

# Biosynthesis of Nano Silver by alga *Pithophora oedogonia*

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## ABSTRACT

The present study aimed at elucidating alga *Pithophora oedogonia* (Montagne) Wittrock (CHLOROPHYTA) on the synthesis of silver nano particles (Ag Nps). The results showed that ability dynamically when dry weight when incubation at room temperature 25 m for 72 hours away from light, and sensitivity test results showed bio-nanotechnology ability of synthetic vital to work as an antidote vital disincensive to the growth of both bacteria *Proteus mirabilis* and more efficiently than inhibition of the bacterium *Staphylococcus aureus* compared with antibiotics (NIT, CTR, IPM, AK), where the results showed that the nano-synthetic vital the most efficient of the Ant NIT in inhibiting bacterium *Proteus mirabilis* while the less efficient inhibition antihistamines.

## INTRODUCTION

Recently, there have been continuous efforts to develop clean, reliable, nontoxic and eco-friendly procedures for synthesis and assembly of silver Nano particle with desired sizes and shaped to expel it biological or medical applications [1]. biosynthesis of (Ag NPs) consider one of the most modern technologies introduced to biology all around the world. This biotechnology depend on many factors related with tested organisms like higher Plant, Algae or Microorganisms [2]. Nano silver is one of most important Nano materials at commercial scale, the median production about 320 tons per year (1). It may due to highly antimicrobial activity many of products related with (Ag NPs) like, household water filters, clothing, cosmetics, smart phones detergents, clothes and toys. Many products exploit the anti-microbial properties of (Ag NPs) [3]. *Pithophora oedogonia* (CHLOROPHYCEAE) is one of attached (sometimes free floated) filament algae inhabits the shallow waters like ponds, rivers which contain high level of phosphorous and nitrates [4] [1]. continuous Needing to developed new alternative substances to reduce, control pathogenic microorganisms, in this, study silver

nano particles (Ag NPs) synthesis from *Pithophora Oedogonia* was test against *Staphylococcus aureus* and *Proteus mirabilis* in the presence of some antibiotics as control [6.5]

## Experimental

Collection of algae, the eukaryotic filamentous green alga *pithophora oedogonia* (montagne) wittrock

was collected from rice fields of Al-Mishkhab in Al-Najaf Province, Iraqi which belong to Phylum Chlorophyta  
Subphylum Chlorophytina  
Class Ulvophyceae  
Order Cladophorales  
Family Pithophoraceae  
Genus Pithophora. [8,9]

The multiple-antibiotic, resistant bacterial isolates of *Staphylococcus aureus* and *Proteus mirabilis* collected from AL-Diwaniya laboratory hospital. Axenic culture of *Pithophora Oedogonia* was obtained according to (7) figure (1):



Algae cultivation: These steps were done by inoculating the algae filaments in (250) ml flasks of Modified CH-10 medium table and incubated at (25)°C and 37 Mex for days. The growth curve of studied algae

was followed by optical density (O.P) at 650 nm by using TRSP-721 spectrophotometer. Nano silver Production, the algae biomass were harvested at stationary phase (1) days of cultivation as mentioned

conditions . (10) gram of dried wet of biomass was resuspended in 100 ml of D.W in 250 ml flasks then heated at 60 c<sup>0</sup> for 15 min, at oven the resulted filtrate were incubated with 1 Molar of silver nitrate

solution ( AgNO<sub>3</sub> ) at room temperature 25 C<sup>0</sup> for 72 hours in the dark condition . The resulted brown color solution refers to production of ( Ag PNs ) Figure ( 2 )



B-

-A -

**Figure: ( 2 ) Leaky alga *Pithophora Sp.* With added 1 Molar irrigation of silver nitrate solution AgNO<sub>3</sub> - A - before incubation -B - after incubation nano solution .**

the fennel step was the purification of (Ag PNs) by centrifugation at 15 rpm for 20 min . for (VIS-UV), (FTIR) spectral analysis 5 ml at the samples of suspension were experimented after 5 day . [ 8.9].

