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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MORINDA COREIA BUCH.-HAM.* AND IT'S ANTIBACTERIAL ACTIVITY

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Keywords:

Morinda coreia, Scanning Electron Microscope, Antibacterial activity, Silver nanoparticles

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ABSTRACT: The synthesis of nanoparticles has become the matter of great interest in recent times due to its various advantageous properties and applications in various fields. Though physical and chemical methods are more popular for nanoparticle synthesis, the biogenic production is a better option due to eco-friendliness. *Morinda coreia* leaf ethanol extracts was screened for the synthesis of Silver nanoparticle using 0.1mM concentration of silver nitrate. The peak of 482 nm in UV-Vis Spectroscopy confirmed the presence of silver nanoparticle synthesis. Biosynthesized silver nanoparticles characterized by Scanning Electron Microscope (SEM), Fourier Transform Infra Red Spectroscopy (FT-IR) and Energy-dispersive X-ray spectroscopy (EDX). The silver nanoparticles around 90 nm were formed. Biologically synthesized silver nanoparticles were further examined for antimicrobial activity against both Gram-negative and Gram-positive bacteria. It was observed that a clear zone of growth inhibition was against in *E. coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus sps*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus vulgaris*, *Staphylococcus mutans* and *Salmonella typhi* confirms the antibacterial property of biologically synthesized nanoparticles.

INTRODUCTION: Nanotechnology is foreseen to significantly influence science, economy and everyday life in the 21st century and also to become one of the driving forces of the next industrial revolution¹. The nanomaterials can be synthesized by physical, chemical and biological methods. Although the physical and chemical methods produce pure, well defined particles, these methods are not cost effective and ecofriendly. This drawback can be exhausted by biological method where the microorganism or plant extracts or plant biomass is used as reducing agent^{2, 3}. Now-a-days biological synthesis of metallic nanoparticles is gaining importance as it is reliable and ecofriendly.

Silver nanoparticles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnosis, antimicrobials and therapeutics, catalysis and microelectronics⁴. However, there are many problems and toxicity of using metal oxide nanoparticles on the human health. The development of new chemical or physical methods has resulted in environmental contaminations since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts⁵.

Morinda coreia belongs to the family Rubiaceae. It is one of the largest and the most widely distributed plants in approximately 400 genera in this family. It is known by the Vernacular (Tamil name) as Nuna. This species is found predominantly in the tropical countries. It is found in dry forest throughout the greater part of India and it is also found in the region southwards to Ceylon and

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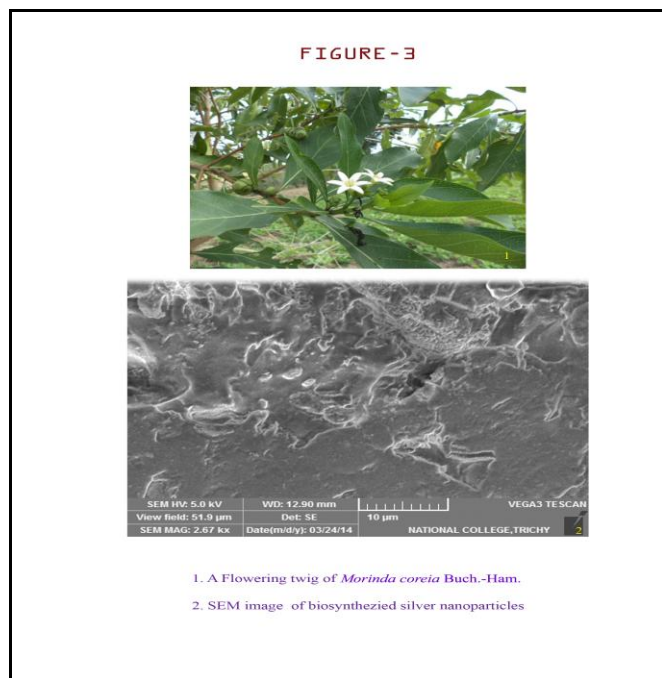
Malaca. A few are found in sub Arctic zone. It is also cultivated in some places⁶. The plant is small to medium sized tree with a straight cylindrical stem 3.6 to 4.2 m in length and ca.90 cm in girth.

