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FACULTY OF AGRICULTURE

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Jabatan : Guru Besar Bidang Mikrobiologi
Fakultas Pertanian Universitas Gadjah Mada
Yogyakarta

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Production of silver nanoparticle from *Chlorella vulgaris* and *Dunaliella salina*

dalam jurnal Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry ISSN 1582-540X\19(4) pp.385-394

adalah sesuai dan sangat relevan dengan bidang biologi karena pada saat ini ada irisan antar bidang yang sangat penting justru untuk pengembangan biologi itu sendiri karena biologi tidak lagi sekadar ilmu tentang jasad hidup tetapi juga dimanfaatkan untuk pengembangan bidang-bidang *new frontier*, seperti bioteknologi, bioengineering, pengembangan material baru berbasis jasad hidup, misalnya penggunaan bakteri sebagai pengganti superkonduktor komputer.

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Prof. Ir. Triwibowo Yuwono, Ph.D.



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SURAT KETERANGAN
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Nama : Prof. Widodo, Ph.D Med Sc.
Jabatan : Guru Besar Bidang Biologi Kanker, Jurusan Biologi, FMIPA,
Universitas Brawijaya

Menerangkan bahwa Karya Ilmiah yang telah di publikasikan oleh:

Nama : Dr. Hermin Pancasakti Kusumaningrum, S.Si., M.Si.
Jabatan : Calon Guru Besar Bidang Biologi, Universitas Diponegoro

Dengan judul "Production of silver nanoparticle from *Chlorella vulgaris* and *Dunaliella salina*" yang diterbitkan di Jurnal Scientific study & Research Chemistry and Chemical Engineering, Biotechnology, Food Industry; ISSN 1582-540X/19(4) pp.385-394

adalah sesuai dan relevan dengan bidang biologi dan arah perkembangan ilmu biologi baik di dalam dan luar negeri. Materi karya ilmiah tersebut sebenarnya masih pada tataran pengembangan keilmuan dan metodologi sebagaimana filosofi dasar dari ilmu Biologi untuk menjadi landasan perkembangan ilmu-ilmu terapan dan teknologi baru. Pada era sekarang, Ilmu Biologi berkembang sangat pesat dengan beririsan dengan berbagai disiplin ilmu lainnya sehingga melahirkan konsep keilmuan baru seperti nanobiologi, bioteknologi, bioinformatika, sintetik biologi dan biomedika. Terjadinya transformasi keilmuan tersebut merupakan bentuk perkembangan ilmu biologi dalam menjawab permasalahan manusia sesuai dengan zamannya. Artikel tersebut diatas menunjukkan bahwa ilmu biologi mampu menyiapkan fondasi penemuan-penemuan penting dibidang material sains, yang akan melahirkan teknologi baru yang akan datang.

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CALON GURU BESAR

Yang bertanda tangan dibawah ini :

Nama : Prof. Dr. Tri Retnaningsih Soeprbowati, MAppSc.
Jabatan : Guru Besar bidang Biologi
Fakultas Sains dan Matematika Universitas Diponegoro Semarang

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Jabatan : Calon Guru Besar bidang Biologi

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“Production of silver nanoparticle from *Chlorella vulgaris* and *Dunaliella salina*”

dalam jurnal Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry ISSN 1582-540X\19(4) pp.385-394

adalah sesuai dan sangat relevan dengan bidang biologi karena merupakan penelitian dalam ranah aplikasi pemanfaatan biologi (dalam hal ini mikroalga *Chlorella vulgaris* dan *Dunaliella salina* dalam produksi nano partikel silver, sehingga lebih ramah lingkungan di bandingkan dengan metode fisik dan kimia. Bionanoteknologi perlu dikembangkan seiring dengan pengembangan nanoteknologi lainnya. Dengan demikian penelitian inipun mendukung visi Biologi Universitas Diponegoro yaitu menjadi pusat pendidikan berbasis penelitian yang unggul di bidang pemanfaatan dan pengembangan sumber daya alam hayati yang berkelanjutan.

