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Biological reduction of silver nanoparticles using plant leaf extracts and its effect on increased antimicrobial activity against clinically isolated organism

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ABSTRACT

The biological synthesis of nanoparticles emerges as an eco-friendly and exciting approach in the field of nanotechnology. In the present investigation, we report the extracellular biosynthesis of silver nanoparticles (AgNP`s) using plant leaf extracts (Artocarpus heterophyllus) for the reduction of aqueous Ag^+ ions and its increased antimicrobial activity. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant leaf extracts as reducing agent for reduction of Ag^+ ions .The quantitative formation of synthesized nanoparticles monitored by UV-visible spectroscopy. After the characterization, these nanoparticles are evaluated for increasing the antimicrobial activities of different antibiotics against clinically isolated organism. The antibacterial activities of Vancomycin, Amoxyclav and Tetracycline increased in the presence of Ag- NPs against Salmonella paratyphi. Norfloxacin,Ciprofloxacin and Erythromycin antibacterial activities increased in the presence of Ag- NPs against Klebsiella pneumonia.

Key words: Bioreduction, silver nanoparticles, Plant leaf extracts, Antibiotics, clinical isolates

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INTRODUCTION

Nanotechnology is an enabling technology that deals with nano-meter sized objects [1]. It is expected that nanotechnology will be developed at several levels like materials, medical devices and systems. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology [2, 3]. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial

activity in silver nanoparticles. As specific surface area of nanoparticles is increased, their biological effectiveness can increase due to the increase in surface energy [4]. Nanoparticles of Free metals have been extensively researched because of their unique physical properties, chemical reactivity and potential applications in catalysis, biological labelling, bio sensing , drug delivery, antibacterial activity, antiviral activity , detection of genetic disorders , gene therapy and DNA sequencing [5].

Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [6].The most widely used and known applications of silver and silver nanoparticles are in the medical industry. These include topical ointments and creams containing silver to prevent infection of burns and open wounds [7]. Other widely used applications are medical devices and implants prepared with silver-impregnated polymers [8]. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using microorganism [9, 10], enzyme [11], and plant or plant extract [12] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [12].

The antimicrobial property of silver is related to the amount of silver and rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as its binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane and nuclear membrane leading to cell distortion and death. Silver also binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication [13, 14]. Silver possess some antifungal and antiviral activities. Silver metal and silver dressings, when used in reasonable amounts, has no negative effects on the human body towards many pathogens such as bacteria, viruses, fungi, yeast etc [14].

In our study, *Artocarpus heterophyllus* plant leaf extracts used for the synthesis of silver nanoparticles and its quantitative formation monitored by UV-visible spectroscopy. Also the silver nanoparticles formations were confirmed by yellowish brown colour formation and our results showed that silver nanoparticles exhibited discrete increased antibacterial activity against clinically isolated *Klebsiella pneumonia* and *Salmonella paratyphi*

MATERIALS AND METHODS

Collection and Extract Preparation

The plant leaves (*Artocarpus heterophyllus)* collected from the campus itself, and allowed to dry for 2 weeks at room temperature. Leaves were shade dried. The plant leaf broth solution was prepared by taking 5 g of thoroughly washed and finely cut leaves in a 300 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiling the mixture for 5 min. They were stored at 4^{o}C and used within a week.

Synthesis and Characterization

For the synthesis of Ag-NP`s (Silver nanoparticles) , two boiling tubes were taken, one containing 10ml of 1mM silver nitrate solution as control and the second flask containing 9ml of

1mM silver nitrate solution and 1ml of plant leaf extracts as test solution were incubated at room temperature for 1-2 hours. The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant is discarded and the pellet is dissolved in deionised water.

The synthesized silver nanoparticles confirmed by yellowish brown colour appearance and Characterized by UV- visible spectrophotometer on a Perkin Elmer (Lamda 25)

Antibacterial activity

Microorganisms for antibacterial activity

Antibacterial activity was carried out using two different strains. The micro organisms were *Klebsiella pneumonia* and *Salmonella paratyphi*. The cultures were maintained on Nutrient agar at room temperature.

Disc Diffusion method

The antibacterial activities of silver nanoparticles were investigated by disc diffusion method [16]. Agar plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. Four wells are made in each of the Petri plates. On the Petri plate that was swabbed with *Klebsiella pneumonia,* antibiotic disc was placed on the first well, on the second well antibiotic disc along with synthesized silver nanoparticle solution was added. To the third well synthesized silver solution was added and to the last well leaf extract was added. The similar procedure was repeated with same antibiotic discs for *Salmonella paratyphi* .The Petri plates were incubated at room temperature. After incubation various zones of inhibition were measured. The resulting zones of inhibition will be uniformly circular. The diameter (mm) of the zones of complete inhibition was measured, including the diameter of the disc. The zones were measured to the nearest whole diameter using a ruler.

RESULT AND DISCUSSION

The formation of silver nanoparticles by plant leaf extracts was investigated. It is well known that silver nanoparticles exhibit yellowish-brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The appearance of yellowish-brown colour in the reaction vessels suggested the formation of silver nanoparticles [3] (Fig 1). Gold and silver nanoparticles exhibit strong absorption of electromagnetic waves in the visible range due to surface plasmon resonance (SPR). SPR is caused due to collective oscillations of the conduction electrons of nanoparticles upon irradiation with visible light [15]. The SPR is highly influenced by shape and size of the nanoparticles.