Its bark is corky. Its leaves are pale brown, long fissured elliptic or lanceolate. Its flowers are indense ovoid heads and white scented. Its fruit is drupe, globes or ovoid, ca.2 cm, in diameter and is edible. The leaves are usually 4.8 cm broadly or narrowly elliptic acute on both ends; glabrous or pubescent; peduncles solitary or 2-nate leaf-opposed a rarely terminal; flowers 5-merous; fruit of many drupes⁷. In the present study, we have demonstrated a suitable green method for the synthesis of silver nanoparticles using ethanolic leaf extract of *Morinda coreia* as reducing agent. The antibacterial activity of silver nanoparticles has been tested against various pathogenic bacteria.

MATERIALS AND METHODS:

Source of Plant Material

Healthy leaves of *Morinda coreia* were collected from Purathakudi, Tiruchirappalli district in Tamil Nadu, India **Fig. 3**.



Extract Preparation

Morinda coreia green leaves were washed and dried in shade. The dried leaves were then ground into powder, stored in dark glass bottles at -20°C until further analyses. The finely ground leaves (10

g) were extracted with ethanol (ratio 1:6 w/v) overnight at 40°C using a shaking water bath (Protech, Malaysia). After filtration with Whatman filter paper No 1 using vacuum pump, the residue was re-extracted. The solvent was completely removed using a rotary vacuum evaporator (Buchi, Flavil, Switzerland) at 40°C . The concentrated extract was then kept in dark bottles at 4°C until used.

Synthesis of Ag/ *Morinda tinctoria* Emulsion

Ethanol extract of *Morinda coreia* (0.5 ml) was added to 50ml of AgNO_3 (1×10^{-1} M) and mixed at room temperature (25°C) for 48 h. Silver nanoparticles were gradually obtained during the incubation period.

Characterization Methods and Instruments

The reduction of silver ions was monitored by measuring double beam UV-VIS spectra of the reaction medium at different wavelength from 360 -700 nm at different functional time (Perkin Elmer, Singapore). The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 7000 rpm for 15 min and dried at 100°C .

The morphology and size of the silver nanoparticles was found by Scanning Electron Microscope (Philip model CM 200). Elemental analysis of silver carried out by EDAX (Philips XL-30). Functional biomolecules associated with silver nanoparticles was confirmed by FT-IR, which is involved in the reduction of silver ions into silver nanoparticles.

Antibacterial activity

Antibacterial activities of the synthesized Ag nanoparticles were determined by using the agar disc diffusion assay method and well method. Approximately 20mL of nutrient agar medium (NA) was poured in sterilized petri plates. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. Chloromphenicol was used as standard and positive controls. The plates containing the test organism and Ag nanoparticles were incubated at 37°C for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of such zones of inhibition was measured using a ruler and the mean

value for each organism was recorded and expressed in millimeter⁴.

RESULT AND DISCUSSION:

Screening of *Morinda coreia* for the Synthesis of Silver Nanoparticles

Morinda coreia leaf ethanol extract was screened for the synthesis of Silver Nanoparticles. The plant extract was treated with 0.1mM Silver Nitrate in 48 hours of incubation. The color change was observed visually as well as by using a spectrophotometer. The leaf extract concentration was also standardized using 0.5 ml from 5 gm of plant material with Silver nitrate solution making the final volume to 50 ml.

The change in color of the reaction mixture was noted by visual observation. The leaf extract incubated with Silver Nitrate, at the beginning of the reaction showed straw yellow color, and gradually increased in color intensity to brown, with the increasing period of incubation. The color of the reaction mixture changed to intense brown after 6 hours of incubation. Control without leaf extract did not exhibit any color change.

UV-Vis Spectrophotometry

It is well known that silver nanoparticles exhibit brown color in ethanol solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The leaf extract was mixed in the ethanol solution of the silver ion complex; it started to change the color from watery to brown due to reduction of silver ion, which may be the indication of formation Ag- NP's. The UV- spectrum of *M. coreia* Ag-NPs was recorded from the reaction medium. The results showed maximum absorption peak at 482 nm **Fig. 1**. Scanning of absorption spectra of the mixture was continually recorded for one month time period, yielded no significant change in the intensity of absorption maxima suggested a stable nanoparticle formation.

