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SYNTHESIZED SILVER NANOPARTICLES FROM PSEUDARTHRIA VISCIDA EXERT HIGH ANTI-MICROBIAL ACTIVITY AGAINST PATHOGENIC BACTERIA

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ABSTRACT

In recent decades the use of plant based antimicrobial formulation is on increase due to microbial resistance to chemical drugs. The present study attempted to synthesize and evaluate Silver Nanoparticles (AgNPs) from methanolic extract of *P. viscida* under diverse temperature against pathogenic bacteria. AgNPs were prepared from a mixture of 1mM to5mM silver nitrate and 1-5% extract of *P. viscida.* Optimum proportion and concentration of extract and silver nitrate was determined and used for production of stable AgNPs. The characterization of stable AgNPs was done by UV-vis spectroscopy and Transmission Electron Microscope (TEM). Loss of organic content and specific characteristic of AgNPs were measured by Differential Scanning Calorimetry (DSC). The spectroscopy showed presence of AgNPs at 430 nm absorbance peak. The TEM analysis revealed AgNPs of good quality, dominant spherical in shape and particle size range from 59-95 nm. AgNPs shows similar inhibitory activity against 4 (67%) out of 6 bacteria tested. 1mM silver nitrate and 2% extract mixture prepared at 40˚c and pH 12-13 was found to be optimum for production of stable AgNPs with induced inhibitory activity that crude extract can't gives alone. The study concludes that AgNPs of good quality, stability and antimicrobial activity can be obtained from methanolic extract of *P. viscida*.

KEYWORDS: Silver Nanoparticles, Pseudarthria viscida, pathogenic bacteria, anti-microbial activity.

INTRODUCTION

P. viscida belongs to fabaceae family and it exhibits wide range of medicinal properties. The decoction is used in pain, hysteria, rheumatism, asthma, heart diseases, paralysis and urinary stones. In India, it is commonly called as *Salaparni* and it is a keen stimulant for digestive system and is used in digestive ailments like anorexia, flatulence, diarrhea, vomiting and piles. It is good as a pain-killer in general body ache. It also possesses anti-inflammatory and wound healing activity.[1] Indian forests are major and cheap source for medicinal plants and its product. Many medicinal plants are gaining importance due to their unique phyto-constituent and versatile application in field of development.[2] The use of environment friendly material like plant extract for synthesis of AgNPs offers several advantages which include eco friendliness, pharmaceutical capability and biomedical application as this synthesis is free of any toxic chemicals.[3] It is a well-known fact that silver ions and silver-based compounds are highly toxic to micro-organisms which include sixteen major species of bacteria.[4] Hence, investigation on synthesis of plant based silver nano particles has regained more importance due to overuse of antibiotics which have caused resistance in many microbes. In this study we have formulated AgNPs with the help of methanolic extract of *P. viscida* by reduction of $Ag+$ to Ag^{-} from silver nitrate solution under optimized condition. Due to wide range of its application, numbers of synthetic methods have been developed.[5] Many literatures show different methods for synthesis of gold and silver particles of different size and shape in polar and non-polar organic solvents. The nano particles synthesized this way shows excellent stability for long period. The smaller particles have the greater effect.[6]

MATERIALS

Silver nitrate ($AgNO_3$) and Nutrient agar were purchased from Hi-Media, India. Mature and healthy entire plant of *Pseudarthria viscida* was collected from Waghai Botanical Garden, NH360, Ambapada, Gujarat-394730, India, and was taxonomically identified and authenticated by Dr. Bimal Desai, Assistant professor, Forestry department, Navsari Agricultural University, Navsari, Gujarat, India. A voucher specimen (voucher number: -UTU/CGBIBT/16-17/01) were kept in Botany lab, Biotech department, UTU, Bardoli, Gujarat for future utilization. All the microbes used in this study were obtained from MTCC, Pune, Gujarat.

METHODS

Preparation of plant extract

Whole plant of *P. viscida* was washed for three times with distilled water. Whole plant materials were shed dry and fine powder was obtained using grinding mill, approx 200 gm whole plant powders of *P. viscida* was used for extraction process by Soxhlet apparatus using methanol as a solvent. Then these methanolic extract was concentrated by rotary evaporator. These concentrated methanolic extract of *P. viscida* was stored at 4˚c and used for this study.

