GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM LEAVES OF *Neolamarckia cadamba* AND MONITORING THEIR PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY.

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ABSTRACT

Neolamarckia cadamba is indigenous to the warmer parts of India. It grows to 15-20 meters tall. Branches of the tree are horizontal, leaves large, shining, opposite, elliptic, oblong. It is a large deciduous tree. Formation of silver nanoparticles by the reduction of silver nitrate during exposure to *Neolamarckia cadamba* leaf powder was easily monitored from the change in the colour of the reaction mixture. Silver nanoparticles synthesized were observed to show the characteristic dark brown colour and it is due to the excitation of surface Plasmon vibrations. The change in the colour of the reaction mixture was observed after 2 hours. Silver nanoparticles obtained were subjected to UV-Visible spectroscopy in the range of 200-800nm and maximum absorbance was found to be at the wavelength of 300nm which is also found to be absorption maxima for AgNPs. Silver nanoparticles were subjected to FTIR analysis where the spectrum showed distinct peaks ranging from 400cm^{-1}to 4000cm^{-1} showed peaks at 3425.94cm^{-1}, 2359.77cm^{-1}, 1732.07cm^{-1} and 1384.32cm^{-1} which indicates the presence of Alkaloids, Tannins, Resins which mainly help in capping and stabilization of synthesized SNPs. The SNP sample was subjected to SEM analysis, the size of the nanoparticles was 150nm and was spherical in shape. The qualitative phytochemical test for the methanol and aqueous extract of the plant *Neolamarckia cadamba* showed positive result for carbohydrates, terpenoids, saponins, alkaloids, tannins, saponins and resins. The AgNPs of *Neolamarckia cadamba* displayed inhibitory activities against *E. coli* ATCC 25922 , *Pseudomonas aerugenosa, Staphylococcus aureus* ATCC 29213, *Klebsiella pneumonia, Lactobacillus acidophilus* MTCC 10307, *Streptococcus mutants* MTCC 497 and results of comparative antibacterial activity of silver nanoparticles were recorded. It was observed that silver nanoparticles showed the more inhibitory zone than the methanol and aqueous extract.

Key words: *Neolamarckia cadamba*, silver nitrate, SEM, FTIR, phytochemical analysis.

I INTRODUCTION

Nanotechnology is one of the most active research areas in the modern science. Based upon the specific characteristics of the nanoparticles such as size, distribution and morphology, Nanoparticles of the material

have the distinct properties than when it is in bulk form of the same material. A nanoparticle is a microscopic particle with at least one dimension with size less than 100 nm. The reducing agents reduce silver ions (Ag^{+}) and lead to the formation of metallic silver (Ag^{0}), which is followed by agglomeration into clusters. These clusters lead to the formation of metallic colloidal silver particles (3). Silver nanoparticles are ultra-fine particles of silver. They are 10-100 nanometres large, and differ from the bulk silver as they have different colours such as yellow, as opposed to the silver. Incident light rays create oscillation in free electrons on the surface of nanoparticles, causing them to absorb electromagnetic radiation, creating different colours reflected (2).

To characterize the synthesized nanoparticles, many analytical techniques have been used, which includes ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) (6).

Neolamarkia cadamba

Kingdom : Plantae

Order : Gentianales

Family : Rubiaceae

Subfamily: Cinchonoideae

Genus : *Neolamarckia*

Species : *cadamba*

Neolamarckia cadamba is indigenous to the warmer parts of India. It grows to 15-20 meters tall. Branches of the tree are horizontal, leaves large, shining, opposite, elliptic and oblong. It is a large deciduous tree. Golden balls of yellow flowers are borne in rounded inflorescence a little smaller then a tennis ball. The fruits are acidic but pleasantly flavoured fruit. The trees grow up to 3 m a year, valued for matchwood or plywood.

II MATERIALS

Materials used were silver nitrate (HIMEDIA, Maharashtra), Muller Hinton Agar (HIMEDIA, Maharashtra), Penicillin (Pfizer, Gujarat)

II METHODS

2.1 COLLECTION OF PLANT LEAVES

Fresh leaves of *Neolamarckia cadamba* were collected from Bandaru village in Belthangady, Dakshina Kannada, India. The leaves were rinsed with sterile water and air dried under shade for 10 days. Then the plant leaves were ground into fine powder. The powder was then processed for extraction by Soxhlet extraction method and solvents used were methanol and single distilled water.

2.2 GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM LEAF POWDER

In order to obtain AgNPs from *Neolamarckia cadamba* leaves, fine leaf powder was used. 5gm of the leaf powder was mixed with the 0.01 mille molar of 100ml silver nitrate solution. Then the solution was centrifuged at 2500rpm for 10 minutes and filtered using the filter paper. And kept at room temperature and observed for the colour change. Leaf powder was mixed with $AgNO_3$ solution in order to reduce pure Ag^+ ions to Ag^0. The formation of AgNPs can be easily observed by monitoring the colour change to dark brown.