Fourier Transform Infrared Spectroscopy (FTIR)

For FTIR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/enzymes that are not capping the silver nanoparticles. The samples were dried and grinded

with mortar and pestle and analyzed on a JASCO FT/IR-5300 model in the diffuse reflectance mode operating at a resolution of 4 cm⁻¹. **Figure 2** shows the FTIR spectra of ethanol silver nanoparticles prepared from the *Morinda coreia* leaf extract.

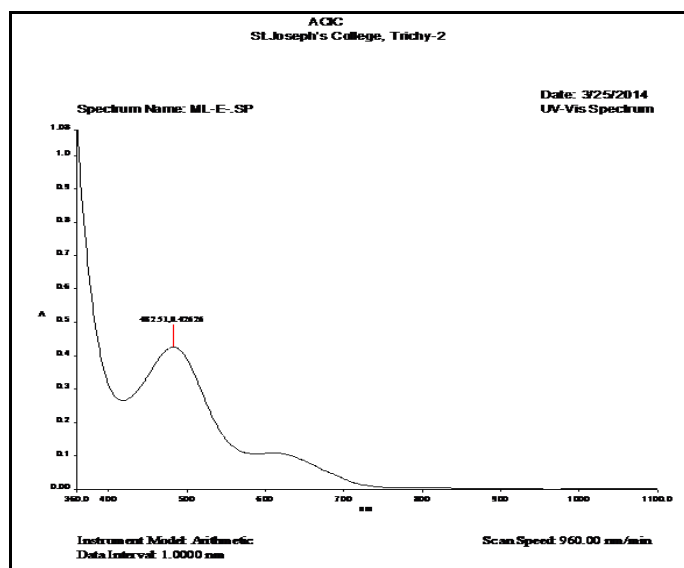


FIGURE 1: UV-VIS SPECTROSCOPY

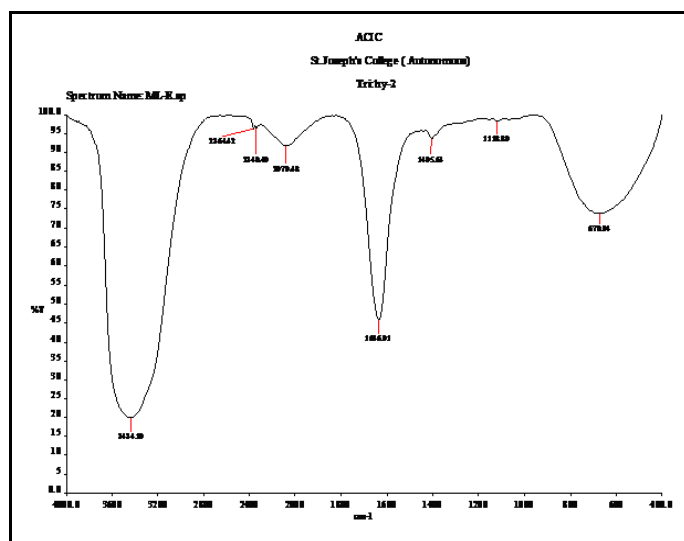


FIGURE 2: FOURIER TRANSFORM INFRARED SPECTROSCOPY

The representative spectra of nanoparticles obtained obvious absorption peaks located at about 2364, 2340, 2079, 1405 and 1118, as well as some intensity peaks decreased like 3434, 1636 and 670. shows the band at 3434 correspond to O-H stretching vibrations of 1°, 2° amines, amides, alcohol and H-bonded to phenols. The absorption bands at 1118 in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates. The peak at 1636.91 indicates C-O stretching vibrations

and it corresponds to the presence of alcohols, carboxylic acids, ethers, esters. The band at 1405 corresponds to C–C bend stretching vibrations to aromatics.

The band around 2200-2400 would indicate the possible presence of a C-N or a C-C triple bond. So FTIR analysis of TBPs in Native and organic acid pretreated form were taken in 400-4000 range. The main functional groups are Amine, Carbonyl Hydroxyl group, and Carboxylic groups. IR spectroscopic study confirmed that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the ethanol medium.