Optimization of various parameters for synthesis of eco-friendly silver nano particles

Concentration of plant extract

To synthesize AgNPs from whole plant extract of *P. viscida*, different concentration of methanolic extract were prepared ranges 1% to 5%.

Concentration of silver nitrate

For optimization of silver ion concentration, different concentration of silver nitrate (range-1mM to 5 mM) were mixed with fixed concentration of plant extract and incubated in dark condition at different temperature.

Temperature

Mixture of plant extract and silver nitrate were kept at different temperature ranges 12˚c, 28˚c, 37˚c and 60˚c under shaking condition keeping other parameter constant.

Reaction time

Time interval was optimized in terms of properties and production by examine the sample at various time interval (0, 15, 30, 60, 90 and 120 minutes) using spectrophotometer. Different concentration of methanolic extract of plant, concentration of silver nitrate, temperature and reaction time was optimized for the synthesis of AgNPs under controlled pH (12.5).

Characterization of AgNPs

TEM (Transmission Electron Microscopy) analysis

TEM of the methanolic extract of *P. viscida* was carried out at Sardar Patel Center for Science and Technology, CharutarVidyaMandal, VallabhVidyanagar, Anand. Analysis report was given by Sophisticated Instrumentation Centre for Applied Research and Testing, Govt. of India. Crude methanolic extract of *P. viscida* was mixed with silver nitrate solution. 2mM

silver nitrated solution was used to prepare mixture of 2%. Then this mixture was used for TEM analysis for confirmation of formation of silver nanoparticles.

DSC analysis

Thermal decomposition behavior of the AgNPs has been studied using Netzsch (STA 449C) DSC/TG. The DSC/TG patterns were collected as a function of temperature up to 900˚c under N2 atmosphere. The heating rate was 10˚c /min. in N2. Alpha alumina was used as reference material.

Zeta potential

The size distribution or average size of the synthesized AgNPs were determined by dynamic light scattering (DLS) and zeta potential measurements were carried out using DLS (Malvern, UK). For DLS analysis the samples were diluted 10 folds by 0.15M phosphate buffer (pH 7.4) and the measurements were taken in the range between 0.1 and 10,000 nm. Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in de-ionized water and then for ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 25˚c with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer.

Antimicrobial study

The comparative antimicrobial activity of the both plant extracts and AgNPs synthesized from plant extract was effectively tested against Gram (+) ve and Gram (-) ve bacteria. The antibacterial activity testing was determined by disc diffusion method. Sterile discs of uniform size were soaked in distilled water, bulk silver, crude extract, AgNPs including standard drug separately. Then the discs were air dried under sterile condition. The plates of Mueller Hinton agar media were prepared and then the soaked discs were placed on respective place. Plates were incubated at 27˚c for 24-48 hrs. Streptomycin was used (10µg/ml) as a standard antibacterial drug.

RESULT

Preparation of extract

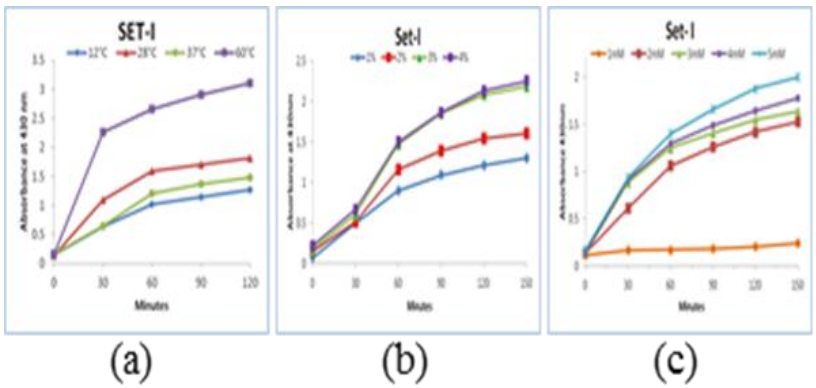
Soxhlet extraction process gave 18.92 gm (12.61%) yield from 150 gm powder of whole plant of *P. viscida*. This yielded powder was sticky and dark greenish brown in colour.

Synthesis and optimization of AgNPs

As a result of simple distinctive process of AgNPs preparation, when silver nitrate is mixed with crude methanolic extract of plant, color started to change from colorless to yellow brown and then dark brown over the time. Change in color was characterized for all five tests by spectrophotometer at 430nm on interval of 15 min as shown in Graph-I.