2.3 CHARECTERIZATION OF SILVER NANOPARTICLE

The nanoparticles were characterised using UV-Visible Spectrophotometer (SYSTRONICS® PC based double beamed Spectrophotometer 2202), Scanning Electron Microscopy ZEISS with OXFORD EDS with a magnification of 100X and Fourier Transform Infrared Spectroscopy.

2.4 PREPARATION OF PLANT EXTRACT

Solvent extraction Crude plants extract was prepared by Soxhlet extraction method. About 20 grams of powdered leaves were uniformly packed into a thimble and extracted with 250 ml of solvents separately. Solvents used were methanol and water. The process of extraction continues for till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30–50ºC till all the solvent gets evaporated. Dried extracts were stored in refrigerator at 4ºC. for their future used for phytochemical analysis.

2.5 PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

Qualitative tests were carried out for the screening of phytochemicals like carbohydrates, alkaloids, tannins, resins and saponins in the aqueous and methanol extracts.

2.6 ANTIBACTERIAL SCREENING OF THE LEAF EXTRACTS

PREPARATION OF BACTERIAL CULTURES

Freeze dried culture (*E.coli* ATCC 25922 , *Pseudomonas aerugenosa*, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumonia*, *Lactobacillus acidophilus* MTCC 10307, *Streptococcus mutants* MTCC 497) was inoculated into nutrient broth and incubated at room temperature for 24 hours to obtain a bacterial culture. This procedure was carried for the selected bacterial cultures to obtain inoculums of particular broth cultures.

2.7 ANTIBACTERIAL ACTIVITY TEST BY WELL DIFFUSION METHOD

The method was employed to assay the plant materials for antimicrobial activity. Petri dishes were plated with Muller Hinton Agar (HIMEDIA, Maharashtra) media and allowed to solidify for 30minutes. The test organisms were then spread on the surface of the media using sterile swab stick. This was allowed to stay in room temperature for 10 minutes. The different extracts of the plant of different concentrations (50µl, 100µl, 150µl, 200µl) were dispensed into the wells using a micropipette. A negative control of water was kept if aqueous extract was used, methanol was kept as negative control if the extract used was methanol extract of the leaves and silver nitrate was kept for silver nanoparticles. Positive control of *Penicillin* was taken. Extract was allowed to diffuse for 30 minutes at room temperature. Then plates were incubated at room temperature for 24 hours.

Zones of inhibition were measured by ruler method, by measuring the distance from one end of the inhibition zone, across the disc to the other end. This was measured by millimetre(mm).

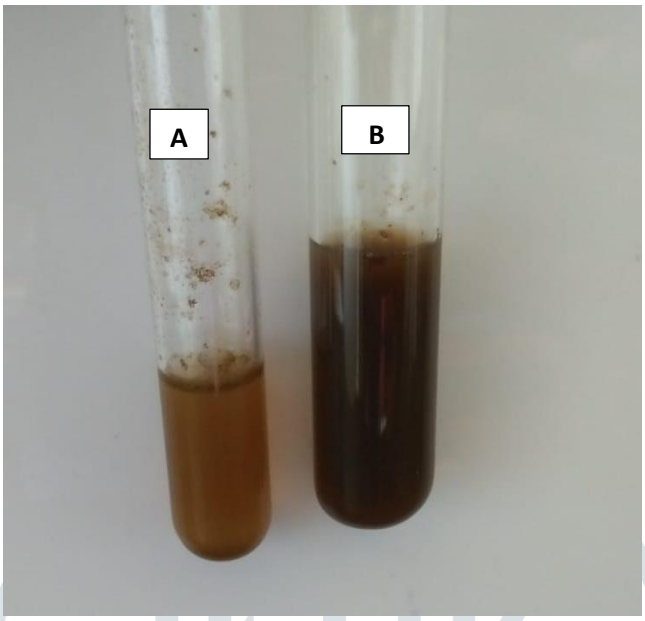
III RESULTS

3.1 COLLECTION OF PLANT LEAVES

Plant leaves were collected from Bandaru village in Belthangady, Dakshina Kannada.

