JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

STUDY OF COMBINED EFFECT OF SILVER & CATHARANTHUS SILVER NANOPARTICLES ON CLINICAL PATHOGENS P. AERUGINOSA AND S.AUREUS CAUSING SKIN INFECTION

Sayed Sanjeeda [1] Hitesh Gaurav [2]
1 Postgraduation student at Department of Microbiology , Chitisak Samuha's S.S & L.S Patkar college of Arts & Science & V.P Varde College of Commerce.
2 Professor in Department of Microbiology , Chitisak Samuha's S.S & L.S Patkar college of Arts & Science & V.P Varde College of Commerce.

ABSTRACT :

Antimicrobial drug resistance has been identified as one of the threat in treatment of infectious diseases. With an aim to achieve alternative classical antibiotic treatments there have been strong research focus on nanoparticles particularly silver nanoparticles. Green synthesis of silver nanoparticles is usually preferred over chemical synthesis since it environmental and eco friendly method. The present work evaluates the antibacterial, antioxidant, antibiofilm potency of biosynthesized silver nanoparticles from Catharanthus roseus leaf extract. The biosynthesized silver nanoparticles exhibit a notable potential for producing spherical and stable AgNPs which was characterized by UV- Vis Spectroscopy , Scanning electron microscopy and Fourier transformed infrared spectroscopy .The antioxidant activity of *C.roseus* and biosynthesized AgNPs was determined by DDPH method. Both *C.roseus* extract and biosynthesized AgNPs showed strong antioxidant activity .The biosynthesized silver nanoparticle was tested against the pathogenic organisms *Staphylocccus.aureus*, *Pseudomonas aeruginosa* by agar well diffusion method. The AgNPs exhibited effective antibacterial activity by creating a zone of inhibition against these micro-organisms. Biofilms formation of these micro- organisms was attained using Congo red agar method . Among these bacterial strains *S.aureus* exhibited strong biofilm formation by producing black coloured colonies on Congo red agar medium while *P.aeruginosa* being the weak biofilm producers. The biofilm eradication was evaluated by which showed 70% and 74% biofilms reduction of *S.aureus* and *P.aeruginosa* respectively. However, the efficiency of biosynthesized silver nanoparticles from *C.roseus* was found to have great antioxidant, antibacterial and antibiofilm effect against the *P.aeruginosa*, and *S.aureus* .

KEYWORDS : Catharanthus.roseus , Biofilm eradication , Antibacterial activity , Silver nanoparticles , antioxidant activity ,Green synthesis

Introduction :

Microbial infection and microbes' resistance to commonly used antibiotics are the leading cause of death worldwide and this lead tremendous impact on health care / public health economy. Most of pathogenic species tends to aggregate, adhere, multiply and form 3D complex network on biotic or biotic surface. This complex 3D multilayered network is known as biofilms. (Roy tiwari, 2018) Microbial biofilms that are sticky to exopolymeric substance (EPS) carrying adherence of micro organism to biotic surface such as host cell ,abiotic surfaces such as medical devices ,catheters, can cause antimicrobial resistance ,due to its molecular content such as EDNA and exoenzymes (β- lactamase, toxins, etc), limited diffusion of antimicrobial through biofilms matrix ,persistent cells content and limited nutrient and oxygen ,surface protein and polysaccharide intercellular adhesion play an important role in biofilms development and product . It is more difficult to treat biofilm-embedded bacteria than a planktonic form. These biofilms particularly have effective shield against the antibodies ,slowing down their penetration , provoking their enzymatic degradation and providing shielded bacterial cells with an opportunity to develop resistance via genetic changes. (Fulaz, 2019)

Biofilms components have been recognised as an excellent target for antimicrobial therapy since these biofilms disruption would render bacterial cells more vulnerable to antibiotics and the immune system. To overcome the limitation /resistance of antibiotics based treatment; new microbial therapies have been developed. These include small molecules, immune –modulators, anti-virulence agents, antibodies, vaccines and metallic nanoparticles. (Koo, 2017)

Nanotechnology is an emerging field of scientific research which has many technological developments and applications it is gaining more and more attention due to their applicability especially in biomedical fields (e.g. in diagnosis and treatment of human cancer). Nanoparticles have also become an area of interest in research due to their unique properties such as electrical conductivity, ductility, toughness and formality of ceramics, increasing the hardness and by increasing luminescent efficiency of semi- conductors. (M.Rittner, 1998) The special characteristics of nano materials and their biologic effect suggested that nanoparticles can use as an alternative treatment of diseases. Gold , silver ,copper ,zinc, platinum are commonly used nanoparticles in products .Among all the metallic nanoparticles, silver has gained popularity since silver and its compounds have been known for its antimicrobial properties throughout history which have been successfully employed for prevention of microbial growth. The most important application of silver and silver nanoparticles in medical industry is topical ointments to prevent infection against wound and burns. (IP.el al, 2006)

To date many reports have been published that showed the action of silver nanoparticles against the bacterial cells as well as biofilms of multi drug resistance bacteria. (Mathur P, 2018) Nanobiotechnology has enhanced the production of minor AgNPs with little toxic effect to humans and more effectiveness among the bacteria. In recent years, nanoparticles have been addressed to play a vital role in medical science and various biotechnology fields as these silver nanoparticles have remarkable biologically application such as antioxidant, anticancer, anti inflammatory, antibiofilm, wound healing and antimicrobial properties.Previous studies demonstrates that *Staphylococcus .aureus* , *P.aeruginosa* , *Acinetobacter baumannii* , *Klebsiella pneumoniae* and *E.coli* are the major contributors which usually form the biofilms in the infected wound and make them more difficult to treat . Therefore these nanoparticles can be used as an alternative therapeutic treatment. (N.p singh, 2017)