Characterization of Ag NPs

**-UV-VIS spectrophotometer**

Silver ion Ag<sup>+</sup> - ion reduction was monitored by measuring the UV-VIS ( SHIMADZU , UV – 1650 pc , Japan ) . spectrum of reaction medium after 30 . As small aliquot of the sample was taken for this Analysis at ( 30 – 700 ) nm .

**-Fourier Transform Infrared Spectroscopy analysis (FTIR) :**

This analysis was carried out using ( SHIMADZU , FTIR – 8400S , Japan ) . the range from ( 4800 – 400 ) cm<sup>-1</sup> .

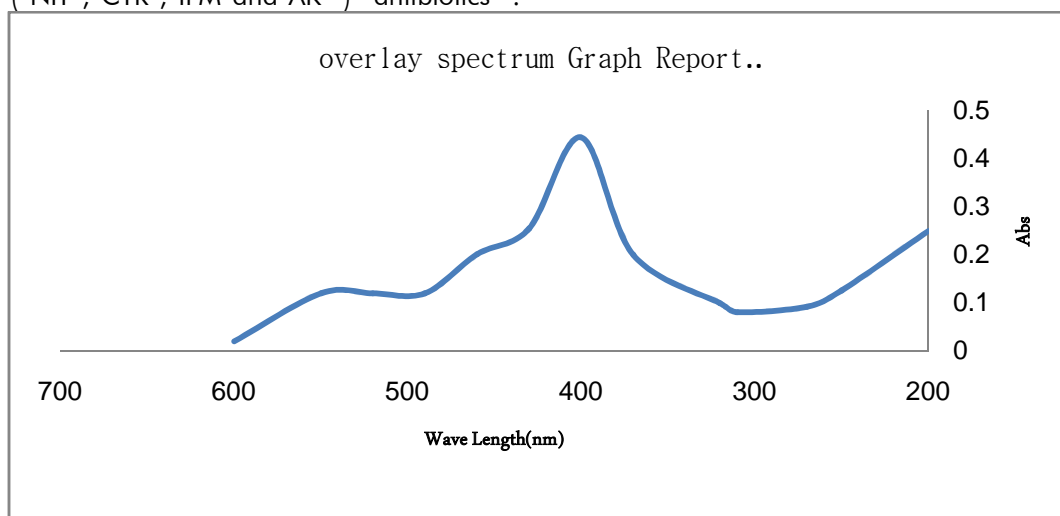
**- Antibacterial Activity**

The Testing of antibacterial activity of ( Ag NPs ) was carried out by Agar diffusion method by dried filter paper discs 0.6 cm diameter impregnated with Ag PNs ( NIT , CTR , IPM and AK ) antibiotics .

The inoculate were prepared by diluting 13 gm. in 250 ml at deionized D.W then autoclaved at 100 c<sup>0</sup> , 121 bar for 30 min , then diluted by (Na cl 0.85%) to produce ( 10<sup>7</sup> back , cm<sup>3</sup> ) conic . these tested discs ( Ag NPs ) and Antibiotics , them distributed on tectorial plants and incubated at 37 c<sup>0</sup> for 24 hrs. the zone of inhibition was measured and expressed in mm .

**Results and Discussion**

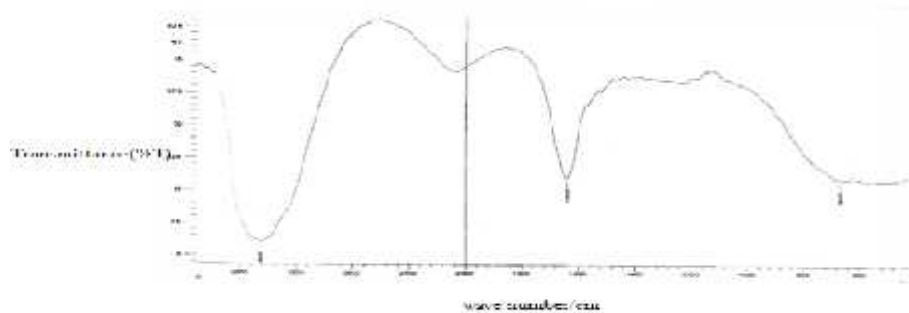
The current study showed that *P. oedogonia* has the feasibility to biosynthesize nano Silver after 72 hours of incubation with silver nitrate (AgNO<sub>3</sub>) at room temperature the reduction of Silver ion Ag (I) to Ag(0) during incubation period, this result was visually noted by color changing of solution from green to pale brown , this may be due to formation of surface Plasmon resonant (SPR) phenomenon fig:2(B) , the UV-Vis spectroscopy exhibit an absorption peak at wave length 420-430 nm as shown in Fig: (3), which is typical of silver nano particles and corresponds to silver reduction [10]



