Biosynthesis, characterization and antifungal activity of the green silver nanoparticles synthesized by a marine alga *Digenia simplex*

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Digenia simplex. **Green biosynthesis. Marine algae. Silver nanoparticles**

Abstract::

Silver nanoparticles synthesized biologically have been widely used in medicinal field, the synthesis of silver nanoparticles has been carried out by using the extracellular filtrate of the algal extract of ***Digenia simplex***, The synthesis of silver nanoparticles was identified primarily by changing the color of the extracellular filtrate and confirmed with the help of the study of XRD (x-ray diffraction),UV visible analysis and IR spectroscopy, it was confirmed that the silver nano particles were constituted extracellularly by an extracellular reductase like enzyme.
The results obtained from the study of antifungal activities of the silver nanoparticles are very significant and indicate that the synthesized silver nanoparticles may have an important advantage over conventional antifungal antibiotics.

1-introduction:

In last few years, research in nanotechnology has been carried out extensively as nanoparticles possess increased structural integrity as well as unique chemical, mechanical, optical, electronic and magnetic properties compared to large particles of bulk materials [1]. In the synthesis of nano particles, the various conventional processes like a number of chemical and Physical methods including chemical reduction in aqueous or non-aqueous solution [2], micro emulsion [3], sono-chemical [4] and microwave-assisted [5] methods have been applied. Since the chemicals like organic solvents, hydrazine, sodium borohydride and N, N-dimethyl formamide utilized in the synthesis of metal nano particles are found to be highly reactive and biological hazardous, the chemically synthesized metal nano particles could not accomplished with the biomedical field. On the other hand, the microbiologically synthesized metal nanoparticles are found to be eco-friendly, reliable, biocompatible and economic. It is reported from the ancient time that silver among the various metals has been considered as an effective antimicrobial agent, food preservative agent and water purifying agent [6]. Accordingly microorganisms such as an algae [7, 8],bacteria [9], actinomycetes [10] and plants [11, 12] have been used for the study of biosynthesis of silver nanoparticles. Synthesis of nanoparticles occurs intracellularly or extracellularly of which
the extracellular process could be used preferably as it is less laborious and also is less costly. It is found that algae are more suitable than bacteria for the use of the synthesis of nano particles.

2-Materials and method:
2.1 The source of the algae;
The marine alga *Digenea simplex* (Wulfen. C.Agardh, 1822) one of red algae of the family Rhodomelaceae was brought from the red sea from Egypt.

2.2 Preparation of silver nanoparticles:
10gm of the algae was powdered and extracted by using 95% ethyl alcohol Centrifuged to get the supernatant and condensed to get the algal extract active compounds "soxhelt apparatus method" [13].
.the silver nitrate solution 10^-3M and the algal extract were added with ratio 9:1 with magnetic stirring for 45min, the color changes from the colorless of algal extract to the grey red color indicating the formation of Ag-NPs.[14]

2.3Characterization of the Ag-NPs produced by the using of the investigated alga was characterized by the X-ray diffraction (XRD)[15], UV.Visible analysis to detect the silver nanoparticles and the FT-IR spectrum to know the active components present in the extract and responsible for the converting of Ag to Ag-NPs[15].