There are various methods from which nanoparticles can be synthesized. They are categorised as top-down or bottom up approaches .The bottle up approaches includes "building of silver nanoparticles from silver atom in an aqueous solution" while Conventional method used for the synthesis of silver nanoparticles includes radiation, chemical –photochemical methods, electrochemical techniques. However these methods are extremely expensive and time – consuming and can be dangerous to human health and environment because of their application of hazardous substances. Therefore, there is a growing need to develop coat –effective and environmental friendly approaches for the synthesis of nanoparticles. (Singh P et al, 2018)

Biological approaches to the synthesis of metal nanoparticles using microorganism and plant extract have been suggested as a valuable alternative to chemical and physical methods. Several reports have been published on the use of natural materials such as plants, bacteria, fungi, yeast, which are used for the synthesis of silver nanoparticles. ((A ,Nandhini R , Aruna Kartikeyan V, Bose P, 2014)

The rate of synthesis of nanoparticles by plants extract is higher than of chemical methods and green synthesis by micro –organisms. The use of plant materials for the synthesis' of nanoparticles does not required an elaborate process such as intracellular synthesis, multiple step purification or the maintenance of microbial cultures. Also plant extract have enzymes such as hydrdrolses , reductases and phytochemicals such as terpenoids , flavanoids , phenol and dihydric phenols and so on to act as reluctant in the presence of metal salts for the nanoparticles synthesis . Moreover the synthesis' of nanoparticles from plants is found to be a rapid, low cost, eco friendly and single step process. Many previous studies reported by using various plants including *Helianthus annus* , *Oryza sativa* , *Saccharum officinarum* , *Zea mays* , *Azadirachta indica,* (Shankar et. al, 2004) *Medicago sativa* (Liu, 2019), *Aloe vera, Diopyros* (Song AND Kim, 2008), *Carica papaya* ((Jain et al , Kumar daima , S.Kachhwaha ,S.l.kothari , 2009)) *and Catharanthus roseus* (Gomma SE ,Yahayu M, Dailin DJ, Enshasy HL, 2021) *are found to promising* substance for the green and facile synthesis of silver nanoparticles AgNPs).

Catharanthus roseus is an erect procumbent herb or under herb containing latex that grows up to 1m tall in sub tropical areas. This perennial herb is grown commercially for medicals uses in India, Australia Africa and Southern Europe .Catharanthus .roseus (Madagascar periwinkle or sadabhar) belong to the family Apocynaceae has been widely used to treat different disease in folk medicine. (Appidi JR ,Grierson DS ,Afolayan AJ , , 2008)

It is also known for its antifungal, antimicrobial, wound healing, anti plasmodium, antioxidant, and antiviral activities due to the presence of vincristine, vinblastine and ajmalicine. The leaves and the rootsof Catharanthus roseus are rich in their phytochemicals; for instance alkaloids, which have demonstrated to have anticancer and anti- hypertive effect. (El -Sayed M , Verpoote R ,, 2005).The alkoids vinblastine and vincristine in C.roseus have been used as anticancer drugs in the treatment of different types of cancers such as lymphomas, Hodgkin's lymphomas, breast cancer, leukaemia, soft tissue sarcomas, multiple myelomas and neuroblatoma. (Parakh MP Swati S, 2019) (Poonarululselvam S, 2012)Successfully synthesized silver nanoparticles from C.roseus leaves extract and tested it antibacterial activity.

The present study aimed to determine the biology synthesis of silver nanoparticles produced from Catharanthus .roseus extract and was characterized by UV-VIS spectrophotometer; Fourier transformed infrared spectroscopy and scanning electron microscopy. Moreover the antioxidant, anti biofilm and anti bacterial activity of biosynthesized silver nanoparticles against the skin infection causing organisms (S.aureus, *P.aeruginosa)* was also evaluated.

Literature survey :

Nanotechnology is an engrossing area of scientific research which has generated various new applications in field of biotechnology and nanomedicine leading to the development of new kind of nanoparticles. The current development of nanotechnology has led to nano-medicine, a field which includes many diagnostics and therapeutics applications such as nano materials and nano devices. (Mohanty S, 2017). There are various methods such as physical, chemical and biological methods used for the synthesis of the nanoparticles .Since the physical & chemical method involved to be expensive, produce low yield and does have high toxic compounds, biological method is preferred where plants and microorganisms are used for the formation of nanoparticles.

The green synthesized nanoparticles using plant materials has gained lot of attention since it embrace to have great advantages such as rapid synthesis , easy availability , cost effectiveness , easy handling , negligible toxicity and can simply scaled great range production . Also the rate of synthesis of nanoparticles by plant extracts is higher than of green synthesis by microorganisms, since the use of plant materials for the synthesis of nanoparticles does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures.Various reports have confirmed that the plant extract can synthesized nanoparticles such as gold ,silver , zinc ,platinum etc .Among the different nanoparticles , silver nanoparticles has found to be more effective as it does consist of good antimicrobial activity against various bacterial organisms and can kill the bacterial biofilms. Due to its inhibitory effect on microbes it has been recognized in medical industry as an application such as ointment which can be used to prevention in open wounds and burns. (IP.el al, 2006)
Different plants such as Lantana camara, Orange peel, Onion, Banana, Neem, O. Tenuiflorum, Coffee, Tea, Capsicum, Garlic and Catharanthus.roseus can be used for the synthesis of silver nanoparticles.

Prior to the antimicrobial , antioxidant, wound healing activities the green silver nanoparticles are characterized using UV-visible spectrophometry, Transmission Electron Microscopy, Scanning Electron Microscopy and Fourier Transform Infrared spectroscopy.

