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A STUDY ON PHYLLANTHUS RETICULATUS GREEN SYNTHESISED SILVER NANOPARTICLES AGAINST ENTEROBACTERIACEA ISOLATED FROM CLINICAL SAMPLES.

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ABSTRACT

Nanoparticles are now a days increasingly used to target bacteria, as an alternative to antibiotics, where the particles extend in nanosciences and turn out to be more responsive and to overcome challenge posed by multidrug resistance in bacteria. Green synthesis of nanoparticles aims at minimizing generated waste and implementing sustainable processes at minimal cost. In this study the aqueous extract of Phyllanthus reticulatus plant leaves was used for the synthesis of silver nanoparticles. Synthesised silver nanoparticles were analysed by U.V Vis spectrophotometry and SEM. It is observed that synthesized nanoparticles are spherical and regular in shape having 260nm in size. Antimicrobial assay was done with E.coli, Klebsiella pneumonia, Shigella sp and salmonella typhi. Minimum inhibitory concentration was determined and noted.It is highly effective against Shigella sp and E coli ,and less effective against Salmonella typhi.

KEY WORDS: Phyllanthus reticulatus ,Greensynthesis Nanoparticles, SEM,UV.Vis spectrophotometry, Antimicrobial assay,MIC.

INTRODUCTION

Frequent use of antibiotics results in resistance of pathogens against them. This is the health and the life-threatening reality. It is therefore necessary to look for new sources of effective potent drugs. Nature is an inexhaustible source of health-promoting substances. Combination of knowledge in natural medicine with modern technology leads to the discovery of new drugs. One of the most promising sources in recent years has been shown to be plant extracts, which are rich in antioxidant and antimicrobial compounds that have been used as a nanoparticle synthesis agent .Green synthesis of nanoparticles while comparing to chemical synthesis is cost effective and ecofriendly and this demands the further studies on this techniques and commercial implementation of nanoparticles as an alternative to antibiotics.

Nanotechnology and nanoscience have been established recently as an interdisciplinary subject dealing with biology, chemistry, physics, and engineering. The term “nano” is derived from the Greek word dwarf in the meaning of extremely small. Unique biological, chemical, and physical properties of silver nanoparticles (AgNPs) lead to the wide range of applications in spectroscopy, sensors, electronics, catalysis, and pharmaceutical purposes . It is well known that silver has an inhibitory effect toward many bacterial strains . Here we used Phyllanthus reticulatus plant leaves extract for the green synthesis of silver nanoparticles.

Phyllanthus reticulatus plant is a common weed seen all over the world. It is included in the family Phyllanthaceae.This plant varies in habit .Phyllanthus reticulatus is a shrub, sometimes partially scrambling and usually only up to 5m high,with light reddish-brown or grey-brown with hairy stems when young,which become smooth with age.

The various parts of the Phyllanthus reticulatus plant is traditionally used as medicine for many diseases. Leaves are highly active against gram positive and gram negative bacteria, and are effective against candida sp. Leaves are used as diabetic and cooling medicine.It is reported to be leaves used as diuretic, anti inflammatory, alternative attentuant, astrigent and as antidiarrhoel.

Chemical components of phyllanthus reticulatus leaves were identified as lupeol, lupeolacetate, stigmasterol, scopoleten, tannic acid, friedelin,epifriedelinol, betulin, taraxerone, beta-sitosterol, glochidonol, octacosanol, taraxerylacetate , 21-alpha-hydroxyfriedelan-3-1 betulinic acid etc.

This study deals with the possibilities of the synthesis of AgNPs with respect to green synthesis of Phyllanthus reticulatus plant leaves, analysis and characterisation of the synthesised nanoparticles, as well as their antimicrobial activity against clinically isolated Enterobacteriacea species and determined their Minimum inhibitory concentration.

MATERIALS AND METHODS

SAMPLE COLLECTION AND EXTRACTION

Collect the plant samples and clean it by washing several times with running tap water and subsequently with distilled water. Dry the sample at room temperature in shade. Grind the sample to yield coarse powder. Take 10gm of dried sample in a 250ml beaker along with 100ml of double distilled water. Boil it for 15 min. Cool the aqueous extract to room temperature, filtered the extract using Whatsmann No.1

SYNTHESIS OF SILVER NANOPARTICLES

Prepare 1mM of silver nitrate solution. Take 45ml 1mM of silver nitrate solution in an Erlenmeyer flask and add 5ml of plant extract for bio reduction process at room temperature. Brown-yellow solution will form, indicating the formation of silver nanoparticles.

