International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 206-215 www.ijprajournal.com ISSN: 2456-4494

# Green Synthesis of Silver Nanoparticles Using Herbal Extract: Characterization and its Biological Activity.

Deena Jose[1],Divya K R[2], R. Bino kingsley[1], Soniya P Davis[1], Midhila.E[1], Hisana[1], Havva NH[1], Nagalekshmi R[*1]

[1]*ELIMS College of Pharmacy, Thrissur-680631, Kerala.*

[2]*Research Scholar, Department of Chemical Oceanography, School of Marine Sciences, CUSAT, Kochi-682016, Kerala.*

Submitted: 01-07-2022 Accepted: 10-07-2022

**ABSTRACT**

Synthesis of silver nanoparticles from plant extracts is one of the most convenient, environmentally friendly, simple and economical method that mitigates any involvement of toxic chemicals. This work summarizes the green synthesis of silver nanoparticles using whole plant extract of Plectranthusamboinicus and evaluation ofit's antimicrobial and antioxidant activity. The prepared herbal nanoparticles were characterized by UV–Visible spectroscopy scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDX) and Fourier transforms infrared spectroscopy(FT-IR) method. The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV–visible spectrophotometric analysis. The FT-IR spectroscopy studies confirmed the capping and stabilization of synthesized nanoparticles. The antioxidant activity using DPPH confirmed the Nano formulation has greater activity when compared to the herbal extract. Hence from the present study we found that the herbal nanoparticles prepared from whole plant extract of Plectranthusamboinicus exhibited better antimicrobial and antioxidant properties when compared with whole plant extract.

**KEY WORDS**:Plectranthusamboinicus, antimicrobial, nanoparticles, SEM, FT-IR, EDX, Antioxidant.

## I. INTRODUCTION

NovelDrugDeliverySystems(NDDS) is a system used for delivery of a drug other than conventional drug delivery systems.The aim of NDDS is to deliveratherapeuticamountofdrugtotheappropriatesiteinthebodytoaccomplishdesiredpharmacologicalaction.Silvernanoparticleshaveagreatpotentialforusein variousbiologicalactivitylikeantimicrobialactivityandantioxidantactivity.Antimicrobialcapabilityofsilvernanoparticlesallowsthemtobesuitablyemployedinnumeroushouseholdproductssuch as textiles,foodcontainers,homeappliancesandmedical devices.Silveris aneffectiveantimicrobialagentwhichexhibitslowtoxicity[1]. Attribution of small size and high surface to volume ratio of metal nanoparticles enhances its bactericidal property which allows them to have an effect on microorganisms membranes and it is not simply due to release of metal ions in solutions [2].

The present study explored the suitability of utilizing Plectranthusamboinicus whole plant extract as a promising material for the synthesis of silver nanoparticles. P. amboinicusspreng belongs to Lamiaceae family and is known as Indian borage in English. It is commonly grown in Ceylon, Moluccas throughout India. The leaves are familiarly known to have medicinal values especially for the treatment of cough, sore throat and nasal congestion. It is used against various bacteria,viruses and fungi infections traditionally.We have synthesized AgNPs using P. amboinicusresulting in reduction of Ag+ to Ag and investigated its antibacterial and antioxidant activity of synthesized nanoparticles and plant extract against different microorganisms.

## II. EXPERIMENTAL

### Preparation of Plectranthusamboinicus whole plant extract

The freshly collected p.amboinicusspecimen where taxonomically identified and authentified by Dr.sreekumar v b (senior scientist) Kerala Forest Research Institute (an institution under kerala state council for science, technology and environment), Peechi, Thrissur, Kerala, India. The 100 gm powder is placed in 1000ml beaker and poured 500ml pure ethanol and kept for 7 days (Maceration method). The extract was filtered and the resulted extract was concentrated in sonicator and dried in