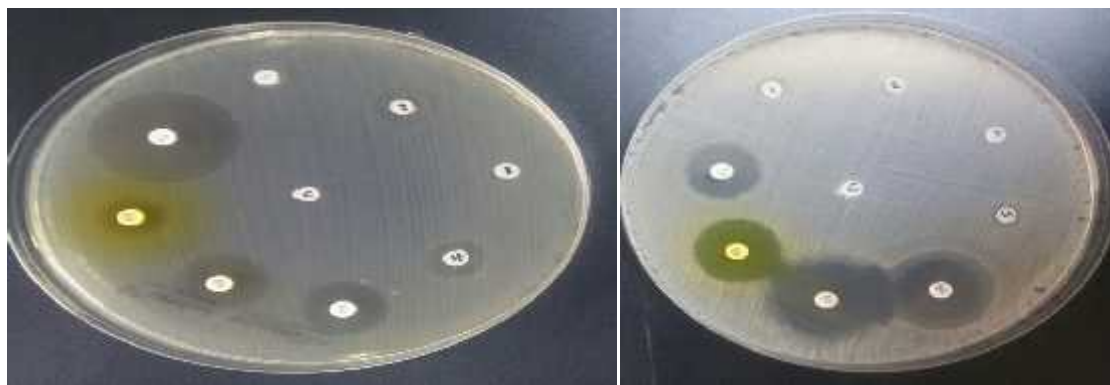
**Figure 2 UV-Vis spectra of absorbance of biosynthesized silver nanoparticles by alga *P. oedogonia* after 72 hours of incubation.**

The result of FTIR analyses exhibit that there are different stretches bond appeared in different peaks **3450.65** assigned with HN and Other peak **1635.64** related to C = C, as well as the peak near **667.37** appointed as the shades of the FTIR for nanosilver

biosynthetic showed strong and sharp absorption band at **1635.64** assigned to the stretching vibration of C = O (NH) group as shown in Figure 3.



**Figure (3): FTIR analysis for nano silver biosynthesized by *algae P. oedogonia* after 72 hours of incubation**



**Figure 4: illustrates the sensitivity Cub on two types of bacteria tested were: A - - Staphylococcus aureus - B-. proteus mirabilis Indicates where No. (4) of the effect of nano silver and an antidote to both types of bacteria.**

On the other hand sensitivity test results showed that AgNPs synthesized by *P. Oedogonia* had an antibacterial activity against tested micro organisms *P. mirabilis* and *S. aureus*, this inhibitory activity represented by inhibition zone which was 9mm and 4mm respectively in compare with NIT ,CTR and AK antibiotics. So the AgNPs was more efficient than NIT antibiotic against *Proteus mirabilis* fig(4). The mechanism of inhibitory effects of silver ion (Ag<sup>+</sup>) on microbes is partially known ,the positive charge on Ag<sup>+</sup> is decisive for its antibacterial activity by electrostatic attraction between negative charge of cell membrane of microorganism and positive one of AgNPs [ref.]. For gram negative bacteria the explanation is differ and it may depends on the conc. of AgNPs and it was closely related with the formation of "pit" in the bacterial membrane, [20, 21, 22] to be assumed that changes in the bacterial cell wall structure as a result of interactions with Ag NPs as an integral part of the activity of any bactericidal, thereby increasing the permeability of the membrane and the one that lead to death Ag NPs reacted with biomaterials sulfur and phosphorus, as well as

