

Biosynthesis of Two Nanoparticles using *Ficus auriculata* Lour. Fresh Fruit extract and their Antibacterial Activity

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ABSTRACT: The bio synthesis of plant-mediated based nanoparticles is increasingly used to target bacteria as an alternative to antibiotics. The use of nanoparticles in antibacterial vaccines for the control of bacterial infections, antibiotic delivery systems for the treatment of many disease, the bacterial detection systems for the generation of microbial diagnostics, and antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing. The present study was focused on studying the in antibacterial activity of nano synthesized *Ficus auriculata* against the gram-negative and gram-positive pathogenic bacteria *Escherichia coli*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Enterobacter faecalis*, *Staphylococcus aureus*, *Shigellasonnei*, *Klebsiella pneumonia*, and *Vibrio cholerae*. The nanoparticles were characterized by UV-visible and FT-IR spectroscopy. The fruit extracts of *F. auriculata* have potent activity against bacterial pathogens. The result of the study will help to design the drug against selected human pathogens in the pharmaceutical industry.

Keywords: *Ficus auriculata* Antibacterial activity, UV-Visible spectroscopy and CuNPs, AgNPs.

INTRODUCTION

In current centuries, nanotechnology has attracted a significant number of researchers from a wide variety of domains, including biological, physics, chemistry, the material sciences, engineering, and medicine, among others. Traditional physical and chemical approaches for synthesising nanoparticles have limitations, including the need for hazardous reaction conditions, extended reaction durations, expensive and rare chemicals, and a tedious isolation process (Lanje *et al.*, 2010; Yang *et al.*, 2012). Therefore, there is room for innovation in the synthesis of NPs towards methods that are less resource- and energy-intensive. Nanoparticles' unique physical, chemical, and biological properties make them applicable in almost any field. As an example, metal nanoparticles have been put to use in the medical and industrial sectors (Mercado *et al.*, 1981; Shaheen *et al.*, 2019) because of their potent antibacterial capabilities. Synthesis techniques for nanomaterials that start with naturally occurring compounds are a rapidly growing area of research (Pelle *et al.*, 2018). By boosting the biocide qualities of nanoparticles, the application of natural extracts that function as both reducers and stabilisers can boost the microbiological activity of nanoproducts. Herbal, fruit, and vegetable extracts are commonly

employed in nanoparticle manufacturing (Zain *et al.*, 2014). Nanoparticles of silver and copper are frequently employed as antibiotics and antifungals. It is well-known that numerous extract types can be employed in the creation of nanoparticles. Antibacterial activity of silver nanoparticles synthesised with banana peel extracts was investigated (Yasir *et al.*, 2018). Used fresh leaves of *Syngonium podophyllum* for silver nanoparticle production; (Padma *et al.*, 2018) looked at extracts of *Punicagranatum* for copper nanoparticle synthesis. One of the major subfields of nanoparticle biosynthesis is the incorporation of plant extracts into the biosynthesis reaction. Because of its decreased qualities, plant extracts like those found in leaves and fruits can be employed as a capping and reducing agent in nanoparticle manufacturing. The current study used *Ficus auriculata* fruit extracts to biologically synthesise nanoparticles. Atthi, or *Ficus auriculata* (Moraceae), has been used for thousands of years in Ayurveda, the ancient Indian medical system, to treat a wide range of illnesses and conditions. UV-visible and FT-IR spectroscopy were used to characterise the synthesised silver and copper nanoparticles mediated by the fruit. Eight different harmful bacterial strains were used to test the nanoparticles' antimicrobial efficacy.

In this study, nanoparticles were biologically synthesised utilising *Ficus auriculata* fruit extracts. It has been used in Ayurveda, the ancient Indian medical system, for a wide range of conditions, including cancer, diabetes, liver disorders, diarrhoea, inflammatory conditions, haemorrhoids, respiratory diseases, and urinary diseases. The fruit-mediated silver and copper nanoparticles were synthesized using standard procedures and characterized by UV-visible and FT-IR spectroscopy. The antibacterial activity of the nanoparticles was also studied.

MATERIALS AND METHODS

A. Material

The fresh fruit of the Moraceae family plant, which belongs to the genus *F. auriculata*, was chosen as the experimental material for this particular investigation. The Marthandam region of the Kanyakumari district in Tamil Nadu was the collection location for these fruits. The taxonomic characteristics were confirmed with the 'Flora of the Presidency of Madras' (Gamble, 1928) and the 'Flora of the Tamilnadu Carnatic' (Mathew, 1981), as well as with the 'Flowering plants of western Ghats India' (Nayar *et al.*, 2014). The fruit parts of *F.*

auriculata under were selected for the present investigations showed in Fig. 1.