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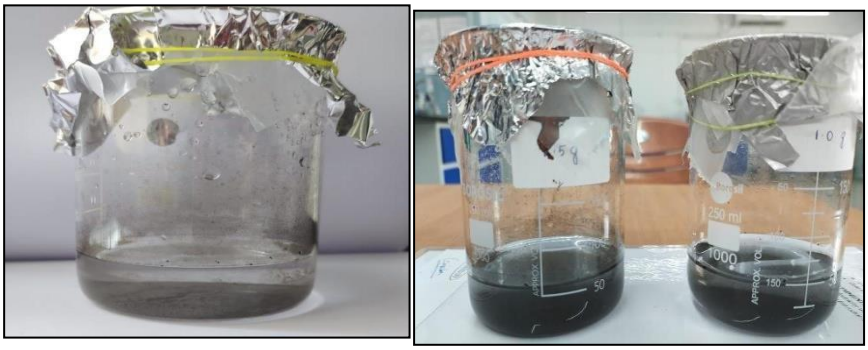
dessicator. Calculate the yield of extract.

**Biosynthesis ofSilver Nanopartcles**

Different concentration of silver nanoparticles in ethanolic solvent were prepared. Weighed 0.5, 0.1, 2.5 g of dried P.amboinicuspowder, added 50ml of 40% ethanol and kept in dark in the air at room temperature for a week and concentrated the solvent[3].From the different concentration of ethanolic extract, 2.5 ml were collected and 50ml 1mM aqueous silver nitrate was added with stirring. Then, the solution was kept in the light for 2 days in air at room temperature.



**Schematic synthesis procedure of green synthesized Ag Nps.**

**QUALITATIVE PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS**

Ethanolic extract were subjected to qualitative phyto-chemical tests for detection of different constituents

**CHARACTERIZATION OF SYNTHESIZED SILVERNANOPARTICLES**

The extract was concentrated and dried by freeze drying, then the following test was carried out.

**Ultraviolet Radiation (UV)**

Instrument: Shimadzu 1900i

Procedure : The sample was directly placed into a quartz cuvettes using ethanol as diluent. The spectra was recorded in the range of 200-400nm.

**Fourier transform infrared (FTIR)**

Instrument : Alpha II

Procedure:Attenuated total reflection-fourier transform infrared spectrometry (ATR_FTIR) Infrared spectra of sample were recorded in Bruker ATR alphakeptataambienttemperatureof25.0+_0.5oC.Theanalytical procedurewassimpleanddidnotneedanysamplepreparation.Thespectra were recorded by placing the samples on a zinc solenoid crystal plate and screwing the anvel over the sample carefully and scanning the sample in region of 4000-400 $cm^{-1}$ to determine various functional groups. The IR spectra of the samples was checked for any possible drug excipients interaction and confirm chemical integrity of given sample.

**Scanning electron microscope (SEM IMAGING)**

Instrument **:**Hitachi SU 3500 Procedure**:** Thepowderswereimagedbyascanningelectronmicroscope(SEM)runat anacceleratingvoltageof10kvusingHitachiSU3500. Thepowderinfew microgram were fixed on to stub by a double sided sticky carbon tape and kept inside the SEM chamber and analyzed at different magnification such as 60X, 200X, 500X, 1.10X and 2.50X respectively to obtain better clarity on the particlemorphology/topology.

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Why gold sputtering?

1. The materials should be non-conductive as like diamond, glass, rubber plasticetc.
2. Evenaminutequantityofwater\moistureinthesamplewill result in non-uniformity in the coating and finally leads to flashing and poor imagequality.
3. Fornanoparticulatesgoldsputteringwillbedonesincemineisamicro- particulate system there is no need of any sputtering as the image qualityis not disturbed by direct exposure of the material to the applied voltage inside thechamber.

**X-ray diffraction analysis (XRD)**

This test method is performed by directing an x-ray beam at a sample and measuring the scattered intensity as a function of the outgoing direction. Once the beam is separated, the scatter, also called a diffraction pattern,indicates the sample's crystalline structure.

**ANTIMICROBIAL ACTIVITY**

The extract were tested for antibacterial activity in well diffusion method[4] by using standard procedure The bacterial species used for the test were bacillus subtilis, pseudomonas fluorescens, E.coli, lactic acid bacilli, staphylococcusaureus.Allthestockculturewereobtainedfrom MTCClab. The microorganism were grown overnight at $37^0$C in nutrientbroth(PH 7.4)

**ANTIOXIDANT ACTIVITY**

**DPPH RADICAL SCAVENGING ASSAY:**

6.34 mM DPPH in methanol[5] was prepared and diluted to 0.634 mM to obtain working standard. Different concentration (0,10,25,50,100µg/ml) of extracts of Plant(2.5g) extract and Nanoparticle (2.5g) extract was added to a series of test tube and mixed with 100µl DPPH and made up to 1ml with distilled water. Kept for 20-30 minutes at room temperature in dark. The decolourisation at 515 nm was measured.

