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Green Biosynthesis of Silver Nanoparticles Using Marine Red Algae *Acanthophora specifera* and its Antimicrobial Activity

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Abstract

Silver has been known since ancient history for its antimicrobial property. Synthesis it by eco-friendly method is a good thing due to its cost effective, stability and environmentally favorable. In this study silver nanoparticles (AgNPs) were obtained by a green synthesis method. Alcoholic extraction of *Acanthophora specifera* was used to reduce silver nitrate salt to AgNPs. Structure of synthesized AgNPs which observed by the dark brown color development was characterized by X-Ray diffraction (XRD), where the crystalline nature of silver nanoparticles were cubic and its size was range in 33 nm to 81 nm. Moreover, infrared spectroscopy (FT-IR) was identified. Additionally, the anti-microbial activity of AgNPs was recorded by using disk diffusion methods. AgNPs significantly reduced the growth of both gram positive *Staphylococcus aureus, Bacillus subtillis*, and gram negative *Salmonella spp., Escherichia coli* in addition to the unicellular fungus *Candida albicans*.

Keywords: Marine algae; *Acanthophora specifera*; Silver nanoparticles; XRD; Antibacterial activity

Introduction

In this era nanotechnology has gained wide acceptance and importance. The outcome of which is the production of nanomaterials like nanoparticles, carbon nanotybes, quantum dots and many more. A nanomaterial is any material that is synthesized under control conditions in the size range of 1:100 nm [1]. Particles within this size range exhibit superior optical, electrical and mechanical properties and therefore its extensive application [2]. Nanoparticles are commonly synthesized using top-down and bottom-up strategies [3,4]. In top-down approach, the bulk materials are gradually broken down to nanosized materials, whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometer range. Bottom-up approach is commonly used for chemical and biological synthesis of nanoparticles.

A wide variety of physical and chemical processes have been developed for the synthesis of metal nanoparticles, but these methods are expensive and require the use of toxic and aggressive chemicals as reducing and/or capping agents [3]. Moreover, Synthesis of silver nanoparticles by biological method using algae has more advantages due to their benign process and ability of large scale production over chemical and physical methods [4]. Therefore, green chemistry should be integrated into nanotechnologies especially when nanoparticles are to be used in medical applications, which include imaging, drug delivery, disinfection, and tissue repair [5]. Nanoparticles are one of the nearly every one sought materials for the future significant in many of the fields. Noble metals are silver, palladium, platinum and gold they exhibit a particular wide range of material behavior along the atomic to bulk transition [6]. Among these noble metals silver have wide applications in jeweler, dental alloy and health additive in traditional Chinese and Indian medicine [7]. Silver nanoparticles have received considerable attention because of their unique chemical and physical properties, which differ greatly from those of bulk materials as well as their potential for technological applications [8], Silver nanoparticles exhibit tremendous application in drug delivery [9], wound healing [10], sensor applications, textile industry, cosmetics and also used as antimicrobial agent [8], also it involved in the medical science due to their antimicrobial action in food pathogens [11]. The literature survey found that the marine red algae are rich sources of phenolic compounds especially bromophenols. Phenolic substances were reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilator actions. Furthermore, tannis and flavonoids are defined as naturally occurring seaweed polyphenolic compounds which have been found only in marine algae [12]. Marine red algae *Acanthophora specifera* is one of the macro algae that come under the order Ceramiales and family Rhodomelaceae. The algae extraction is rich with a large scale of biomolecules compounds used as an antioxidant, antiviral, antifungal and antimicrobial activities have been detected in red algae [13]. Reduction of $AgNO_3$ by using of the red alga *Acanthophora specifera* is an effective method for the synthesis of silver nanoparticles. This alga is a very abundant biomass in nature, easy performance at room temperature using dead biomass and environmental friendly compared to other chemical methods that use toxic chemicals.

