mm wells, different CL-AuNP concentrations (10, 20, and 30 g/mL) were put into the well. The agar plates were then incubated in an incubator for 24 hours at the ideal temperature. Zone of inhibition was monitored and evaluated following incubation. The outcomes were contrasted with a conventional control of 30 g/mL of ampicillin.

Analysis of statistics

Multiple comparisons in the statistical analysis of the data were done using one-way analysis of variance (ANOVA), and post hoc analysis was done using the LSD test. P 0.05 was used as the cutoff point for statistical significance. The data was analysed using SPSS (version 11).



*Bacillus subtilis*

*S.aureus*

- 1- Negative control; 2- CL-AuNPs (10 µg); 3 - CL-AuNPs (20 µg); 4- CL-AuNPs (30 µg);  
5- STD

Zone of Inhibition (mm)			
	Conc ( $\mu\text{g/ml}$ )	B.subtilis	S. aurues
CL-AuNPs	10	$9.8 \pm 0.56^{*** a}$	$1.2 \pm 0.43^a$
	20	$12.5 \pm 1.1^{*** b}$	$3.4 \pm 0.23^{* a}$
	30	$14.3 \pm 0.87^{***}$	$10.5 \pm 0.47^{** b}$
Ampicillin	30	$15.6 \pm 0.91^{***}$	$14.3 \pm 0.82^{***}$
Negative control	-	NI	NI

NI stands for "no inhibition zone." The mean SEM ( $n = 3$ ) of each number is used to express it. statistically significant when compared to the negative control at  $*p0.05$ ,  $**p0.01$ , and  $***p0.00$ . statistically significant when compared to STD at  $ap = 0.001$  and  $bp = 0.05$ .

## Results and Discussion

Thus, synthesized gold nanoparticles from To create an antibacterial agent, citrus medica may be a viable choice against the *Bacillus sps* and *Staphylococcus aurues* microorganism. Each and every one of the synthesised AgNPs exhibited both +ve and -ve bacteria are susceptible to their antibacterial activities. Even at lower extract concentrations, the synthesised AgNPs can be employed as a potential antibacterial agent. (Lebogang Mogole *et al.*, 2021) *Escherichia coli* and *Staphylococcus aureus*, two types of bacteria with different gramme sizes were both susceptible to AgNPs' broad-spectrum antibacterial action. The zone of inhibition was an effective way to show that silver nanoparticles have antibacterial properties. +is approach of creating AgNPs is economical and environmentally beneficial. (Moir Carmalita Dharsika Niluxsshun *et al.*, 2020)

## CONCLUSION

This work closes by demonstrating how to make gold nanoparticles (AuNPs) in a sustainable manner using Citrus medica extract as a reducing and stabilising agent. The synthesised AuNPs were examined using (TEM), (FTIR), and UV-Vis spectroscopy. The creation of spherical AuNPs with an average size of 18 nm was verified by these techniques. Four distinct bacterial strains, including *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and

*Staphylococcus aureus*, were employed to investigate the antibacterial effectiveness of the synthesised AuNPs. The AuNPs considerably reduced the activity of all of the tested bacterial strains, with *S. aureus* having the biggest inhibition zone. The (MIC) and (MBC) values for AuNPs against the tested bacterial strains were also determined. Overall, this study suggests that *Citrus medica* extract may be a practical and environmentally sound technique for creating AuNPs with potent antibacterial action against several bacterial species. The synthesised AuNPs may be used in a range of biomedical applications, including medicine administration and wound healing, because of their unique properties and biocompatibility. The potential applications of these AuNPs must be further investigated, as well as the mechanisms that underlie their antibacterial action. We created nanoparticles based on our research work, and these extract nanoparticles demonstrated antibacterial activity.

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