## III. RESULTS AND DISCUSSIONS

**Yield ofextracts**

| EXTRACTS | Solvent | CONCENTRATIONS (gm) | YIELD (%W/W) |
|---|---|---|---|
| Plecthrantusamboinicus (lour) spreng | Ethanol | 0.5 | 96 |
| | | 1.0 | 98.4 |
| | | 2.5 | 98 |

**Selection ofNano-formulaion**

From the antioxidant and anti-microbial activity 2.5 gm nano-formulation showed maximum activity which was selected for characterization procedure and the selected formulation were freeze dried.

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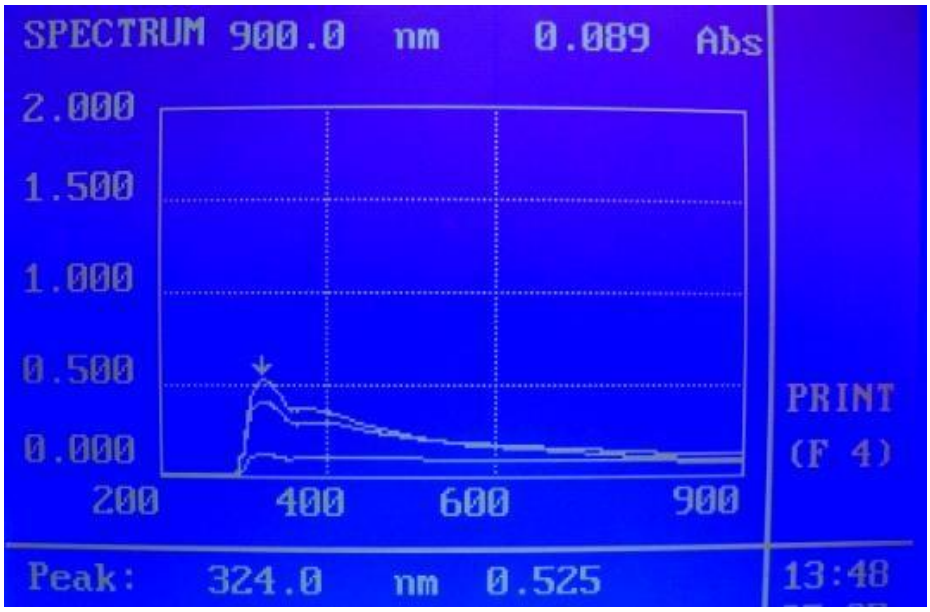
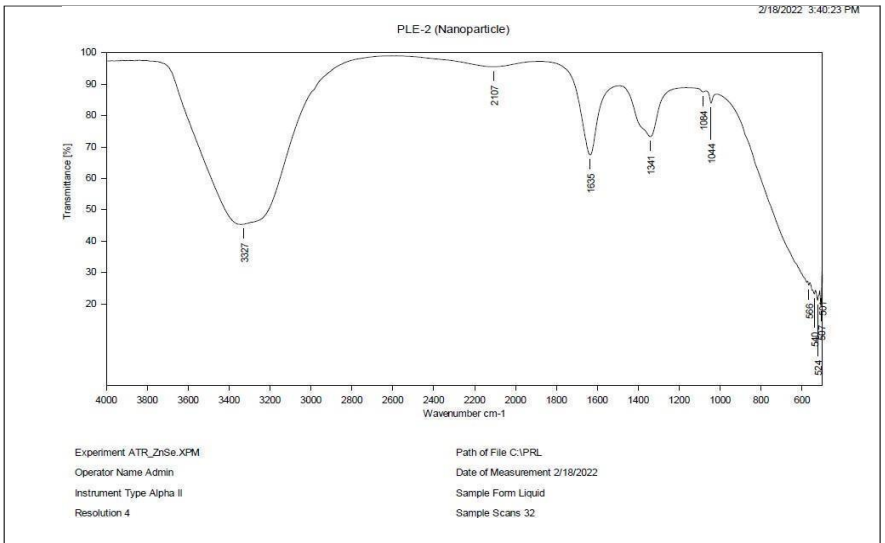
**UV GRAPH**