Graph-I: Highest peak of silver nitrate at 430 nm with different concentration of plant extract 1% to 5%.

Pure silver nitrate and methanol were used as blank to nullify its interference in test sample. Effect of different parameters like different temperature, concentration of plant extract and concentration of silver nitrate are shown in Graph II on interval of 30 minutes.



Graph-II: Effect of (a) Temperature, (b) extract concentration and (c) silver nitrate concentration on synthesis of AgNPs.

As shown in Graph-II (a), 1% and 2% concentration of plant extract produced stable AgNPs, which were not getting precipitated on long storage, in contrast AgNPs from 3% - 5% of

plant extracts were getting precipitated after 120 minutes. 2% plant extract produced higher amount of AgNPs compare to 1%, hence the optimized concentration for stable AgNPs production was found to be 2%. Same way as shown in Graph-II (b), minimum 2% silver nitrate was found to be adequate for stable AgNPs production. Both of these parameters were optimized under different temperature and 28˚c was found to be the best optimum temperature for synthesis of such stable AgNPs as shown in Graph-II (c).

Result of TEM

Morphology and size of produced AgNPs were determined by TEM analysis. Figures I show the obtained TEM images which confirmed that AgNPs were produced from methanolic extract of *P. viscida*.

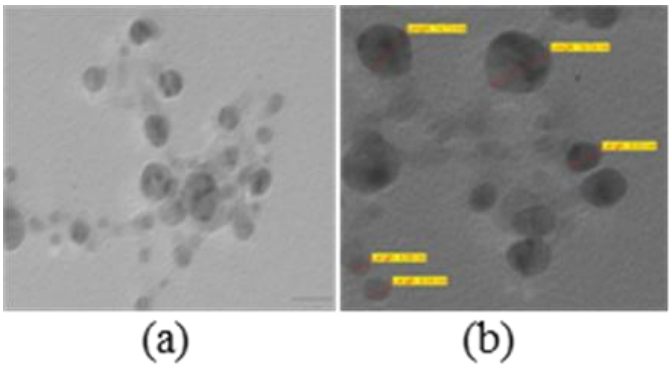
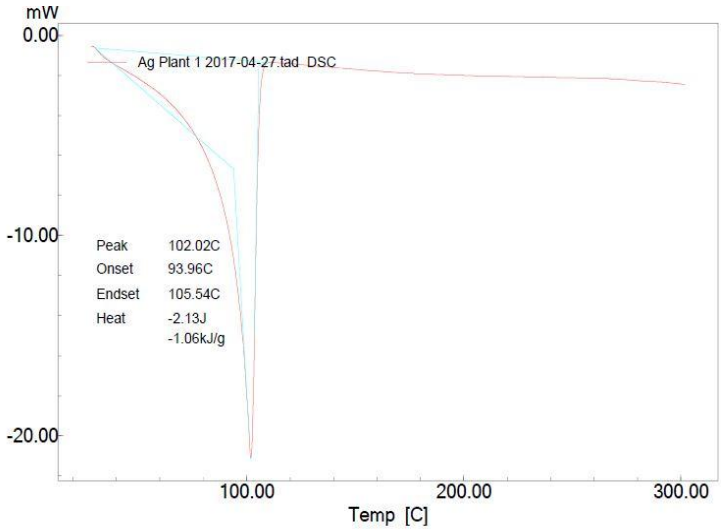


Image I: TEM images of AgNPs from extract of *P. viscida.*

It was also observed that the produced AgNPs were predominantly spherical in shape. The overall morphology of the produced AgNPs was composed of almost uniform nanoparticles which can be clearly seen in Image 1.[7] which was produced by reaction between 2% methanolic extract of *P. viscida* and 2mM silver nitrate mixture.

DSC analysis

DSC study shows the nature of the plant extract compound loaded on the AgNPs. Different compound shows their particular characteristic endodermic peaks in DSC. The endodermic peak of AgNPs was found at 102.02˚c, range is 93.96˚c to 105.54˚c as shown in Graph-III, due to transition temperature.