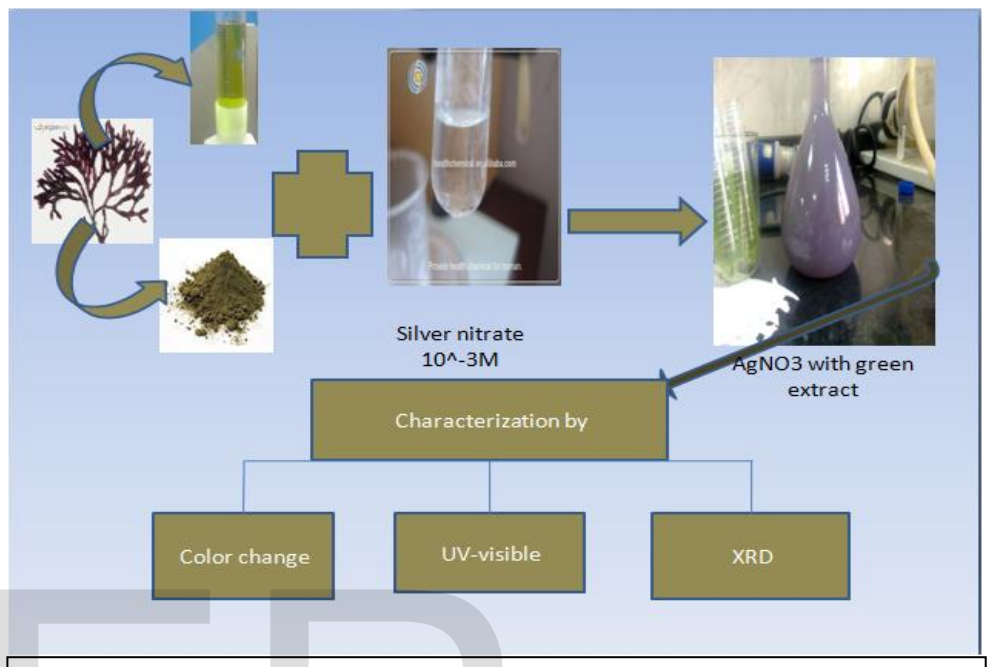
2.4The antifungal activity by the prepared Ag-NPs:
The antifungal activity of the biosynthesized Ag-NPs was tested by the standard agar well diffusion method. Discs (5mm) were used and loaded by 10microAg-NPs under sterile conditions. Then these discs were placed onto the plates which were previously seeded by the fungus of interest. Then plates were incubated at 30 ◦C for 3 days. The zone of inhibition was measured by a ruler and expressed in mm.

2.4.1The fungus; *Fusarium solani* from the family Nectriaceae, is human and plant infecting fungus.

2.4.2 Media prepared: (PDA) potato dextrose agar, 200gm of potato was boiled in a liter of water till 10min, then 20gm of glucose and 20gm of the agar were added to the potato filtrate then completed to one liter [16].

3- Results and Discussion:

3.1 Characterization of the Ag-NPs formation was by the change of color from the colorless of the alga to the grey red color, also by using the XRD detector give the size of silver oxide formed 55nm and 90nm [bookmark 1].



Summary of the green biosynthesis of Ag-NPs by using marine alga ***Digenia simplex***

Figure (1)

The crystalline size of the detected silver nanoparticles measured by XRD 'red colored line":
Main Graphics, Analyze View: (Bookmark 1)

Pos. [°2Th.]	Height [cts]	d-spacing [Å]	Rel. Int. [%]	Crystallite Size only [Å]	Micro Strain [%]
31.6998	72.07	2.82273	100.00	935.851300	0.150811
32.1564	31.44	2.78368	43.62	563.767700	0.246882