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Semarang, 4 Agustus 2019

**Berikut kami lampirkan beberapa contoh paper author lain
sebidang yang memperkuat ketiga surat tersebut.**

Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed *Ulva lactuca* *In vitro*

J. Saraniya Devi^{1,2} and B. Valentin Bhimba^{2*}

¹Department of Microbiology, Asan Memorial College of Arts and Science, Chennai-100, India

²Department of Biotechnology, Sathyabama University, Chennai, India

Abstract

Introduction: A novel process for the rapid synthesis of AgNPs using the aqueous extract of marine macroalgae, *Ulva lactuca*.

Methods: The nanoparticles were synthesized by subjecting the reaction media at 121°C for 10 min. and were assessed for *in vitro* cytotoxic activity on Hep-2, MF7, HT29 and Vero cell lines.

Results: The synthesized AgNPs were characterized by observing a peak at 434nm using UV-visible spectroscopy. Fourier Transform Infra Red spectroscopic analysis was carried out to identify the functional groups responsible for the synthesis of AgNPs. The synthesized AgNPs were further demonstrated and confirmed by the characteristic peaks observed in the XRD image. SEM and TEM images revealed that AgNPs were spherical in shape and measured 20-56nm in size. Qualitative as well as quantitative status of elements that might be involved in the formation of AgNPs is stressed by EDAX analysis.

Conclusion: The synthesized nanoparticles were potently cytotoxic against Hep 2 cell lines and mildly cytotoxic against MCF 7 and HT 29 cell lines. The synthesized AgNPs were found to be a cytotoxic against human (Hep2, MCF7 and HT29) cancer cell lines.

Keywords: Silver nanoparticles; Marine macroalgae-*Ulva lactuca*; *In vitro* cytotoxicity; Hep2 cell lines; MCF7 cell lines; HT29 cell lines; Vero cell lines

Introduction

With the terrestrial resources being greatly explored and exploited, researchers turn to the oceans for numerous reasons. The oceans cover more than 70% of the world surface housing 34 living phyla out of the 36 and more than 300,000 known species of fauna and flora. The marine environment is known to contain over 80% of world's plant and animal species. In recent years, many bioactive compounds have been extracted from various marine plants, marine animals and marine organisms [1].

Seaweeds are marine plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water. In India seaweeds are mainly exploited by industries for phycocolloids but very poorly explored for their beneficial application in pharmacology [2]. Seaweeds are important sources of protein, iodine, vitamins, minerals [3] and polyphenols [4] with their metabolites showing wide range of promising biological activities. Over the past several decades, seaweeds and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential [5]. The macro algae reported to contain various significant compounds with antibacterial [6], antiviral [7] and antitumor activity [8]. AgNPs have been synthesized using marine microalgae and screened for its antibacterial activity [9].

Discovery of anticancer drugs that must kill or disable only tumor cells without undue toxicity is an extraordinary challenge [10]. Toxicity of plant or microbial material is considered as the presence of antitumor compounds [11]. In recent years, an increasing number of marine natural products have been reported to display antimicrobial activities [12] and anti-tumor compounds have been isolated from sponges, tunicates, algae and other organisms [13]. In most cases, the evaluation of the anti-cancer potential of crude extracts from different

sea organisms has been carried out by *in vitro* cytotoxicity tests in malignant cell cultures [14]. Hundreds of potential anti-tumor agents have been isolated from marine origin especially from marine algae [15,16]. Isolation of cytotoxic anti-tumor substances from marine organisms has been reported in several references during the last 40 years [17].

In this study, AgNPs were synthesized using *Ulva lactuca* and its cytotoxicity was assessed against Hep-2, HT-29, MCF-7 and Vero cell lines.