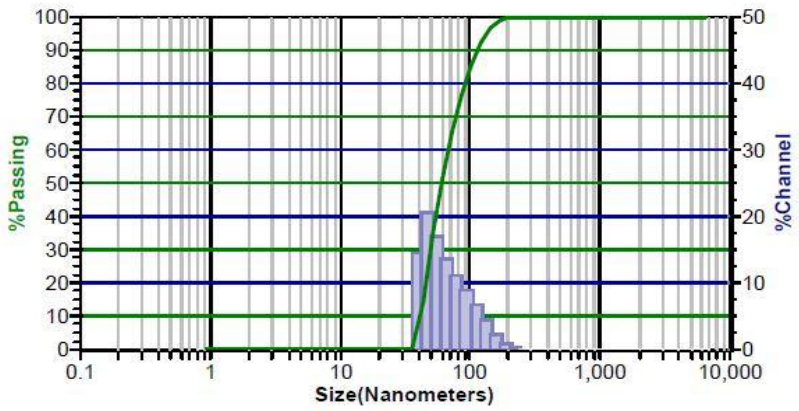


Graph-III: DSC analysis of AgNPs synthesized from extract of *P. viscida*.

These findings are in agreement with the literature report of.[8] The plot showed a steady weight loss due to moisture loss, between 100-750˚c the weight loss was due to degradation of organic compounds then after no degradation and remaining content accounts for silver weight.

Zeta sizer

The particle size range of AgNPs synthesized from *P. viscida* monitored by using particle size analyzer Mastsizer 2000. The result showed that AgNPs average size range was found to be 59.50 nm as shown in Graph-IV.

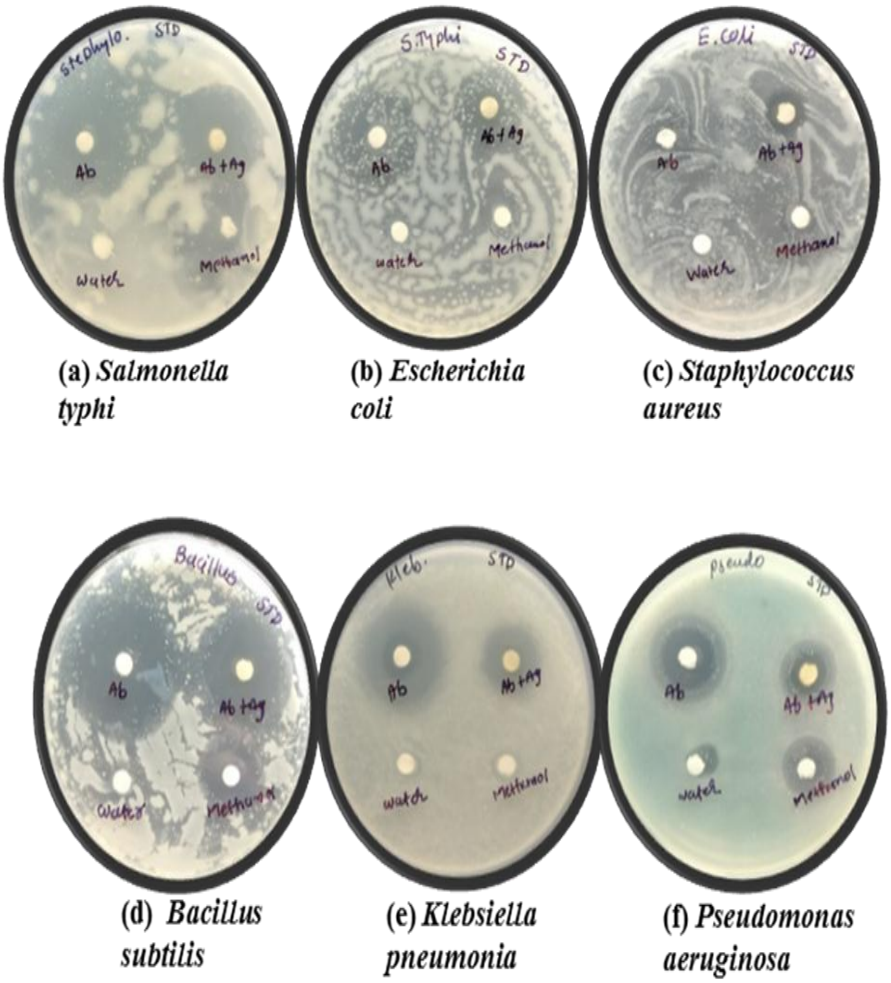


Graph IV: Particle size distribution of AgNPs from extract of *P. viscida*.