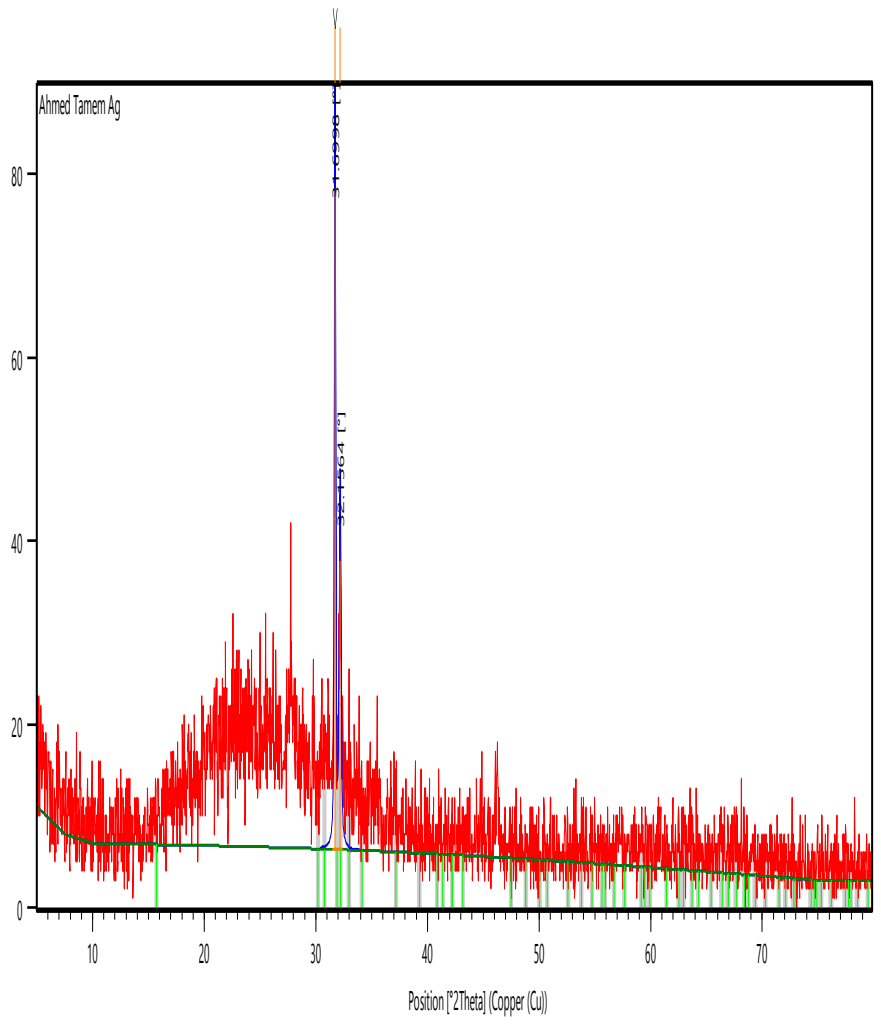
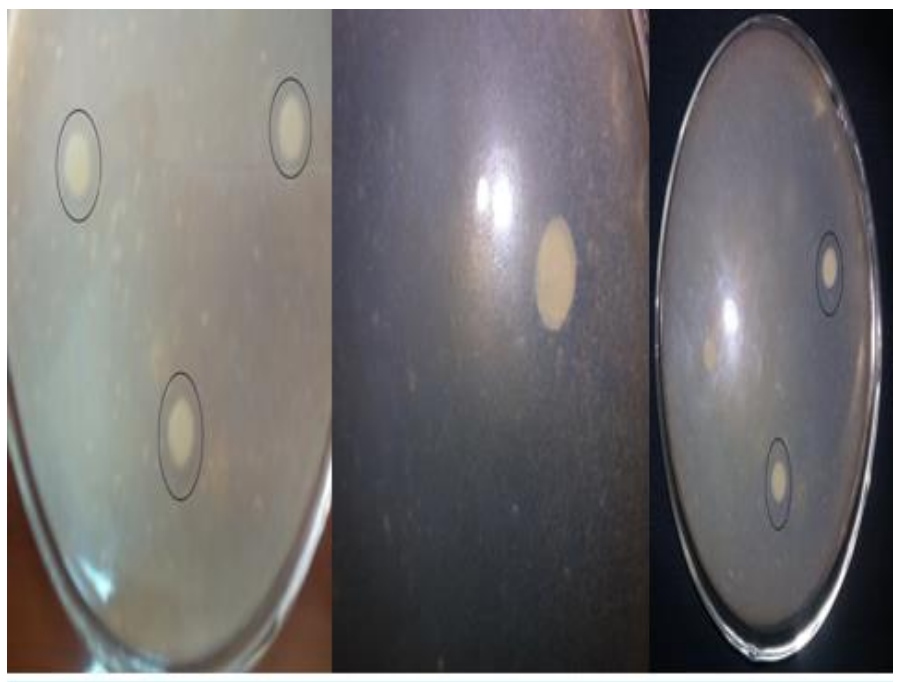


Figure (2) indicate the crystalline nano silver oxide of the single peak.