UV–visible spectroscopy is one of the established methods to monitor the continuous synthesis of AgNPs where strong and broad peak developed within the 420–430 nm range and can be frequently observed, which the peak range characteristic is for AgNPs (Nayak D, 2015)

Scanning Electron Microscope (SEM) and Transmission Electron Microscope is an invaluable tool which is used to obtain structural information about nanoparticles such as size and morphology of the biosynthesized AgNPs. The analysis of SEM and TEM micrographs of the synthesized AgNPs indicated that the synthesized nanoparticles were distinct, uniform in shape, spherical, and well-separated and the average size of the particles ranged between 52 and 96 nm.

The Fourier transformed infrared spectroscopy (ATR-FTIR, Brucker) analysis of spectra within the range of 500-4000 cm^{1} is conducted to determine the role of phytoconstituents present in nanoparticles synthesis.

Methods such as Agar well diffusion, Minimum Inhibitory Concentration and Bacterial Growth Inhibition, Biofilm inhibitory concentration methods was studied for the determination of the
antibacterial and antibiofilm activity of silver nanoparticles. To check the synergistic effect of conjugated silver nanoparticles fractional inhibitory concentration index method can be used. (SC De la Fuente –Nunez, 2014)

Since ,multi – drug resistant pathogens are on rise and are creating serious health risk , there has been strong research focus where numerous, new antibiotics therapeutic in amalgamation with nanoparticles have been discovered and developed in order to treat and kill Gram-negative, as well as Gram-positive, human biofilms forming pathogens. (Mohanty S, 2017)

Comprehensive studies of antimicrobial resistance have revealed that the bacterial infections are resistance to antibiotics are not due to free bacteria but rather to bacteria existing within a biofilm . (Liu, 2019) These biofilm-forming bacteria are resistant to conventional antimicrobials due to: (1) the inability of the antimicrobial to penetrate the biofilm, (2) evolution complex drug resistance properties, and (3) biofilm mediated inactivation or modification of antimicrobial enzymes. (Elbourne et al, 2019)Usage of silver nanoparticles and antibiotics in combination can help to prevent and eradicate both planktonic and biofilm-forming antibiotic-resistant bacteria. (SC De la Fuente –Nunez, 2014).Fortunately, continuous researches have been conducted to develop eco-friendly nanoparticles-based antimicrobials which can utilize phytochemicals and can eradicate both planktonic and as well as biofilm-forming antibiotic-resistant bacteria.

A research based on Biosynthesized of silver nanoparticles extract from Catharanthus.roseus plant was conducted. Catharanthus.roseus plant belongs to family Apocynaceae is an herbaceous herb that is grown commercially for medicinal uses in India, Australia, Africa, and Southern Europe. It carries certain properties such antimicrobial, antifungal, antioxidant, wound healing, antiviral, antiplasmodium activities. The alkaloids vincristine, vinblastine and ajmalicine compound present in Catharanthus.roseus can be used as a chemotherapeutic regime as anti cancer treatment. (Gomma SE ,Yahayu M, Dailin DJ, Enshasy HL, 2021) Due to its above medicinal properties it has gained focus on the green synthesis of silver nanoparticles.The green synthesized AgNPs also manifested good antibacterial property against various pathogens such as gram-negative bacteria, such as *E. Coli, K. Pneumonia, P. Aeruginosa, and S. Aureus* and also showed antifungal activity against C. Albicans. It is found that the silver nanoparticles do have significant effect similar to an antibiotic than the use of plant extract or silver nitrate only (Gomma SE ,Yahayu M, Dailin DJ, Enshasy HL, 2021)

Similar to green synthesis of silver nanoparticles from Catharanthus.roseus, the green synthesis of silver nanoparticles using Withania coagulans extract was studied for its antibacterial activity in combination with the Antibiotics was studied. The antibacterial activity in silver nanoparticles was confirmed by zone of inhibition and pores on the surface of bacteria while the conjugated silver nanoparticles with Levofloxacin found to have synergy and additive behaviour against the tested bacteria *E.coli, S.aureus, K.pneumoniae, and P.vulgaris*). Furthermore, the bactericidal and bacteriostatic effect was reported which was depends upon its concentration. Thereby, proved that the conjugation of silver nanoparticles with an antibiotic to be beneficial due to its synergy and additive effect against the bacteria. (Anand kumar keshari, 2021)
A research based on the antibacterial activity of silver nanoparticles (AgNPs) synthesized using Areca catechu extracts against three species of antibiotic-susceptible and three species of resistant bacteria was investigated which resulted that the effects of this plant were more promising when compared with other medicinal plants tested which was tested against antibiotics resistance bacteria i.e *Enterococcus faecalis ,Vancomycin-resistant Enterococcus faecalis (VRE), Pseudomonas aeruginosa (P. Aeruginosa), Multidrug-resistant Pseudomonas aeruginosa (MRPA) ,Acinetobacter baumannii (A. Baumannii) ,Multidrug-resistant Acinetobacter baumannii (MRAB)* . (Jeong Su Choi, 2021)

Thereby , various research have been conducted where the extracts of several plants including ginger, garlic, capsicum and their mixtures which were successfully able to produce AgNPs which does have a remarkable antimicrobial properties against *E. Coli* and *S. Aureus* and a mild effect on *C. Albicans*. (Hamed Abu Sara, 2019)