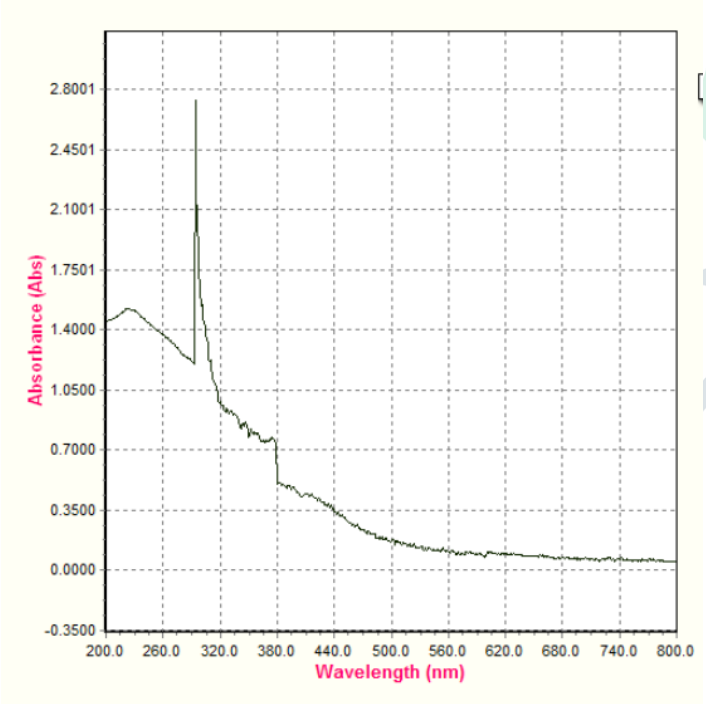
3.2 GREEN SYNTHESIS OF SILVER NANOPARTICLES

Before reaction (**A**) After reaction (**B**)