Methods

Seaweed

Fresh seaweed sample were collected in polythene bags from the intertidal regions of the Mandapam coastal regions (Lat.09° 17.417 N; Long.079° 08.558 E) of Gulf of Mannar. Samples were cleaned thoroughly by washing with running tap water to remove stones, epiphytes, extraneous matter and necrotic parts. Then cut into small pieces and rinsed with sterile distilled water. The cleaned macro-algae were shade dried at room temperature for a week. The shade-dried thalli were powdered and aqueous extract was prepared.

Rapid synthesis of AgNPs

1gm of seaweed powder was extracted with water and filtered. 1mM

***Corresponding author:** B. Valentin Bhimba, Department of Microbiology, Asan Memorial College of Arts and Science, Chennai-100, India, E-mail: bvbhimba@yahoo.co.in

Received February 03, 2012; **Published** August 24, 2012

Citation: Devi JS, Bhimba BV (2012) Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed *Ulva lactuca* *In vitro*. 1: 242. doi:[10.4172/scientificreports.242](http://dx.doi.org/10.4172/scientificreports.242)

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Algal production of nano-silver and gold: Their antimicrobial and cytotoxic activities: A review



Mostafa M. El-Sheekh ^{a,*}, Hala Y. El-Kassas ^b

^a Botany Department Faculty of Science, Tanta University, Egypt

^b National Institute of Oceanography and Fisheries, Marine Environmental Division, Hydrobiology Laboratory, Alexandria, Egypt

Received 12 July 2016; revised 7 September 2016; accepted 20 September 2016

Available online 6 October 2016

KEYWORDS

Algae;
Antibacterial;
Antifungal;
Antiviral;
Gold-nanoparticles;
Silver-nanoparticles

Abstract The spreading of infectious diseases and the increase in incidence of drug resistance among pathogens have made the search for new antimicrobials inevitable, similarly is the cancer disease. Nowadays, there is a growing need for biosynthesized nanoparticles (NPs) as they are one of the most promising and novel therapeutic agents of biological origin. The unique physico-chemical properties of the nano silver (Ag-NPs) as well as nano gold (Au-NPs) when combined with the growth inhibitory capacity against microbes lead to an upsurge in the research on NPs and their potential application as antimicrobials. The phytochemicals of marine algae that include hydroxyl, carboxyl, and amino functional groups can serve as effective metal reducing agents and as capping agents to provide a robust coating on the metal NPs. The biosynthesis of Ag-NPs and Au-NPs using green resources is a simple, environmentally friendly, pollutant-free and low-cost approach. The biosynthesized NPs using algae exerted an outstanding antimicrobial and cytotoxic effect.

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* Corresponding author.

E-mail address: mostafaelsheikh@science.tanta.edu.eg (M.M. El-Sheekh).

Peer review under responsibility of National Research Center, Egypt.

<http://dx.doi.org/10.1016/j.jgeb.2016.09.008>

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Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency

Jayshree Annamalai¹ · Thangaraju Nallamuthu¹

Received: 18 January 2015 / Accepted: 4 March 2015 / Published online: 20 March 2015
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Abstract Silver ions (Ag^+) and its compounds are highly toxic to microorganisms, exhibiting strong biocidal effects on many species of bacteria but have a low toxicity toward animal cells. In the present study, silver nanoparticles (SNPs) were biosynthesized using aqueous extract of *Chlorella vulgaris* as reducing agent and size of SNPs synthesized ranged between 15 and 47 nm. SNPs were characterized by UV–visible spectroscopy, scanning electron microscopy, transmission electron microscopy, X-ray diffraction and Fourier infrared spectroscopy, and analyzed for its antibacterial property against human pathogens. This approach of SNPs synthesis involving green chemistry process can be considered for the large-scale production of SNPs and in the development of biomedicines.

Keywords Silver ions · Green chemistry · Human pathogens · Biomedicines

Introduction

Nanotechnology is an exploitation of strange properties of materials smaller than 100 nm (nm) to create new useful objects. Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic material. Their uniqueness arises specifically from higher surface-to-volume ratio and

increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications.

