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ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM *BOERHAAVIA DIFFUSA* LINN.

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ABSTRACT

The present study focused the synthesis, characterization and multifacet properties of silver nanoparticles obtained from *Boerhaavia diffusa* Linn. Silver nanoparticles were subjected to UV- Spectrophotometry, SEM, EDAX, FTIR and XRD confine nanoparticles synthesis.DPPH assay and Hydrogen Peroxide assay were used to analyse its antioxidant property. Finally the anticancer activity was carried out using Human hepatic carcinoma cell lines (HepG2). The silver Nanoparticles obtained from the leaf extract of *Boerhaavia diffusa* had a great antimicrobial activity against pathogenic strains of bacteria and fungi, exhibited fine free radical scavenging activity and showed superior anticancer activity against Human hepatic carcinoma cell lines (HepG2).

KEYWORDS: Nanotechnology, *Boerhaavia diffusa*, UV- Spectrophotometry, FTIR, antimicrobial, antioxidant, anticancer.

INTRODUCTION

Medicinal plants are important to the global economy, as approximately 85% of traditional medicine preparations involve the use of plants or plant extracts.[1] Plants are an important source of medicines and play a key role in world health.[2] A new branch of nanotechnology is nanobiotechnology. Plants are important source of natural products used in pharmaceutical industries. Increase in demand for natural medicines has resulted in the production of these products by alternative approaches such as biotechnological approaches especially plant tissue Cultures. Almost 80% of the world population depends on traditional medicine which predominantly based on plant material. Medicinal plants are relied upon by 80% world's population and in India; the use of medicinal plants as therapeutic agents remains an important component of the traditional system.[3] It is a multidisciplinary field involving research and development of technology in different fields of science like biotechnology, nanotechnology, physics, chemistry and material science. It deals with biofebrication of nano-objects of bi-functional macromolecules usable as tools to construct or manipulate nano-objects.[4] Silver nanoparticles have proved to be most effective because of its good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms.[5, 6] They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries, for water treatment, sunscreen lotions etc.[4, 7]

BOERHAAVIA DIFFUSA

Boerhaavia diffusa Linn. is an herbaceous member of the family Nyctaginaceae. The major active principle present in the roots is alkaloid and is known as punarnavine. In the traditional system of medicine, *B. diffusa* roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, and abdominal pain and as an antistress agent.[8] The worldwide use of *B. diffusa* roots to treat liver disorders was validated when researchers demonstrated, in 1980 and 1991, that its root extract had anti-hepatotoxic properties. Pharmacological studies have demonstrated that *B. diffusa* possesses anti-diuretic, anti-inflammatory, antifibrinolytic, anticonvulsant and antibacterial properties, which makes it a very useful medicinal plant.[9]

Figure 1: Photograph of *Boerhaavia diffusa.*

The genus *Boerhaavia* has several species and is distributed in the tropical, subtropical and temperate regions of the world.[10] The aqueous extracts of roots are also a rich source of a basic protein, known as systemic resistance inducing protein (BD-SRIP). The aqueous solution of this protein, when applied before virus infection/inoculation, induces strong systemic resistance in several susceptible plants against commonly occurring viruses.[11]

MATERIAL AND METHODS

Collection of Plants

Boerhaavia diffusa plant was collected from Vemboor of Tuticorin District, Tamilnadu.

PREPARATION OF LEAF EXTRACT

Fresh and healthy leaves were collected and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted foreign particles, cut into small pieces and dried at room temperature. About 10 g of these finely incised leaves of each plant type were weighed and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min. The extracts were then filtered through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments.

Synthesis of Silver Nanoparticles

10ml of leaf extract was added into 90 ml of aqueous solution of 1Mm silver nitrate ($AgNo_3$) for the reduction of silver nitrate into Ag^+ ions and kept in dark room at 37^o C for 24 hours. After 24 hours, color of the solution changed from green to dark brown indicating the formation of silver nanoparticles. The bioreduced silver nanoparticles solution was measured using UV- Visible absorbance.

Purification of Silver Nanoparticles

The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 7000rpm for 20minutes. It is followed by redispersion of the pellet in deionized water to get rid of any uncoordinated biological molecules.

CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES

UV- visible spectrophotometry

Initial characterization of silver nanoparticles was carried out using UV-Visible spectroscopy. Change in color was visually observed in the silver nitrate solution incubated with leaf extract of *Boerhaavia diffusa.* The bioreduction of precursor silver ions was monitored by sampling of aliquots (Silver nanoparticles diluted with distilled water) at different time intervals. Absorption measurements were carried out on UV-Visible Spectrophotometer at a resolution 1nm between 200 and 800nm. Distilled water used as a blank. The spectrum was recorded then plotted.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of synthesized and stabilized silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min and the sample was analyzed for morphology and size of the silver nanoparticles.