Fig. 1. Natural Habit of *Ficus auriculata* Lour. and Fruit.

B. Preparation of Plant Extract

Fruits were picked while still firm and fresh, then washed in both tap water and distilled water to eliminate any dust or debris that could be apparent. Small chunks of fresh fruit (10g) were weighed out and diluted into 100 ml of distilled water, then heated for 15 minutes. The obtained extracts were filtered using Whatman no. 1 filter paper, and the resulting filtrates were kept at 4°C until needed (Manisha *et al.*, 2013).

C. Synthesis of Silver and Copper Nanoparticles

Table 1: Methodology for Synthesis of Nanoparticles using Different Metals.

AgNO ₃ Mediated Synthesis (Alagumuthu and Kirubha 2012; Dwivedi <i>et al.</i> , 2013)	CuSO ₄ Mediated Synthesis (Susrutha and Karthikeyan 2006)
<ol style="list-style-type: none"> 1 mM AgNO₃ (0.1619g) solution was prepared. 10 ml of aqueous extract is added with 90 ml of 1 mM AgNO₃ solution. Kept in dark room at 24 hours. The colour change was observed from pale yellow to dark brown. 	<ol style="list-style-type: none"> 1mM CuSO₄ (0.06243g) solution was prepared. 20 ml of aqueous extract is added with 80 ml of 1 mM CuSO₄ solution. Kept in dark room at 24 hours. The colour change was observed from light blue to dark blue.

D. Concentration of phyto nanoparticles

A colour change was observed after 24 hours of incubation. Aqueous solution synthesised the nanoparticles from plants. This solution was centrifuged at 10,000rpm for 20min. After centrifugation, pellets were combined with petroleum ether for quick drying and collected in a micro centrifuge tube with ethanol for characterisation and antibacterial tests.

E. Characterization

(i) UV-VIS-Spectrophotometer analysis. To monitor nanoparticle synthesis, 1ml of the sample suspension was taken in a quartz tube, diluted with 2ml of distilled water, and scanned in UV-Vis spectra between 200 and 800 nm in a UV-visible spectrophotometer. For maximum absorption, 24h UV-Vis spectra were obtained (Banerjee *et al.*, 2014).

(ii) FT-IR spectrophotometer analysis. FT-IR is the best method for identifying chemical bonds (functional groups) in compounds. FT-IR analysis employed nanoparticle powders. For translucent sample discs, 10mg of dried extract powder was encapsulated in 100 mg of KBr pellet. The powdered sample of each particle was placed into an FT-IR spectroscope

(SJASCO FTIR 410 Spectrophotometer) with a scan range of 400–4000 cm and a resolution of 2 cm⁻¹.

E. Antibacterial Activity of Nanoparticles

The antibacterial activities of isolated plant nanoparticle pellets were tested by agar disc diffusion method. The test organisms used for the assay are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio cholera*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Streptococcus pyogenes* and *Shigella sonnei*. The antibacterial activities of the synthesized nanoparticles were evaluated by measuring the zone of inhibition method. The samples for each bacterial strain were sub-cultured in individual agar slants.

(i) Maintenance of Microbial Strains. Testing microbes were isolated from pure cultures by streaking them onto agar plates. Pure cultures were streaked on Muller–Hinton agar slants and kept at 4°C to preserve microbial strains. Filter paper (Whatman No. 1) disc diffusion measured antibacterial activity 8. Pre-sterilized discs. Sterile discs received nanoparticle solution extracts. Each sterilized disc received 200–500 microliters of nanoparticle extract solution by micropipette. Ten test tubes contained Muller–Hinton Agar broth. Cotton-plugged and autoclaved. 18 hours at 37±1.5°C incubated Muller–Hinton Agar broth. After

incubation, microbial strains were smeared on sterile Muller–Hinton agar plates and incubated at 37°C for 18hrs. After incubation, disc inhibition zones were measured and recorded. Each concentration included three replicates.

RESULT AND DISCUSSION

A. Synthesis and characterization of Nanoparticles

The production of nanoparticles in *F. auriculata* fruit extract after treatment with different metal compositions (Silver Nitrate and Copper Sulphate) resulted in a colour change from colourless to dark brown and pale yellow. UV-Visible Spectroscopy and FT-IR Spectroscopy, both of which are commonplace in biological research, also confirmed the presence of nanoparticles.