Silver nanoparticles are nontoxic, safe inorganic antibacterial agent used for centuries and are capable of killing about 650 types of diseases causing microorganisms. Silver has been described as oligodynamic because of its ability to exert a bactericidal effect at minute concentrations. The first scientific papers describing the medical use of silver report the prevention of eye infection in neonates in 1881 and internal antisepsis in 1901 (Russell and Hugo 1994). After this, silver nitrate and silver sulfadiazine have been widely used for the treatment of superficial and deep dermal burns of wounds and for the removal of warts (Rai et al. 2009).

SNP mode of action is presumed to be dependent on Ag^+ ions that strongly inhibit bacterial growth through suppression of respiratory enzymes and electron transport components and through interference with DNA functions (Li et al. 2006). Silver in a nanometric scale (less than 100 nm) has strong toxicity to a wide range of microorganisms (Elechiguerra et al. 2005). Morones et al. (2005) defined the antibacterial activity of SNPs in four types of Gram-negative bacteria *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Salmonella typhi*, and suggested that SNPs attach to the surface of the cell membrane and disturb its function by penetrating into bacterial cell and release of silver ions. Silver nanoparticles have also been found to exert antibacterial activity against some drug-resistant bacteria (Birla et al. 2009; Inoue et al. 2010).

Chlorella vulgaris is a medicinal unicellular green microalgae, which contains more than 20 different vitamins and minerals, 19 of 22 amino acids and high content of

✉ Jayshree Annamalai
jayshreeannamalai@yahoo.com

¹ Centre of Advanced Study in Botany, School of Life Sciences, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

Inhibition effect of engineered silver nanoparticles to bloom forming cyanobacteria

Thi Thuy Duong¹, Thanh Son Le¹, Thi Thu Huong Tran²,
Trung Kien Nguyen¹, Cuong Tu Ho¹, Trong Hien Dao¹,
Thi Phuong Quynh Le³, Hoai Chau Nguyen¹, Dinh Kim Dang¹,
Thi Thu Huong Le⁴ and Phuong Thu Ha⁴

¹Institute of Environmental Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

²Faculty of Environment, Hanoi University of Mining and Geology, Hanoi, Vietnam

³Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

⁴Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

E-mail: duongthuy@ietvn.vn and thuhp@ims.vast.ac.vn

Received 21 May 2016

Accepted for publication 24 June 2016

Published 24 August 2016



CrossMark

Abstract


Silver nanoparticle (AgNP) has a wide range antibacterial effect and is extensively used in different aspects of medicine, food storage, household products, disinfectants, biomonitoring and environmental remediation etc. In the present study, we examined the growth inhibition effect of engineered silver nanoparticles against bloom forming cyanobacterial *M. aeruginosa* strain. AgNPs were synthesized by a chemical reduction method at room temperature and UV-Vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscope (TEM) showed that they presented a maximum absorption at 410 nm and size range between 10 and 18 nm. *M. aeruginosa* cells exposed during 10 d to AgNPs to a range of concentrations from 0 to 1 mg l⁻¹. The changes in cell density and morphology were used to measure the responses of the *M. aeruginosa* to AgNPs. The control and treatment units had a significant difference in terms of cell density and growth inhibition ($p < 0.05$). Increasing the concentration of AgNPs, a reduction of the cell growths in all treatment was observed. The inhibition efficiency was reached 98.7% at higher concentration of AgNPs nanoparticles. The term half maximal effective concentration (EC50) based on the cell growth measured by absorbance at 680 nm (A680) was 0.0075 mg l⁻¹. The inhibition efficiency was 98.7% at high concentration of AgNPs (1 mg l⁻¹). Image of SEM and TEM reflected a shrunk and damaged cell wall indicating toxicity of silver nanoparticles toward *M. aeruginosa*.