Researchers suggested different mechanisms of the AgNPs action onto bacteria. (Hamed Abu Sara, 2019). (Vahvaselka, 2008) demonstrated that the membrane permeability and respiration function, which can lead to cell death. In addition, the AgNPs does not only attach to the bacterial cell surface but also enter inside the bacteria, which results in a disruption of adenosine triphosphate (ATP) production and DNA replication, generation of ROS and direct damage to cell structures (Sahayaraj and Rajesh, 2011)Furthermore, the bactericidal effect of silver also attributed to the inactivation of phospho-mannose isomerase, which catalyzes the conversion of mannose-6-phosphate to fructose-6-phosphate which is an important intermediate of glycolysis, and a most common pathway in bacteria for sugar catabolism (Beddy D, 2004)

The in vivo assessment of wound healing in (Catharanthus.roseus) CR-AgNPs-treated mice was also found to effective in closuring and reducing size of wounds. (Chatterjee AK, 2014)**.**Similarly, the effect of AgNPs in dermal contraction and epidermal re- epithelialisation during wound healing was investigated which suggested that the AgNPs could increase the rate of wound closure. (Lee et al., 2010)**.** C.roseus does have antioxidant properties which are beneficial for the management of many deleterious diseases because of their scavenging ability. Since it react with nitric oxide to form peroxynitrite, and can generate toxic radicals, such as the hydroxyl radical. (Halliwell, 1992) .Although the mechanism of action is still needed to study in more detailed level with more advanced experimental proofs, since, AgNPs promise to have a potential biologic.

The antioxidant activity of biosynthesized AgNPs can be evaluated using DPPH scavenging assay. DPPH is a stable compound which accepts hydrogen or electron from AgNPs. The colour changed from purple to yellow after reduction, can be quantified by its decreased absorbance at wavelength of 517nm. The dose-dependent increase in the inhibition percentage of synthesized AgNPs at various concentrations such as 50, 150, and 300gmL where the 300gmL1 concentration was found to exhibit a higher inhibition (82%) compared to that of the other two concentrations. The disappearance of purple colour when synthesized AgNPs were added might be caused by the presence of antioxidant in the medium al label. (Anand kumar keshari, 2021)

Such potent bioactivity of green synthesis silver nanoparticles may justify their biomedical use as antioxidant, antimicrobial agents for controlling various health-related diseases, particularly in wound healing.Hence, plant extracts, derived from different medicinal plant species, was able to synthesize AgNPs and their use of the plant extracts has an advantage over chemical or physical synthesis of AgNPs due to their ability to stabilize AgNPs, their own antibacterial properties, their high level of efficacy, and their low toxicity and their use which represents an eco-friendly approach to the synthesis of AgNPs. This high throughput Nano biotechnology curated with silver nanoparticles can offer a new approach to treat microbial pathogens that are resistant to current treatment practices and for the treatment of biofilms therefore, demonstrating the potential of using plant-derived AgNPs to inhibit biofilm formation for therapeutic treatments represented a new method of effectively treating a variety of multi drug resistance pathogens. (V.E Kangan, 2005)

Considering all the fact , the plant-derived AgNPs was found to exhibited strong antibacterial and anti-biofilm activity against different clinically important human pathogens and thereby, indicating it is safer and more advantageous to biosynthesize metal nanoparticles using the natural reducing agents present in plants and microbes along with the conjugation of AgNPs to natural, antimicrobial molecules produced by microbes or plants.

Materials & Methods :

1) Preparation of Catharanthus .roseus extract :

   C.roseus leaves was collected from garden, Mumbai (India). C.roseus was dried for 7 days in hot air oven. Then it was powdered using grinder. 2 % extract were prepared by soaking two grams of powder into methanol for 24-48 hours. The prepared extract was filtered out using Whatman filter paper no 1. The filtrate was then kept on boiling water bath to evaporate the methanol and then the dried extract was collected and dissolved it in 10ml of dimethyl sulfoxide (DMSO).It was then further use for synthesis of silver nanoparticles

2) Green synthesis of silver nanoparticles using C.roseus extract :
   10 ml C.roseus extract was mixed with 90ml of $AgNO_3$ (1mM) in 250 ml of flask and the colour of the solution was observed. The colour of solution was changed from light yellow to dark brown colour. This colour change indicates the conversion of silver nitrate ($AgNO_3$) into silver nanoparticles.

3) Chemical synthesis of silver nanoparticles using tri-soduim acetate:
   1M solution of silver nitrate was dissolved in 100 ml of distilled water and was heated till boil .5 ml of 1% tri- sodium citrate was added drop wise and mixed vigorously during the process.The solution was heated until the colour changes to pale yellow.The solution was removed and cooled with stirring and was stored at dark

4) Characterization of green synthesized silver nanoparticles :

- Uv-vis spectrophotometer: To observe the optical density of synthesized silver nanoparticles, the sample was analysed for UV-Visible Spectroscopic studies at room temperature which was operated at 20nm with range between 200nm to 600nm.

- Scanning electron microscope (SEM): To analyze the surface morphology of the biosynthesized silver scanning electron microscopic was carried out. For SEM analysis, thin film of silver nanoparticles was prepared and was sent at C.R.C.T.S., Rayat Shikshan Santha's Karmaveer Bhaurao Patil college ,Vashi, Navi Mumbai – 400703 for analysis

- Fourier transformed infrared spectroscopy (FTIR): FTIR was carried out by scanning silver nanoparticles over the range of 4000-450 cm^1 at resolution of 4 cm^{1} where the functional groups responsible for the formation of silver nanoparticles can be detected. The sample was prepared was send to SAIF-IIT BOMBAY POWAI for the analysis .

5) Antibacterial activity of biosynthesized silver nanoparticles and chemically – synthesized silver nanoparticles :

Comparative Antibacterial activity analysis of biosynthesized and chemically synthesized silver nanoparticles was determined using agar well diffusion method.The antimicrobial activity of both the silver nanoparticles was tested against the gram –positive (*S.aureus*) & gram – negative (*P.aeruginosa, K.pneumoniae*) organisms on Muller Hinton agar.After incubation of 24 hours at 37 'C the zone of inhibition was measured using ordinary scale and their antibacterial activity was determined.