B. UV- visible spectrum Analysis

The colour shift and subsequent UV-vis spectroscopy may follow the reduction of the metal ion to metal nanoparticles when exposed to the plant fruit extract of *F. auriculata*. Surface plasmon vibrations were excited,

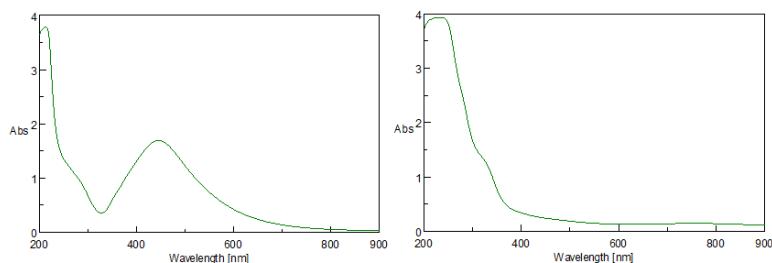


Fig. 2. UV- Vis spectrum of Silver and Copper Nanoparticles of *F. auriculata*.

(ii) Copper nanoparticles analysis of *F. auriculata*.

UV-Visible Spectroscopy is a method that has been considered useful for studying the synthesis of copper nanoparticles. A 265 nm absorption peak was identified in the spectra of copper nanoparticles generated in reaction fluids, and this peak is indicative of copper nanoparticles (Fig. 2). Absorption peak sharpness appears to be concentration ratio dependent in UV-Vis spectra (Sheny *et al.*, 2011).

C. FT-IR spectrum analysis

FT-IR Analysis peak values and functional groups of silver and copper nanoparticles fruit extracts of *F. auriculata* were represented in following Figures and Tables. The FT-IR Spectrum confirmed the presence of functional groups such as NH₂ Amines, C-C, NH₂& N-H, S-OR Alkane, Amines, Esters, C-O, C-O, O-C, C-N, C=S, P-H, Si-OR Alcohol, Ether, Anhydrides, Amines, Thiocarbonyl, Phosphine, Silane, C-O, C-O aromatic, P=O, P=O, Si-CH₃ Carboxylic Acids, Ester, Amine oxide (N-O), Phosphonate, Phosphoramidate, Silane, S=O Sulfate, NH₂ mines, N=C=O -N=C=S, -N=C=N-, -N₃, C=C=O, C-C, Si-HIsocyanates, Isothiocyanates, Diimides, Azides, Ketenes, Alkyne, Silane, P-H, Si-H Phosphine, Silane, C-H, O-H Alkane, Carboxylic Acids, O-H Carboxylic Acids, O-H, N-H Alcohol, Amines etc.

leading to a shift in colour. Nanoparticles of a specific size and shape can be studied in aqueous solutions using UV-Vis spectroscopy (Wiley *et al.*, 2006). Using a UV-visible spectrophotometer with an absorbance range of 200-900 nm (Fig. 2 and 3), we were able to validate the existence of nanoparticles by getting a spectrum in the visible range.

(i) Silver Nanoparticles analysis of *F. auriculata*.

Absorption spectra of silver nanoparticles created in the reaction medium have showed the absorbance peak in the range of 250 to 600 nm, and the absorption peak found at 455 nm is the characteristic peak of silver nanoparticles (Fig. 2). Our results corroborated those of 6 who investigated the impact of leaf extract concentration on the size of silver nanoparticles. The synthesis of silver nanoparticles has been shown to be significantly impacted by the concentration of metal salts (Dubey *et al.*, 2010). Similar findings on the impact of biomass quantity on nanoparticle synthesis have been published previously (Arunachalam *et al.*, 2013).

The results of FT-IR spectrum of selected *F. auriculata* synthesized silver and copper nanoparticles were displayed in (Fig. 3 & 4) and functional groups corresponding to the peak values tabulated in (Tables 2 & 3).

