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RESEARCH ARTICLE

GREEN SYNTHESIS AND BACTERICIDAL ACTIVITY OF SILVER NANOPARTICLES USING TRYPTOPHAN WITH MICROWAVE ASSISTED METHOD.

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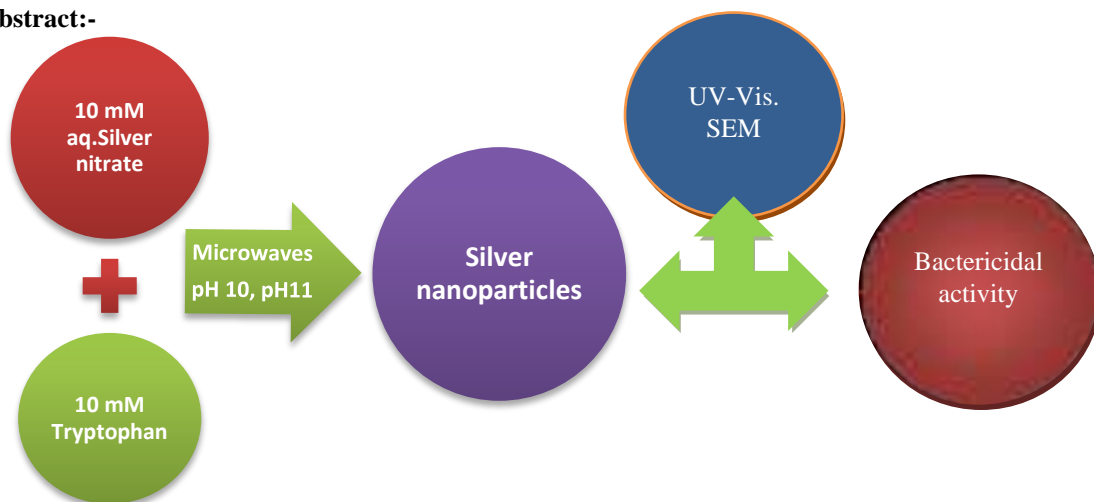
Abstract

Colloidal silver nanoparticles are utilized in various high end technology applications and in efficient drug delivery. The silver nanoparticles were synthesized using alkaline 1mM tryptophan solution under microwave irradiation. The green method yields approximately 16 - 21 nm silver nanoparticles of uniform morphology. Synthesis of colloidal silver nanoparticles was monitored by UV-Visible spectroscopy by taking UV spectra in between 300 to 600 nm and Scanning Electron Microscopy (SEM). The efficiency of synthesized silver nanoparticles was studied by agar well diffusion method against standard culture of *Escherichia coli* and *Staphylococcus aureus*.

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Introduction:-

Graphical Abstract:-



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Background:-

The emergence of nanotechnology, a multidisciplinary field has influenced all branches of science forming an impact on biological sciences. Nanoparticles synthesized using various metals has become an important field of study. Silver nanoparticles have become an important discovery in the modern world as these particles have proved to carry catalytic, polarizing and conductive properties as well as their antimicrobial abilities¹. Colloidal silver nanoparticles being utilized for centuries in various fields. Currently those are utilized in various high end technology applications like organic photovoltaics, sensory probes, therapeutic agents, drug delivery in biology². It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganism. This aspect of silver makes it an excellent choice for multiple roles in the medical field.

Various methods of synthesis of silver nanoparticles have been reported by researchers using chemical reducing agents such as polymers, dendrimers, hydrogels etc. The use of a capping agent such as synthetic polymers, proteins, natural macromolecules like starch or amino acids like tryptophan could be an alternative which is safe and without any toxic effects³. These capping agents reduce the oxidative stress as well as stabilize the nanoparticles⁴. The use of microwaves for the production of silver nanoparticles is a very promising method⁵. In the microwave frequency between 300MHz to 300GHz, polar molecules like water try to orient with the electric field while the dipolar molecules reorient with the alternate electric field, with loss of energy⁶.

The spectrum of applications of silver nanoparticles is vast, spreading from physical sciences, chemical sciences as well as biological sciences. Silver is non toxic to living animal cells yet exhibits antibacterial effects. This ability is extensively used by researchers to produce silver based antimicrobial drugs. The nanoparticles of noble metals exhibit a high surface to volume ratios, making it a cost effective option⁷. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death⁸.