Material and Methods

Collection of *Acanthophora specifera* and chemicals

Alga was collected from red sea (Hurghade, Egypt), $AgNO_3$ and the test microorganisms were kindly supplied by the Phycological Lab., Botany and Microbiological Department, Faculty of Science, Beni-Suef University.

Preparation of alga extract

Alga was dried well after washing it by distilled water and puffing it from other associated materials. After drying, alga was grind into fine powder. The extract was prepared by taking 10 gm of powder

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and soaking it on 50 ml ethanol 95% for 24 hour with shaking. Then making centrifuge for 20 minute at 4000 rpm at room temperature. Finally keep the supernatant for using it on the reduction of silver salt to synthesis AgNPs.

Syntheses of AgNPs

It was achieved by adding 10 ml of algae extraction to 90 ml 0.001 M of $AgNO_3$ at room temperature. You note at once the color change to greenish yellow color on mixture ($AgNO_3$ solution + Algae extraction), which change gradually till become dark brown color which indicates the formation of AgNPs.

Characterization of synthesized AgNPs

Crystalline nature of the silver nanoparticles and its size were detected by XRD. FT-IR was used to identify the possible biomolecules responsible for the reduction of the Ag ions and capping of the bio-reduced silver nanoparticles synthesized by *Acanthophora specifera*.

Antibacterial activity

Is done by using disc diffusion method on both gram positive bacteria *Staphylococcus aureus* and *Bacillus subtillis* and gram negative bacteria *Salmonella sp* and *Escherichia coli* in addition to the unicellular fungus *Candida albicans*. These bacteria were freshly cultivated for 24 hour on nutrient agar and then each bacterial culture was inoculate on Muller Hinton agar, then preparing discs (6 mm) and loaded it by 200 µ of nanoparticles by the help of micropipette under aseptic conditions and plates were incubated at 37°C for 24 h. The zone of inhibition was measured using a ruler and expressed in mm [14,15].

Results and Discussion

Before adding algae extract to $AgNO_3$ solution, algae extract was green and $AgNO_3$ solution was colorless, after addition the algae extract to $AgNO_3$ solution the color change till be dark brown (Figure 1). Dark brown color was observed indicate the formation of AgNPs. Such a color transition is often indicative of changes in the metal oxidation state. In this case, Ag^+ was reduced to Ag^0 by some biomolecules in algae extract. So as the reaction time increasing the reaction rate was gradually increased. This clear in increasing the brown color gradually (Figure 2). Thus the synthesized silver nanoparticles by algae extract increased, which still stable for months due to the presence of stabilizing agents in algae extract. The results were analyzed by XRD (Figure 3) to ensure from the final product and crystalline nature of AgNPs. That was cubic crystalline, also the size of silver nanoparticles were obtained range of (33 to 81 nm). The 2θ values of XRD pattern was range from 5° to 80°. There are two peaks for AgNPs at 45.2° and 58.5° and other unknown peaks were observed it may be due to the fewer biomolecules of stabilizing agents are enzymes or proteins in the algae extract and other peaks don't identified it may belong to biomolecules of algae extract.

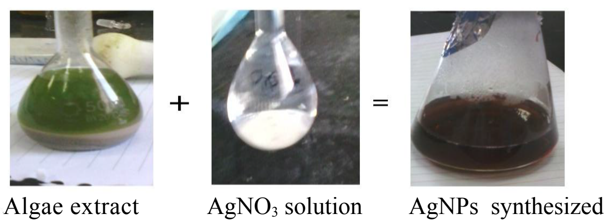


Figure 1: Formation of dark color after adding algae extract.

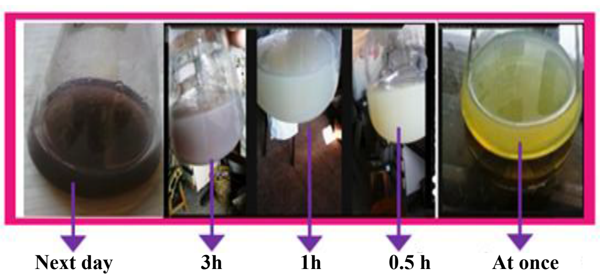


Figure 2: Dark brown colour increase with time.