Energy Dispersive X-ray Spectroscopy (EDAX)

Boerhaavia diffusa leaf extract reduced silver solution was dried; drop coated on to carbon film and tested using Hitachi S-4500 SEM instrument equipped with a Thermo EDAX attachments. Energy dispersive X-ray spectroscopy (EDAX) analysis for the conformation of elemental silver was carried out for the detection of elemental silver. EDAX was sample composition of the analyzed for the sample composition of synthesized nanoparticles.

Fourier Transfrom Infrared Spectroscopy (FTIR)

For FTIR measurements, the Ag nanoparticle solution was centrifuged at 10000 rpm for 30min. The pellet was washed three times with 20ml de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed.

XRD Analysis

A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks, using the Debye-Scherrer formula,

$$D=0.94\lambda/ \beta \cos\theta$$

Where, D is the average crystallite domain perpendicular to the reflecting planes, λ is the X-ray wave length and β

is the full width at half maximum and θ is the diffraction angle.

Antimicrobial Activity Assay

Bacterial and fungicidal activity was using standard agar well diffusion method against human pathogenic bacteria (*Corynebacterium glutamicum, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Pseudomonas aeruginosa*) and fungi (*Aspergillus japonicas, Penicillium citrinum, Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus*).

Procedure

Nutrient agar (NA) and Potato Dextrose Agar (PDA) were prepared for cultivation of the bacteria and fungi respectively. Approximately 20ml of molten and cooled media was poured in sterilized petridishes. The plates were left overnight at room temperature to check for any contamination to appear. Then under an aseptic condition, placed a sterile swab into the broth culture of a fresh overnight grown cultures of the bacteria and fungi then gently removed the excess liquid by gently pressing or rotating the swab against the inside of the tube and spread it on nutrient agar and potato dextrose agar containing petri plates respectively. With a help of sterile borer, 5mm wells punched in the solid agar medium and then different concentration of the solution (50µl, 100µl, 150µl) containing nanoparticles, 1mM silver nitrate was inoculated in these wells and the plates were incubated at 37^0C for 12-24 hours and 2 or 3 days for bacteria and fungi respectively. Further, the plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells surrounding bacteria and fungi growth. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

ANTIBACTERIAL ACTIVITY OF PUS AMPLE AGAINST SILVER NANOPARTICLES

Collection of pus samples

A pus sample was collected from diabetic wound of patients at government hospital, Sivakasi. Wound samples were collected using sterile cotton swabs. The pus specimen was inoculated on blood and MacConkey agar plates. The streaked plates were incubated at 37^0C for 24 hours.

Preparation of bacterial strains inoculums

The bacterial strains of pus samples were inoculated into the inoculums were prepared in 50ml of nutrient broth and the inoculums were incubated at 37°C for 24 hours.

Procedure[12]

Antibacterial assay was carried out by disc diffusion technique. The plant extracts and synthesized nanoparticles were tested against pus samples using the Mueller Hinton agar. Medium was prepared, pH was adjusted and poured 20 ml per plate. After solidification, the pus sample culture was swabbed on the plates. Then sterile discs were loaded with the plant extract and nanoparticles (100µl). The plates were incubated for 24 hrs. After incubation period the zone of inhibition was measured.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay[13]

The antioxidant activity of synthesized nanoparticles was determined on the basis of their scavenging activity of the stable 1, 1- diphenyl-2-picryl hydrazyl (DPPH) free radical. The sample and ascorbic acid were mixed with 95% ethanol to prepare the stock solution (5mg/ml). Here ascorbic acid was taken as standard.[14]

Procedure

At first, 5 tubes were taken to make aliquots of 5 concentrations (20-100µl) with the samples. DPHH was weighed and dissolved in ethanol to make 0.004% (w/v) solution and to dissolve homogeneously magnetic stirrer was used. After making the desired concentrations 3 ml 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 minutes to complete the reactions. DPPH was also applied on the blank test tubes the same where only ethanol was taken as blank. After 30minutes, the absorbance of each test tube was taken by a UV spectrometer at 517 nm.