(i) Silver Nanoparticles of *F.auriculata*. The FT-IR spectrum of Silver nanoparticles of *F. auriculata* confirmed the presence of functional groups such as NH₂ & N-H Amines, NH₂& N-H, S-OR Amines, O-C, C-N, S=O, P-H, P-OR, Si-OR, Anhydrides, Amines, Sulfoxide, Phosphine, Esters, Silane, S=O Sulfate, C=C, N-H, nitroso, nitro, Aromatic, Amides, N=O, N=O, NH₂, nitroso, C=C, C=O, C=N Amines, N=O, Alkene, Amides, Oxime (=NOH), P-H, Si-H Phosphine, Silane, C-H, O-H Alkane, Carboxylic Acids, O-H, N-H Alcohol, Amines in the peak values are 1043.41, 1213.64, 1327.81, 1354.77, 1481.12, 1550.50, 1529.51, 1634.21, 1760.42, 2087.34, 2307.72, 3247.30, 3282.25, 3343.12 and 3580.74 respectively (Fig. 3 and Table 2). This suggests presence of phenolic compound in the extract (Silverstein and Webster, 2006). These interactions may occur between free amino groups, carboxylate group, or cysteine residues in the proteins (Prathna *et al.*, 2010).

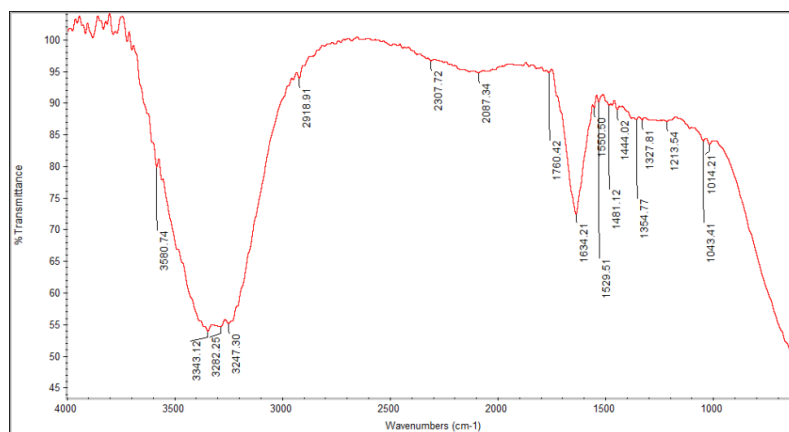


Fig. 3. FT - IR Spectrum of Silver Nanoparticles of *F. auriculata*

Table 2: FT-IR spectral Values and Functional Groups of Silver Nanoparticles of *F. auriculata*.

Sr. No.	Peak values	Functional Group	Class
1.	1014.21	C-N	Amines
2.	1043.41	C-N, O-C	Amines, Anhydrides
3.	1213.64	C-N, C-O, P=O	Amines, Carboxylic Acids, Phosphoramidate
4.	1327.81	C=C	Aromatic
5.	1354.77	C=C	Aromatic
6.	1481.12	C=C	Aromatic
7.	1529.51	N-H, C=C	Amides, Aromatic
8.	1550.50	N-H, C=C	Amides, Aromatic
9.	1634.21	C=C, C=O	Alkene, Amides
10.	1760.42	C-O	Ether
11.	2087.34	Unknown	Unknown
12.	2307.72	C-C	Alkyne
13.	2918.91	C-H, O-H	Alkane, Carboxylic Acids
14.	3247.30	O-H	Carboxylic Acids
15.	3282.25	O-H	Carboxylic Acids
16.	3343.12	N-H	Amines
17.	3580.74	O-H	Alcohol, Oxime(=NOH)

(ii) Copper nanoparticles of *F. auriculata*. The FT-IR spectrum of copper nanoparticles of *F. auriculata* confirmed the presence of functional groups such as NH₂& N-H Amines, C-C, NH₂& N-H, S-OR Alkane, Amines, C-N, S=O, Si-OR Ester, Anhydrides, Amines, Sulfoxide, Silane, Aromatic, Amides, N=O, C=C, C=O, NH₂ Alkene, Amides, Amines, C=O, C=O Carboxylic Acids, Ketone, N=C=O, -N=C=S, -N=C=N-, -N₃, C=C=O, C-C, Si-H Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes, Alkyne, Silane, P-H, Si-H Phosphine, Silane, C-H, C-H, O-H, Alkane, Aldehyde, Carboxylic Acids, O-H Carboxylic Acids, C-H, O-H, N-H Alkyne, Alcohol, Amines, O-H Alcoholin the peak values are 1043.92, 1216.13, 1481.61, 1550.38, 1531.68, 1637.21, 1761.45, 2143.97, 3249.91, 3360.52, 3316.77, 3386.53, 3682.93, 3661.29 and 3882.10 respectively (Fig. 4 and Table 3).