FTIR measurements were to identify some of the possible biomolecules responsible for the reduction of the Ag⁺ ions and the capping of the bio-reduced silver nanoparticles synthesized⁸. The broth after reduction of Ag⁺ was centrifuged at 12,000 rpm for 20 minutes to isolate the silver nanoparticles free from proteins or other compounds present in the solution.

Scanning electron microscope (SEM)

SEM micrographs of the synthesized silver nanoparticles using the ethanol extract of leaves of

Morinda coreia fabricated on a glass substrate. The synthesized nanoparticles morphology was characterized by scanning electron microscope. The silver nanoparticle formed was predominantly less fiber with ununiform shape **Fig. 3**. It is known that the shape of metal nanoparticle considerably change their optical and electronic properties. The SEM image exposed that the formed nanoparticle was fiber in shape formed with the size range of 90 nm. Scanning electron microscopy has provided further insight into the morphology and size details of the synthesized nanoparticles. The synthesized silver nanoparticles were not well dispersed. It is with aggregation, possessing fiber shape.

EDAX The EDAX attachment on the SEM provided chemical analysis of the field of view as well as spot analyses of minute particles and confirmed the presence of specific elements. The presence of elemental silver in the reaction mixture was confirmed by EDAX analysis. EDAX exposed strong signal in the silver region and confirmed the formation of silver nanoparticles. Metallic silver nanocrystals generally show typical absorption peak approximately at 1.050, 1.256, 6.431 keV due to surface plasmon resonance. And also a strong signal for C and some other weak signals are recorded possibly due to elements (Ca, O, Si, Ag) from enzymes or proteins present within the leaf extract **Fig. 4**. The EDAX analysis displayed signature spectra for silver and thus convincingly evidenced the presence of this noble metal in the plant. Other Molecules like CaCO₃ and SiO₂ are also present in large amount.

Spectrum processing:

Peaks possibly omitted: 1.050, 1.256, and 6.431 keV

Processing option: All elements analyzed (Normalized)

Number of iterations = 4

Standard:

C CaCO₃ 1-Jun-1999 12:00 AM

O SiO₂ 1-Jun-1999 12:00 AM

Si SiO₂ 1-Jun-1999 12:00 AM

Ca Wollastonite 1-Jun-1999 12:00 AM

Ag Ag 1-Jun-1999 12:00 AM

| Element | Weight% | Atomic% |
|---------|---------|---------|
| C K | 57.09 | 71.01 |
| O K | 27.43 | 25.61 |
| Si K | 1.93 | 1.03 |
| Ca K | 2.04 | 0.76 |
| Ag L | 11.50 | 1.59 |
| Totals | 100.00 | |