**FT-IR GRAPH**



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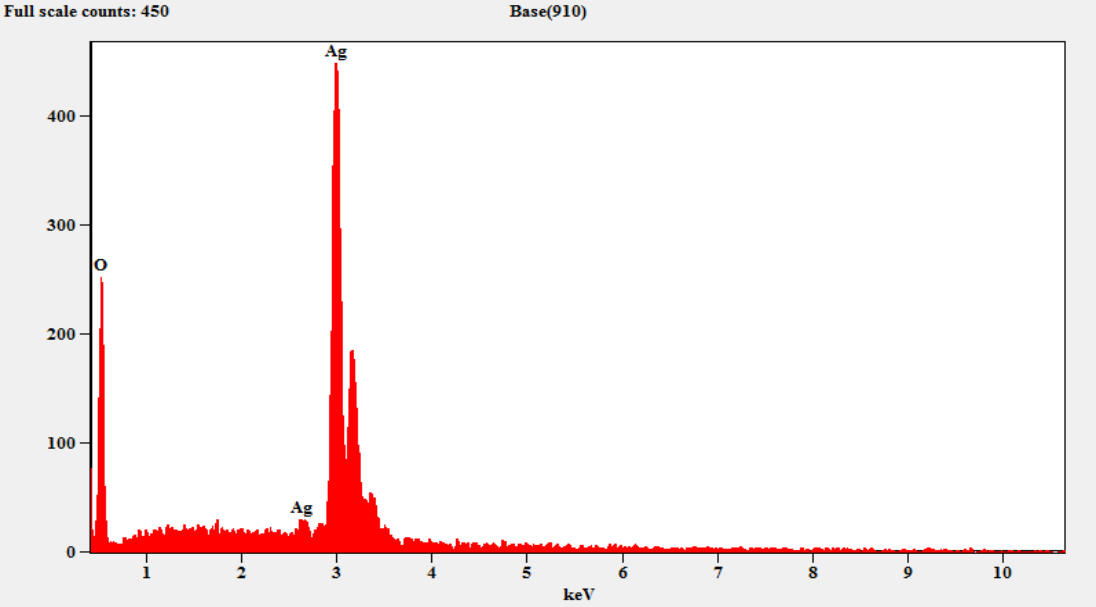
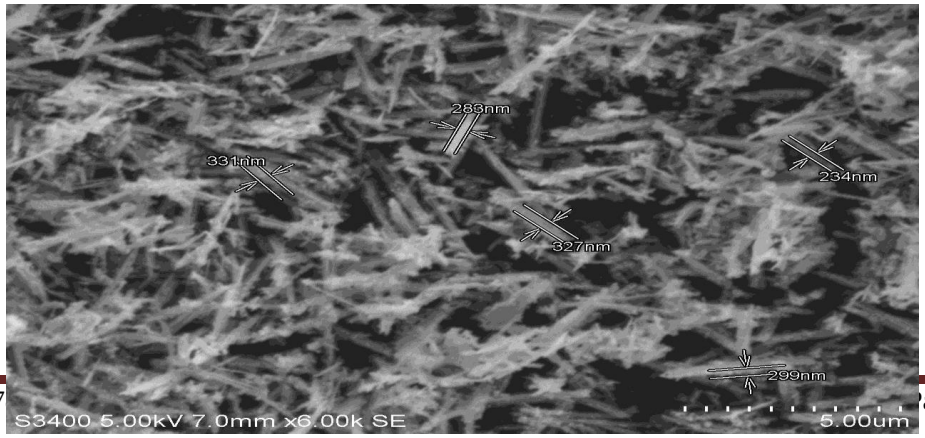
**SEM EDX**