D. Antibacterial Activity

The findings suggested that several metal nanoparticles exhibit potent antibacterial action against a wide range of microbes. As microbial medication resistance rises,

there has been a recent uptick in the usage of metal nanoparticles against bacteria. Human diseases caused by pathogenic bacteria have long been treated using synthetic medications. Human bacterial infections are commonly treated with a variety of plants that have been found to have therapeutic properties. Eight different pathogens were tested for *F. auriculata*'s antibacterial activity, and the results are tallied and depicted in Fig. 5. The various nanoparticle extracts of *F. auriculata* shown good effectiveness for the zone of inhibition against eight human diseases (Table 4 and Fig. 5). The zone of inhibition against *Staphylococcus aureus* was largest for copper (10 mm) and silver (18 mm) nanoparticles. Copper nanoparticles exhibited a 10-mm zone of inhibition against *Klebsiella pneumonia* and *Enterococcus faecalis*. Very little action (4 mm) was shown in silver nanoparticles against *Vibrio cholerae*, *Enterococcus faecalis*, and *Escherichia coli*. The zone of inhibition for *Vibrio cholera* was reduced to 6 mm in copper nanoparticles.

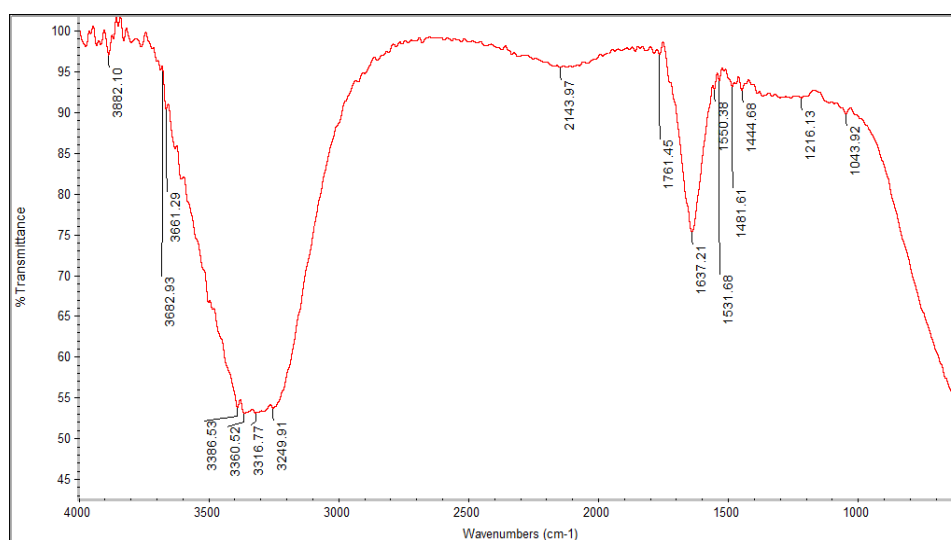


Fig. 4. FT - IR Spectrum of Copper Nanoparticles of *F. auriculata*.

Table 3: FT-IR spectral Values and Functional Groups of Copper Nanoparticles of *F. auriculata*.

Sr. No.	Peak values	Functional Group	Class
1.	1043.92	C-N, O-C, Si-OR	Amines, Anhydrides, Silane
2.	1216.13	C-O, C-N,	Carboxylic Acids, Amines
3.	1481.61	C=C	Aromatic
4.	1550.38	N-H	Amides
5.	1531.68	C=C, N-H	Aromatic, Amides
6.	1634.21	C=C	Alkene
7.	1761.45	Unknown	Unknown
8.	2143.97	-N=C=O, -N=C=S, -N=C=N-, -N ₃ , C=C=O	Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes
9.	3249.91	O-H	Carboxylic Acids
10.	3316.77	C-O, N-H, O-H	Ether, Amines, Alcohol
11.	3360.52	O-H	Alcohol
12.	3361.29	O-H	Alcohol
13.	3386.53	C-H, O-H	Alkane, Carboxylic Acids
14.	3682.93	Unknown	Unknown
15.	3882.10	Unknown	Unknown

Because of their small size, metallic nanoparticles have a lot of exposed surface area that might interact with bacteria. Such extensive exposure to the outside world is likely to increase the rate of bacterial eradication (Russell *et al.*, 1994; Parameswari *et al.*, 2010). This may be because gram-negative bacteria have an abundance of negative charges that help copper nanoparticles connect with their cell walls (Ruparelia *et*

al., 2008; Nino-Martinez *et al.*, 2008). Furthermore, the outer membrane of the bacteria is captured by the nanoparticles, which then kills the germs inside. Silver's antibacterial characteristics have made it a staple in the medical industry for many years. Proof exists that it can block HIV from attaching to host cells as well (Alt *et al.*, 2012; Leon *et al.*, 2015).