Anti bacterial activity

The silver nano particles with narrow size distribution obtained in this study were tested as antibacterial agent. Individual and combined effects of bioreduced AgNPs were investigated

against different pathogenic bacterial strains. Standard antibiotic drug showed zone of inhibition in almost all the bacterial strains as showed in Images-II.

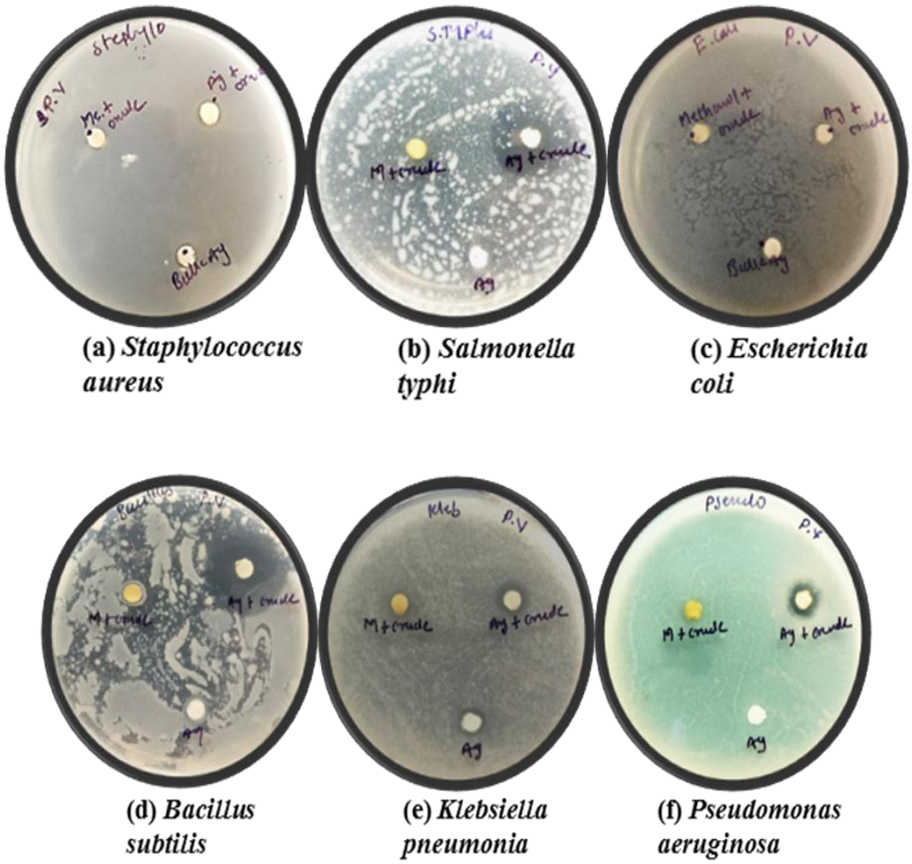


(a) *Salmonella typhi* (b) *Escherichia coli* (c) *Staphylococcus aureus*

(d) *Bacillus subtilis* (e) *Klebsiella pneumonia* (f) *Pseudomonas aeruginosa*

Image II: Zone of inhibition of standard drug in all selected bacterial strains.

Crude extract alone shows no inhibitory activity against all selected bacterial strains as shown in Images-III. While, combined with silver nitrate and crude extract of *P. viscida i.e.* AgNPs, showed wide variation in result as shown in Images-III.



Images III: Zone of inhibition of AgNPs.

These AgNPs showed greater synergy and was found to be superior against *Salmonella typhi* and *Bacillus subtilis* as it showed greater of zone of inhibition. These AgNPs showed comparatively low inhibitory activity against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and no activity against *Escherichia coli* and *Staphylococcus aureus* as small and no zone of inhibition was observed respectively. The diameter of zone of inhibition of all tested sample were measured and fold increase in inhibition were also calculated, data are exert in Table- II.

Table II: Diameter of zone of inhibition and fold increase in inhibition by AgNPs.