Quantitative Results for: Base(910)

| Element Line | Weight% | Weight% Error | Atom% |
|---|---|---|---|
| **OK** | 31.49 | ±2.47 | 75.60 |
| **AgL** | 68.51 | ±1.95 | 24.40 |
| **AgM** | - | --- | - |
| | - | | - |
| | - | | - |
| **Total** | 100.00 | | 100.00 |

**SEM**



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**Antimicrobial activity**

Anti microbial activity in plant extract

| Extracts | Staphylococcus aureus | Bacillus subtilus | Lactic acid bacilli | Pseudomonas fluoresence | Escherichia coli |
|---|---|---|---|---|---|
| 0.5g | 10mm | 18mm | 18mm | 16mm | 20mm |
| 1.0g | 11mm | 20mm | 19mm | 18mm | 20mm |
| 1.5g | 13mm | 21mm | 20mm | 19mm | 21mm |
| 2g | 14mm | 22mm | 21mm | 19mm | 21mm |
| 2.5g | 15mm | 24mm | 22mm | 20mm | 22mm |

| Nanoparticle | Staphylococcus aureus | Bacillus subtilus | Lacticacid bacilli | Pseudomonas fluoresence | Escherichia coli |
|---|---|---|---|---|---|
| 2.5g | 49mm | 18mm | 21mm | 41mm | 43mm |

Anti microbial activity in nanoparticle

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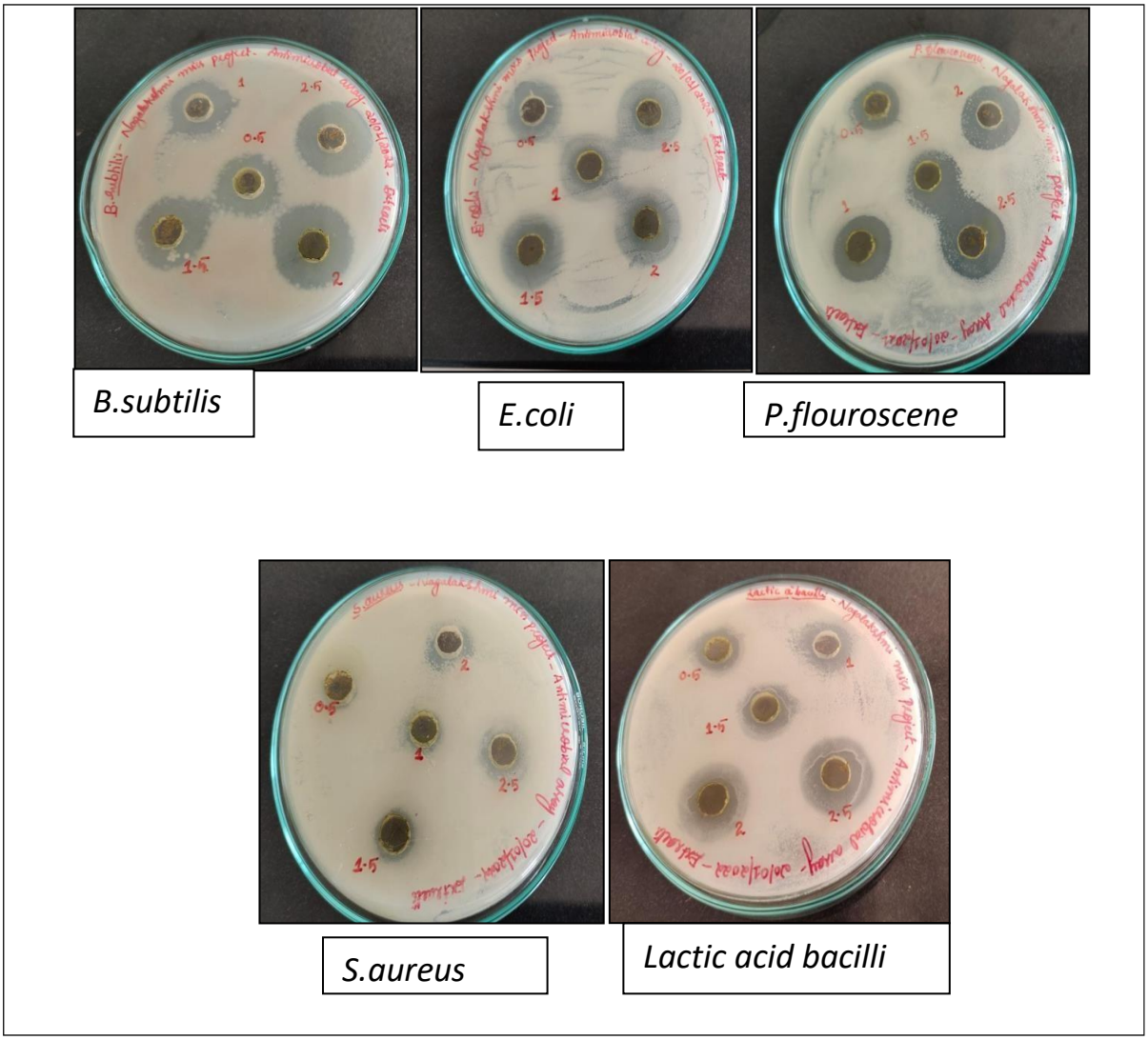