6) DETERMINATON OF BIOFILMS USING CONGO RED :

It is a qualitative method used to detect the biofilms production by using congo red agar medium.3.7 grams of brain heart infusion broth and 3 grams of agar was dissolved in 100 ml of distilled water and 0.08 grams Congo red was dissolved in 10ml of distilled water was used to prepared Congo red agar media.Both of them were autoclaved at 121'C.The selected bacterial strain was cultured on Congo red agar medium and was incubated at 37'C for 24 hours. Development of black coloured colonies with dry crystalline consistency are regarded as biofilms - positive while those showing pink coloured colonies was considered to be biofilm non-producers.

7) Biofilms eradication assay :

Isolates of respective organisms that is *S. aureus, P. Aeruginosa* and *K.pneumoniae* was inoculated in tryptic soy broth (TSB) and was incubated at 37' C for 24 hours.The tubes of respective organisms was decant and was washed with phosphate buffer solution with ph 7.4 so that it leaves only biofilms.Biosynthesized silver nanoparticles was added to it and was incubated at 37'C for 24 hours.The isolates of which biofilms formed on the wall of test tubes was stained with 1% of crystal violet for 30 minutes and then after discharge of planktonic cells it was washed with phosphate buffer and with alcohol. 30% acetic acid was added to the tubes and was kept for 15 minutes.The optical density (O.D) was measured at wavelength of 595 nm .The percentage of biofilm eradication was calculated using the above formula : 1 – [O.D $_{595}$ of cell treated with AgNPs] / O.D$_{595}$ of non- treated control X 100

8) Analysis of antioxidant activity of Catharanthus .roseus and biosynthesized silver nanoparticles by DDPH method :

4 ml of 2, 2 Diphenyl-1-picrylhydrazyl (DDPH) was prepared in 0.004 % methanol and was mixed with 1 ml of C.roseus extract and biosynthesized silver nanoparticles.It was kept at room temperature for incubation for 30 minutes.After incubation for 30 minutes, it's absorbance was recorded using UV-VIS spectrophotometer at 517 nm.The percentage of antioxidant activity was calculated using above formula : O.D OF CONTROL / O.D OF SAMPLE X 100

Results and Discussion:

1. Green synthesis of silver nanoparticles :
   When 10 ml of the Catharanthus. Roseus leaves extract was added to 90 ml of $AgNO_3$ (1mM), it was found to be responsible for the formation of silver nanoparticles. The synthesis of silver nanoparticles was started after addition of extract which was observed visually by development of brown colour which was further confirmed by UV-VIS Spectrophotometer.

2. Chemical synthesis of silver nanoparticles:
   When 5 ml of 1% trisodium citrate was added to 90 ml of 1M silver nitrate $AgNO_3$, it was found to act as a reducing agent for the formation of silver nanoparticles .The appearance of pale yellow colour and then resulting into grey colour indicated the formation of silver nanoparticles which was chemically synthesized with the help of tri sodium citrate and was further confirmed by UV-VIS Spectrophotomter.

3. Characterization of silver nanoparticles :

Uv – vis spectrophotometer : The biosynthesized and chemically synthesized of the silver nanoparticles was monitored by recording the absorption spectral at wavelength range of 200 -800 nm . The spectral analysis observed by UV-VIS spectrophotometer confirmed that the synthesis of biosynthesized silver nanoparticles have taken place with the occurrence of the highest peak at 414nm while the spectral analysis of chemically synthesized silver nanoparticles have taken place with the occurrence of the highest peak at 325 nm. (Figure 1& 2). Past studies suggested that a SPR (surface plasmon resonance) peak located between 410 & 450 nm have been observed for AGNPs and might be attributed to spherical nanoparticles. (Zaheer, 2012)

Scanning electron microscopy : Characterization of silver nanoparticles with scanning electron microscope (SEM) was done in order to determine the structural information that the size and morphology of the silver nanoparticles. Results obtained from the analysis of SEM micrographs indicated the formation of spherical and variable size of biosynthesized silver nanoparticles. (Figure 3).

Fourier transformed infrared : The FTIR analysis was carried out in order to identify the presence of various functional groups in biomolecules responsible for the reduction of silver nitrate. The FTIR analysis result of biosynthesized indicated the absorption peak location. The FTIR signals of AGNPs were observed 3437,2924, 2852,1736,1688,1637,1460,1382,1166,1034 and 569. It confirmed the present of different functional group such as hydroxyl ,carbonyl ,amine , conjugated ketones, aldehydes responsible for the reduction of Ag^+ ions to

Ag^0 nanoparticles . The strong absorption peak at 3437 was observed caused by the presence of N-H bond for amine groups was used for stabilization of AGNPs. However, the presence of amine group characteristics proteins / enzymes is responsible for the reduction of $AgNO_3$ to Ag. (Mohamed NH, 2014) .All the functional groups present on the surface AgNPs provides stability and compressed in nano size. (Figure 4).