DPPH radical scavenging activity (%) = [(control absorbance)-(sample absorbance)]/ [control absorbance]*100

Hydrogen Peroxide Assay

The antioxidant of the synthesized nanoparticles was determined on the basis of their scavenging activity of the stable hydrogen peroxide free radicals. The samples and hydrogen peroxide were mixed with phosphate buffer (pH-7.4) to prepare stock solution. Here ascorbic acid was taken as standard.

At first, 5 test tubes were taken with aliquots of 5 concentrations (20-100µl) of the synthesized silver nanoparticles. To that, 0.6 ml of H_2O_2 in phosphate buffer was added. The reaction mixture was incubated at room temperature for 10minutes. Absorbance was read at 230nm against the blank. Then the percentage of inhibition was calculated by the following equation.

H_2O_2 radical scavenging activity (%) = [(control absorbance)-(sample absorbance)]/[control absorbance]*100

ANTICANCER ACTIVITY

Cell Line

The human hepatic carcinoma cell lines (HepG2) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 hrs the cells were treated with serial concentrations of the test samples. The nanoparticles *B.diffusa* was dispersed in phosphate buffered saline. An aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT ASSAY

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

RESULT AND DISCUSSION

Visible observation of silver nanoparticles synthesis

The silver nanoparticles were synthesized using leaf extract of *B.diffusa* plant. The reduction of silver ions into silver particles during exposure to the plant extract is followed by color change. As the leaf extracts was mixed in the aqueous solution of silver ion complex, it started to change the color from yellowish brown to dark brown (Figure 2). Almost all the herbal mediated silver nanosolutions after incubation time were showed the color change from light to dark color. In this present study, the leaf extract of *Boerhaavia diffusa* has the potential to reduce silver nitrate to silver nanoparticles. The color of the reaction medium changed into dark brown color after 24 hours. In the present study the color changes was observed in both plants within 30 minutes. For, instance, the color change was in 5minutes incubation period of using leaves of *Prosopis juliflora*.[15]

Figure 2: Visible observation of silver nanoparticle from *B.Diffusa* leaf extract. A) Silver nitrate, B) plant extract, C) Initial reaction mixture D) Reaction mixture after 24 hours.

UV-visible spectroscopy analysis

The synthesis of silver nanoparticles in the mixture of solution was further analyzed and confirmed by UV-visible spectroscopy. The UV spectra peaks of *B.diffusa* showed in figure.3. The broad Plasmon resonance was observed after 24 hrs and 48 hrs at 418nm for *B.diffusa*. UV –Vis specra recorded for the green synthesis silver nanoparticles of leaf extract of *Canjancus cajan* at 470nm.[16]

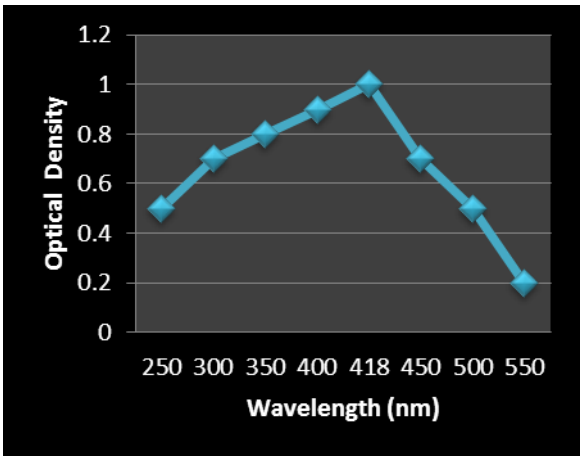


Figure 3: UV-Visible absorption spectrum of silver nanoparticles from *B.diffusa* leaf extract.

SEM analysis of silver nanoparticles

SEM image was showed that the information about the morphology and size of the synthesized silver nanoparticles of *B.diffusa* in figure 4 (A, B, C&D) respectively. From the SEM images the nanoparticles are

more or less crystal in shape in the maginification of 20.00KX, 50.00KX, 100.00KX. The *B.diffusa* exhibit the uniform sized cubic grains, the size of the cubes range from 10-20nm. Similarly, silver nanoparticles synthesized from leaves of *Allium cepa*[16] exhibited crystal shape.