	Crude (a)	AgNPs (b)	Fold increase (c)
Salmonella typhi	2	19	89.3
Bacillus subtilis	2	20	99.0
Klebsiella pneumonia	2	5	5.3
Pseudomonas aeruginosa	2	6	8.0
Escherichia coli	2	2	0.0
Staphylococcus aureus	2	2	0.0

DISCUSSION

In this study we have successfully verified and confirm the quick and proficient route for synthesis of silver nanoparticles using extract of *P. viscida*. The progression of intense yellowish brown colour confirms the synthesis of silver nano particles which is completely due to surface plasmon resonance. According to literature *P. viscida* is in rich source of flavanoids and phenolic acid derivatives.[9,10] Flavanoids are widely known for reduction reaction for successful synthesis of nano particles.[11] Hence, according to result of phytochemical analysis, presence of high flavanoids and phenolic content in *P. viscida* extract rationally increase the potential of *P. viscida* extract for bioreduction of Ag+ to Ag^0. Many literatures revealed *P. viscida* contains ascorbic acid and citric acid in notable amount.[9,10] In addition to that ascorbic acid and citric acid are widely used in synthesis of AgNPs, it is a strong justification to use *P. viscida* extract for synthesis and shaping of silver nano particles.[12] Presence of Diosgenin as a dominant saponins in *P. viscida* is also reported which might contribute to surfactant properties of *P. viscida* extract.[13,14] Plant surfactants are already been in use for synthesis of silver nano particles. similarly the starch content reflects the capping properties of the extract and starch is widely used in various synthetic process for capping and stabilizing AgNPs.[15,16] Thus phytochemical studies support the use of *P. viscida* extract as a suitable agent to facilitate the synthesis of AgNPs. The complete reduction of Ag+ with in 4 hrs indicates that synthesis is much faster compared with other plants, which takes more than 24 hrs for complete synthesis of AgNPs to occur. Optimization studies showed that the sharpness of the peak increased with an increase in temperature. Most likely this occurs due to an increase in the reaction rate of conversion of metal ions to nanoparticles at higher temperature.[17] *P. viscida* is also known to contain various bioactive compounds like flavanoids, terpenoids, amino acid, protein and glycosides. It is speculated that the alcohol groups are oxidize to carbonyl groups in the course of reduction, which support the suggestion that polyol groups are primarily responsible for the bioreduction process. Thus the water soluble fraction of *P. viscida* played key roles in the bioreduction of the precursors and evolution in shape in the AgNPs. Silver ion and silver based compounds are highly toxic to micro-organisms, showing a strong biocidal effect against microbial species because they are highly reactive species with larger surface area.[18,19,20] AgNPs produced using microbes and plant extract are known to exhibit potent anti microbial activity. Anti bacterial activity determined using disc diffusion method confirms that combining crude extract with silver nano particles resulted in higher bactericidal effect on the test pathogen than the either crude or silver used alone. This experiment provides solid evidence of the antibacterial synergy

between crude extract and silver nitrate. It is very interesting observation that some pathogens are resistant to almost all the antibiotic and medicinal plants. Therefore this combinational approach of crude extract and silver nitrate would provide a strategy for effective control on such pathogens. From the result of our study we can conclude that the antibacterial effect of silver nitrate is the key contributor of the synergistic effect observed with a combination of silver nitrate and crude extract.

Mode of action of all known antibiotics have been recognized by the scientist.[21,22] Silver has been in use since century for burn wounds but still there is not any reliable explanation on mechanism of silver.[23] Silver at low concentration cannot penetrate into the cells but it can be adsorb on bacterial cell wall. Respiration in bacteria occurs across the cell membrane hence adsorption of silver in cell wall prevent the dehydrogenation. According many hypotheses, the catalytic oxidation of silver nitrate reacts with bacterial cell membrane which causes cell death. As discussed above, the killing mechanism of crude extract and silver nitrate is different. Therefore synergistic effect can be a powerful alternative against drug resistant micro-organism. Silver nitrate shows the selective approach towards phospholipids and glycoproteins which are present in cell membrane, consequently concentration of anti bacterial agents at specific point on the cell membrane will increase due to reaction between crude extract and silver nitrate. In addition we can say that silver nitrate aid in transportation of bio-active compounds to the cell surface. Silver nitrate is also specific towards sulfur in protein of bacterial cell and as a result it increases membrane permeability and enhanced infiltration of the bio active compounds. Due to the larger size of AgNPs in comparison to silver ion, more molecules can react with them which will directly increase antibacterial activity.

CONCLUSION

From this study we conclude that, synthesis of plant based silver nano particles is speedy and economical approach as it produced numbers of highly stable nano particles. *P. viscida* is rich in source of flavanoids and phenols, which may play important role for in synthesis of nanoparticles. These synthesized nanoparticles are capped with phenolic compound and can be used to reduce free radicals.

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