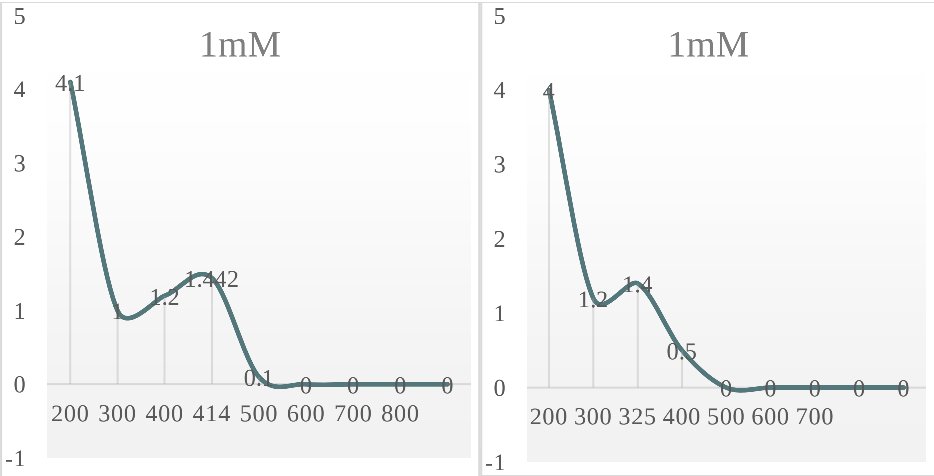

Figure 1& 2 : UV-VIS spectral analysis of biosynthesized and chemically synthesized AGNPs

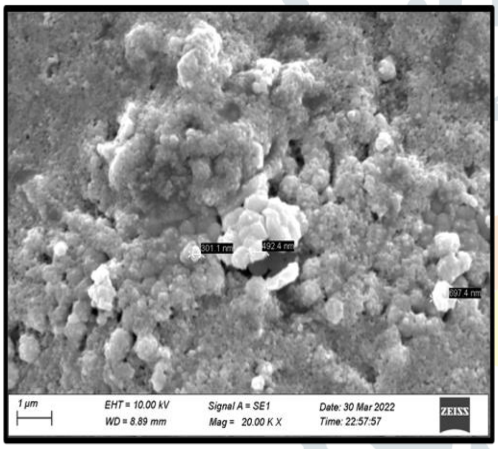

Figure 3: SEM images of biosynthesized AGNPs

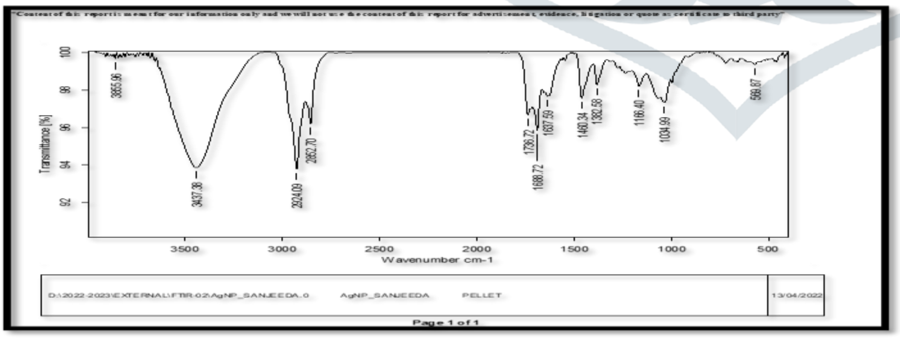

Figure 4 : FTIR analysis of biosyntheized AGNPs

4. Determination of antibacterial activity of biosythesized & chemically synthesized AgNPs :

The antibacterial activity of biosynthesized silver nanoparticles and chemically synthesized nanoparticles was analysed against gram-positive (*S.aureus)* and gram negative (*P.aeruginosa* , *K.pneumoniae*) using agar well cup method. The results confirmed that the antibacterial activity was present in both biosynthesized as well as chemically synthesized silver nanoparticles against the respective organisms i.e. are *S.aureus* and *P.aeruginosa*. By the comparing the results, it was indicated that biosynthesized silver nanoparticles exhibits greater antibacterial activity than of chemically synthesized silver nanoparticles which was confirmed through their visible zone of inhibition on Muller Hinton agar plates. (Figure 5&6). (Vahvaselka, 2008) demonstrated the AGNPs bacterial cell membrane permeability and respiration function which lead to cell death. In addition, silver nanoparticles not only attach to bacterial cell surface but also, enter inside the bacteria,which result in a disruption of adenosine triphosphate (ATP) production, dna replication ,gernartion of ROS and direct damage to cell structures. (Sahayaraj and Rajesh, 2011).

5. Determination of biofilms formation using Congo red agar method :

The selected bacterial strain *S.aureus, P.aeruginosa* and *K.pneumoniae* which was cultured on Congo red agar medium after incubation of 48 hours at 37' C was observed to determine the biofilms formation of respective micro-organisms. According to given results it was observed that S.*aureus & K. pneumoniae* can produced black colonies indicating strong biofilms formation while *P .aeruginosa* produced dark red & black colonies indicating weak biofilms formation.(Figure 7). (C.R. Arciola, 2002) established a colorimetric scale ranging from very red to very black with 6 kinds of nuances – very red , red , Bordeaux , almost black , very black and black for the classification of biofilms production of the micro -organisms. The results of the present study thereby confirmed, the production of biofilms produced by *S.aureus, P.aeruginosa & K.pneumoniae* on Congo red agar medium.