Keywords: silver nanoparticle, electrochemical synthesis, chemical synthesis, cyanobacteria bloom, *Microcystis aeruginosa*

Classification numbers: 2.04, 4.00, 4.02, 5.08

1. Introduction

Nowadays, the occurrence of toxic cyanobacterial (CB) blooms in eutrophic freshwater, brackish and marine ecosystems is receiving increasing attention worldwide as a

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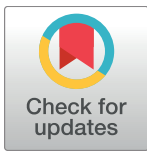
RESEARCH ARTICLE

Silver nanoparticles as a control agent against facades coated by aerial algae—A model study of *Apatococcus lobatus* (green algae)

Paulina Nowicka-Krawczyk^{1*}, Joanna Żelazna-Wieczorek¹, Tomasz Koźlecki²

1 Laboratory of Algology and Mycology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland, **2** Department of Chemical Engineering, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

* paulina.nowicka@biol.uni.lodz.pl



OPEN ACCESS

Citation: Nowicka-Krawczyk P, Żelazna-Wieczorek J, Koźlecki T (2017) Silver nanoparticles as a control agent against facades coated by aerial algae—A model study of *Apatococcus lobatus* (green algae). PLoS ONE 12(8): e0183276. <https://doi.org/10.1371/journal.pone.0183276>

Editor: Amitava Mukherjee, VIT University, INDIA

Received: May 13, 2017

Accepted: August 1, 2017

Published: August 14, 2017

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by National Centre for Research and Development in Poland, project AgNASIL (PBS3/B1/10/2015) is gratefully acknowledged. Publication is financially supported by Wrocław Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018, and by the University of Łódź task grant for young researchers no. B161100001139.02 (2016) and no

Abstract

Aerial algae are an important biological factor causing the biodegradation of building materials and facades. Conservation procedures aimed at the protection of historic and utility materials must be properly designed to avoid an increase of the degradation rate. The aim of the present study was to investigate the effect of silver nanoparticles (AgNP) synthesized with features contributing to the accessibility and toxicity (spherical shape, small size) on the most frequently occurring species of green algae in aerial biofilms and thus, the most common biodegradation factor—*Apatococcus lobatus*. Changes in the chloroplasts structure and the photosynthetic activity of the cells under AgNP exposure were made using confocal laser microscopy and digital image analysis and the estimation of growth inhibition rate was made using a biomass assay. In the majority of cases, treatment with AgNP caused a time and dose dependant degradation of chloroplasts and decrease in the photosynthetic activity of cells leading to the inhibition of aerial algae growth. However, some cases revealed an adaptive response of the cells. The response was induced by either a too low, or—after a short time—too high concentration of AgNP. Taken together, the data suggest that AgNP may be used as a biocide against aerial algal coatings; however, with a proper caution related to the concentration of the nanoparticles.

Introduction

Progress in research on the effects of silver nanoparticles (AgNP) on biological systems has led to the application of these molecules to the public and commercial use [1, 2]. Experiments on different bacterial and fungal strains [3–8] have proven that AgNP, used either solely or as a compound combined i.e. with titanium dioxide, have a biocidal effect on heterotrophic microorganisms [9–17]. AgNP penetrate bacterial, fungal and animal cells [18, 19] and interfere with membrane proteins, activating a biochemical cascade that leads to an inhibition of cell division [20]. When passing through the cell wall and plasma membrane by diffusion or endocytosis, nanoparticles cause a mitochondrial dysfunction resulting in an increase of the reactive

Biosynthesis characterization of silver nanoparticles using *Cassia roxburghii* DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity

