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# Structural studies of a water insoluble β-glucan from *Pleurotus djamor* and its cytotoxic effect against PA1, ovarian carcinoma cells

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ABSTRACT

A water insoluble β-glucan (PS), with molecular mass ~9.16 × $10^4$ Da was isolated from the 4% alkaline extract of an edible mushroom, *Pleurotus djamor* and found to consist of (1→3)-β-D-glucopyranosyl moiety. The structure of the PS was elucidated on the basis of total hydrolysis, methylation analysis, periodate oxidation, and NMR experiments ($^1$H, $^{13}$C, DQF-COSY, DEPT-135, and HSQC). The structure of the repeating unit of the polysaccharide was established as:

→3)-β-D-Glcp-(1→.

The water insoluble β-glucan showed cytotoxic effect against PA1 cells, where ~50% population was destroyed at 100 μg/mL concentration, and almost all cells at 250 μg/mL concentration. The wound healing assay showed significant anticarcinogenic effect against ovarian carcinoma PA1 cells after 48 h of treatment.

## 1. Introduction

Edible mushrooms provide a significant source of nutritional as well as medicinal compounds and used for the development of drugs (Breene, 1990; Moradali, Mostafavi, Ghods, & Hedjaroude, 2007; Wang et al., 2014). Mainly, edible mushrooms are consumed by the people as delicious food ingredient and measured as a source of biomolecules such as proteins, fats, carbohydrates, amino acids, vitamins, minerals but the energy value of the mushroom varies for different species (Bello & Akinyele, 2007). Edible mushrooms are recognized as not only functional foods but also their bioactive compounds that have various valuable impacts on human health (Khan et al., 2016; Ren, Perera, & Hemar, 2012). Among the different bioactive polysaccharides, β-glucan is the most important as functional compounds in mushrooms (De Silva et al., 2013). Generally, β-glucans are a long chain polymers of glucose units linked by α- (alpha) and β- (beta) type glycosidic bonds present in the cell wall of oat, barley, yeast and mushrooms (Brown & Gordon, 2003; Chan, Chan, & Sze, 2009). It was found that β-glucans from different sources have different linkage types, branching manners and molecular weights (Du, Bian, & Xu, 2014). The structural diversity of mushroom β-glucans are linked by only β-(1→3) and linear β-(1→3), (1→6) linkages (Misaki & Kakuta, 1995; Ojha, Chandra, Ghosh, & Islam, 2010; Synytsya et al., 2009) and nonlinear form with β-(1→3) back bone branched at *O*-6 (Mizuno et al., 1990, Rout, Mondal, Chakraborty, & Islam, 2008), and β-(1→6) backbone branched at *O*-3 (Maji et al., 2012; Sen et al., 2013).

However, the β-D-glucans are biologically important polysaccharide for enhancing and stimulating the immune systems of human body (Blascheck, Kasbauer, Kraus, & Franz, 1992; Chan et al., 2009; Kidd, 2000; Kiho, Sakushima, Wang, Nagai, & Ukai, 1991; Kulicke, Lettau, & Thielking, 1997; Smiderle et al., 2008). They have antitumor (Wasser, 2002) as well as antioxidant activities (Blokhina, Virolainen, & Fagerstedt, 2003; Kofuji et al., 2012; Papas, 1998). Several mushroom polysaccharides such as Lentinan (from *Lentinus edodes*, Japan), Schizophyllan (from *Schizophyllum commune),* Krestin (from turkey tail mushroom *Trametes versicolor*), Agarican (from *Agaricus blazei*, USA), and Grifron-D (from *Grifola frondosa*, Japan) have been established as pharmaceutical agents and anticancer drugs (Chihara, Maeda, Hamuro,

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# Bioactive polysaccharides from natural sources: A review on the antitumor and immunomodulating activities

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ABSTRACT

Polysaccharides are a structurally diverse group of biological macromolecules of well-known occurrence in nature. The mushroom, plant and other polysaccharides draw a lot of attention due to their several difficult biological properties, such as, anticancer, antiviral, immunomodulating, antimicrobial, anticoagulant, antidiabetic, antioxidant, and antitumor activities. Several bioactive glucans and heteroglycans were isolated from different mushroom, plant and bacterial cell wall. Polysaccharides have highest ability for carrying biological information comparison with other biopolymers such as proteins and nucleic acids due to the structural variability. It is the focus of this review to bring together the available knowledge of the structure, and function of the different polysaccharides of the mushroom, plant and bacterial cell wall.

