Biochemistry and Biophysics Reports 14 (2018) 69–77

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Synthesis, characterization and evaluation of antimicrobial efficacy and brine shrimp lethality assay of *Alstonia scholaris* stem bark extract mediated ZnONPs

Nookala Supraja[a], T.N.V.K.V. Prasad[b], Arumugam Dhanesh Gandhi[a], Devipriya Anbumani[a], P. Kavitha[c], Ranganathan Babujanarthanam[a,*]

[a] Department of Biotechnology, Thiruvalluvar University, Serkkadu, Vellore 632115, Tamil Nadu, India
[b] Acharya N G Ranga Agricultural University, Nanotechnology Laboratory, RARS, Tirupati 517502, Andhra Pradesh, India
[c] Department of Biochemistry, Sri Abirami College for Women, Gudiyattam 632803, Tamil Nadu, India

ARTICLE INFO

Keywords:
Zinc oxide nanoparticles
Fungal *sp*
Alstonia scholaris bark extract
Gram negative and Gram positive bacteria
Brine shrimp assay

ABSTRACT

Alstonia scholaris is one of the most important medicinal plants and herein, we present the synthesis of zinc oxide nanoparticles using the bark extract of *Alstonia scholaris,* and evaluation of their antimicrobial efficacy. Stable ZnO nanoparticles were formed by treating 90 mL of 1 mM zinc nitrate aqueous solution with 10 mL of 10% bark extract. The formation of *Alstonia scholaris* bark extract mediated zinc oxide nanoparticles was confirmed by UV–visible spectroscopic analysis and recorded the localized surface plasmon resonance (LSPR) at 430 nm. Fourier transform infrared spectroscopic (FT-IR) analysis revealed that primary and secondary amine groups in combination with the proteins present in the bark extract is responsible for the reduction and stabilization of the ZnONPs. The crystalline phase of the nanocrystals was determined by XRD analysis and morphology was studied using transmission electron microscopy (TEM). The hydrodynamic diameter (26.2 nm) and a positive zeta potential (43.0 mV) were measured using the dynamic light scattering technique. The antimicrobial activity of *Alstonia scholaris* ZnONPs was evaluated (*in-vitro*) using disc diffusion method against fungi, Gram-negative and Gram-positive bacteria which were isolated from the biofilm formed in drinking water PVC pipelines. The results obtained suggested that ZnO nanoparticles exhibit a good anti-fungal activity than bactericidal effect towards all pathogens tested in *in-vitro* disc diffusion method (170 ppm, 100 ppm and 50 ppm). Further, the toxicity of biosynthesized ZnONPs was tested against *Alstonia scholaris* to evaluate the cytotoxic effect that displayed LC_{50} value of 95% confidence intervals.

1. Introduction

Nanobiotechnology, a branch of nanoscience has been playing a decisive role in 21st century in deciphering diverse tribulations particularly in the fields of farming, medicine and electronics. Nanoscience poses a basic scientific challenge as it requires a control over the connections between atoms. All physiochemical methods of nanoparticles synthesis are having inherent limitations up to a certain extent which impose an important hurdle in the maturation of this science. The possibility of utilizing biological materials for nanoparticles synthesis has appeared as the most efficient and greener approach [1]. Nanomaterials exhibit unique and considerably changed physical, chemical, and biological properties compared to their bulk counterparts [2]. Although physical and chemical methods [3] are more popular for nanoparticle synthesis, the use of toxic compounds limits their applications [4]. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for production of metallic nanoparticles [5]. Green synthesis of metallic nanoparticles from plants [6] is been an interesting aspect as the process is ecofriendly and non-toxic. Plant and plant materials have become potential sources for the synthesis of metallic nanoparticles recently. A number of researchers have reported on synthesis of metallic nanoparticles including silver [7], gold [8], titanium dioxide [9], tungsten oxide [10], and copper oxide [11] using different plant materials.

Due to the amenability to biological functionalization, the biological nanoparticles are finding important applications in the field of medicine. The antimicrobial potential of metal based nanoparticles has led to its incorporation in consumer, health-related and industrial products. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. The discovery, development

* Corresponding author.
E-mail address: babukmg@gmail.com (R. Babujanarthanam).

https://doi.org/10.1016/j.bbrep.2018.04.004
Received 26 January 2018; Received in revised form 6 March 2018; Accepted 12 April 2018