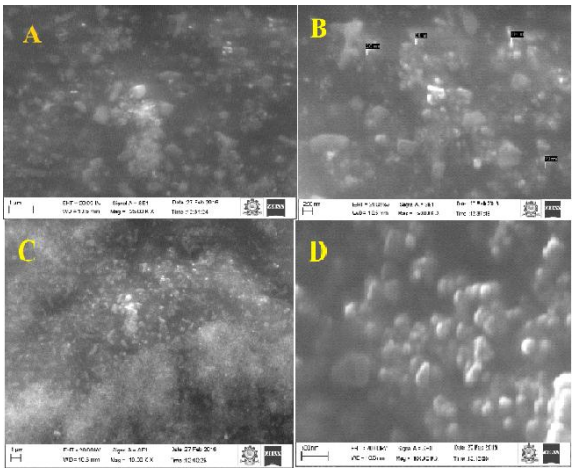


Figure 4: SEM images of silver nanoparticles synthesized from *B.diffusa* A) 20.00KX B) 50.00KX C) 10.00KX D) 100.00KX.

EDAX analysis of silver nanoparticles

Energy Dispersive Absorption Spectroscopy (EDAX) of synthesized nanoparticles of *B.diffusa* was showed in the figure 5. The presence of expected element in the final products Ag was confirmed. The present analysis revealed that the nano-structures were formed as peak at 3 keV solely of silver nanoparticles for *B.diffusa*. The other spectral signals such as Ca, K, Cl, O, Mg and Si were also noticed in the EDAX spectrum.

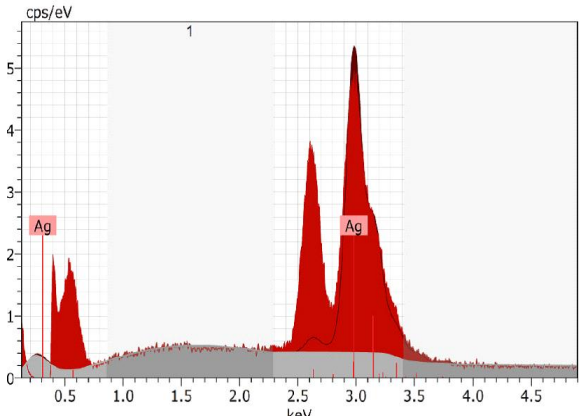


Figure 5: EDAX Spectrum of Silver nanoparticles from *Boerhaavia diffusa.*

FTIR spectrum

FTIR spectrum of produced silver nanoparticles were showed many absorption bands and the absorption bands for *B.diffusa* seen at 3732.97cm^{-1} 2922.92cm^{-1} 2850.59cm^{-1} 1742.57cm^{-1} 1511.12 cm^{-1} 1368.40cm^{-1} 1229.54cm^{-1} 978.81cm^{-1} 679.86cm^{-1} 653.82cm^{-1} 517.85cm^{-1} were assigned to the Amide N-H Stretch, alkanes C–H stretch, alkanes C–H stretch, carbonyls C=O stretch, nitro compounds N–O asymmetric stretch, alkanes C–H rock, aliphatic amines C–N stretch, alkenes =C–H bend, alkynes -C≡C–H:C-H bend, alkynes C-H bend, C–Br stretch alkyl halides (Figure 6). FTIR spectrum indicates the leaf extract of *B.diffusa* assisted production of silver nanoparticles by showing functional groups like Amide, Alkanes, Carbonyls, Alkyl halides, Alkynes, Aliphatic amines, Nitro groups, Aldehydes present at different position. From this observation, the identified biomolecules present in the plant extract of *B.diffusa* was responsible for reduction and stabilization of silver nanoparticles. Functional residues have stronger capability to bind silver nanoparticles to prevent agglomeration and provides longer stability and also stated that the biomolecules present in the plant extract may play the dual role that is silver nanoparticles synthesis as well as stabilization of synthesized particles from *Azadirachta indica*.[17]

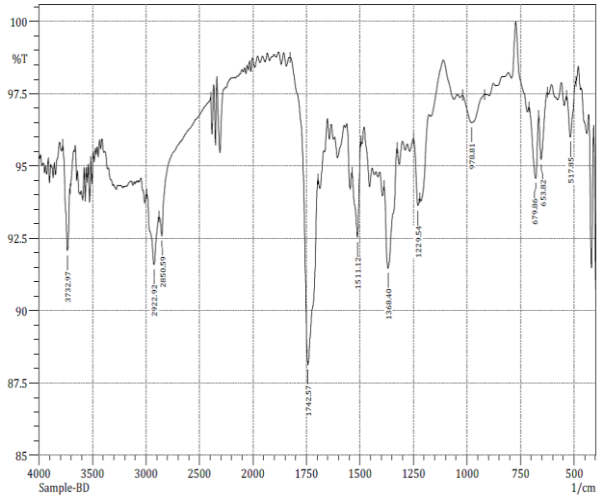


Figure 6: FTIR spectrum of silver nanoparticles synthesized by using *B.diffusa* leaf extract.