## 1. Introduction

The great bulk of the carbohydrates in nature are present as polysaccharides, which have relatively large molecular weights (Maity et al., 2014a, 2015; Xu et al., 2016). Polysaccharides have been produced as the first biopolymer on Earth (Tolstoguzov, 2004). These biopolymers are complex carbohydrates and made up of monosaccharides joined together by glycosidic linkages (Maity et al., 2014b; Nandi et al., 2014; Shakhmatov et al., 2016). Their structures may be linear or they may contain various degrees of branching (Bhanja et al., 2013; Manna et al., 2017; Patra et al., 2012a). The high structural diversity reflects the functional diversity of these molecules (Maity et al., 2017; Meng et al., 2014; Wang et al., 2016). There is a clear correlation between allowed conformations and linking pattern (Li et al., 2018). For example, the chains in amylose tend to adopt single coiled helical (D.E.C. Cambridge, 2013) conformations while some $(1\rightarrow3)$-, $(1\rightarrow6)$-β-D-glucan chains adopt triple helical (Giese et al., 2013) conformation.

Polysaccharides exist in an enormous structural diversity as they are produced by a vast variety of species; including microbes, algae, plants, and animals (Denman and Morris, 2015; Ji et al., 2003; Ghorai et al., 2009; Kanagasabapathy et al., 2011; Li et al., 2017a; Wu et al., 2006). They are able to offer the highest capacity for carrying biological information because they have the greatest potential for structural variability (Liu et al., 2014; Popov et al., 2007). Polysaccharide related technologies have played a leading role in the development of a wide range of products that include foods, pharmaceuticals, textiles, papers and biodegradable packaging materials (Licht et al., 2010; Wu et al., 2016a,b). The medicinal properties of mushrooms and plants have been confirmed through an intensive research conducted worldwide (Fan et al., 2012; Jiang et al., 2015; Oliveira et al., 2019). Different type of antioxidant, antitumor and immunomodulating polysaccharides were isolated from edible mushrooms, bacterial cell wall, and plants ((Bhanja et al., 2012; Feng et al., 2016; Mandal et al., 2015; Patra et al., 2012b; Patra et al., 2013; Smiderle et al., 2008; Siu et al., 2016). These polysaccharides do not directly attack to the cancer cells. They generate their antitumor effect indirectly, through stimulation of various defensive

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Research Article

# GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MANGROVE FRUIT POLYSACCHARIDE FOR BACTERIAL GROWTH INHIBITION

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**ABSTRACT**

**Objective:** The objective of this study was to find out the antibacterial activity of the silver nanoparticles (Ag-NPs) using a low-cost green synthesis approach for the formulation of Ag-NPs applying polysaccharide extracted from the fruits of a mangrove plant of Sundarban.

**Methods:** Fresh and healthy fruits were collected from *Ceriops decandra* plant. Sufficient amount of carbohydrates was extracted from those fruits and the physicochemical characterization of the polysaccharide was analyzed by gas chromatography–mass spectrometry and Fourier-transform infrared spectrophotometry. The respective polysaccharide was further applied to generate the Ag-NPs which were characterized by UV visible, dynamic light scattering, transmission electron microscopy, EDAX, and X-ray diffraction. The antibacterial efficacy of the Ag-NPs was also determined against some pathogenic Gram-negative and Gram-positive bacteria using the microdilution method.