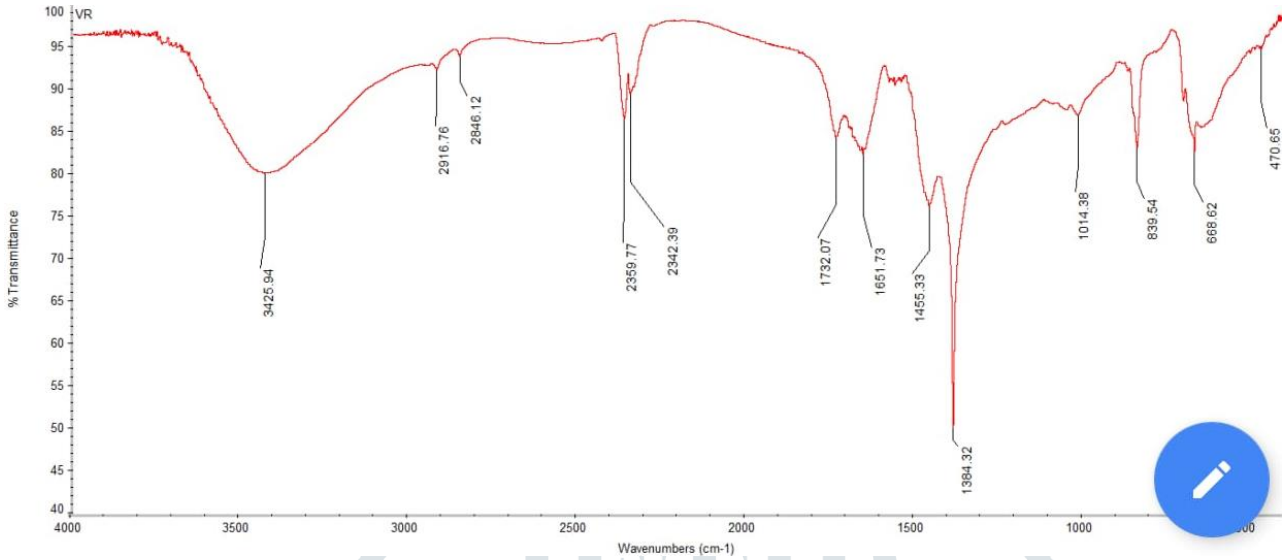


3.3 CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES

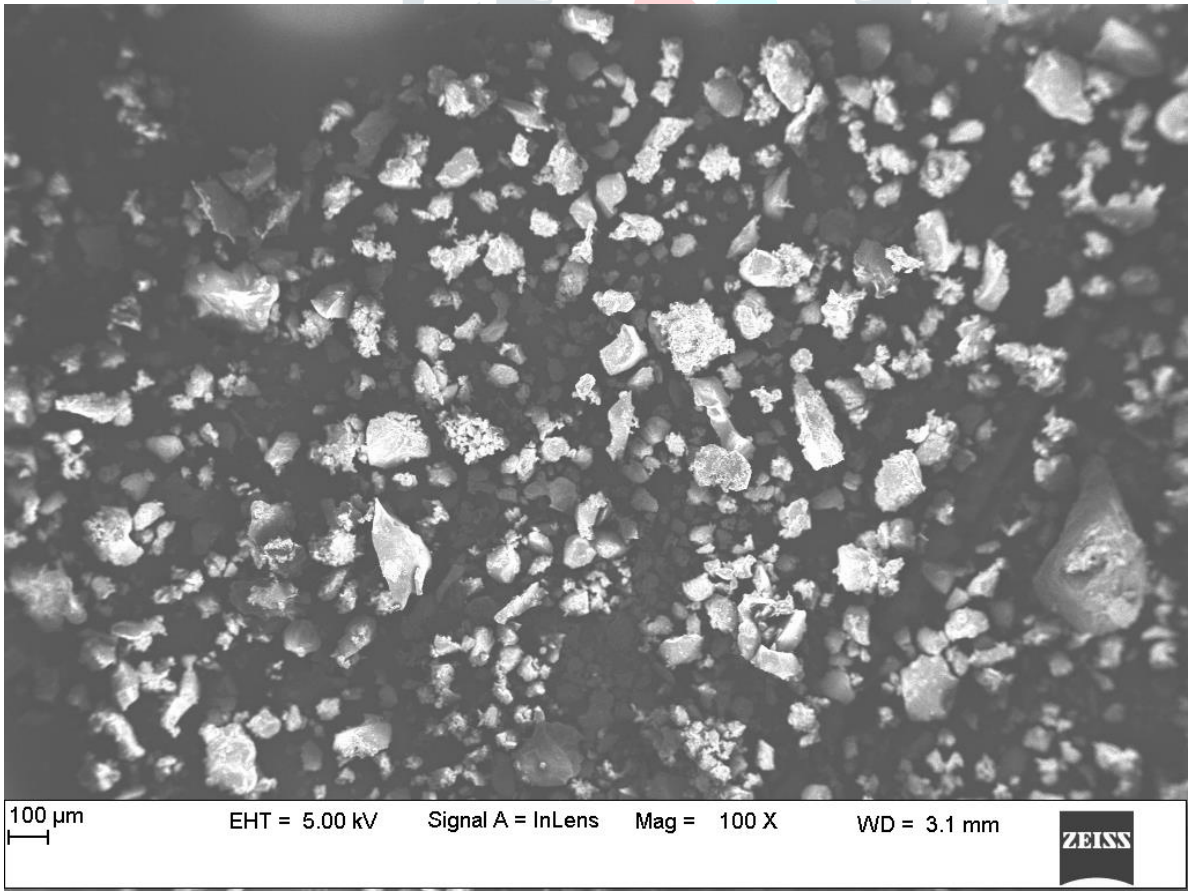
3.3.1 UV-VISIBLE SPECTRUM



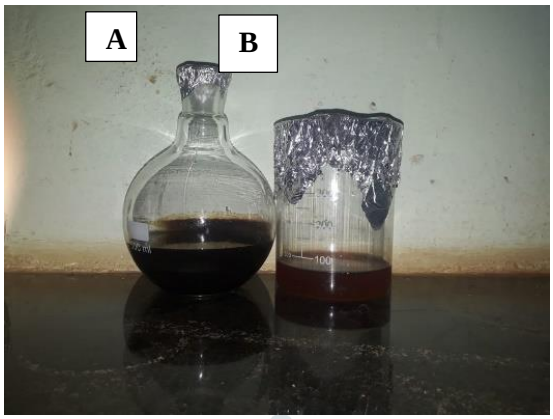
3.3.2 FOURIER TRANSFORM INFRA-RED SPECTROSCOPY