XRD analysis

The XRD pattern showed the intense peaks in the whole spectrum of 2Ɵ values ranging from 10-70 for the silver nanoparticles. The synthesized silver nanoparticles were in the form of nanocrystals *B.diffusa* 2Ɵ values were 27.811, 29.964, 30.245 and 32.706 corresponding to the diffraction exhibited from 10 to 70 range of 2Ɵ (Figure 7). XRD pattern obtained for the silver nanoparticles showed number of Bragg's reflections that may be indexed on the basis of the face centered cubic structure of silver.[18]

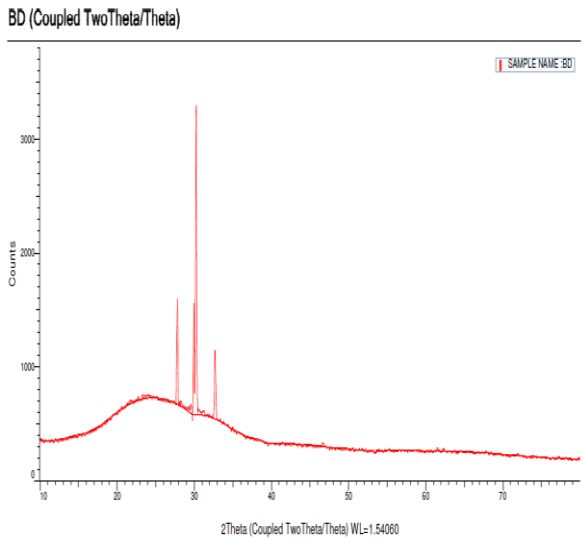


Figure 7: XRD patterns of silver nanoparticles synthesized from *Boerhaavia diffusa*.

Antimicrobial activity

Silver is said to be a universal antimicrobial substance for centuries. Though, silver ions or salts have limited usefulness as an antimicrobial agent. Such as, the interfering effects of salts and antimicrobial mechanism of continuous release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using silver nanoparticles. However, to use silver against microorganisms, it is essential to prepare it with environmentally friendly and cost- effective methods. Besides, it is also important to enhance the antimicrobial effects of silver ions.[19]

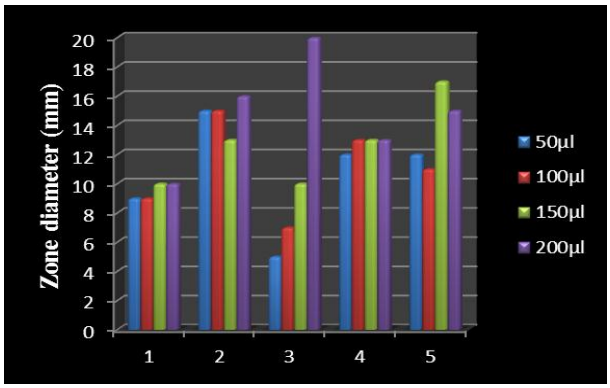


Figure 8: Antibacterial activity of silver nanoparticles of *B.diffusa* with different concentrations at 50µl (1), 100µl (2), 150µl (3), 200µl (4).

Antibacterial activity of silver nanoparticles from leaf extract of *B.diffusa* was performed by Agar-well diffusion method. In this study, five bacterial cultures were used namely *E.Coli, K.pneumonia, P.vulgaris, S.aureus and C.glutamicum* in 50µl, 100µl, 150µl, 200µl. In that plates the silver nanoparticles were added and incubated for 24 hrs (Figure 9). After incubation, the zone of inhibition was well was measured and showed in figure 8.

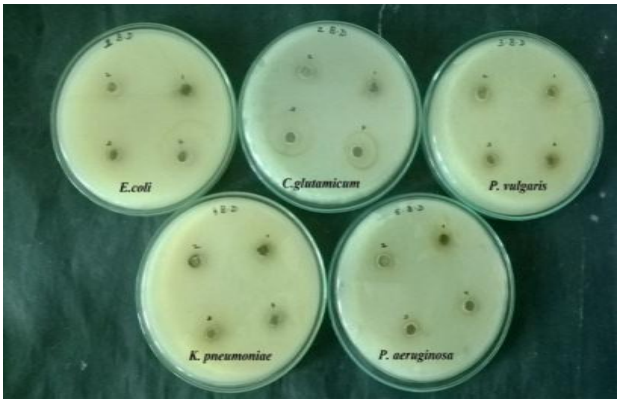


Figure 9: *B.diffusa* silver nanoparticles against bacterial colonies. 1) *E.coli* 2)*K.pneumoniae* 3) *P.vulgaris* 4) *P.aeruginosa* 5) *C.glutamicum*.