**Results:** Glucose and galactose are the major monomers among the extracted carbohydrates. Various types of spectral analysis confirmed the formation of Ag-NPs. The green synthesized Ag-NPs have the average diameter of about 28 nm. Furthermore, the green synthesized Ag-NPs exhibited strong antibacterial activity against some pathogenic Gram-positive (*L. cytomonogenes, Bacillus Subtilis,* and *Staphylococcus aureus*) and Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria.

**Conclusion:** The green synthesis of Ag-NPs using plant polysaccharide was an environment-friendly and cost-effective method as compared to the conventional physical and chemical synthesis techniques.

**Keywords:** Green synthesis, *Ceriops decandra,* Polysaccharide, Silver nanoparticle, Bacterial growth.

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## INTRODUCTION

Since 1000 B.C., Ag has been applied for the treatment of burns, wounds, and several bacterial infections in the form of metallic silver, silver nitrate ($AgNO_3$), as well as silver sulfadiazine. However, the use of silver compounds in medicinal systems declined to a great extent after the introduction of penicillin during the 1940s [1]. However, over the recent past, the field of nanotechnology has introduced a new era of science which involves the creation of materials near-atomic scale with unique chemical, physical, thermal, and optical properties [2]. As a consequence, metallic silver made a remarkable comeback in the form of Ag-NP with powerful antimicrobial effects. Investigations have revealed that after exposure to bacteria, the nano-silver gets attached to the cell membrane, penetrates inside, and attacks respiratory chain that ultimately leads to cell death. In addition, these particles release silver ions inside cells which inhibit bacterial replication ability and cause the deactivation of proteins containing thiol groups [1,3]. This has unfolded novel strategies to use pure silver against a wide range of antibiotic-resistant microorganisms, and as a result, it has been adopted in many commercial products such as topical ointments, toothpaste, soap, and socks [4,5]. In addition, Ag-NP has also emerged up as a promising agent for wastewater purification system [6]. In recent work, the use of silver compounds as antimicrobial compounds against coliform bacteria found in wastewater has been reported [7].

So far, a number of techniques are available for Ag-NP synthesis; although most of them are expensive, complicated, and involve the use of hazardous chemicals [8]. Conversely, the biological techniques are considered as an alternative and advancement over other methods as it involves natural reagents such as sugars, biodegradable polymers, plant extracts, and microorganisms [4,9-19]. In this context, the mangrove plant, *Ceriops decandra* (Griff.) W. Theob., could be an effective alternative as it was scientifically proved to contain several bioactive components. It has been traditionally used as remedial measures for hepatitis, diabetes, wounds, ulcers, boils, angina, dysentery, and diarrhea [20-23]. Recent investigations have reported that *C. decandra* possesses antioxidant [24], antinociceptive [25], antidiabetic [26], and antimicrobial properties [27-30]. Thus, the present work was aimed for the synthesis of Ag-NP by a greener method using polysaccharide extracted from fruits of *C. decandra*, characterization of the particles, and assessment of antibacterial efficacy against some pathogenic microorganisms.

## MATERIALS AND METHODS

### Plant material

The plant parts of *C. decandra* (Griff.) W. Theob. were collected from the mangrove forest of Sundarban (21.9497° N, 89.1833° E) of the district South 24 Parganas, West Bengal, India. The respective plant specimen was self-identified and binomially jointly by the Department of Botany,

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# EFFICACY OF ANTIMICROBIAL ACTIVITY OF GREEN SYNTHESIZED AG-NANOPARTICLES USING POLYSACCHARIDE ISOLATED FROM THE LEAVES OF *HERITIERA FOMES*

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***Key words:*** *Ag-nanoparticles, Polysaccharide, Antimicrobial activity, Heritiera fomes*

**Abstract –** The synthesis of silver nano particles was done by using the polysaccharide from leaf extract of *Heritiera fomes* and $AgNO_3$. Thus synthesized Ag-nanoparticles are characterized by using UV- VIS spectrum band at 446 nm. Here, the polysaccharide acts as reducing and also stabilizing agent. The synthesized Ag-nanoparticles were found as very effective an antimicrobial agent against some important human pathogens such as *S. typhi. S. typhimurium, Pseudomonas* sp. These Ag-nanoparticle can also reduce the microbial load by destroying the microorganisms present in sewage water .