In order to determine the functional groups on *Acanthophora specifera* ethanolic extract and predict their role in the synthesis of silver nanoparticles, FTIR analysis was performed. The spectrum showed a number of peaks thus reflecting a complex nature of *Acanthophora specifera*. FT-IR absorption spectra of ethanol extract after reduction of Ag^+ ions indications the capping ligand of the silver nanoparticles may be an aromatic and/or alkanes compounds (Figure 4). Whereas the band at 3279.78 (cm^{-1}) assigned to O-H, or COOH stretched, the band at 2129.628 (cm^{-1}) assigned to CO_2, the band at 1639.579 (cm^{-1}) assigned to C=C, the band at 1016.139 (cm^{-1}) assigned to C-C and the band at 607.638 (cm^{-1}) assigned C-O group. The groups of monosaccharide, polysaccharide, uronic acids and biological molecules such as secondary metabolites found on marine algae [16,17]. Could possibly play major role in the synthesis and stabilization of metal nanoparticles. 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 [16].

Antibacterial activity

The antimicrobial activity against these types done by disc diffusion

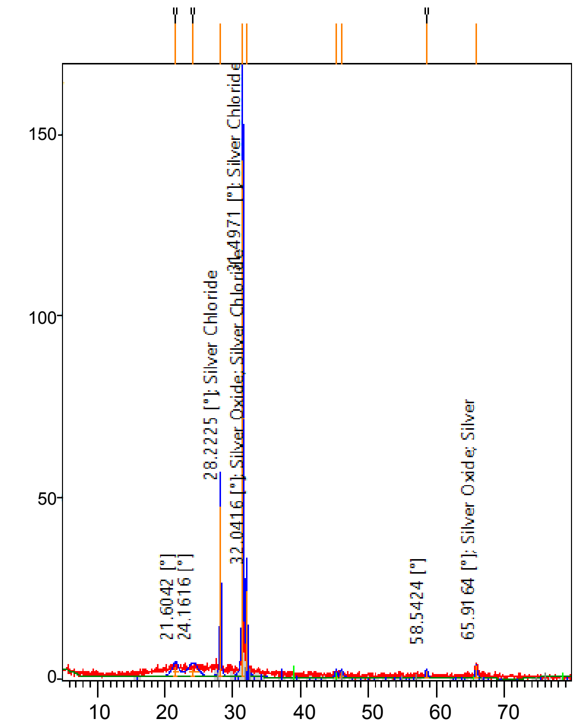
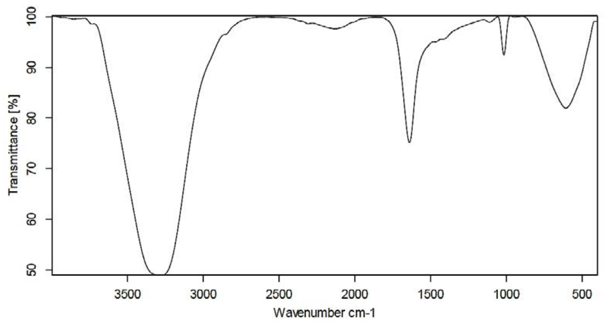


Figure 3: XRD show Ag-NPs synthesized by alcohol extraction.

method. Ag-NPs significantly reduced the growth of the tested bacteria (Figures 5-9).