Reduction of the silver ion to silver nanoparticles during exposure to the plant leaf extracts could be followed by color changes [16]. The silver nanoparticles were characterized by UV- visible spectrophotomèter (Fig. 2). This technique has proved to be very useful for the analysis of nanoparticles. The UV- visible spectra showed a strong plasmon resonance which was centered approximately at 430nm. It is observed that the maximum absorbance occurs at 430 nm. Since the peak wavelength did not shift during the reaction, we could quantitatively monitor the concentrations of silver nanoparticles and thus conversion by measuring the absorbance at 430 nm.

The combination effect of these nanoparticles with different antibiotics was investigated against *Klebsiella pneumonia* and *Salmonella paratyphi* using disk diffusion method. The diameter of inhibition zones (in millimetres) around the different antibiotic disks with or without Ag-NPs

against test strains are shown in Table1 and Table2. The antibacterial activities of Different antibiotics increased in the presence of Ag- NP`s against this two test strains. The antibacterial activity of Vancomycin, Amoxyclav and Tetracycline increased in the presence of Ag- NPs against *Salmonella paratyphi* but not much enhanced noticed with other antibiotics. Similarly, the antibacterial activity of Norfloxacin, Ciprofloxacin and Erythromycin increased in the presence of Ag- NPs against *Klebsiella pneumonia* but not much enhancement with other antibiotics.

CONCLUSION

In conclusion, the silver nanoparticles were synthesized using plant leaf extracts of *Artocarpus heterophyllus* as reducing agent. According to José Guilherme S.,et al (2003) *Artocarpus heterophyllus* lam analysis by capillary GC and GC-MS, 54 components of which 37.4%, were alcohols and 32.2% carboxylic acids. The main constituents were 3-methylbutanoic acid (28.2%) and 3-methylbutan-1-ol (24.3%). Other important flavour compounds include 2-acetyl-1-pyrroline and the tentatively identified 2, 5-dimethyl-4-hydroxy-3(2*H*)-furanone[18]. Compared to all the other volatile components, alcohols were the mostly found compounds and with this we can conclude that it may be one of the reasons for the reduction of silver nanoparticles.

The combination effect of these nanoparticles with different antibiotics was investigated against *Klebsiella pneumonia* and *Salmonella paratyphi* using the disk diffusion method. The diameter of inhibition zones (in millimetres) around the different antibiotic disks with or without Ag-NPs against test strains are shown in Tables. The antibacterial activities of Different antibiotics increased in the presence of Ag- NPs against test strains. This biosynthesis of silver nanoparticles is an economical, efficient, eco-friendly and a simple process and the nanoparticles formed are biocompatible. With this, we can conclude that even though this kind study is published in research papers, our approaches also may be one of the platforms for the development in nanomedicine for the threatening diseases.

Fig.1. 1mM $AgNO_3$ solution before adding extract(Artocarpus heterophyllus) (left), after adding extract to the solution.

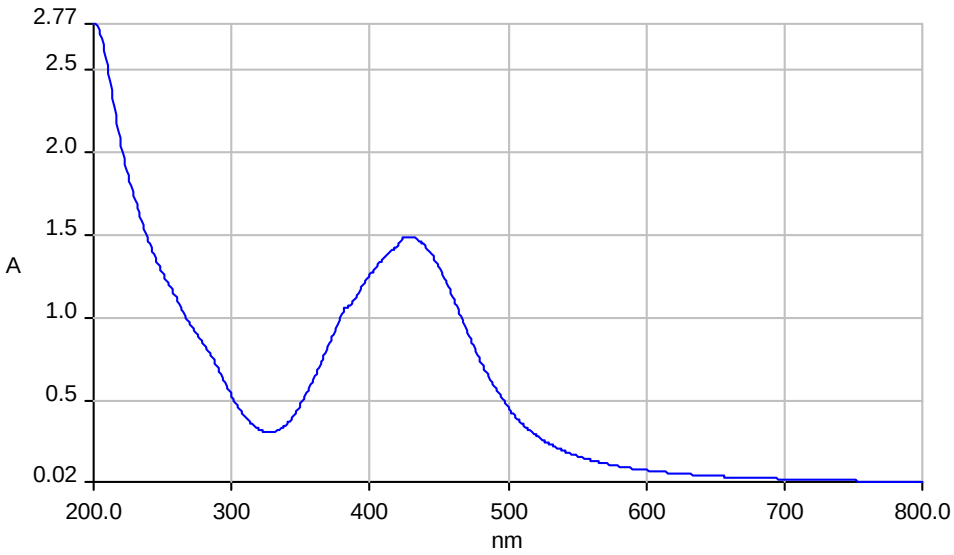

Fig. 2.UV- Vis spectra recorded after adding Silver Nitrate to the Plant Leaf Extract (*Artocarpus heterophyllus*)

TABLE 1: Zone of Inhibition (mm) of different antibiotics against *Klebsiella pneumonia*

ANTIBIOTIC DISC (µg/disc)	*Klebsiella pneumonia*	
	ANTIBIOTIC ONLY	ANTIBIOTIC + Ag-NPs
Norfloxacin	17	29
Vancomycin	16	20
Ciprofloxacin	19	29
Kanamycin	14	20
Erythromycin	19	29
Nitrofurantoin	11	16
Methicillin	11	14
Amoxyclav	10	17
Tetracyclin	19	23
Nalidixic Acid	19	23

TABLE 2: Zone of Inhibition (mm) of different antibiotics against *Salmonella paratyphi*

ANTIBIOTIC DISC (µg/disc)	*Salmonella paratyphi*	
	ANTIBIOTIC ONLY	ANTIBIOTIC + Ag-NPs
Norfloxacin	19	24
Vancomycin	8	19
Ciprofloxacin	21	26
Kanamycin	16	19
Erythromycin	22	29
Nitrofurantoin	14	16
Methicillin	10	12
Amoxyclav	8	18
Tetracyclin	19	27
Nalidixic Acid	20	24

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