## INTRODUCTION

In recent years, metal nanoparticles have wide ranging implications in a variety of areas, including physics, chemistry, electronics, optics, materials science, the biochemical sciences, catalytic and magnetic field for their unusual properties (Cao, *et al.*, 2001; Hayward *et al.*, 2000; Pradhan and Pal, 2001, 2002; Zeng *et al.*, 2007; Fendler, 1987; Schmid, 1992; Kamat, 1993). There are various methods for nanoparticles formation such as sol-process, micelle, sol-gel process, chemical precipitation, hydrothermal method, pyrolysis, chemical vapour deposition, bio-based protocols etc. A common method for the preparation of metal nanoparticles involves the treatment of nobel metal (Ag, Pt, Au, and Pd) salt with a chemical reducing agent such as citrate salt, borohydride, formaldehyde, and solvent like N,N-dimethyl formamide (DMF), ethylene glycol or other organic compounds (Leela, *et al.*, 2008; Chou *et al.*, 2005; Schukin *et al.*, 2003; Khanna *et al.*, 2003; Pastoriza-Santos and Liz-Marzan, 1999; Rivas, *et al.*, 2001; Zeng *et al.*, 2007). In spite of the novel properties exhibited by the metal nanoparticles due to quantum size effects, their synthesis protocol poses a major environmental problem (Lewis, 1993; Alivisators, 1996). Use of nontoxic chemicals, environmentally benign solvents, and renewable materials are some of the key issues that merit important consideration in a green synthetic strategy (Anastas and Warner, 1998; Raveendran *et al.*, 2003; Poliakoff *et al.*, 2001; De Simone, 2002). Nowadays biomolecules and living organisms are useful sources for the synthesis of these nanomaterials where they act as both reducing and stabilizing agents (Esumi *et al.*, 2003; Mucic *et al.*, 1998). Huang *et al.*, 2007). Among the nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine (Parashar, *et al.*, 2009). Silver nanoparticles (Ag NPs) having high specific surface area and high fraction of surface atoms are gaining importance as an antimicrobial agent against several antibiotic-resistant bacterial strains (Chastre, 2008; Lara *et al.*, 2010). Among many possible natural products, polysaccharides represent an excellent scaffold for this purpose. The use of polysaccharides like starch (Vigneshwaran *et al.*, 2006) and chitosan (Huang *et al.*, 2004) for the synthesis of Ag NPs has been reported in recent years. So far, there have been no reports on the synthesis of nanoparticles by using leaves of *Heritiera fomes* [sundari]. Therefore, the