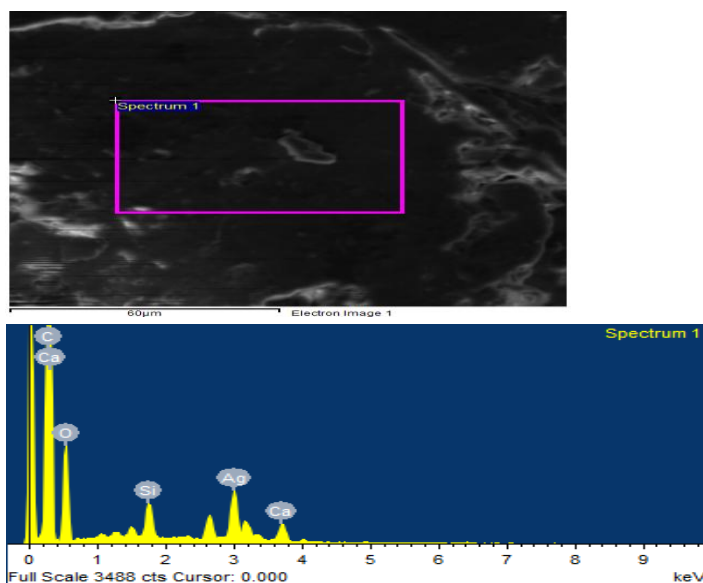


FIGURE 4: ENERGY-DISPERSIVE X-RAY SPECTROSCOPY

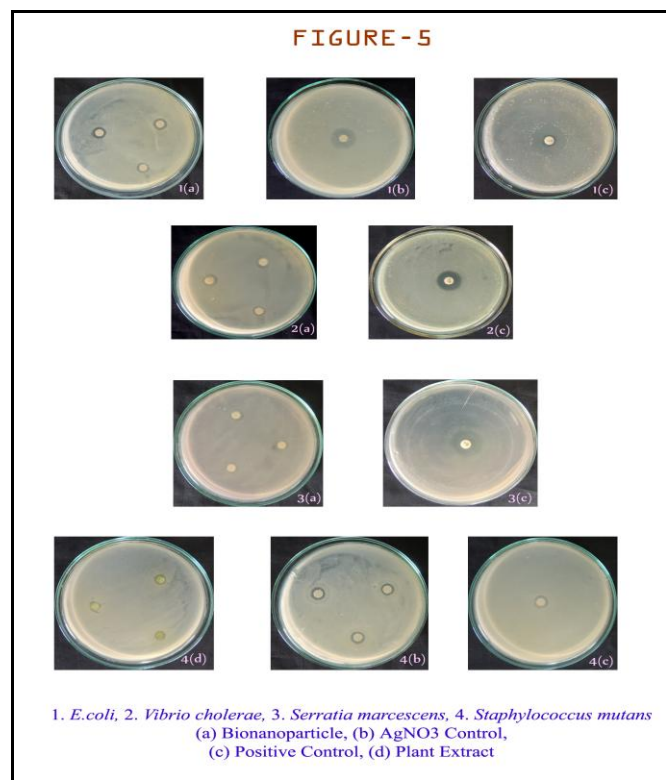
Antibacterial activity

Antibacterial activity of the synthesized Silver nanoparticles was determined using disc diffusion method. This was confirmed by the inhibitory effect on bacterial growth as reflected by the

inhibition zone compared to known antibiotics **Table 1**. The sterile nutrient agar medium (20 ml) in petri dishes was uniformly smeared using sterile cotton swabs with test pure cultures of pathogenic bacteria **Fig. 5**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF MORINDA COREIA

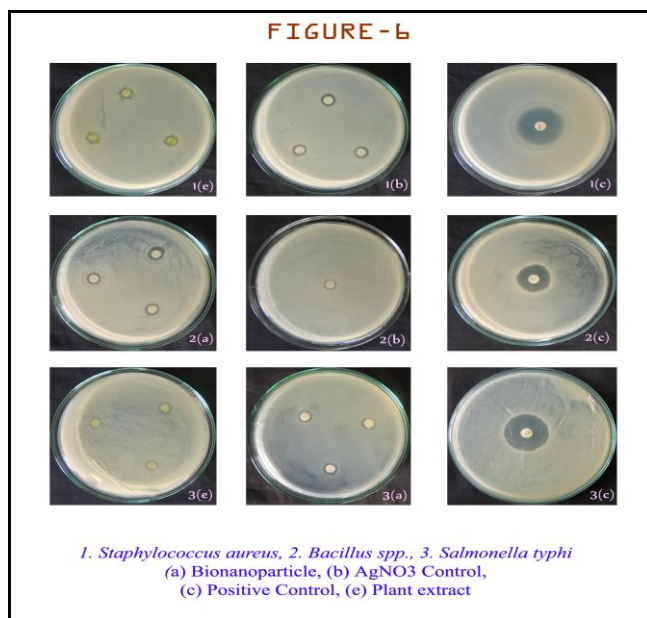
| Ex. No. | Test Bacteria | Ethanol extract (mm) | Biosynthesized nanoparticles (mm) | AgNO ₃ control (mm) | Positive control (mm) | Negative control (mm) |
|---------|-------------------------------|----------------------|-----------------------------------|--------------------------------|-----------------------|-----------------------|
| 1 | <i>E. coli</i> | - | 10.66±0.57 | 15 | 17 | - |
| 2 | <i>Vibrio cholerae</i> | - | 10.33±1.52 | - | 17 | - |
| 3 | <i>Serratia marcescens</i> | - | 11.33±1.04 | - | 17 | 7.5 |
| 4 | <i>Staphylococcus mutans</i> | 7.00±0.50 | 10.83±1.04 | 9 | 21 | - |
| 5 | <i>Staphylococcus aureus</i> | 7.66±0.57 | 11±0.5 | 8.5 | 28 | 8 |
| 6 | <i>Bacillus spp.</i> | - | 11.33±1.52 | 9 | 20 | - |
| 7 | <i>Salmonella typhi</i> | 7.00±1.00 | 10.16±0.57 | - | 25 | - |
| 8 | <i>Klebsiella pneumoniae</i> | - | 9.16±0.76 | 11 | 27 | - |
| 9 | <i>Proteus spp.</i> | - | 9.16±0.28 | 9 | 22 | - |
| 10 | <i>Pseudomonas aeruginosa</i> | - | 9.83±0.28 | 12.5 | 19 | - |