3.2 The antifungal activity of Ag-nps;
F. solani is known to infect soybeans, sometimes causing Sudden Death Syndrome (SDS) like what happen with *Pisum sativum* fig4[17], the results obtained from the study of antifungal activities of the silver nanoparticles are very significant and indicate that the synthesized silver nanoparticles may have an important advantage over conventional antifungal antibiotics figure 3, as it gives clear zones 4-5mm around the discs were injected by Ag-NPs, several mechanisms of Ag-NPs antifungal activity the silver ions react with SH groups of proteins [19,20]and play an essential role in bacterial

inactivation . Micromolar levels of silver ions have been reported to uncouple respiratory electron transport from oxidative phosphorylation, which inhibits respiratory chain enzymes or interferes with membrane permeability to protons and phosphate[18], also the presence of silver ions and sulfur in the electron-dense granules observed after silver ions treatment in the cytoplasm of fungal cells suggests an interaction with nucleic acids that probably results in impairment of DNA replication[18].



Figure(3) ; the antifungal activity of the prepared algal Ag-NPs

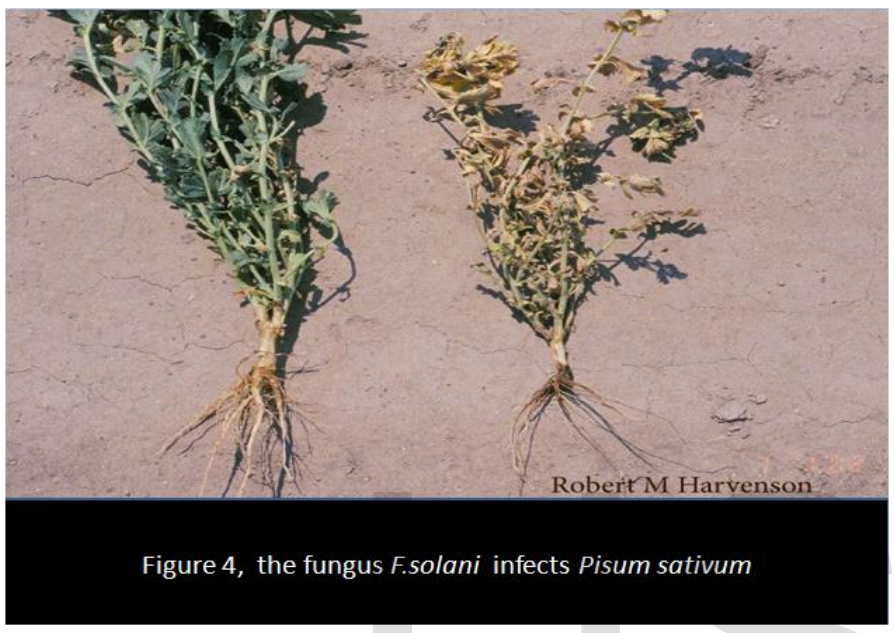
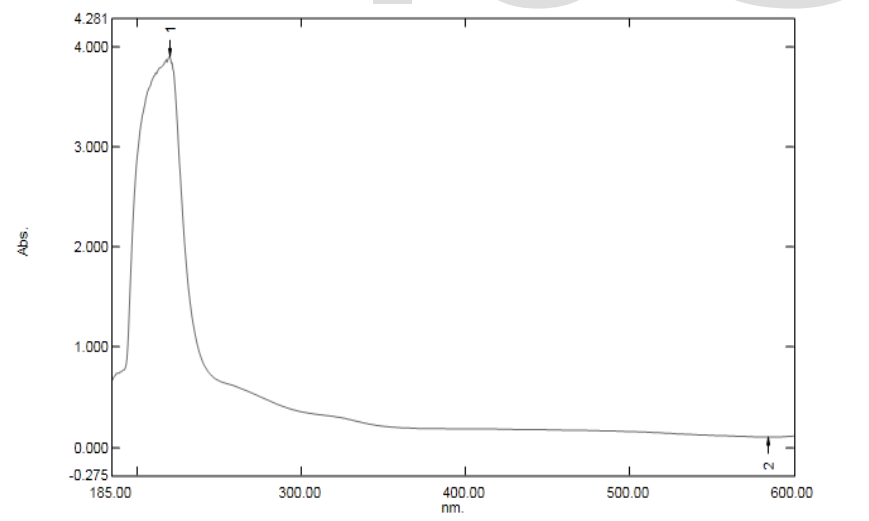