3.3.4 SCANNING ELECTRON MICROSCOPY



3.3 PLANT EXTRACT OBTAINED BY SOXHLET EXTRACTION



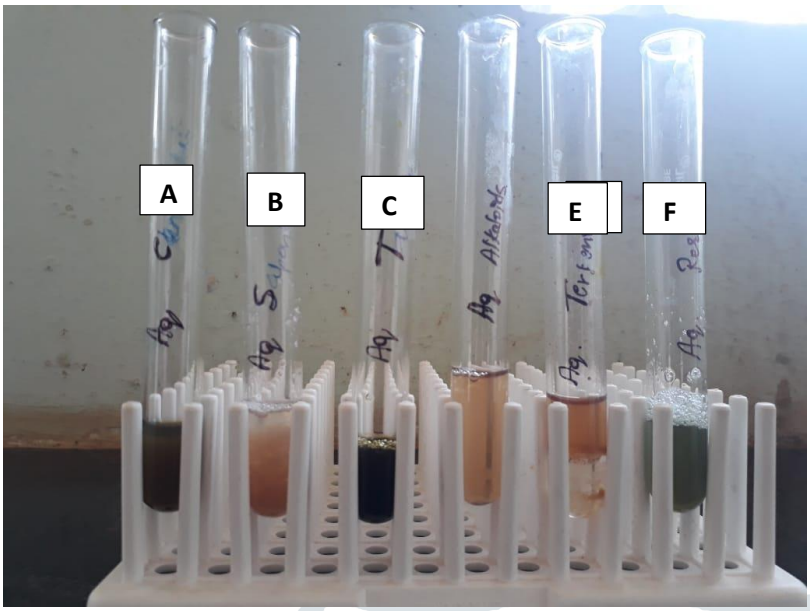
A: Methanol extract, B: Aqueous extract

3.4 RESULTS OF PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF *Neolamarckia cadamba:*

	Aqueous extract	Methanol extract
Carbohydrates	+	+
Tannins	+	+
Alkaloids	+	+
Terpenoids	+	+
Resins	+	+
Saponins	+	+

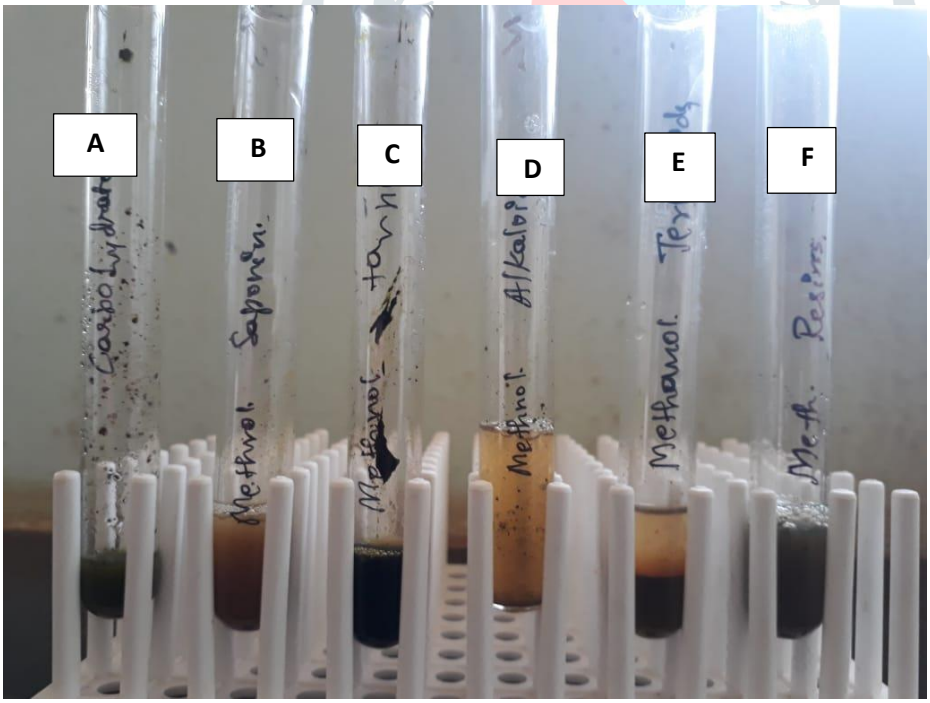
'+' = corresponds to the positive result . ' –' = corresponds to the negative result.