The AgNPs exhibited good antibacterial activity against both Gram-negative and Gram-positive bacteria. In the *in-vitro* antimicrobial assay, chloramphenicol, an antimicrobial agent that is widely used against many bacterial infections, was used as positive control. The antibacterial effects of biologically synthesized silver nanoparticles have been investigated against *E. coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus spp.*, *Pseudomonas*

aeruginosa, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus vulgaris*, *Staphylococcus mutans* and *Salmonella typhi*.

It was observed that a clear zone of growth inhibition was recorded against *E. coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus spp.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus vulgaris*, *Staphylococcus mutans* and *Salmonella typhi* which confirms the antibacterial property of biologically synthesized nanoparticles **Fig. 6**. The maximum antibacterial activity was observed against *Bacillus spp.*, *Serratia marcescens* followed *Staphylococcus aureus* **Fig. 8**.



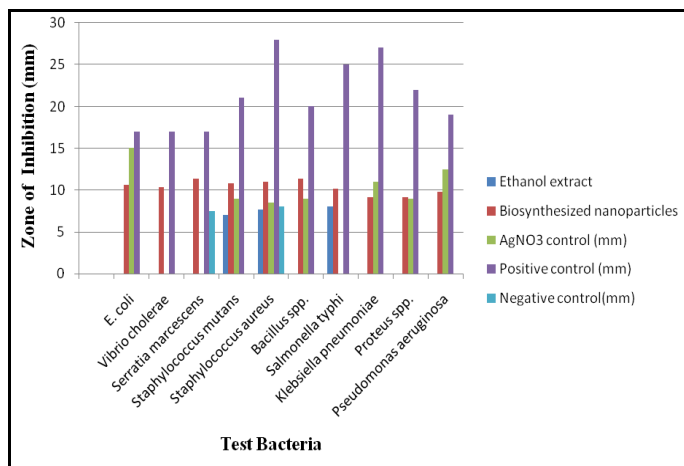
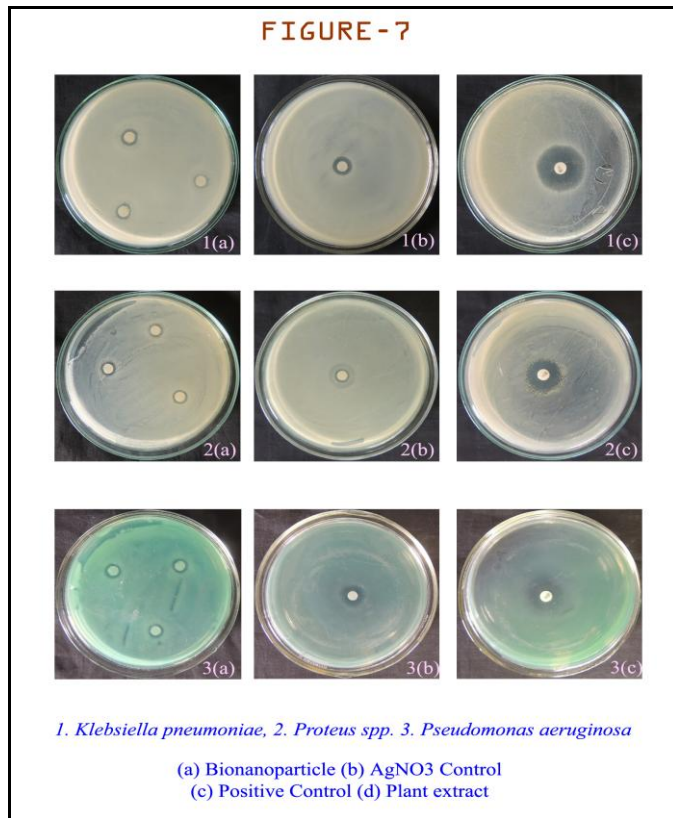