Zone of inhibition in plant extract

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E.coli

B.subtilis

P.flourescene

Zone of inhibition in silver nitrate extract

Antioxidant Activity

| Sample | | Concentration (µg/ml) | Percentage inhibition (I%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| Plant extract | | 0 | 0 | 24 |
| | | 10 | 16.77 ± 3.78 | |
| | | 25 | 51.39 ± 4.72 | |
| | | 50 | 54.10 ± 3.78 | |
| | | 100 | 58.19 ± 3.31 | |
| Nanoparticle | | 0 | 0 | 17.5 |
| | | 10 | 23.56 ± 4.72 | |
| | | 25 | 66.89 ± 7.18 | |
| | | 50 | 77.10 ± 7.00 | |
| | | 100 | 79.11 ± 4.09 | |

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## IV. DISCUSSION

From the ethanolic extract of different concentration (0.5, 1.0, 2.5) of P.amboinicus, percentage yield calculated was (96, 98.4, 98) respectively. From the phytochemical screening of ethanolic extract, found the presence of alkaloids and absence of saponins, flavanoids, phenolic compounds, steroids respectively.

Prepared silver nano-formulation of different concentration of ethanolic extract and from visual inspection, the change in solution color from colorless to reddish-brown was a clear indication of the formation of green mediated AgNPs in the reaction mixture. The occurrence of color change in the reaction mixture was due to the excitation of surface plasmon resonance in the AgNPs.FromUV-visible absorption spectroscopy, Plant extract of ofP. amboinicus was used for the biosynthesis of PA-AgNP. During the preparation, the colourless silver nitrate solution turned brown indicating indicating the formation of silver nanoparticles (AgNPs). The occurrence of brown color can be attributed to the surface plasmons, arising from the collective oscillations of valance electrons in the electromagnetic field of incident radiation. The UV-V is spectra of the biosynthesized AgNPs, shows plasmon resonance at 350 nm indicating formation of PA-AgNP. The position and shape of the surface plasmon absorption is dependent on the shape and size of particles formed, their interparticle distance, and the dielectric constant of the surrounding medium.From Energy-dispersion X-ray (EDX) spectroscopy, Energy-dispersion X-ray (EDX) spectroscopy study was employed to detect the existence of elemental silver. The results clearly indicate an intense signal at approximately 2.98 KeV corresponding to the presence of metallic silver nanocrystals, occurring due to surface plasmon resonance (SPR). The other intense signal at around 0.0– 0.5 Kev represents the characteristic absorption for oxygen and carbon. This indicates the presence of P.amboinicus plant extract as a capping ligand on the surface of AgNPs. From this spectrum, the presence of elemental signal of the metallic silver was confirmed. Major emission energy identification peaks for silver displayed and these correspond with peaks in the spectrum at approximately 3 keV, thus attesting that silver has been correctly identified in Ag NPs. These results therefore indicated not only the successful formation of AgNPs, but also confirmed the phytoconstituents presented in PA leaf extract acted as reducing and capping agents for the synthesis and the stabilization of AgNPs. From FTIR spectroscopy, FTIR measurements were carried out to identify the various functional groups in biomolecules responsible for the reduction of silver ions to AgNPs and capping/stabilization of AgNPs. The band intensities in different region of spectra for P. amboinicus extract and biosynthesized silver nanoparticles were analysed. The similarities between the two FTIR spectra, with some marginal shifts in peaks clearly indicate the plant extract is also acting as a capping agent. The P. amboinicus plant extract showed a number of peaks reflecting a complex nature of the plant extract. FT-IR analysis results of methanolic extract of P. amboinicus proved that presence of phenols, alkanes, alkanes, aromatics, aromatics and alkyl halide. The strong peak at 3327 can be due to O-H bond of phenol, while the peak at 2107 corresponds to C≡C stretch stretching of alkynes. The C=C stretch of alkene is confirmed by peak 1636, peak at1341 shows $NO_2$ stretch of nitro compounds, peak at 1064 confirms the C-F stretching of alkyl and aryl halide, while peak at 1044 is due to =C-H bend of alkanes. These functional group present in plant extract was involved in the capping and stabilization of AgNPs. From Scanning Electron Microscopy, The SEM image shows that relatively spherical and uniform nanoparticles are formed. Some of the larger particles seen may be due to aggregation of nanoparticles induced by evaporation of solvent during sample preparation. From the micrographs, it was observed that the synthesized nanoparticles were well dispersed. The aggregation of nanoparticles was attributed to the Van der Waals forces.