and clinical use of antimicrobials during the 20th century have substantially reduced mortality from microbial (bacterial, fungal, viral and parasitic) infections. An antimicrobial kills the microorganisms and inhibits their growth. They are classed according to their function as anti-bacterial, anti-fungal, anti-viral and anti-parasitic. Antimicrobials that kill microbes are called microbicidal and those inhibit their growth are called micro biostatic. The use of higher plants and their preparation to treat infectious and non-infectious disease is an age old practices and are the only method available in the past. Though the use of natural sources like plant material for curing diverse forms of ailments leads to human civilization, the scientific analysis of different natural sources for their possible medicinal potency is comparatively recent origin. The emergence and spread of antibiotic resistance microorganisms triggered the search of new materials through diverse sources including investigations on plants. Higher plants can serve as both potential antimicrobial crude drugs and a source of new anti-infective agents. Like many other medicinal plants, *Alstonia scholaris* (L.) R.Br. (Apocynaceae) is an evergreen tropical tree native to Indian subcontinent and South East Asia having grayish rough bark and milky sap rich in poisonous alkaloid. The bark also called dita bark is traditionally used by many ethnic groups of Northeast India and also other parts of the world as an antimicrobial agent against fungal infections, malarial fever, toothache, rheumatism, snake bite, dysentery, bowl disorder, etc., and the latex is used in treating coughs, through sores and fever [12,13]. Among the several genera of *Alstonia*, only *scholaris* species has been studied for antimicrobial potency [14]. Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [15]. Many attempts have been made to use silver nanoparticles as an anticancer agent, and they have all turned up positive [16,17]. The role of ZnO nanoparticles as an anticancer agent should open new doors in the field of medicine.

However, hardly reports are available on antimicrobial activity of zinc nanoparticles synthesized using the aqueous extract of this plant. There are no reports on antibacterial, antifungal activity and brine shrimp assay of aqueous extract of this *Alstonia scholaris* plant through Zinc oxide nanoparticles. The present work therefore, attempts to evaluate the antimicrobial activity of the aqueous extract towards pathogenic bacteria and fungi by *in-vitro* disc diffusion method.

2. Materials and methods

2.1. Chemicals

Zinc nitrate (> 99% pure) was purchased from Sigma Aldrich, India. Potato dextrose broth, Potato dextrose agar, Nutrient broth, Nutrient agar plate, was supplied by Hi-media, India.

2.2. Collection of biofilm formed in poly vinyl chloride (PVC) pipes

The PVC Biofilm samples were collected from four different regions located in and around Tirupati, (Chittoor District) Andhra Pradesh, India. The samples were collected from drinking water PVC pipelines and taken in the sterile container. The collected samples were in amorphous form. These samples were stored in an ice box for further characterization.

2.3. Collection of plant material

Healthy plant of *Alstonia scholaris* was collected from Mumbai, Maharashtra, India. The identity of the plant was confirmed by Agarkar Research Institute, Pune, India. A voucher specimen (No. AHMA-23537) has been deposited for future reference. From the selected plant bark was collected by scrapping the trunk using neat and clean knife during the month of May, 2016 and collected material was carefully washed and dried at 45 °C to constant weight. The dried bark of plant material were powdered, passed through a BSS no. 85-mesh sieve and stored in air tight container.

Fig. 1. Photograph showing *Alstonia scholaris* stem bark.

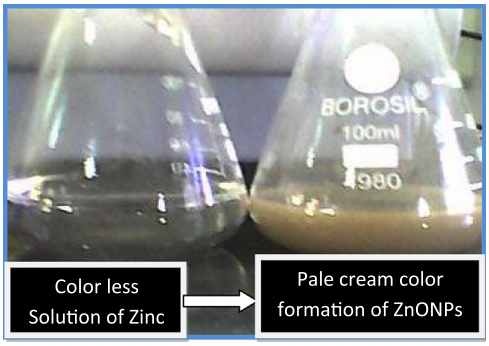

Fig. 2. Synthesis of Zinc oxide nanoparticles by *Alstonia scholaris* stems bark.

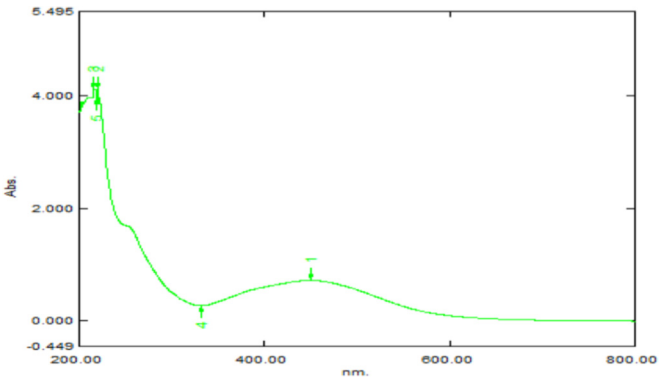

Fig. 3. UV-visible spectroscopic micrograph showing the localized surface plasmon resonance (LSPR) of ZnO nanoparticles synthesized using *Alstonia scholaris* bark extract.

2.4. Preparation of aqueous bark extract

The collected *Alstonia scholaris* bark was allowed to shade dried for 48 h and was ground to get fine powder. Then, 10 g of powder was mixed with 100 mL of distilled water and boiled for 40 min. After that, the extract was filtered by using Whatman No. 1 filter paper and collected the filtrate in plastic bottle and stored at 4 °C for further characterization and experimentation.

2.5. Isolation of fungal and bacterial sp. from drinking water pipeline

Eight fungal species and ten bacterial samples were isolated from drinking water supply PVC pipelines in Tirupati, Chittoor district, AP, India. Through serial dilution pour plate technique, fungal sp. was isolated using potato dextrose agar (PDA) medium and Gram-negative and Gram-positive bacteria were isolated from nutrient agar medium. Further, it is maintained in potato dextrose agar slants (fungi) and