Antifungal activity of *B.diffusa* derived silver nanoparticles were performed by Agar-well diffusion method. In this study, five fungal cultures were used namely *A.japanicus, P.citrinum, A.niger, A.flavus, A.oxrasicus.* The silver nanoparticles were taken in different concentration such as 50µl, 100µl, 150µl, 200µl showed in figure11. After the incubation of 48 hours the zone of inhibition was found in Fig.10.

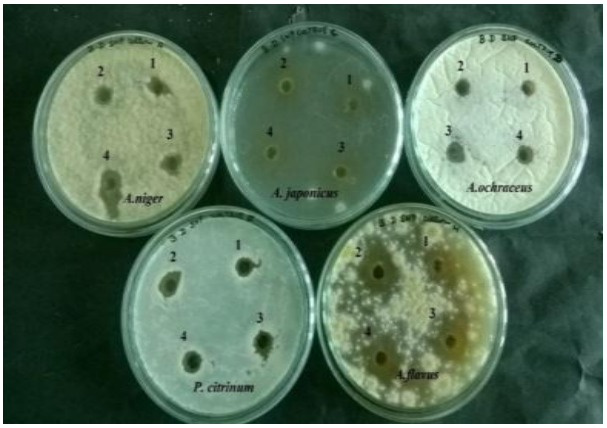


Figure 10: *B.diffusa* silver nanoparticles against fungal culture 1) *A.japonicas 2) P.citrinum 3) A.niger 4) A.flavus 5) A.ochraceus.*

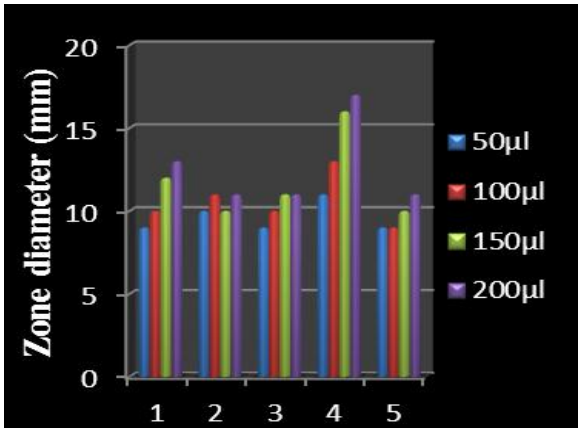


Figure 11: Antifungal activity of silver nanoparticles of *B.diffusa* with different concentrations at 50µl (1), 100µl (2), 150µl (3), 200µl (4).

Antibacterial activity of pus sample

The antidiabetic activity of the silver nanoparticle was performed by disc diffusion method. In this study, the pus sample was brought from Government hospital of Sivakasi from the diabetic patients. The patients sample was inoculated separately in blood agar and MacConkey agar plates. Then the pus sample was plated with the silver nanoparticles were added and incubated for 24 hours in incubator. After incubation, the zone of inhibition was around the disc was measured and it was showed Figure11. The zone of inhibition against the pus sample of diabetic patient was 10 mm in diameter. Wound infections are a major complication in diabetic patients and also a major healthcare burden.[20] Patients with chronic diabetic conditions are prone to showed process of wound healing and seem to be at particularly high risk for soft tissue infections, urinary tract infections and surgical site infections caused by *staphylococcus aureus, streptococcus haemolyticus, pseudomonas aeruginosa*.[21, 22, 23,24]

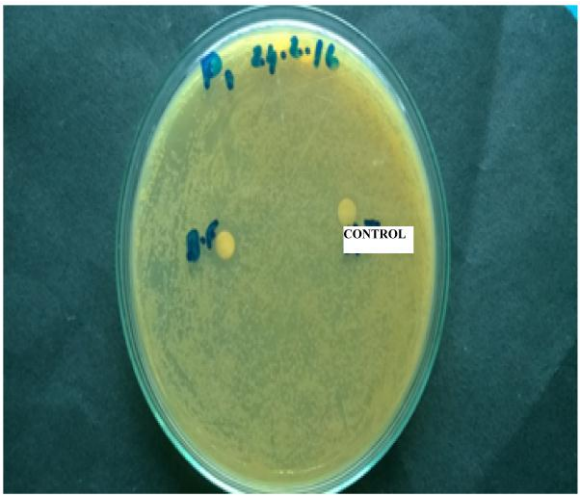


Figure 12: Antibacterial activity of pus sample against *B.diffusa* derived silver nanoparticles.