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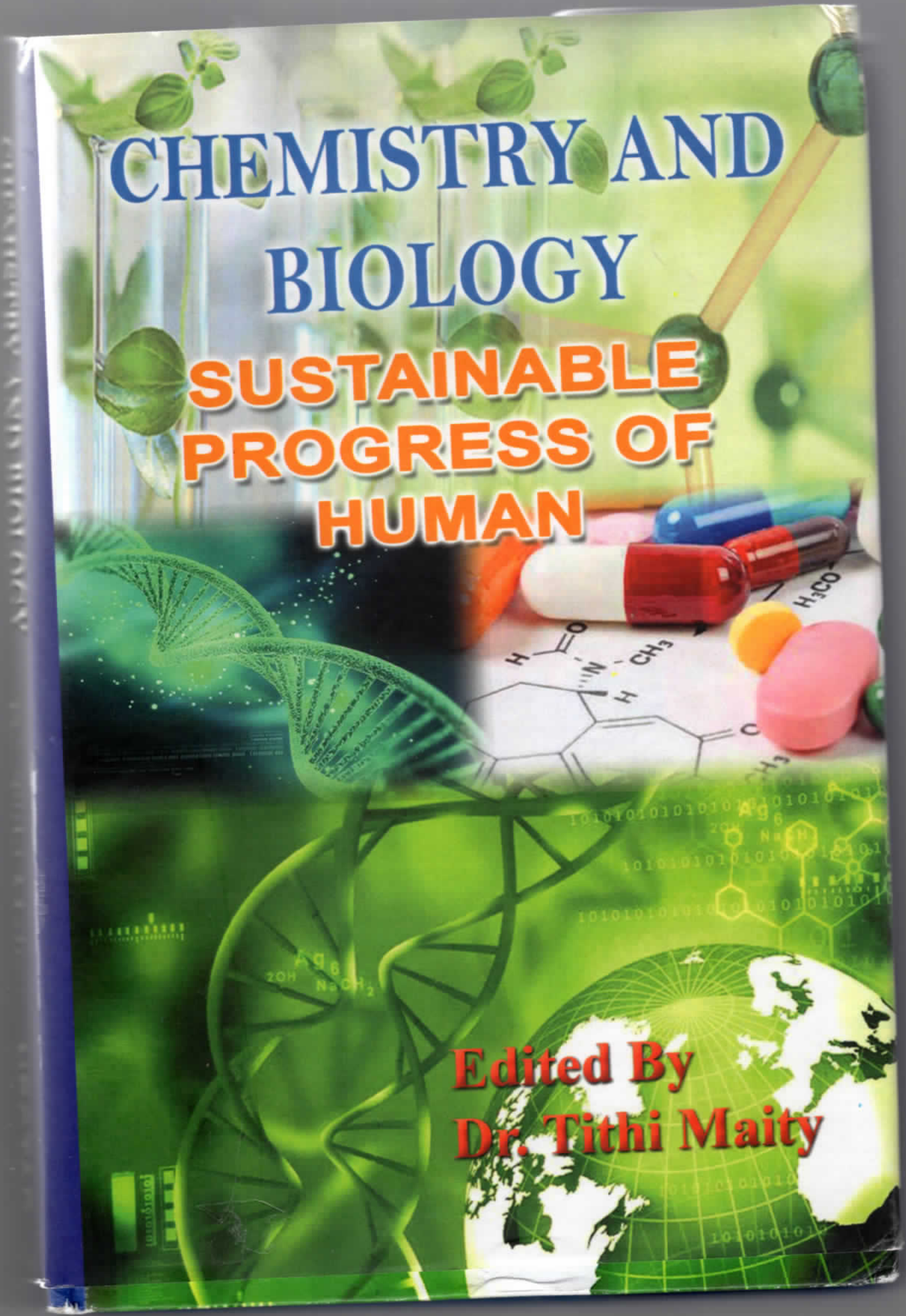
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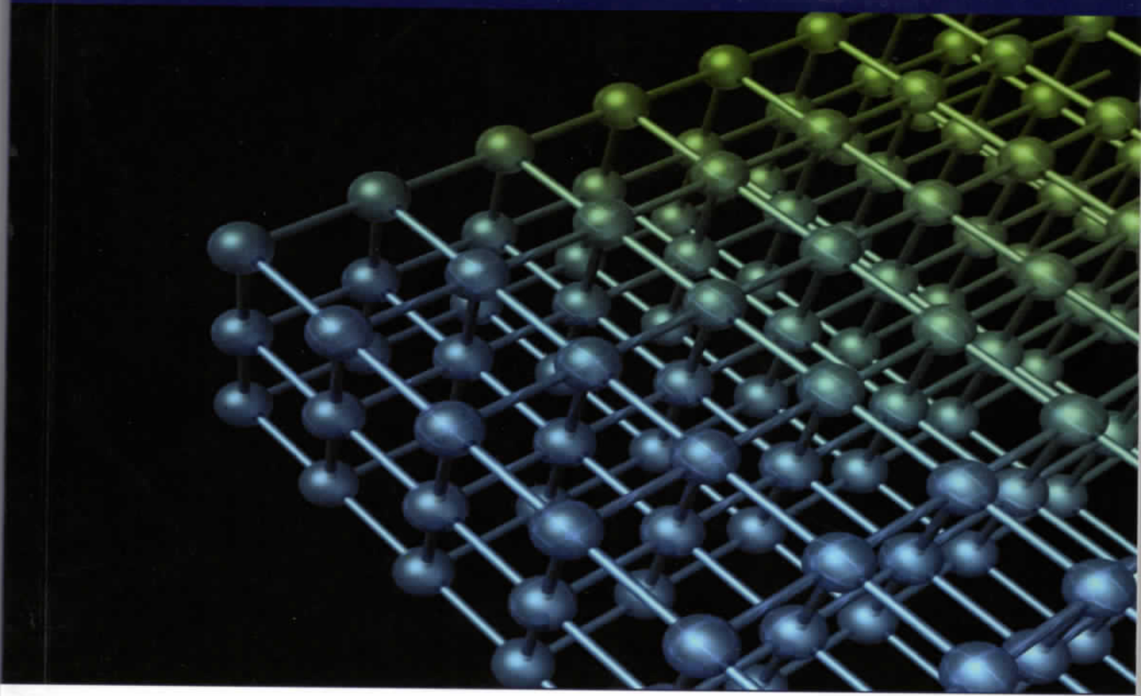
# Chapter 2