CHARACTERISATION OF SYNTHESISED NANOPARTICLES

In the present work the synthesized nanoparticle were characterized by 2 methods, namely

- UV-Visible spectroscopy
- Scanning electron microscopy(SEM)

UV-VIS spectrophotometer

The Silver nanoparticles were characterized in a UV-VIS spectrophotometer to know the kinetic behaviour of nanoparticles at a scanning range of 300-600 nm.Thespectrophotometer (Systronics UV-Visible Spectrophotometer 119) was equipped with a software to record and analyze data. UV visible spectrum of preparednanoparticle is analysed and the peak is compared with standards

SEM analysis

The SEM analysis of nanoparticles was done in JOEL JSM-6480 LV SEM machine at SAIF,Cochin University of Science And Technology (CUSAT),Kochi .

ANTIMICROBIAL ASSAY

Method: Agar well diffusion method

Test Organisms : *E.coli, Klebsiella pneumoniae, Shigella sp, Salmonella typhi*

Procedure:

15-20 mL of Mueller-Hinton agar was poured on glass petri plates of same size and allowed to solidify. Standardized inoculum of the test organism was uniformly spread on the surface of these plates using sterile cotton swab. Five wells with a diameter of 8 mm(20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. One well was loaded with plant extract and another one with silver nitrate and the other one loaded with Chloramphenicol as positive control. Remaining wells were loaded with compound solution of desired concentration (10mg/50mg) .Then,the agar plates were incubated under 37 degree celsius for 24 hours.After incubation,clear zone was observed.Inhibition of the bacterial growth was measured in mm.

MINIMUM INHIBITORY CONCENTRATION (MIC)

Preparation of Bacterial Culture

Using aseptic techniques a single colony was picked from each plates of different organism (*Salmonella typhi, E.coli, Shigella sp and Klebsiella pneumoniae*) was inoculated into 5ml nutrient broth and incubated for 37˚C .

Preparation of Resazurin solution(0.02%)

0.002g of Resazurin salt powder is dissolved in 10ml of distilled water and vortexed. The mixture was filtered by millipore membrane filter (0.2µm). The Resazurin solution can be kept at 4˚C for 2 weeks.

Preparation of the plates

Plates were prepared under aseptic conditions. A sterile 96 well plate was labelled. A volume of 100µl of test material in 10% sterile water was pipetted in descending concentrations (100µl,80µl,60µl,40µl,20µl) into the plates. Broth(50µl), indicator(Resazurin dye)(10µl) and bacteria (10µl) was added to the respective wells. Three controls C1,C2,C3 were kept. In C1 test material was serially diluted along with broth, saline and indicator as per above mentioned concentrations were added without any bacteria. In C2 bacteria, broth and indicator was added without drug. In C3 chloramphenicol was serially diluted along with broth,indicator and bacteria.

RESULTS

In this study on mixing the plant extract with silver nitrate and kept at room temperature brown- yellow color was observed

Figure 1 : Brown- yellow solution indicating the formation of Silver nanoparticles.

CHARACTERIZATION OF SYNTHESIZED NANOPARTICLE

UV -Vis spectral analysis

The spectrophotometric analysis of silver nanoparticle has done in a range of 300-600nm. This gives the maximum absorbance range of synthesized silver nanoparticle and it is estimated to be 380nm