Radical scavenging activity of DPPH assay

The free radical scavenging activity of silver nanoparticles of *B.diffusa* was determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. The DPPH radical scavenging effects of nanoparticles of *B.diffusa* was showed in Figure13 and the silver nanoparticles showed the significant free radical scavenging activities when compared to with standard ascorbic acid. In various concentration of synthesized silver nanoparticles (20µl to 100µl) about 37% to 59% 0f free radicals was observed in *B.diffusa*'s silver nanoparticles.

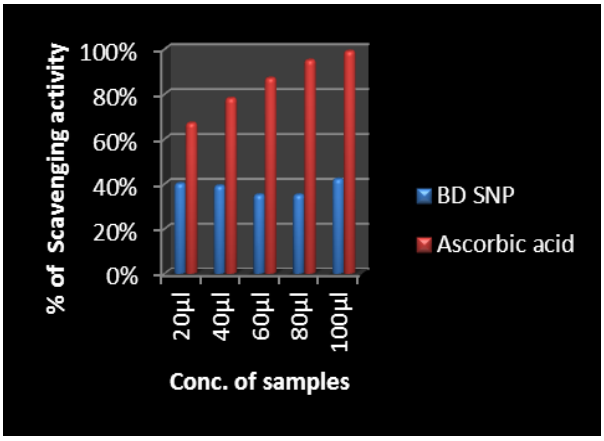


Figure 13: Percentage of antioxidant activity of ascorbic acid and *B.diffusa* silver nanoparticles by DPPH assay.

Free radical activity of hydrogen peroxide

The H_2O_2 scavenging activity of the silver nanoparticles of *B.diffusa* was performed. H_2O_2 scavenging activity of silver nanoparticles compared with standard ascorbic acid. The percentage of inhibition of free radicals increased with increase in concentrations of substrates. The concentration from 20µl to 100µl of silver nanoparticles showed 20% to 52% for *B.diffusa*'s silver nanoparticles (Figure14). The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for *in vitro* antioxidant study to understandthe pharmacological role of medicinal plant and it's isolate. *In vitro* techniques have been used for detection of antioxidants, which are based on the ability of compounds to scavenge peroxy radicals.[25]

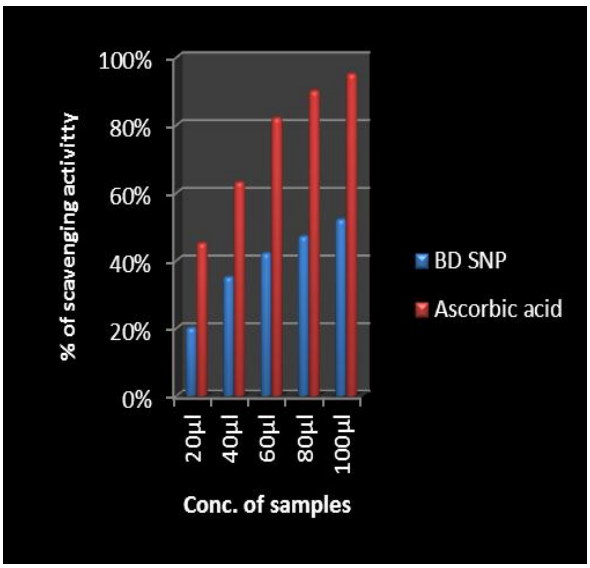


Figure 14: Percentage of antioxidant activity of ascorbic acid and *B.diffusa* silver nanoparticles by H_2O_2 assay.

Anticancer activity

The present study was conducted to screen cytotoxic potential against human hepatic carcinoma cell line

(HepG2) with silver nanoparticles of leaf extracts of concentration A)0.25µg B)2.5µg C)25µg D)50µg E) 100µg F) Control. *B.diffusa* plants (Fig.14) The microscopic examination of the human hepatic carcinoma cell line (HepG2) revealed characteristic growth pattern after 24 hours were treated with serial concentrations of test sample (0.25, 2.5, 25, 50, 100µg) of *B.diffusa*. Separate control was maintained to check the viability of the procedure. The present study clearly led to the confirmation that, the % of inhibition was increased in the reaction mixture. In the concentration like 50µg and 100µg the proliferation of the cells were completely inhibited in *B.diffusa*'s silver nanoparticles. Simultaneously, % of cell inhibition was also evaluated. Least inhibition was observed in the concentration like 2.5µg, it gives 0.562193% of cell inhibition. Similarly 76.66901% of the cells were inhibited when the concentration of *B.diffusa*'s silver nanoparticles has reached around 25µg (Table1 and Figure15).

