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Integrated Science

Comparative study of antibacterial activity of standard antibiotic with silver nanoparticles synthesized using *ocimum tenuiflorum* and *garcinia mangostana* leaves

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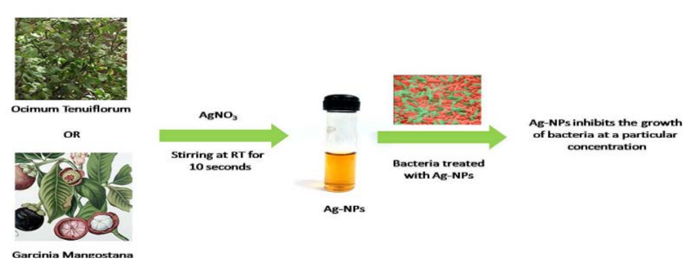
ABSTRACT



This study investigates an efficient and sustainable route of Ag-NPs preparation using leaf extracts of two plants, *Ocimum tenuiflorum* and *Garcinia mangostana*, well adorned for their wide availability and medicinal property. Silver nanoparticles (Ag-NPs) have been synthesized at room temperature following a green approach by reducing the corresponding salt ($AgNO_3$) with plant leave extract. The reaction was instantaneous and average diameter of the particles formed was around 15nm and 20nm (for *ocimum tenuiflorum*) and 25nm and 38nm (for *garcinia mangostana*) as measured by HRTEM and DLS techniques respectively. Electron diffraction pattern showed that the particles were crystalline in nature. The silver nanoparticles were highly stable for more than four months at room temperature in both the cases. These nanoparticles were used for assessing antibacterial activity studies against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria.

Keywords: *Green approach, silver nanoparticles, room temperature, antibacterial activity, plant extract.*

INTRODUCTION

Metal nanoparticles have a high surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, such as high catalytic activity and enhanced optical, electronic, magnetic and antibacterial properties, they are drawing the attention of scientists for their novel methods of synthesis.[1-2] Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum antimicrobial activities, which has been exploited for centuries to prevent and treat various diseases, most notably infections. Silver nanoparticles play a profound role in the field of biology and medicine due to their remarkable physiochemical properties. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities. In medicine, silver and silver nanoparticles have

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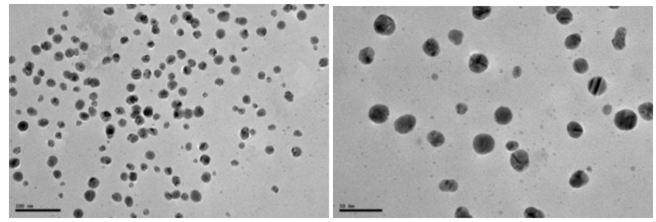
shown a wide range of applications including ointments and creams containing silver to prevent infections due to burns and open wounds. Silver nanoparticles, both spherical and non spherical, are reported to possess anti-fungal, anti-inflammatory, anti-viral, anti-angiogenesis and anti-platelet activity.[3-8] Indian flora is the huge source of cost-effective non-hazardous reducing and stabilizing compounds. Nowadays, the use of different types of medicinal plants by traditional medical practitioners for the treatments of various types of diseases is increasing. The most common and important medicinal plant i.e., *Ocimum tenuiflorum* have different medicinal properties such as anticancer, antimicrobial, cardio-protective, antidiabetic, analgesic, antispasmodic, antiemetic, hepatoprotective, antifertility, adaptogenic and diaphoretic actions.[9-12] The active constituent of leave of the *Ocimum tenuiflorum* is Eugenol (l-hydroxy-2-methoxy-4- allylbenzene) which is mainly responsible for the therapeutic potentials of cardiovascular system, urinary system, reproductive system, immune system, gastric system, blood biochemistry, central nervous system and also has found significance in various ailments in modern medicines.[13-20] Garcinia mangostana belongs to the family of Guttiferae and is named "the queen of fruits". Experimental studies have demonstrated that extracts of garcinia mangostana have antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral activities. The pericarp of garcinia mangostana is a source of xanthones and other bioactive substances. Prenylated xanthones isolated from this pericarp have been extensively studied; some members of these compounds possess antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, antifungal and antiviral properties. Xanthones have been isolated from pericarp, whole fruit, heartwood, and leaves. The most studied xanthones are α-, β-, and γ-mangostins, garcinone E, 8-deoxygartanin, and gartanin.[21] In this work, green synthesis of silver nanoparticles using ocimum tenuiflorum and garcinia mangostana plant extract is described. The antibacterial efficacy of synthesized nanoparticles was evaluated against both gram-positive and gram-negative bacteria.