Table 4: Antimicrobial activity of the *F.auriculata* fruit extract synthesized nano particles by disc diffusion assay.

Sr. No.	Pathogens	Zone of Inhibition of Nanoparticles (mm)			
		AgNO ₃	CnSO ₄	Control H ₂ O	Antibiotics (Tetracycline)
1.	<i>Escherichia coli</i>	04	08	02	02
2.	<i>Streptococcus pyogens</i>	06	08	03	03
3.	<i>Vibrio cholera</i>	04	06	04	23
4.	<i>Klebsiella pneumonia</i>	08	10	04	04
5.	<i>Shigella sonnei</i>	06	08	04	04
6.	<i>Staphylococcus aureus</i>	18	12	06	22
7.	<i>Salmonella typhimurium</i>	06	08	02	02
8.	<i>Enterococcus faecalis</i>	04	10	04	24

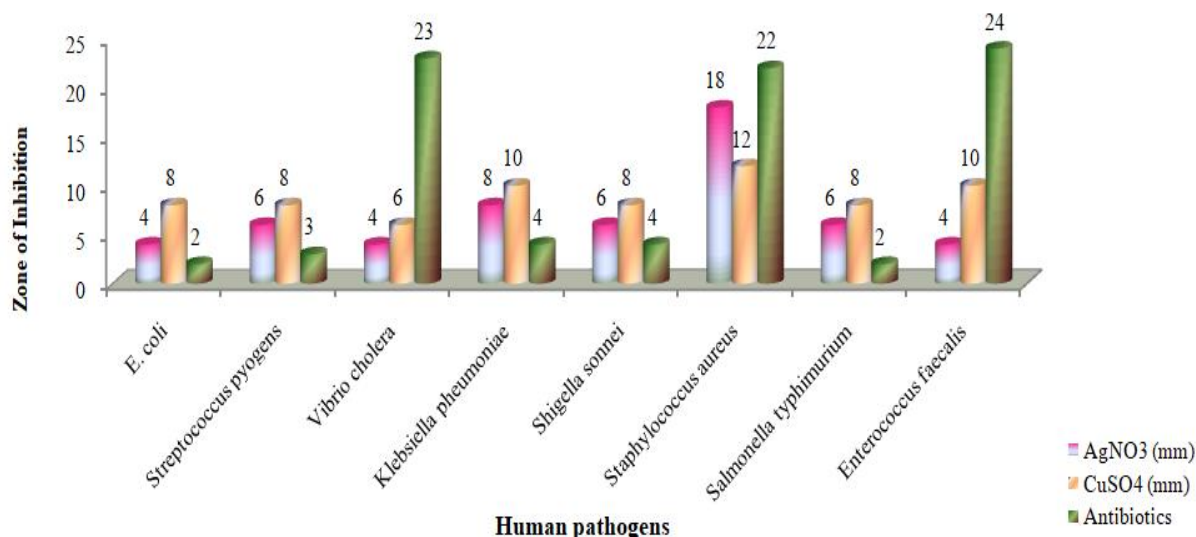


Fig. 5. Antimicrobial potential of Nanoparticles *F. auriculata*.

When Lee *et al.* (2007) looked into the antibacterial properties of *Micrococcus mercurialis*, they found that the fresh leaves and leaf powder-mediated synthesis nanoparticles were most effective against *Bacillus subtilis* (16 nm) and *Aeromonas sobria* (12 nm), respectively. Silver, copper, and zinc nanoparticles were synthesised from *Tiliacora acuminata* Lour and compared for antibacterial efficacy (Vijayakumari *et al.*, 2019). *Vibrio cholerae* and *Enterococcus faecalis* were shown to have a larger zone of inhibition (7 mm).

CONCLUSIONS

For nanotechnology to truly take off, a trustworthy and eco-friendly method of synthesising metallic nanoparticles is required. In the field of nanotechnology, nanoparticles are considered essential building blocks. Both silver and copper nanoparticles were found to have exceptionally efficient zones of inhibition against pathogenic microorganisms. According to the present research, plants are essentially pharmaceutical assembly lines. Additional testing is needed to extract the pure component for use in medication manufacturing. The produced nanoparticles have potential medical uses as bactericidal agents, and the underlying technology is promising for larger-scale nanoparticle manufacturing.

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Conflict of Interest. None.

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