3.4.1 PHYTOCHEMICAL ANALYSIS RESULTS OF AQUEOUS EXTRACT



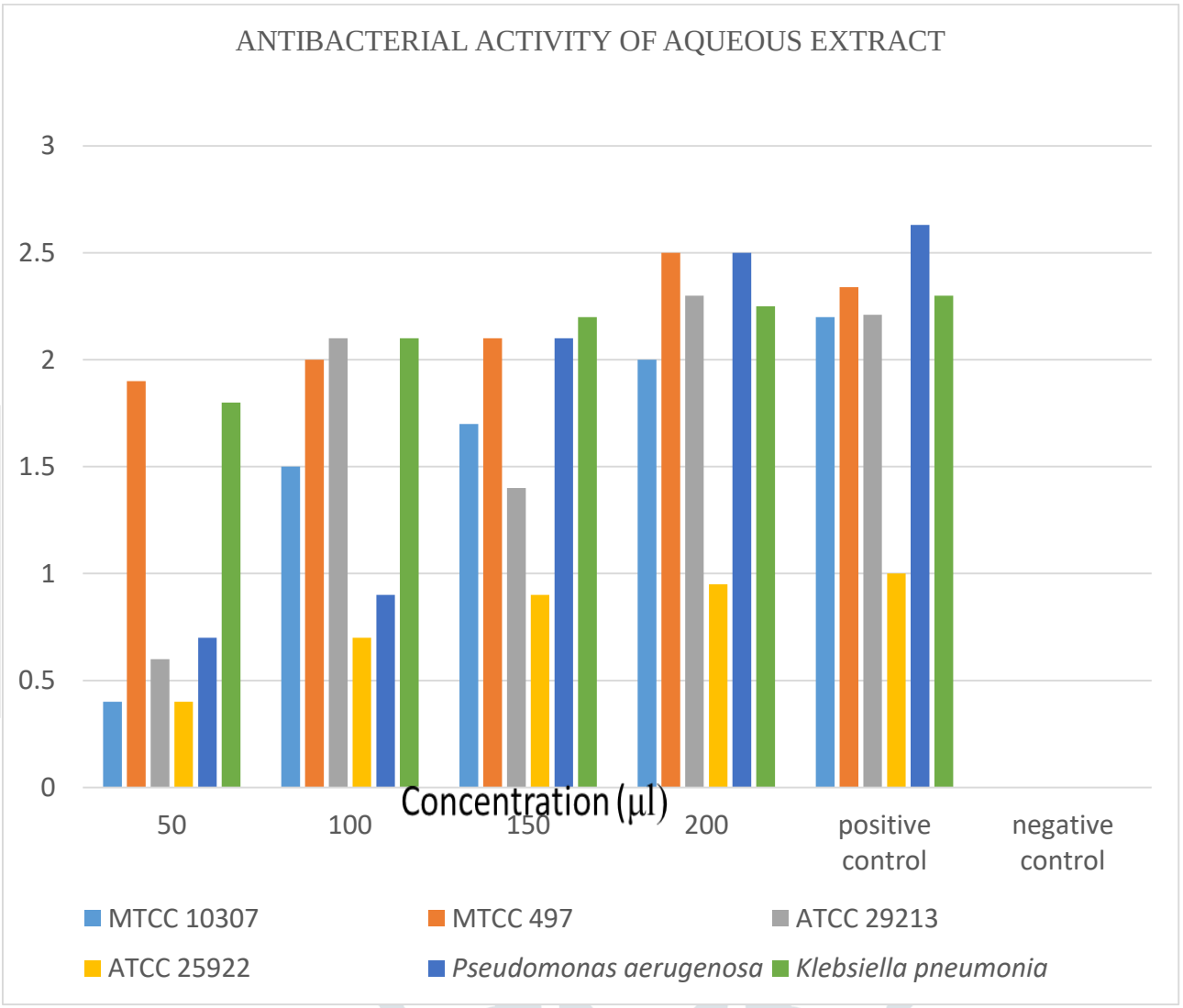
A = Test for Carbohydrates, B = Test for saponins, C = Test for Tannins, D = Test for Alkaloids E = Test for Terpenoids, F = Test for Resins.