include components within cells, for example, proteins or DNA, and cell components such as membrane these components proteins affect breathing, and dividing and ultimately survival surviving cells bacterial cell wall, can be part of the silver ions Ag NPs enter in cells to accumulate damaged DNA and influence on protein synthesis. (23) .In other words, Ag NPs that are filled with polyphenolic components cell walls of bacteria crashes where makes Gram-negative bacteria are sensitive specifically Polyphenolic generate free radicals and other species as a response based on the oxygen reaction, which can make a big damage and toxicity may lead to further damage when they become membranes off including a loss on a wide range of ions + K leading to shortages in the membrane resulting in disruption in the leakage visceral, which includes the discharge of proteins and molecules lipopolysaccharide where the bacterial outer membrane of many fatty sugars and asymmetrical mainly composed while the inner membrane consists from a tight chains of phospholipids, which are semi-permeable (24). Is not

understood fully the precise mechanism of interaction that occurs between Ag NPs and bacteria have Ag NPs attached to the wall of the cell and thus permeable membrane disruption and breathe cells eventually can also be for Ag NPs that cells directly penetrate because they may bind the cell wall containing sulfur with biomolecules proteins containing phosphorus, for example, nuclear acid and therefore it can be easily linked to the constituents of the bacterial cell and destabilize the normal cell function 25)). As may be due to other possible mechanism, a release Alcaitonat Ag, which is antibacterial of Ag NPs (26).

### Conclusion

This investigation showed that the green alga *P. oedogonia* effectively synthesized AgNPs by interacting with aqueous silver nitrate (AgNO<sub>3</sub>), this may be typical candidates for more studies in the future exploring their role in pharmacological and biomedical applications. The biosynthesis of nano particles by algae and cyanobacteria can reduce costs, times, pollution and it may play major role in the shift towards green chemistry and away from the side effects of manufactured drugs.

### References

- Kumarijyoti, Mamta Baunthiyal and Ajeetsinyh . ( 2016) . Characterization of silver Nano particles using uric dietician Linn . leaves and their synergistic effects with antibiotics Jour or Radiation Reteach and Applied screens . q (2016) . 217 – 227 .
- Moghaddam KM. (2010). An Introduction to microbial metal nanoparticle preparation method. J Young Invest 19(19):1–6 .
- Nadagouda MN, Varma RS.(2008). Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chem. ;10(8):859–862.
- Dar MA, Ingle A, Rai M.(2013). Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria* sp. evaluated singly and in combination with antibiotics. Nanomedicine ;9(1):105–110. [PubMed] .
- Kumar V, Yadav SK.(2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol. ;84(2):151–157.
- Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S.(2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids Surf B Biointerfaces . ;65(1):150–153. [PubMed] .
- Rosarin FS, Mirunalini S.(2011). Nobel metallic nanoparticles with novel biomedical properties. J Bioanal Biomed. ;3:085–091 .
- Prescott, G.W. (1962). *Algae of the Western Great Lakes area*. With an illustrated key to the genera of desmids and freshwater diatoms. Revised [Second] edition. pp. [i]-xiii, 1-977. Dubuque, Iowa: Wm. C. Brown Company Publishers 135 South Locust Street.
- Maulood, B.K., Hassan, F.M., Al-Lami, A.A., Toma, I.J. & Ismail, A.M. (2013). *Checklist of algal flora in Iraq*. pp. 1-94. Baghdad: Ministry of Environment.
- Mosulishvili LM, Nadareishvili VS, Kharabadze NE, Belokobylsky AI. Facility for Lyophilization of Biological Preparation. URSS Patent No 779765, Bul. 42; 1980.
- Kalabegishvili T, Kirkesali E, Frontasyeva MV, Pavlov SS, Zinicovscaia I, Faanhof A (2012). Synthesis of gold nanoparticles by blue-green algae *Spirulina platensis*. Proceedings of the International Conference Nanomaterials: Applications and Properties, 1(2): 02NNBM09 (3pp). Sumy State University Publishing .
- Morais PC, Santos RL, Pimenta ACM, Azevedo RB, Lima ECD.(2006). Preparation and characterization of ultra-stable biocompatible magnetic fluids using citrate coated cobalt ferrite nanoparticles. Thin Sol Fil ;515(1):266–270. doi: 10.1016/j.tsf.2005.12.079. [Cross Ref]
- Alexandrova K, Markova-Deneva I, Gigova A, Dragieva I (2008) . In: Dimov S, Menz W (eds) TEM/SEM and FT-IR characterization of biocompatible magnetic nanoparticles. Multi-Material Micro Manufacture., pp 1–4, Cardiff University, Cardiff, UK: Published by Whittles Publishing Ltd.
- CISI( Clinical and laboratory Standards Institute ) . ( 2012) . Performance Standards for Antimicrobial Susceptibility Testing . 22<sup>th</sup> Informational Supplement ., 32 (3) . Wayne ,Pannsylvania , USA .
- Lokini S, Narayanan V.(2013) . Antimicrobial and anticancer activity of gold nanoparticles synthesized from grapes fruit extract. ChemSciTrans ;2:105–110.
- Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M.(2006). Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. BiotechnolProg;22(2):577–583. doi: 10.1021/bp0501423.[PubMed] [Cross Ref] .
- Aromal SA, Philip D. (2012).Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size dependent catalytic activity. SpectrochimActaA ;97:1–5. doi: 10.1016/j.saa.2012.05.083.[PubMed] [Cross Ref] .
- Gericke M, Pinches A.(2006).Microbial production of gold nanoparticles. Gold Bull ;39:22–28. doi: 10.1007/BF03215529. [Cross Ref] .
- Li S, Shen Y, Xie A, Yu X, Qiu L, Zhang L, Zhang Q.(2007). Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem ;9:852–858. doi: 10.1039/b615357g. [Cross Ref] .
- Shameli K, Ahmad M, Al-Mulla EAJ, Ibrahim NA, et al. (2012). Green biosynthesis of silver nanoparticles using *Callicarpamaingayi* stem bark extraction. Molecules. ;17:8506–8517. doi: 10.3390/molecules17078506. [PubMed] [Cross Ref] .
- Gardea-Torresdey JL, Tiemann KJ, Gamez G, Dokken K, Tehuacanero S, Jose-Yacamán M.(1999). Gold nanoparticles obtained by bio-precipitation from gold(III) solutions. J Nanoparticle Res ;1(3):397–404. doi: 10.1023/A:1010008915465. [Cross Ref] .
- Ankanna S, Prasad TNVKV, Elumalai EK, Savithramma N. (2010).Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark. Dig J NanomaterBiostruct ;5:369–72 .