Figure 4, the fungus *F.solani* infects *Pisum sativum*

3.3 The UV-Visible spectrophotometer gives the wave length at 220nm due to the accumulation of some components from the extract on the silver nanoparticles, as the assumed wavelength max of the Ag is 450nm graph 5.



[Measurement Properties]
Wavelength Range (nm.): 185.00 to 600.00
Scan Speed: Fast
Sampling Interval: 0.5
Auto Sampling Interval: Enabled
Scan Mode: Single

No.	P/V	Wavelength	Abs.	Description
1		220.00	3.901	
2		585.00	0.105	

Figure (5) the UV –visible analysis of the extract after the formation of silver nanoparticles

3.4 The FT- IR spectroscopy :
In order to determine the functional groups on *Digenia simplex* (ethanolic extract) and predict their role in the synthesis of silver nanoparticles, FTIR analysis was performed for the silver nanoparticles of the extract of *Digenia simplex*, the FT-IR spectrum is shown in graph (6). The presence of the three bands at 3301.239 nm, 2136.282nm, 1637.861nm may be assigned to carboxylic acid bands of some components in the extract and arises due to carbonyl stretch and free-O–H stretch vibrations in the carbonyl linkages of the acids , The FT-IR spectroscopic study has confirmed that the carbonyl group forms amino acid residues and peptides of proteins have the stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration of the particles and stabilizing in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilizing of the silver nanoparticles in aqueous medium.