The present study aims at synthesis of silver nanoparticles using microwaves⁹ with tryptophan as a reducing and capping agent. The synthesis was carried out at two different pH values. The synthesized silver nanoparticles were analyzed using UV-Visible spectrophotometer and their morphological study was carried out using SEM images. The bactericidal action was studied using clinical isolates of *Escherichia coli* and *Staphylococcus aureus*.

Methods:-

Preparation of Silver nanoparticles:-

Analytical grade Silver Nitrate tryptophan and Sodium hydroxide were used for the preparation of nanoparticles. Aqueous Silver Nitrate (10mM) was used as the precursor for synthesizing silver nanoparticles. 10mM tryptophan solution was used as the reducing agent as well as capping agent. 0.1 N Sodium hydroxide solution was used to adjust the pH of the solution.

The metal precursor (AgNO_3) solution was taken in a clean tube (Solution A). pH of tryptophan solution was adjusted to pH 10 and pH 11 to give a reducing environment (Solution B). Solution A and Solution B at pH 10 were mixed in 1:1 proportion in another tube. The tube was exposed to microwaves at 800W of power for 10 seconds. The colour change was noted to confirm the formation of silver nanoparticles. The process was repeated for the other tube of tryptophan (pH 11).

Purification of silver nanoparticles:-

The nanoparticle containing solutions were transferred into a clean centrifuge tube and the solution was centrifuged at 13,000 r.p.m for 15 minutes. The supernatant was discarded and the pellet was suspended in absolute alcohol. The suspension was centrifuged again for 20 minutes and the pellet was resuspended in double distilled de-ionized water.

Characterization of silver nanoparticles:-**UV-VIS Spectroscopic study of silver nanoparticles:-**

The synthesis of silver nanoparticles in aqueous solution were monitored by sampling of aliquots (0.2 ml) from the suspension. The aliquots were diluted with 2 ml deionized water and UV-visible spectra of the resulting diluents were obtained. The UV-VIS spectroscopic analysis of silver nanoparticles was performed between 300-600 nm and a graph (wavelength v/s absorbance) was plotted based on the readings

Scanning Electron Microscopy studies of silver nanoparticles:-

The further characterization of the silver nanoparticles was accomplished using the scanning electron microscopy (SEM) imaging. A very small quantity of synthesized nanoparticles were dispersed in a small volume of absolute alcohol and the suspension was sonicated to break up agglomerates. A small drop of this solution was placed on was then placed on a Silica semiconductor plate using a micro-pipette and the solvent was evaporated as quickly as possible. This sample was then used for SEM imaging to measure the size of silver nanoparticles.

Study of antibacterial action of silver nanoparticles:-

Anti-bacterial activity of silver nanoparticles was determined by agar well diffusion method against clinical isolates of Gram negative bacteria - *Escherichia coli* and Gram positive bacteria - *Staphylococcus aureus*.

Preparation of inoculums:-

The inoculums for each bacterial culture (10^8 cfu/ml) was prepared from broth cultures grown in sterile Mueller-Hinton broth at 37°C for 18 hours

100µl of suspension of Silver nanoparticles:distilled water in the proportions of 1:3, 1:1 and 3:1 were added in each well with sterile distilled water as negative control and Ciprofloxacin (50 µg/ml) as the positive control. The plates were incubated at 37° C for 24hrs for bacterial cultures and the zone of inhibition was measured. Clear inhibition zones around the wells indicated the presence of antimicrobial activity. All data on antimicrobial activity were obtained as average of triplicate observations.

Results:-

Metallic nanoparticles are synthesized using various methods with a variety of capping and stabilizing agents. The synthesis of silver nanoparticles using tryptophan as a green stabilizing agent was successfully carried out at pH 10 and pH 11.

The particles were characterized using UV visible spectrophotometer as well as scanning electron microscope. The formation of nanoparticles was confirmed by the change in the colour of silver nitrate solution after the microwave exposure in both the solutions of tryptophan. The colour of the solution was colourless which changed to brown with the formation of silver nanoparticles.