23. Ajitha B, Reddy YAK, Reddy PS.(2015). Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract. *Mater SciEng C*. ;49:373–381. doi: 10.1016/j.msec.2015.01.035. [PubMed] [Cross Ref] .
24. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, et al. Antimicrobial effects of silver nanoparticles. *NanomedNanotechnolBiol Med*. 2007;3:95–101. doi: 10.1016/j.nano.2006.12.001. [PubMed] [Cross Ref] .
25. Dibrov P, Dzioba J, Gosink KK, Hase CC.(2002). Chemiosmotic mechanism of antimicrobial activity of Ag (+) in *Vibrio cholerae*. *Antimicrob Agents Chemother*. 2002;46:2668–2670. doi: 10.1128/AAC.46.8.2668-2670 . [PMC free article] [PubMed] [Cross Ref] .
26. Sondi I, Sondi BS. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram negative bacteria. *J Colloid Interface Sci* 2004;275(1):177–82.
27. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO.(2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mat Res* ;52:662–668. doi: 10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3. [PubMed] [Cross Ref].
28. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB. (2009). The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *Int J Antimicrob Agents*. 2009;34(2):103–110. doi: 10.1016/j.ijantimicag.01.017. [PubMed][Cross Ref] .
29. Hajipour MJ, Fromm KM, Ashkarran AA, Aberasturi DJD. Antibacterial properties of nanoparticles.(2012). *Trends Biotechnol*. 2012;30:499–511. doi: 10.1016/j.tibtech. 06.004. [PubMed] [Cross Ref] .
30. Azam A, Ahmed F, Arshi N, Chaman M, Naqvi AH. (2009). One step synthesis and characterization of gold nanoparticles and their antibacterial activities against *E. coli* (ATCC 25922 strain) *Int J TheorAppl Sci*. ;1:1–4.
31. Cao YW, Jin R, Mirkin CA.(2001). DNA-modified core–shell Ag/Au nanoparticles. *J Am ChemSoc* ;123:7961–2 .