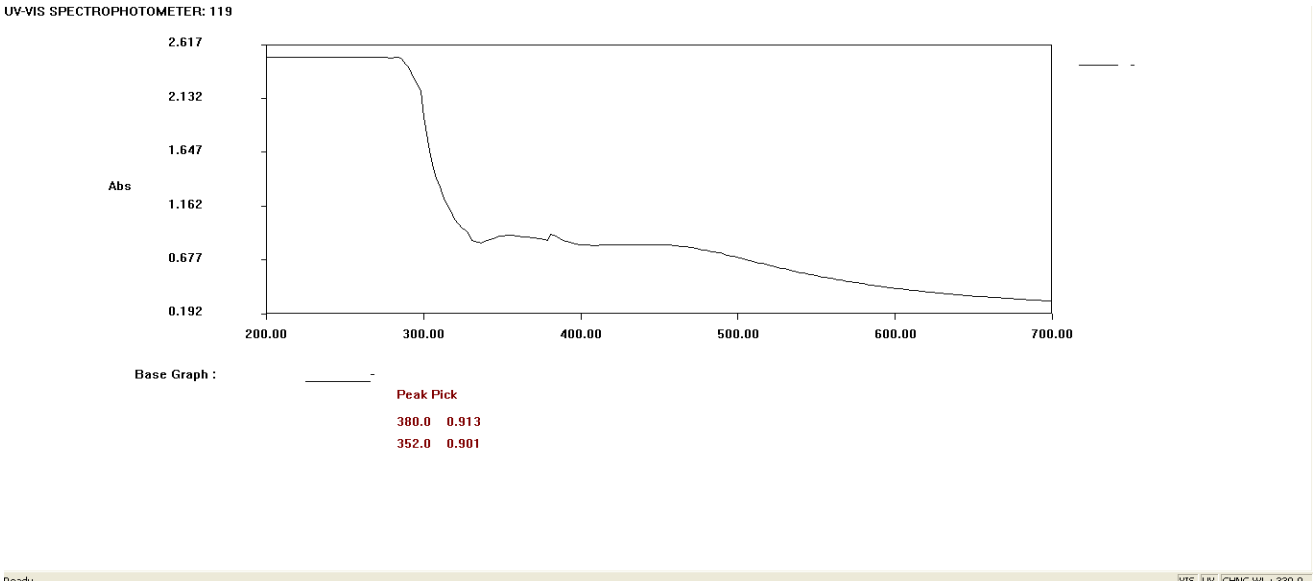
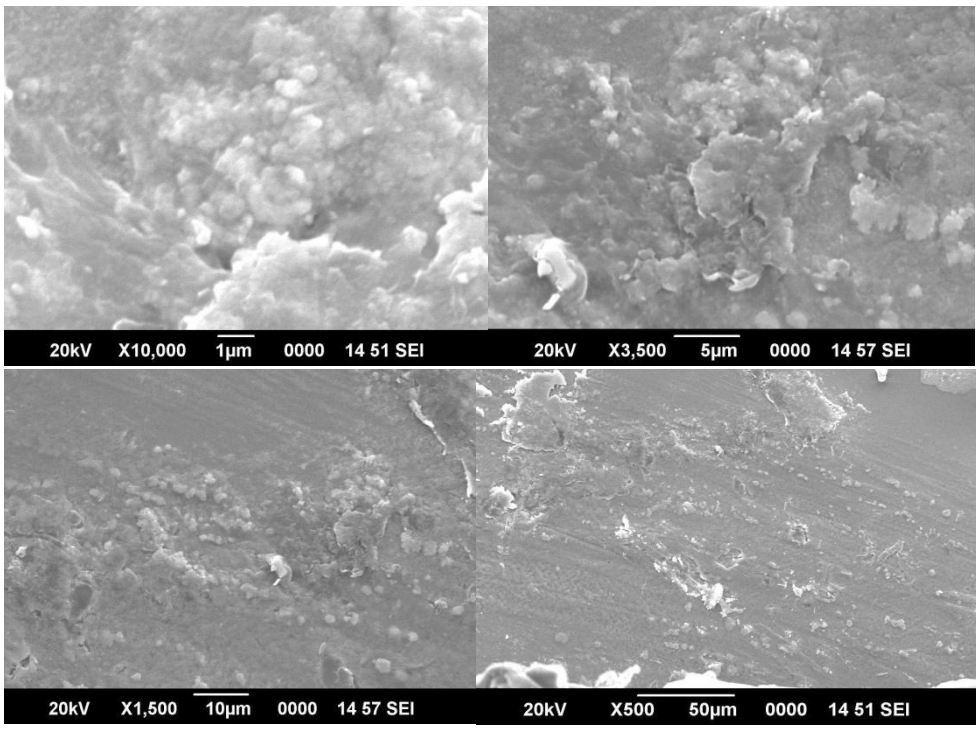


Figure 2: UV spectra of synthesized Silver nanoparticles

In the UV spectra of prepared silver nanoparticles, a peak at 380 nm was observed which indicate the formation of silver nanoparticle and there is only one peak at the spectrum which indicates that the prepared silver nanoparticle is pure.

SEM ANALYSIS

In this work the prepared silver nanoparticles morphology was studied by using SEM. Figure shows the SEM micrographs of silver nanoparticles. From the figure 4, it is observed that silver nanoparticles having spherical morphology and regular in shape having 260 nm particles.