Although gram positive bacteria have a thick cell wall contain peptidoglucon. which sometimes no significant effects of nanoparticles on it. On the other hand, a sharply clear zone was appeared for prepared nanoparticles against the tested gram negative bacteria which contain thin lipid layer. Where nanoparticles easily enter to the cell and disrupt it. Clear zone of *Escherichia coli* were (23.5 mm) and it was the biggest one. That is means that it is more sensitive to Ag-NPs synthesized by *Acanthophora specifera* than other bacteria then come *Salmonella sp* its clear zone was(19.5 mm) those two types are gram negative type. Clear zone on *Bacillus subtillis* was also(19.5 mm). But at *Staphylococcus aureus* was (18.75 mm). Those two types are gram positive type. On *Candida albicans* clear zone was (20 mm). The antimicrobial activity of AgNPs come from its ability to destroy the bacterial membrane. Through binding with protein and it is also reported to uncouple respiratory electron transport from oxidative phosphorylation which



Peak pick

Wave number	Abs. intensity	Rel. intensity	Width	Threshold	Shoulder
3279.780	0.490	0.511	446.951	99.72	0
2129.628	0.976	0.023	249.151	4.43	0
1639.579	0.752	0.249	97.894	48.39	0
1111.892	0.989	0.008	134.250	0.99	0
1016.138	0.925	0.076	34.366	14.72	0
607.638	0.819	0.175	259.007	33.40	0

Figure 4: FT-IR show the functional groups response for $AgNO_3$ reduction.

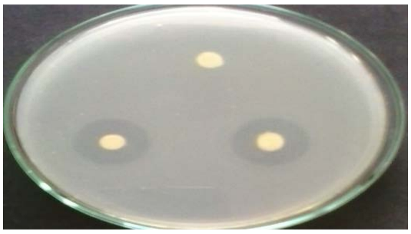


Figure 5: Antibacterial activity of Ag-NPs against *Salmonella sp.*

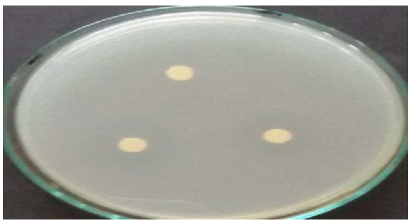


Figure 6: Antibacterial activity of Ag-NPs against *Escherichia coli.*

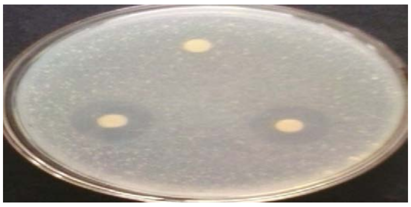


Figure 7: Antibacterial activity of Ag-NPs against *Staphylococcus aureus.*

Figure 8: Antibacterial activity of Ag-NPs against *Bacillus subtillis.*

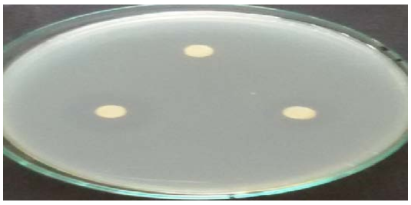


Figure 9: Antibacterial activity of Ag-NPs against *Candida albicans.*

inhibits respiratory chain enzymes or interferes with membrane permeability to protons and phosphate [18]. Three most common mechanisms were suggested on the activity of nano-silver on bacteria up to now: uptake of free silver ions followed by disruption of ATP production and DNA replication, formation of Reactive Oxygen species (ROS) and direct damage to cell membranes [19,20].

Conclusion

Silver nanoparticles were synthesized through an environment friendly method. The ethanol extract of algae acted as a reducing agent and capping. Till prevent its aggregation. These AgNPs were characterized by XRD its size was range in 33 nm to 81 nm and crystalline nature was cubic. In addition to the may possible groups for reduction detected by FT-IR. The anti-microbial activity carried against gram positive *Staphylococcus aureus* and *Bacillus subtillis*, gram negative *Salmonella* sp. and *Escherichia coli* and yeast strain *Candida albicans*. Which give positive results against all. Results also revealed that, the AgNPs synthesized here are a wide distribution anti-microbial agent. So it could use on various medicinal applications as a sterilizing agent, on coating medicinal devices and on dental material.

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