## Silver nanoparticles from the leaves green extract of Aloe vera (*Aloe barbadensis Miller*): Biosynthesis, characterization, and biological activity

***Gajendra Nath Maity*[1], *Ipsita K. Sen*[2], *Prasenjit Maity*[3*]**

A green, simple, and low-cost synthesis of silver nanoparticles (AgNPs) by mixing $AgNO_3$ solution with the aqueous leaves extract of *Aloe vera*, without any harmful reducing and capping agents. The biosynthesis of AgNPs was observed by the color charge from colorless (metal salt solution) to a yellowish brown (nanoparticle colloidal dispersion), which was confirmed by UV–vis spectroscopy. The surface plasmon resonance (SPR) band of UV–vis spectrum around 430 nm confirmed the formation of AgNPs. The synthesized Ag-nano particle was found as very effective against important human pathogenic bacteria such as *S. typhimurium* ATCC14025. These Ag-nano particles can also reduce the microbial load by destroying the microorganisms present in sewage water. This nanoparticles is responsible for the degradation of the bacterial DNA int mononucleotide level and for that reason it shows hyperchromi effect. These Ag-nano particles are very good effective against *typhimurium* ATCC14025 compared with antibiotic Ciprofloxacin.

***Keyword:*** *Aloe barbadensis Miller,* Polysaccharide, AgNPs, Biological activities.



TAPANENDU KAMILYA

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# Green synthesis of silver nanoparticles using *Cheilocostus speciosus* extracted polysaccharide and reports on antimicrobial activity

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## Abstract

A green synthesis of silver nanoparticles has been done by $AgNO_3$ with polysaccharide, isolated from the leaves of *Cheilocostus speciosus*. The polysaccharide plays the role for both reducing and stabilizing agent. The synthesized AgNPs have been characterized by UV–Vis spectroscopy. The surface plasmon resonance (SPR) band of UV–Vis spectrum around ~ 430 nm, confirms the formation of AgNPs. The synthesized AgNPs show antimicrobial activity against some important human pathogenic bacteria such as *E. coli* ATCC 25922, *S. typhimurium* ATCC 14025, *K. pneumoniae* ATCC 70063.The AgNPs are responsible for the degradation of the bacterial DNA into mononucleotide level and shows the hyperchromic effect.

***Keywords:*** *Cheilocostus speciosus,* Polysaccharide, AgNPs, Biological activities

## 1. Introduction

Nanotechnology is the most interesting areas due to its wide application in chemistry, electronics, ecology, medicine as well as to the chemists, biologists/microbiologists for their commercial demand as in biological fields[1,2]. There are various methods for nanoparticles formation including sol-process, micelle, sol-gel process, chemical precipitation, hydrothermal method, pyrolysis, chemical vapor deposition, bio-based protocols, etc. Among them, a common method for the preparation of metal nanoparticles involves the treatment of noble metal (Ag, Pt, Au, and Pd) salt with a chemical reducing agent