6. Biofilms eradication assay : The eradication of biofilms formation activity of biosynthesized silver nanoparticles on *S.aureus and P. aeruginosa* isolates was studied .The results obtained indicated that the biosynthesized silver nanoparticles can eradicate 70 % of biofilm formation of S.aureus isolates while it can eradicate 74% of biofilms formation of P.aeruginosa isolates .Hence, the biosynthesized silver nanoparticle was found to be effective in reducing and eradicating the biofilm formation of the respective micro-organisms. (Table 1). Limited research have been conducted on anti-biofilm activity of silver nanoparticles. (Kalishwarlal, 2010) previously reported the antiobiofilms activity of biosynthesized AGNPs against P.aeruginosa and S.epidermidis.in that study, AGNPs of 100nm in size exhibited biofilm formation by 95-98%. (Goswani S. Sahreen T, 2015) also reported on biofilms eradication of AGNPs and found out 15mg/ml of AGNPs resulted into 89% inhibition of S.aureus & 75% in E.coli. Results of the present study revealed that the silver nanoparticles synthesis from Catharanthus.roseus can effectively eradicate the biofilms formation production by the Gram- positive bacterium *S.aureus* & Gram -negative bacterium *P.aeruginosa*. Recently research has been conducted on conjugating the antibiotics rifampicin with chemically synthesized AGNPs for use in combatting biofilms formation by methicillins resistant S.aureus and K.pneumoniae. (Farooq U, 2019)

7. Analysis of antioxidant activity of Catharanthus leaves extract and biosynthesized silver nanoparticles :

The antioxidant activity of C.roseus extract and biosynthesized silver nanoparticles was determined by DDPH scavenging method. DPPH is an stable compound that accept hydrogen and electron from silver nanoparticles. The colour change of purple colour to yellow colour can quantified by its decreased absorbance at wavelength of 517nm might be due to the presence of antioxidant in the medium which adhered to explained the functional group produced by C.roseus leaf extract .Based on the results and calculations it was confirmed that the C.roseus extract and biosynthesized silver nanoparticles have 61% and 74% antioxidant activity respectively present in them . (Figure 8). Since, the mechanism of action is needed more advanced experimental proofs, AGNPs are promising as potential biological label.

Figure 5& 6 :Antimicrobial activity of biosynthesized & Chemically synthesized AGNPs

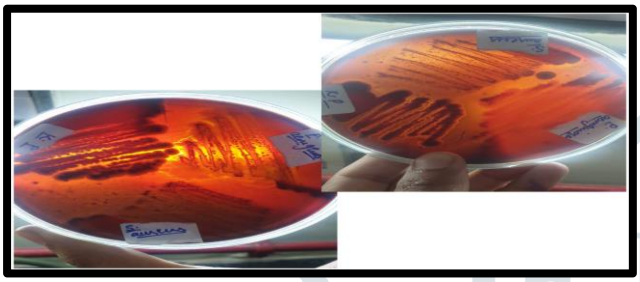

Figure 7 : Biofilms production of tested micro-organisms on Congo red agar medium.

TABLE NO 1 : Percentage of biofilms eradication of micro-organisms by biosynthesized silver nanoparticles.

SR.NO	MICRO-ORGANISMS	O.D $_{595}$ UNTREATED CONTROL	O.D$_{595}$ TRETAED WITH BIOSYNTHESIZED AgNPs	% OF BIOFILMS REDUCTION
1)	*S.aureus*	1	0.30	70%
2)	*P.aeruginosa*	0.9	0.33	74%

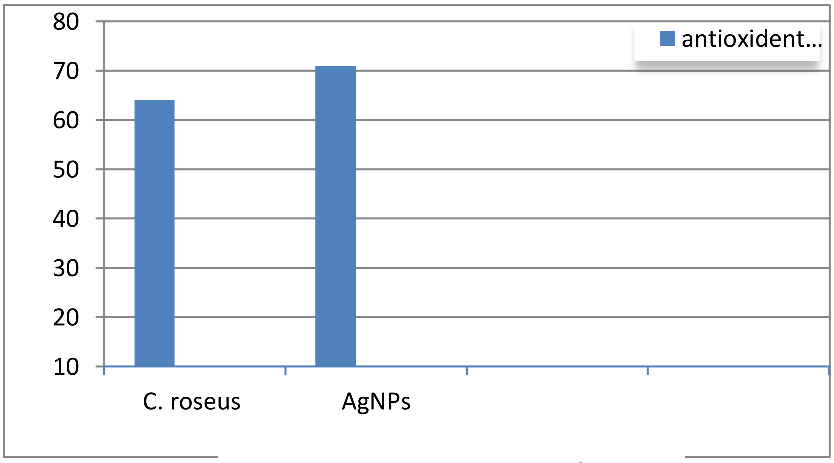

Figure 8 : Antioxidant activity of Biosynthesized Silver nanoparticles.

CONCLUSION & FUTURE SCOPE :

From this current study it is concluded that the biosynthesized silver nanoparticles obtained from Catharanthus roseus found have a good antioxidant activity, antibacterial activity against the gram –positive and gram –negative micro organisms and can also successfully eradicate the biofilm formation produced by *Staphylocccus.aureus* and *Pseudomonas aeruginosa* due to their small size and the presence of capping agent.

Hence, these biosynthesized silver nanoparticles derived from Catharanthus roseus can be used as alternative to the antibiotics and can combat to treat skin infection since it possesses a distinct antimicrobial properties against the clinical pathogens and also can play an crucial role in combating biofilms infection including their prevention , disruption and removal.

Since , this study mainly focus on treating skin infections caused by P.aeruginosa & S.aureus by silver nanoparticles obtained from Catharanthus.roseus , it could potentially have an application in the biomedical industry as a cream or ointment along with the antibiotics which can be further tested on normal cells to study its effect as anti- cancer ,wound healing therapy .

Article I. Bibliography

(A ,Nandhini R , Aruna Kartikeyan V, Bose P. (2014). Sythesis and characterization of silver nanoparticles from inulin plant. *Asian Biomedical Pharma*, 1-6.

Anand kumar keshari, A. S. (2021). antioxidant and antibacterial properties of biosynthesized silver nanoparticles . *nanomedicine research journal* , 37-44.

Appidi JR ,Grierson DS ,Afolayan AJ , . (2008). Ethanobotanical study of plants used in treatment of diarrhoea in eastern cape South Africa. *bioscience*, 1962-1963.