3.4.2 PHYTOCHEMICAL ANALYSIS RESULTS OF METHANOL EXTRACT

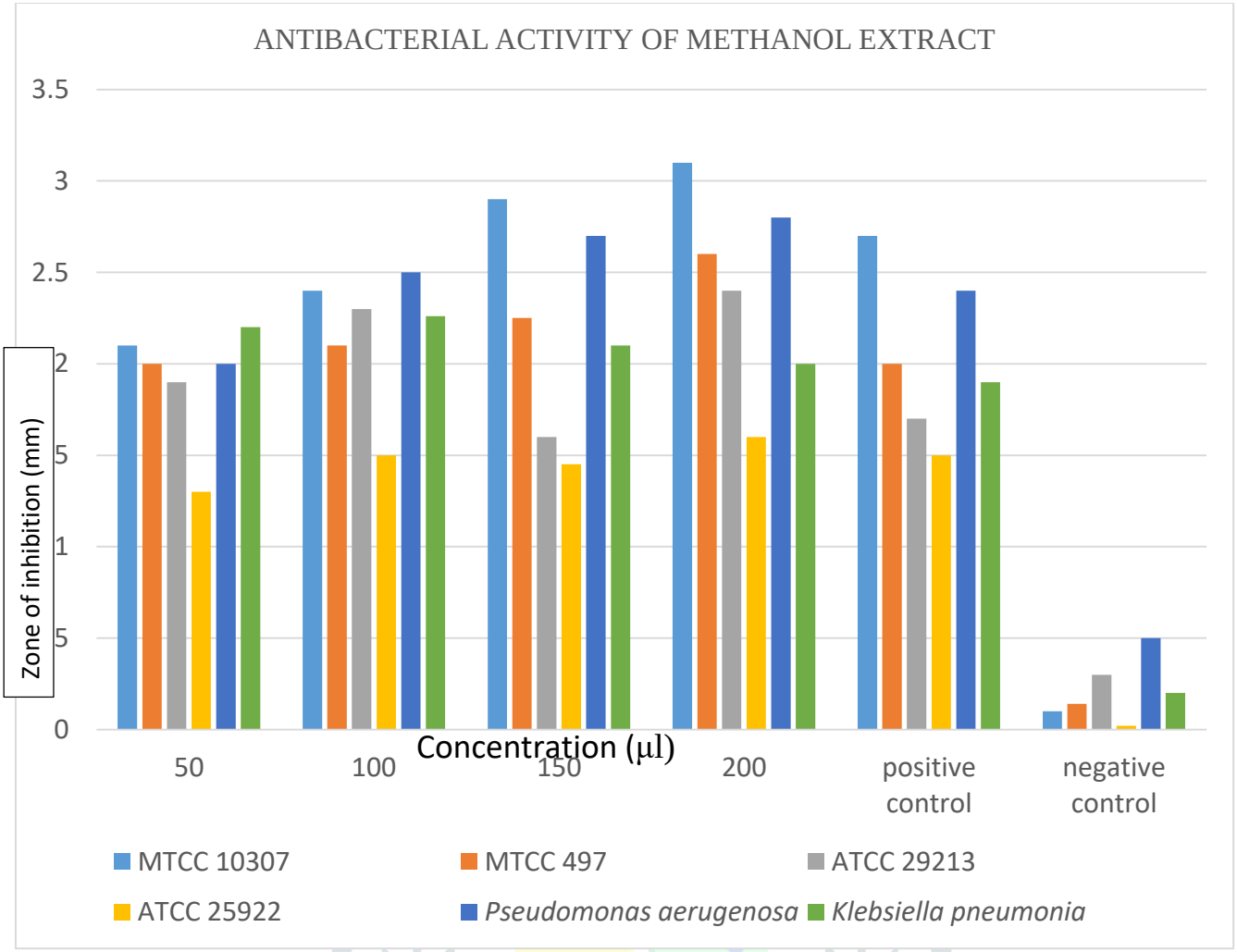


A = Test for Carbohydrates, B = Test for saponins, C = Test for Tannins, D = Test for Alkaloids, E = Test for Terpenoids, F = Test for Resins

3.5 ANTIBACTERIAL ACTIVITY



E.coli=ATCC 25922 *Staphylococcus aureus* = ATCC 29213, *Lactobacillus acidophilus* = MTCC 10307, *Streptococcus mutants* = MTCC 497, negative control = distilled water, positive control = *Penicillin.*



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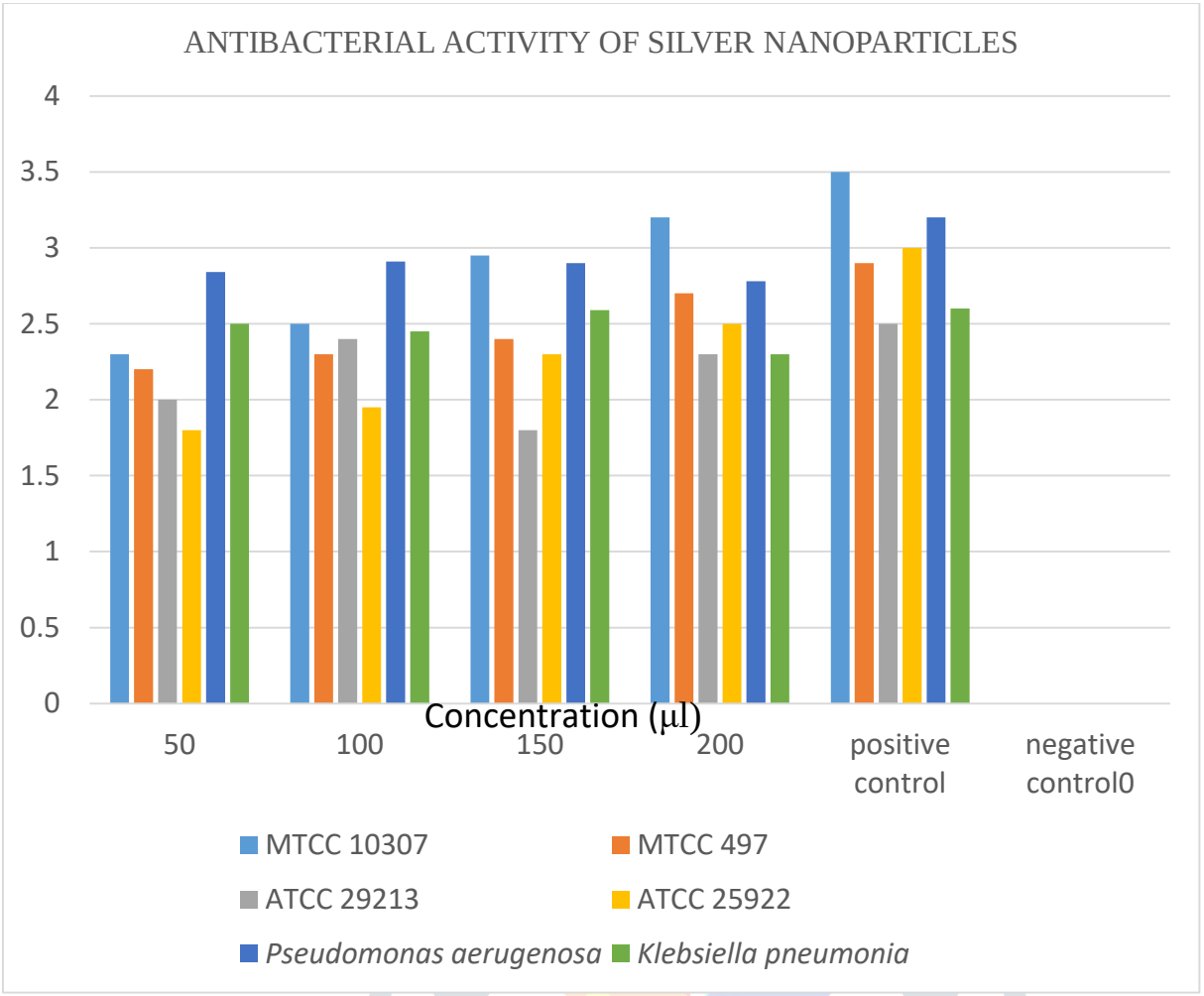