Pannerselvam
Balashanmugam
Pudupalayam Thangavelu
Kalaichelvan

Centre for Advanced Studies in
Botany, University of Madras, Chennai,
India

Abstract: The present study reports the green synthesis of silver nanoparticles (AgNPs) from silver precursor using a plant biomaterial, *Cassia roxburghii* DC., aqueous extract. The AgNPs were synthesized from the shade-dried leaf extract and assessed for their stability; they elucidated characteristics under UV-visible spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, high-resolution transmission electron microscopy, and energy dispersive X-ray spectroscopy. The synthesized AgNPs exhibited a maximum absorption at 430 nm, and the X-ray diffraction patterns showed that they were crystal in nature. Fourier transform infrared spectroscopy analysis confirmed the conversion of Ag⁺ ions to AgNPs due to the reduction by capping material of plant extract. The HR-TEM analysis revealed that they are spherical ranging from 10 nm to 30 nm. The spot EDAX analysis showed the presence of silver atoms. In addition, AgNPs were evaluated for their antibacterial activity against six different pathogenic bacteria: three Gram-positive bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus*, and three Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter aerogenes*. They were highly sensitive to AgNPs, whereas less sensitive to AgNO₃. Furthermore, the green synthesized AgNPs were immobilized on cotton fabrics and screened for antibacterial activity. The immobilized AgNPs on cotton cloth showed high antibacterial activity. Therefore, they could be a feasible alternative source in treating wounds or may help in replacing pharmaceutical band-aids.

Keywords: bioreduction, stability, immobilization, cotton cloth, minimum inhibitory concentration

Introduction

Nanomaterials are at the leading edge of the rapidly developing field in nanotechnology. Their unique size makes them superior and indispensable in many areas of human activity. Nanotechnology encompasses the production and application of physical, chemical, and biological systems at scales ranging from individual atoms or molecules to submicron dimensions, as well as the integration of the resulting nanostructures into larger systems. Science and technology research in nanotechnology promises breakthroughs in areas such as materials and manufacturing, nanoelectronics, medicine and health care, energy, biotechnology, and information technology. Nanoparticles are usually referred to as the particles with a maximum size of 100 nm. Nanoparticles exhibit new properties when compared to larger particles of the bulk material. The novel properties are derived due to the variations in specific characteristics, such as size, distribution, and morphology of particles. The nanoparticles have a wide range of applications, such as in combating microbes, drug delivery, catalysis,¹ water purification,² treatment

Correspondence: Pannerselvam
Balashanmugam
Centre for Advanced Studies in Botany,
University of Madras, Guindy Campus,
Chennai 600 025, India
Tel +91 999 463 3730
Email biobala17@gmail.com



Biosynthesis of silver nanoparticles using *Ulva fasciata* (Delile) ethyl acetate extract and its activity against *Xanthomonas campestris* pv. *malvacearum*

S. Rajesh¹, D. Patric Raja², J.M. Rathi³ and K. Sahayaraj^{1*}

ABSTRACT

Metallic nanoparticles have been traditionally synthesized using wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this research, we present a simple and ecofriendly biosynthesis of Ag nanoparticles using *Ulva fasciata* crude ethyl acetate extract as reducing and capping agent. The bionanoparticle characterized with UV-vis Spectroscopy, FTIR, XRD, SEM and EDX. Characterization reveals that the nanoparticles are crystalline in nature, spherical in shape and poly-dispersed with size ranging from 28 to 41 nm. The alkyne group (3424.30 cm^{-1}) of *U. fasciata* crude ethyl acetate extract shifted and reduced the AgNO_3 . GC-MS analysis revealed the presence of 1-(Hydroxymethyl)-2, 5, 5, 8A-tetramethyl decahydro-2-naphthalenol as reducing agent and hexadecanoic acid was found to be a stabilizing agent. *Ulva fasciata* based bionanoparticles inhibited the growth of *Xanthomonas campestris* pv. *malvacearum* (14.00 ± 0.58 mm Zone of inhibition), with a Minimum Inhibitory Concentration of $40.00 \pm 5.77 \mu\text{g/mL}$. The study shows that *U. fasciata* crude ethyl acetate extract could be used as a reducing agent for simple ecofriendly synthesis of silver nanoparticles. However, more studies are essential before recommending them for disease management.