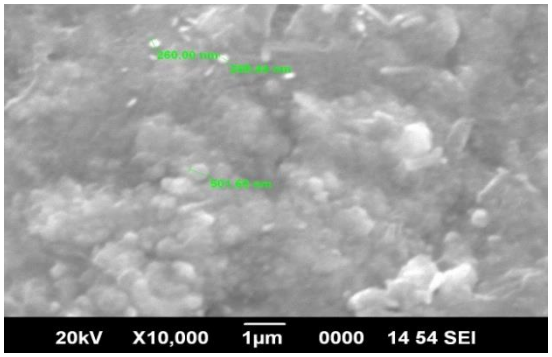


Figure 3 : SEM image of silver nanoparticles synthesised

WELL DIFFUSION METHOD

Antimicrobial activity of synthesized silver nanoparticles

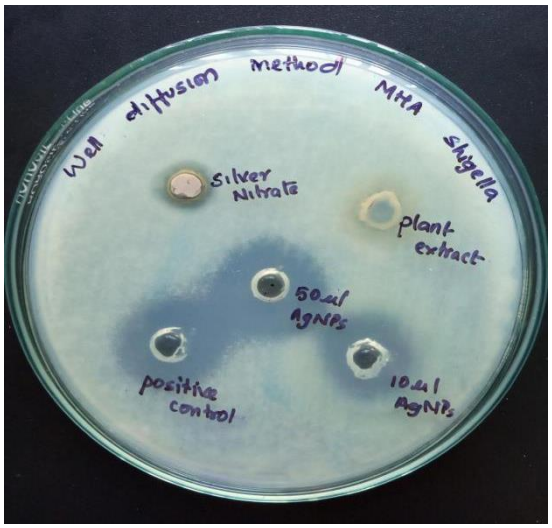


Figure 4 (a) Antimicrobial activity against *Shigella sp*

(b) Antimicrobial activity against *E.coli*

The bacteria inoculated into the agar plates using cotton swab. Well were made in the inoculated medium and 10 and 50µl of silver nanoparticle were dispensed in the respective wells.In one well 50µl of silver nitrate is added, another well 50µl of plant extract were added and positive control(chloramphenicol) was added in another well. After incubation the result were noted. *Shigella sp*

and *E.coli* incubated plate, there was a zone of clearance near the wells filled with both 50 and 100µl of silver nanoparticle. But in *Klebsiella pneumoniae* incubated plate, the zone of clearance was seen only at the well filled with 50µl silver nanoparticle and in *Salmonella typhi* incubated plate, the slight zone of clearance was observed for 50µl silvernanoparticle.

MINIMUM INHIBITORY CONCENTRATION (MIC)

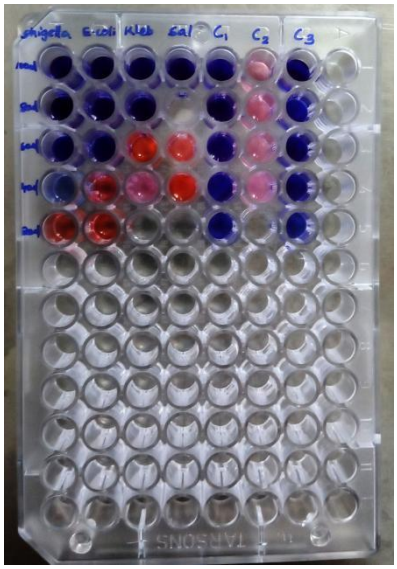


Figure 5 : MIC by broth dilution using Resazurin dye

Plates after 24hr in modified resazurin assay[pink colour indicates growth and blue means inhibition of growth; the test organisms are *Shigella sp, E.coli , Klebisella pneumoniae, and Salmonella typhi*.(A to D, test compound in serial dilution in wells (100,80,60,40,20µl) +broth+indicator+bacteria) C1(test compound in serial dilution + broth + saline + indicator, no bacteria) C2 (control without drug, bacteria + broth + indicator) C3 positive control (chloramphenicol in serial dilution + broth+indicator + bacteria)

SUMMARY

Silver nanoparticles could be synthesised from *phyllanthus reticulatus* plant leaves. It is observed that synthesized nanoparticles are spherical and regular in shape having 260nm in size. This method is cost effective and ecofreindly. Synthesised silver nanoparticles shows very good anti microbial activity against the selected members of *Enterobacteriacea.* Further invitro, insilico, animal tests are needed to confirm its usage as an alternative to antibiotics

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