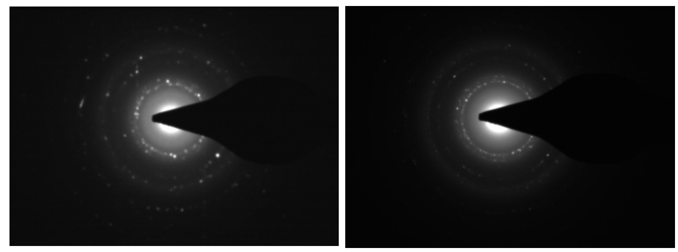
RESULTS AND DISCUSSION

The maximum absorption wavelength (λ_{max}) of the prepared Ag nanoparticles through ocimum tenuiflorum (OT) and garcinia mangostana (GM) leaves was observed at 410nm and 421nm respectively. The average particle size of these silver nanoparticles was around 15nm (OT) and 25nm (GM) measured by high resolution transmission electron microscopy (Figure 1). The particles obtained were spherical in shape and reasonably mono-dispersed as can be seen clearly from their TEM image. The maximum distribution of particles as measured by DLS comes out to be around 20nm (OT) and 38nm (GM). The electron diffraction pattern (SAED) clearly concludes that the particles were crystalline in nature (Figure 2). Silver ion concentration was analyzed by atomic absorption spectroscopy (AAS) which showed the conversion of Ag^+ ions into Ag nanoparticles. Initially, standard solution of $AgNO_3$ was prepared and analyzed with AAS. Now, Ag^+ ion concentration



(a) (b)

Figure 1. High resolution transmission electron microscopy (HRTEM) image of silver nanoparticles synthesized using (a) *Ocimum Tenuiflorum* leaves and (b) *Garcinia Mangostana* leaves.



(a) (b)

Figure 2. Electron diffraction pattern of silver nanoparticles synthesized using (a) *Ocimum Tenuiflorum* leaves and (b) *Garcinia Mangostana* leaves.

in the reaction solution, after adding $AgNO_3$ into leaf extract, was monitored at different time intervals. The result showed decrease in concentration of Ag^+ ions, indicating the conversion of Ag^+ ions into Ag nanoparticles. Biosynthesized silver nanoparticles were observed to exhibit good antimicrobial activity on both gram-negative and gram-positive microorganism. The MIC and IZD of silver nanoparticles against *E. coli* and *S. aureus* is shown in Table 1. The results indicated that silver nanoparticles synthesized from Ocimum tenuiflorum had more activity as compared to garcinia mangostana leaves. These nanoparticles have stronger activity than standard antibiotic (ciprofloxacin or amikacin or penicillin) at lesser concentration.

The proposed mechanism of inhibitory action of silver nanoparticles on micro-organisms showed that, upon treatment, DNA loses its replication ability and expression of ribosomal subunit proteins as well as other cellular proteins and hence enzymes essential to ATP production becomes inactivated. This then suggested the possibility that the silver nanoparticles might have penetrated inside the bacteria and fungi, causing damage by interacting with phosphorus containing compounds such as DNA. Silver tends to have a high affinity to react with such compounds. It has also been hypothesized that Ag nanoparticles primarily affected the function of membrane bound enzymes, in the respiratory chain. The interaction between silver nanoparticles and constituents of the bacterial membrane caused structural changes and damage to membranes, thereby, finally leading to cell death. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger silver particles not only at the surface of cell membrane, but also inside the bacteria.[22-26]

Table 1. Comparison of antibacterial activity of synthesized silver nanoparticles versus standard antibiotics.

Species	*E.coli*		*S.aureus*	
Synthetic Source	*Ocimum Tenuiflorum*	*Garcinia Mangostana*	*Ocimum Tenuiflorum*	*Garcinia Mangostana*
Minimum Inhibitory Concentration (MIC, μg/mL)	0.25	0.38	0.64	0.92
Standard Antibiotic (MIC, μg/mL)	0.58 (Ciprofloxacin)		2.4 (Ciprofloxacin)	
Zone of Inhibition (ID, mm)	30	25	30	28
Standard Antibiotic (ID, mm)	20 (Amikacin - 30μg/ml)		25 (Penicillin - 20μg/ml)	

EXPERIMENTAL SECTION

0.5mL of freshly prepared $AgNO_3$ solution (0.08% w/v) was added to 10mL of filtered plant extract solution (0.520g of leaves were taken and boiled in 20mL of double distilled water for nearly 5 min and filtered to obtain 15ml of light yellowish colour extract). The colour of the solution changes to yellowish-brown within 10 sec, indicating the formation of silver nanoparticles. The above mixture was further stirred for 5 min at room temperature (25 $^{\circ}$C). These as synthesized metal nanoparticles were then used for testing their antimicrobial activity. The pH of the leaf extract was maintained at 8.0.

CONCLUSIONS

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. In the present study, we have demonstrated that Silver nanoparticles (AgNPs) can be successfully obtained from bioreduction of silver nitrate solution using natural and low cost biological reducing agent, Ocimum tenuiflorum and garcinia mangostana leaves extracts. Owing to varying properties of these plant species, AgNPs obtained from them also varied in size, the smallest being yield using ocimum tenuiflorum leaf extracts. The AgNPs were found to have wider antimicrobial activity in gram negative than gram positive organisms as compared to standard antibiotics.

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