Key words: Antibacterial, EDX, FTIR, MIC, silver nanoparticles, SEM, *Ulva fasciata*, *Xanthomonas campestris* pv *malvacearum*, XRD

INTRODUCTION

Silver has been used for many years for its antimicrobial properties. Alexander the Great used silver vessels to store drinking water (Silver *et al.*, 2006). However, the formulation of silver has changed during antiquity, from bulk silver to ionic silver or adsorbed on carrier materials (Zeolite) (Kwakye-Awwah *et al.*, 2008) and now to silver nanoparticles. In order to advance nanotechnology for antimicrobial applications, development of methods to understand and control the behavior of nanomaterials is needed. A nanomaterial may be defined as any material (insulator, conductor or semiconductor), which has been controllably synthesized on the size range of roughly 1 to 100 nm. At this size and dimensional range, essentially any material will exhibit different properties from those it would as an atomic cluster or as the larger bulk materials.

Production of metallic nanoparticles can be achieved through chemical, physical or biological methods. Chemical approaches are the popular mode of synthesis of nanoparticles. However; these methods cannot avoid the use of toxic chemicals. Now-a-days biological synthesis of metallic nanoparticles is gaining importance as it is reliable

and ecofriendly. Previous literature revealed that the nanoparticle synthesis using algae as source has been unexplored and underexploited. Recently there are a few reports that algae is being used as a biofactory for synthesis of metallic nanoparticles. Singaravelu *et al.* (2007), Rajasulochana *et al.* (2010) and Vivek *et al.* (2011) reported the synthesis of silver bionanoparticles using *Sargassum wightii*, *Kappaphycus alvarezii* and *Gelidiella acerosa* crude extracts, respectively. Govindaraju *et al.* (2008) reported the synthesis of silver nanoparticles using microalgae, *Spirulina platensis*. However, no report was available about the synthesis of nanomaterials using the ethyl acetate from marine algae. On the other hand there are numerous works related to green synthesis of metallic nanoparticles using higher plants. Gardea-Torresday *et al.* (2003) first reported the formation of gold and silver nanoparticles by living plants. Shankar *et al.* (2004) reported pure metallic silver and gold nanoparticles synthesis by the reduction of Ag^+ and Au^+ ions using neem (*Azadirachta indica*) leaf broth. There have been recent reports on phytosynthesis of silver and gold nanoparticles by employing lemon grass extract (Shankar *et al.*, 2004, 2005), *Sesbaniadrammandii* (Sharma *et al.*, 2007), green tea (*Camellia*

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S. Rajesh¹, D. Patric Raja², J.M. Rathi³ and K. Sahayaraj^{1*}

¹Crop Protection Research Centre, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India, *Telephone: +91 462 4264376; Fax: +91 462 256 1765; Email: ksraj42@gmail.com

²Department of Plant Biology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

³Department of Chemistry, St. Mary's College (Autonomous), Thoothukudi – 628 002, Tamil Nadu, India.

Received: November 28, 2011

Revised: March 17, 2012

Accepted: March 26, 2012



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MARINE BROWN ALGAE *TURBINARIA CONOIDES* AND ITS ANTIBACTERIAL ACTIVITY

SHANMUGAM RAJESHKUMAR^{1,2}, CHELLAPANDIAN KANNAN² AND GURUSAMY ANNADURAI¹

¹*Environmental Nanotechnology Division Sri Paramakalyani Centre for Environmental Sciences
Manonmaniam Sundaranar University, Alwarkurichi – 627 412, Tirunelveli,
Tamil Nadu, India*

²*Department of Chemistry Manonmaniam Sundaranar University, Abishekapatti – 627 012, Tirunelveli,
Tamil Nadu, India.*