From antimicrobial and DPPH radical scavenging assay, select the best nano-formulation for characterization purpose. The characterization work done was 2.5 concentration respectively. From UV-visible spectra at 350 nm indicate the formation of PA-AgNP. IR range at 3327, 2107, 1636 and 1064 confirm the capping and stabilization of AgNPs. From EDX spectra 2.98 key signal shows the presence of metalic silver nanocrystals. From SEM image showed the formation of spherical and uniform nanoparticles.
From DPPH radical scavenging assay found $IC_{50}$ of plant extract (24) and $IC_{50}$ of nano-formulation (17.5). From antimicrobioal studies least zone of inhibition was recorded against S.aureus. The synthesized silver nanoparticles exhibited better antimicrobial property towards gram negative S.aureusand Escherichia coli.

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## V. CONCLUSION

- Extracts of whole plant of P.amboinicuswas carried out using ethanol as solvent.
- From preliminary phyto chemical studies and TLC, extract shows presence of alkaloids as major secondary metabolite.
- Synthesized the different concentration (0.5, 1.0, 2.5) of silver nanoparticle from ethanolic extract and from change in color solution, found the formation of green mediated silver nanoparticles.
- From the DPPH radical scavenging activity and antimicrobial activity, selected the best nano-formulation (2.5) for characterization purpose.
- The formation of P.amboinicus silver nanoparticles was confirmed from UV visible spectra, energy dispersion x-ray (EDX) spectra, FT-IR spectra and SEM showed formation of spherical and uniform nanoparticles.
- From DPPH radical scavenging assay, nano-formulation shows maximum antioxidant activity.
- From antimicrobioal studies least zone of inhibition was recorded against S.aureus. The synthesized silver nanoparticles exhibited better antimicrobial property towards gram negative S.aureusand Escherichia coli.

## REFERENCES

[1]. Khan, Mohammad. (2017). THE NOVEL DRUG DELIVERY SYSTEM. WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES. 6. 477-487.

[2]. J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez, Nanotechnology 16 (2005) 2346–2353

[3]. Ajitha B, Ashok Kumar Reddy Y, Sreedhara Reddy P. Biosynthesis of silver nanoparticles using Plectranthusamboinicus leaf extract and its antimicrobial activity. Spectrochim Acta A Mol BiomolSpectrosc. 2014 Jul 15;128:257-62.

[4]. Swamy K, Pokharen M, Dahal N et al. Phytochemical and antimicrobial studies of leaf extract of Euphorbia nerifolia. J Med Plants Res.2011;5:5785–8.

[5]. Nagalekshmi R, Menon A, Chandrasekharan DK, Nair CK. Hepatoprotective activity of Andrographis paniculata and Swertiachirayita. Food Chem Toxicol. 2011 Dec;49(12):3367-73.