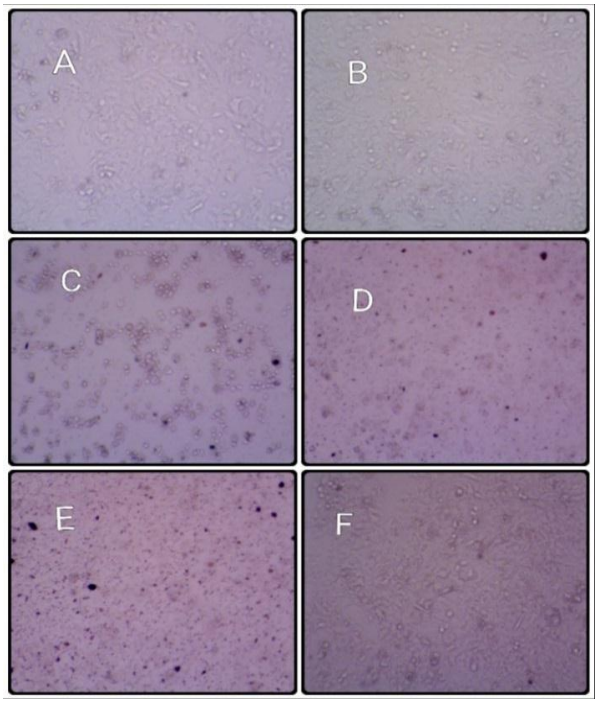
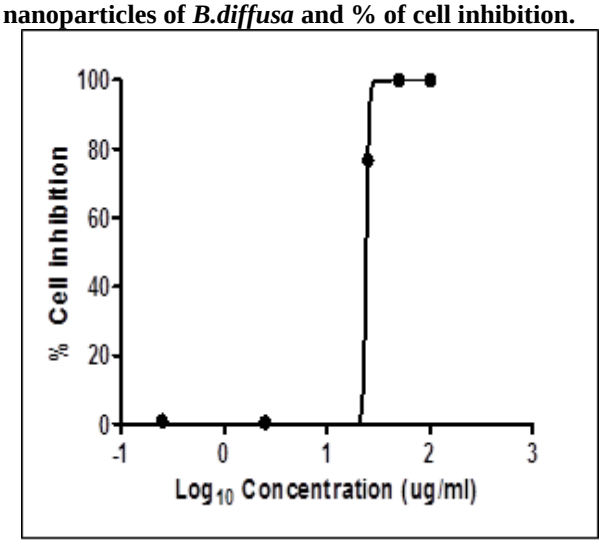


Figure 15: Effect of *B.diffusa* AgNPs on cell inhibition of human hepatic carcinoma cell line (HepG2).

Table 1: Different concentration of *B.diffusa* silver nanoparticles and absorbance against the cell line (HepG2).

Conc(µg/ml)	% Cell Inhibiton		
0.25	0.983837	**IC50**	24.07 µg/ml
2.5	0.562193	**R^2**	0.9999
25	76.66901		
50	100		
100	100		

Table 2: Different concentration of silver nanoparticles of *B.diffusa* and % of cell inhibition.



On the other hand, IC_{50} and R value of the silver nanoparticles suspension were also estimated. The experimental data has clearly revealed that IC_{50}= 24.07 µg/ml for *B.diffusa* silver nanoparticles, this value is quite a least concentration that could control the proliferation of cancer cell lines in the *in vitro* condition (Table 2).

CONCLUSION

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of silver nanoparticles using aqueous *Boerhaavia diffusa* Linn. leaves extracts. The silver ions in an aqueous solution were exposed to the *Boerhaavia diffusa* Linn. leaves extract, the biosynthesis of AgNPs were confirmed by the rapid color change of plant extracts. The natural benign AgNPs were confirmed further by using UV-Vis spectroscopy, SEM, EDAX, FTIR and XRD. Phenols and flavonoids were present in the leaves and they serve as an effective reducing agent. AgNPs biosynthesized from *Boerhaavia diffusa* Linn. leaves also exhibits great antimicrobial activities against pathogenic bacterial and fungal cultures. The synthesized nanoparticles showed dose dependent free radical scavenging activity and anticancer activity against human hepatic carcinoma cell line (HepG2). These biosynthesis silver nanoparticles can potentially be used for different medical application.

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