ABSTRACT

Improvement of eco-friendly procedures for the nanoparticles synthesis is the growing in to the field of nanotechnology. In this report we used the extract of marine brown seaweed *Turbinaria conoides* for the silver nanoparticles synthesis is the new one, cost effective and environmentally favorable. The surface plasmon resonance band positioned at 420 nm for silver nanoparticles was characterized by UV-Visible Spectrophotometer. X-Ray diffraction (XRD) data illustrated the crystalline nature of silver nanoparticles. Scanning electron microscope (SEM) shows the spherical shaped with the average size of 96 nm. The possible biomolecules are amines and poly phenols may responsible for reduction of silver ions was identified through Fourier transmittance infrared spectroscopy (FT-IR). Further these biosynthesized silver nanoparticles were found to be highly toxic against gram positive bacteria *Bacillus subtilis* (MTCC3053) and gram negative bacteria *Klebsiella planticola* (MTCC2277) was analyzed by zone of inhibition.

KEYWORDS: *Turbinaria conoides*, Silver nanoparticles, Scanning electron microscope, Antimicrobial activity



GURUSAMY ANNADURAI

Centre for Environmental Sciences Manonmaniam Sundaranar University, Alwarkurichi –
627 412, Tirunelveli, Tamil Nadu, India

*Corresponding author



Review

Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications

Sadia Saif ^{1,2,*}, Arifa Tahir ¹ and Yongsheng Chen ^{2,*}

¹ Department of Environmental Science, Lahore College for Women University, Lahore 54000, Pakistan; arifa.tahir@gmail.com

² School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

* Correspondence: sadia.saifpk@gmail.com (S.S.); yongsheng.chen@ce.gatech.edu (Y.C.); Tel.: +92-332-4543310 (S.S.); +1-404-894-3089 (Y.C.)

Academic Editor: Thomas Nann

Received: 12 August 2016; Accepted: 7 November 2016; Published: 12 November 2016

Abstract: Recent advances in nanoscience and nanotechnology have also led to the development of novel nanomaterials, which ultimately increase potential health and environmental hazards. Interest in developing environmentally benign procedures for the synthesis of metallic nanoparticles has been increased. The purpose is to minimize the negative impacts of synthetic procedures, their accompanying chemicals and derivative compounds. The exploitation of different biomaterials for the synthesis of nanoparticles is considered a valuable approach in green nanotechnology. Biological resources such as bacteria, algae fungi and plants have been used for the production of low-cost, energy-efficient, and nontoxic environmental friendly metallic nanoparticles. This review provides an overview of various reports of green synthesised zero valent metallic iron (ZVMI) and iron oxide (Fe₂O₃/Fe₃O₄) nanoparticles (NPs) and highlights their substantial applications in environmental pollution control. This review also summarizes the ecotoxicological impacts of green synthesised iron nanoparticles opposed to non-green synthesised iron nanoparticles.

Keywords: iron nanomaterials; sustainable green nanotechnology; environmental pollution; environmental toxicology

1. Introduction

Nanotechnology is the ability to measure, see, manipulate and manufacture things on an atomic or molecular scale, usually between one and 100 nanometres. These tiny products also have a large surface area to volume ratio, which is their most important feature responsible for the widespread use of nanomaterials in mechanics, optics, electronics, biotechnology, microbiology, environmental remediation, medicine, numerous engineering fields and material science [1]. Different protocols have been designed for the production of metallic nanoparticles. Currently, two main approaches are used to synthesize nanoparticles, referred to as the top-down and bottom-up approaches. Briefly, in the top-down approach, nanoparticles are produced by size reduction of bulk material by lithographic techniques and by mechanical techniques such as machining and grinding, etc., while, in bottom-up approach, small building blocks are assembled into a larger structure, e.g., chemical synthesis [2]. However, the most acceptable and effective approach for nanoparticle preparation is the bottom-up approach, where a nanoparticle is “grown” from simpler molecules known as reaction precursors. In this way, it is likely possible to control the size and shape of the nanoparticle depending on the subsequent application through variation in precursor concentrations and reaction conditions (temperature, pH, etc.) [3].

Physical and chemical methods are being used extensively for production of metal and metal oxide nanoparticles. However, this production requires the use of very reactive and toxic reducing agents