FIGURE 8 ANTIBACTERIAL ACTIVITY OF MORINDA COREIA LEAF WITH ETHANOL EXTRACT

Different types of nanomaterials like copper, zinc, titanium, magnesium, gold and silver have arising as antimicrobial materials, especially silver nanoparticles that are more efficient. These materials exhibit antimicrobial activity against bacteria, viruses and other eukaryotic microorganisms⁹. The actual mechanism of formation, for instance, of silver nanoparticles, in all of these microorganisms and plants, is still an open question, even though much research has been attempted to find different ways to investigate the possible mechanisms⁹.

Comparable aspects occurred with the antibacterial activity of silver nanoparticles. One such feature proven for silver nanoparticles is that for antibacterial activity, size, morphology and concentrations are all important. For example, small particles have larger surface areas to be in contact with the bacterial cells, showing a larger activity¹⁰. The antimicrobial efficacy of the nanoparticles also depends on the shape or morphology of the nanoparticles. Ingle has evaluated the antibacterial activity of biosynthesized silver nanoparticles produced by *Fusarium acuminatum* on different human pathogens. These authors reported efficient antibacterial activity of AgNps against multidrug resistant and highly pathogenic bacteria, such as, *S. aureus*, *Salmonella typhi* and *E. coli*. Silver nanoparticles showed significant antimicrobial effects than silver ions (1.4–1.9 folds). The maximum antibacterial activity of silver nanoparticles was against *S. aureus*, followed by *Vibrio cholera*, *S. typhi* and the minimum was for

E. coli **Fig. 7**. This result revealed that specific efficiency of silver nanoparticles can be related with differences due to the strain, which can be related to the bacterial membrane structure¹¹.



Other applications of silver nanoparticles are in association with antibiotics to improve their effects and in wound healing^{12, 13-14}. In this aspect biological synthesized silver nanoparticles and their focused application on the synergistic antibiotic effects, applications on textile industries and their possibilities in the field of cancer studies will be discussed.

CONCLUSION: *Morinda coreia* leaf extracts was screened for the synthesis of Silver nanoparticle using 0.1 mM concentration of silver nitrate. The leaf extract concentration was also standardized using 0.5 ml from 5 gm of leaf powder with Silver nitrate solution making the final volume to 50 ml. The peak of 482 nm in UV- spectrum confirmed the presence of silver nanoparticle synthesis. In FTIR Spectrum, the band at 3434 correspond to O-H stretching vibrations of 1°, 2° amines, amides, alcohol and H-bonded to phenols.

The absorption bands at 1118 in the fingerprint region indicate several modes such as C-H

deformation or C-O or C-C stretching, pertaining to carbohydrates. The peak at 1636.91 indicates C-O stretching vibrations and it corresponds to the presence of alcohols, carboxylic acids, ethers, esters. The presence of active functional groups in leaf extract results in the swift reduction of silver ions to silver nanoparticles. Scanning electron microscopy has provided insight into the morphology and size details of the synthesized nanoparticles. The synthesized silver nanoparticles were not well dispersed. The particle size was found to be less than 90 nm. The EDAX analysis displayed signature spectra for silver and thus convincingly evidenced the presence of this noble metal in the plant. Other molecules like CaCO_3 and SiO_2 are also present in large amount.

The AgNPs exhibited good antibacterial activity against both Gram-negative and Gram-positive bacteria. It was observed that a clear zone of growth inhibition was against in *E. coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus sps*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus vulgaris*, *Staphylococcus mutans* and *Salmonella typhi* confirms the antibacterial property of biologically synthesized nanoparticles. The solvent ethanol does not inhibit the growth of all test bacteria. Chloramphenicol, the standard drug inhibited all the tested bacteria.

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