Beddy D, W. R. (2004). Increased vascular endothelial growth factor production in fibroblast isolated from strictures in patients with Croh's disease . *Biomedial pharmaceutical* , 72-77.

C.R. Arciola, D. C. (2002). Detection of slime production by means of an optimized Congo red agar plate test based on colourimetric scale in Staphylococcus.epidermis clinical isolates genotyped for ICA locus. *Biomaterials* , 4233-4239.

Chatterjee AK, c. R. (2014). Mechansim of antibacterial activity of copper nanoparticles . *nanotechnology* , -.

El -Sayed M , Verpoote R ,. (2005). Methylasmonate accerates catabolism of monoterpenoid indole alkaoids in catharanthus during leaf process. *Filtoterpia*, 83-90.

Elbourne et al, T. V. (2019). The use of nanomaterials for the mitigation of biofilms formation. *Methods in microbiology* , 61-92.

Farooq U, A. ,. (2019). Rifampicin conjugated silver nanoparticles : A New arena for the development of Antibiofilm potential against methicillin resistant Staphylococcus.aureus & Klebshiella.pneumoniae. *International journal of nanomedicine 14*, 3983-3993.

Fulaz, S. V. (2019). Nanoparticle-biofilms interaction: The role of the EPS Matrix . *trend microbiology* , 915-926.

Gomma SE ,Yahayu M, Dailin DJ, Enshasy HL. (2021). Antimicrobial compound from Catharanthus roseus : a review. *international journal science and technology RES*, 113-121.

Goswani S. Sahreen T, S. M. (2015). role of biogenic silver in disruption of cell -cell interaction in S.aureus & E.coli. *chemical engineering*, 73-80.

Halliwell. (1992). Reactive oxygen species and the central nervous system. *journal of neurochemistry*, 1602-1623.

Hamed Abu Sara, Z. E. (2019). " Smart triiodine compound: halogen bonding has inflence on antimicrobial activities . *pathogens*, -.

IP.el al, L. V. (2006). Antimicrobial activities of silver dressing : an invitro comparsion. *Medical microbiology* , 59-60.

Jain et al , Kumar daima , S.Kachhwaha ,S.l.kothari . (2009). sythesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Nanomaterial and biostructures*, 557-563.

Jeong Su Choi, H. C. (2021). silver nanoparticles for surface plasmon resonance in oragnic optoelectrics. *Particle & particle sysyem characterization* , 164-175.

Kalishwarlal, K. B. (2010). Silver nanoparticles impede the biofilm formation by P.aeruginosa and Staphylococcus,epidermidis. *Colloids surface.Biointerfaces 79*, 340-344.

Koo, H. A. (2017). targeting microbial biofilms : current & propective therapeutic strategies. *Nature.Rev. microbiol*, 740-755.

Liu, S. I. (2019). Nanotechnology based on antimicrobial and delivery system of biofilm infection control. *CHEM.SOC*, 428-446.

M.Rittner, T. (1998). Nanostructural materials ; an overview and commericial analysis. *Minerals, Metals matter ,sociology*, 428-446.

Mathur P, J. S. (2018). Pharmaceutical aspect of silver nanoparticles . *cell nanomedicine ,biotechnology* , 115-126.

Mohamed NH, I. M.-M. (2014). Antimicrobial activity of latex silver nanoaprticles using Calotropics procera . *Asian pacific journal tropical biomedicine* , 876-883.

Mohanty S, M. S. (2017). An investigation on the antibacterial , cytotoxic and antibiofilm efficiency of starch stabliziler silver nanoparticles . *Nanomedicine and nanotechnology* , 916-924.

N.p singh, M. r. (2017). changing trends in antimicrobial susceptibility pattern of bacterial isolates in burn unit. 1083-1087.

Nayak D, M. ,. (2015). Synergitics combination of antioxidant of silver nanoparticles and chitosan based formulation. *Colloid interface science, 457*, 142-152.

Parakh MP Swati S, T. T. (2019). Catharanthus roseus : A review. *Acta scientific pharmaceutical*, -.

Poonarululselvam S, P. C. (2012). Sythesis of silver naoparticles using leaves of catharanthus roseus Linn.G.donn and their antiplasmodium activities . *Asian pacific J tropical medical* , 574-580.

Roy tiwari, M. ,. (2018). Strategies for combating biofilms : A focus on antibiofilm agent and their mechanism . *virulence* , 522-554.

Sahayaraj and Rajesh. (2011). bionanoparticles : synthesis and antimicrobial applications. *biology* , 228-244.

SC De la Fuente –Nunez, M. e. (2014). A broad spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. *antimicrobial agents Chemotherapy* , 5363-5371.

Shankar et. al. (2004). biosynthesis of silver and gold nanoparticles from extract of different geranium plant . *nature matter*, 482-488.

Singh P et al. (2018). Anti biofilms effect of gold and silver synthesized by Rhodiola rosea rhizome extract . *cell nanomed biotechnology* ,, 886-896.

Song AND Kim. (2008). Rapid biological sythesis of silver nanoparticles using leaf extract . *Bioprocessing and Biosystem engineering* , 79-84.

V.E Kangan, H. B. (2005). Nanomedicine and nanotoxicology : a two sides of same coin. *nanomedicine: nanotechnology*, 313-316.

Vahvaselka, Y. a. (2008). The formation and charcterization of ultra thin films containing Ag nanoparticles. *Journal of materails and chemistry* , 141-248.

Zaheer, Z. &. (2012). Silver nanoparticles to self assembled films: green synthesis and characterization. *Colloids and surfaces B : Biointerfaces 90*, 48-52.