E.coli=ATCC 25922 *Staphylococcus aureus* = ATCC 29213, *Lactobacillus acidophilus* = MTCC 10307, *Streptococcus mutants* = MTCC 497, negative control = distilled water, positive control = *Penicillin.*

IV DISCUSSION

As our lifestyle is now changing we are moving away from nature. While we cannot go away from nature because we are part of nature. As herbs are natural products they are and free from side effects, they are comparatively safe, eco-friendly and are locally available. Traditionally there are lot of herbs used for benefit of us. There is a need to promote them to save the human lives. *Neolamarckia cadamba* is one of ayurvedic remedy that has been mentioned in many Indian medicinal literatures. The phytochemistry of *Neolamarckia cadamba* helps in the treatment of various ailments like diabetes mellitus, diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial activity. The major constituents of the plant are triterpenes, triterpenoid glycosides, flavanoids, saponins, indole alkaloids; cadambine, cadamine, isocadambine, isodihydrocadambine.

Formation of silver nanoparticles by the reduction of silver nitrate during exposure to *Neolamarckia cadamba* leaf powder can be easily monitored from the change in the colour of the reaction mixture. Silver nanoparticles

bear characteristic dark brown colour due to the excitation of surface Plasmon vibrations. The change in the colour of the reaction mixture after 2 hours.

In this present study leaf extract which was subjected for the synthesis of silver nanoparticles under the ambient room temperature with an apparent visual colour change to dark brown colour which intensified with time. Silver nanoparticles obtained were subjected to UV-Visible spectroscopy in the range of 260-800nm and maximum absorbance was found to be at the wavelength of 300nm which is also found to be absorption maxima for AgNPs. Silver nanoparticles were subjected to FTIR analysis where the spectrum showed distinct peaks ranging from 400cm-1to 4000cm-1 showed peaks at 3425.94cm-1, 2359.77cm-1, 1732.07cm-1 and 1384.32cm-1 which indicates the presence of Alkaloids, Tannins, Resins which mainly help in capping and stabilization of synthesized SNPs.The SNP sample was subjected to SEM analysis, the size of the nanoparticles was 150nm and was spherical in shape.

The phytochemical screening for the methanol and aqueous extract of the plant *Neolamarckia cadamba* showed positive result for carbohydrates, terpenoids, saponins, alkaloids, tannins, saponins and resins.

Silver ion has been known to be effective against a broad range of microorganisms. Silver nanoparticles are well known as one of the most universal antimicrobial substances in the field of biology. The AgNPs of *Neolamarckia cadamba* displayed inhibitory activities against *E.coli* ATCC 25922 , *Pseudomonas aerugenosa, Staphylococcus aureus* ATCC 29213, *Klebsiella pneumonia, Lactobacillus acidophilus* MTCC 10307, *Streptococcus mutants* MTCC 497 and results of comparative antibacterial activity of silver nanoparticles were recorded. It was observed that silver nanoparticles showed the more inhibitory zone than the methanol and aqueous extract.

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