Characterization of silver nanoparticles:-**UV-Visible spectrum of silver nanoparticles:-**

The nanoparticles showed increase in absorption as the wavelengths increased from 300nm to 400nm. The nanoparticles synthesized had absorption maxima at 420nm confirming the formation of silver nanoparticles. The absorption spectrum showed a steep decrease as the wavelengths longer than 450nm. . The peak was symmetrical and there were no other maxima with any other wavelength between the range of wavelengths studied.

Scanning Electron Microscopy studies of silver nanoparticles:-

The size and shape of nanoparticles were studied using bright field scanning electron microscope. The particles were round in shape with a few agglomerer which indicated a possible sedimentation of the particles with time. The size of newly synthesized nanoparticles was found to be between 16nm to 21nm (Figure 1).

Study of antibacterial action of silver nanoparticles:-

Silver is known to be effective against microorganisms. The nanoparticles synthesized in the present study were observed to be effective against the clinical isolates of a representative Gram positive and Gram negative bacterial strain, *E.coli* and *S.aureus* respectively.

The antibacterial activity of silver nanoparticles synthesized using microwaves and 10mM tryptophan at pH 10 and pH 11 was studied using agar well diffusion method. The nanoparticles synthesized at pH 10 were found to be more

effective against *S.aureus* while the particles synthesized using tryptophan pH 11 were more effective against *E.coli*. Among the three dilutions used, the 3:1 dilution showed highest inhibition among all.

As stated in Table 1, the zone of inhibition for *E.coli* was observed to be 12, 14 and 25mm for tryptophan pH 10 and 15, 15 and 16mm and for pH 11 for 1:3, 1:1 and 3:1 dilutions respectively. The zone of inhibition for *S.aureus* at pH 10 was 25, 27 and 36mm which reduced to 20, 24 and 26mm as the pH increased to 11 for the same dilutions used (Graph 1 and 2, Figure 2).

The positive control showed a zone of inhibition of 16mm for *E.coli* and 14 mm for *S. aureus*. The negative control showed no inhibition for both the test organisms. It is proved that the ability to inhibit bacterial cells by silver nanoparticles is due to the silver cations released from silver nanoparticles that lead to changes in the membrane structure of bacteria. The destabilized membrane increases membrane permeability of the bacteria, denaturation of cellular proteins thus inhibiting their growth. According to Muthukumar et al, (2014)¹⁵, silver has a greater affinity to react with sulfur- or phosphorus-containing biomolecules in the cell. Thus, sulfur-containing proteins in the membrane and phosphorus containing elements like DNA are likely to be the preferential sites for silver nanoparticles binding.

Discussions:-

The effect of pH on synthesis of nanoparticles was studied by Mukha et al (2016)⁹ as well as Agnihotri et al, (2013)¹⁰. The absorption spectrum of the particles showed a maxima at 420nm (Graph 1). Similar observations were reported by Haris et al, (2017)¹¹ and Zaheer et al, (2010)¹² with silver nanoparticles showing maxima at 415nm and 425nm respectively. SEM images.

Several researchers have studied the bactericidal action of metallic nanoparticles, specifically silver particles synthesized using plant extracts. Krishnamoorthy and Jayalakshmi (2012)¹³. have reported the silver nanoparticles to be effective against *S. aureus*, *Proteus* sp, *Salmonella* sp. as well as *Bacillus*. Praba et al. (2015)¹⁴ have reported bactericidal action of silver nanoparticles against *E.coli*, *S. aureus*, *Klebsiella pneumoniae*. Silver nanoparticles synthesized using *Ocimum* extract were found to be effective against *Pseudomonas aeruginosa*^{15,16}. The nanoparticles synthesized using *Parthenium* leaf extract were reported to be effective against *Salmonella typhi*¹⁷.

Rapid green synthesis of silver nanoparticles could be carried out successfully using tryptophan as the stabilizing agent. The particles thus synthesized were characterized using UV-Visible spectrophotometer and Scanning Electron Microscope. The nanoparticles were found to be effective against the selected clinical strains of bacteria which proves their potential for the commercial use in medicine in future. Since the method used in the present study is rapid, without the use of any harmful stabilizing agents, it could be easily adopted for further synthesis of metallic nanoparticles.

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