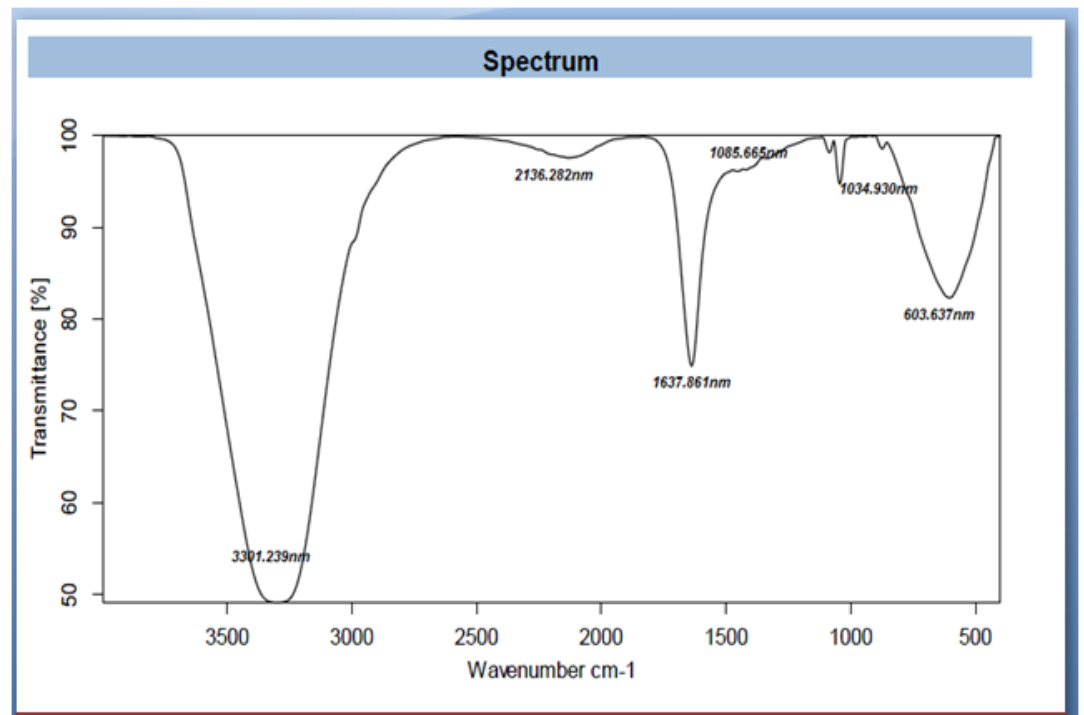


Figure (6) the FT-IR spectrum of the extract after the formation of Ag NPs .

4-Conclusion:

The extracellular filtrate of the algae, *Digenea simplex* could synthesize silver Nano particles from the aqueous solution of silver nitrate. Such type of the synthesis of silver nanoparticles may be considered as eco-friendly as it is free from any toxic chemicals or organic solvents. The synthesized silver nano particles are found to be relatively. So it can be considered as low cost process compared to the other processes, like chemical process as there is no requirement of any sort of capping agent for making the synthesized silver nano particles stable. In the present study it is found that the dimension of the synthesized silver nano particles is in the range of 55-90 nm. Investigation of antifungal activities of silver nano particles against some fungal strains. Indicated that it could be considered as a potential antifungal agent implicating its biomedical application.

5-REFERENCES;

[1] B. Bhushan, in Springer Handbook of Nanotechnology, Spinger-Verlag Berlin,
Heidelberg (2004).
[2] C. Petit, P. Lixon and M. P. Pileni, J. Phys. Chem. **997**, 12974 (1993).
[3] J. N. Solanki and Z. V. P. Murthy, Colloids Surf. A **359**, 31 (2010).
[4] V. G. Pol, D. N. Srivastava, O. Palchik, V. Palchik, M. A. Slifkin,A. M. Weiss and Gedanken,
Langmuir **18**, 3352 (2002).
[5] X. Li, L. Wang and X. Lu, J. Hazard. Mater. **177**, 639 (2010).
[6] S. Silver, FEMS Microbiol. Rev. **27**, 341 (2003).
[7] K. C Bhanisa, S. F, D'Souza, Colloids Surf. B **47**, 160 (2006).
[8] A.R. Binupriya, M. Sathishkumar, and Soon-Il Yun, Ind. Eng. Chem. Res. **49**, 852 (2010).
[9] R. Shahverdi, S. Minaeian, H. R. Shahverdi, H. Jamalifar, A. A. Nohi. Proc. Biochem.
42, 919 (2007).
[10] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar,M. Sastry. Colloids
Surf. B **28**, 313 (2003).
[11] A. Rai, A. Singh, A. Ahmad, M. Sastry, Langmuir **22**, 736 (2006).
[12] M. Sathishkumar, K. Sneha, S. W. Won, C. W. Cho, S. Kim, Y. S. Yun. Colloids Surf,
B **73**,332 (2009).
[13]vivek et al (2011).
[14]Kumar and Steffi(2011).
[15]Singaravelu(2007).
[16]Bennett, J. Michael(2014)
[17]BAM Media M127: Potato Dextrose Agar
[18] J. Braz. Chem. Soc. vol.21 no.6 São Paulo 2010
[19] Liau, S. Y.; Read, D. C.; Pugh, W. J.; Furr, J. R.; Russell, A. D.; *Lett. Appl. Microbiol*. **1997**, *25*, 279.
[20] Feng, Q. L.; Wu, J.; Chen, G. O.; Cui, F. Z.; Kim, T. N.; Kim, J. O.; *J. Biomed. Mater. Res., Part A* **2000**, *52*, 662.