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Research Paper

GREEN SYNTHESIS OF SILVER NANOPARTICLES, IT'S CHARACTERISATION AND DETERMINATION OF ITS ANTI – MICROBIAL PROPERTIES

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Abstract

The biosynthesis of nanoparticles has been proposed as a cost effective and environment friendly alternative to chemical and physical methods. Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology. In the present study, synthesis of silver nanoparticles (AgNPs) or (Green-Silver) has been demonstrated using extracts of *Parmotrema perlatum*, *Cinnamomum cassia*, *Illicium verum* reducing aqueous silver nitrate. The extracts were prepared by Soxhlet extraction. The synthesized AgNPs were characterized using instrumental technique which includes ultraviolet-visible spectroscopy (UV-Vis). The anti-microbial activity of these nanoparticles was studied against Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli* and the fungi *Candida albicans*. Both, the solvent extracts of the three plant materials and the nanoparticle synthesized showed inhibition of growth of these organisms. This work proved the capability of using biomaterial for the synthesis of silver nanoparticle, by adopting the principles of green chemistry. Silver nanoparticles prepared through bioconversion in the study, successfully inhibited the growth of the organisms tested and this anti-microbial property of silver nanoparticles can be explored as a potential therapeutic against microbial infections.

Key words: nanoparticles, green chemistry, biomaterials, cost effective, eco-friendly, therapeutic.

INTRODUCTION

Man has used natural resources for various purposes since time immemorial. The value of medicinal plants to mankind is well proven. As perhaps in other parts across the globe, Indians have been using various plants and plant materials in different forms to

treat different infections. A mixture of extracts from various spices called '*kadha'* has been traditionally used by Indians to cure Respiratory Tract Infections (RTI), Gastro-intestinal Tract Infections (GTI), and other disorders or ailments such as diabetes, jaundice, constipation, etc. Thus, identification and subsequently; extraction of such substances or compounds is essential and it is important to understand how these substances function? What are their target sites? Pathways they interfere in and the actual compound involved. That is studying the compound at its physiological, chemical and molecular level.

The discovery of silver nanoparticles (AgNP's) from different parts of such plants and its role as an anti-microbial agent answered many of these questions. Now, it's clear that many plants exhibit anti-microbial and/or anti-viral properties which can be attributed to the silver nanoparticles (AgNP's) present in them. Also, anti-cancer potential of AgNP's have been reported against various cell types. Silver nanoparticles (AgNP's) have varied applications in the field of medicine, food, textile and many other industries. An increasingly common application is the use of silver nanoparticles (AgNP's) for anti-microbial coatings; and many textiles, keyboards, wound dressings, and biomedical devices now contain silver nanoparticles (AgNP's) that continuously release a low level of silver ions to provide protection against bacteria.

Nanotechnology relates to materials, systems and processes, which operate at a scale between 1- 100 nanometers (nm). Nanotechnology has been also known as having one or more dimensions measuring 100 nm or less, or having at least one dimension at this scale, which affects the materials behavior and properties. For that reason, nanotechnology does not describe a single technology, but a range of technologies.

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for "Green Nanotechnology". Many biological approaches for both extracellular and intracellular

nanoparticles synthesis have been reported till date using micro-organisms including bacteria, fungi and plants.

As reported by Z. Sadowski (2010), Green synthesis of AgNP's requires least time in comparison with other methods. For instance, the Neem (*Azadirachta indica*) leaf broth and aqueous solution of silver nitrate or chloroauric acid were used for the extracellular synthesis of pure metallic silver and gold particles. The time required for Ag^{+} and Au^{3+} ions to reduce was 4h and 2h, respectively, being extremely short compared to both bacteria and fungi (24h and 120h). Surface active constituents of the leaf broth stabilize nanoparticle suspensions- an aqueous suspension showed stability even after 4 weeks.

India, the "Land of Spices", produces 75 of the 109 varieties of spices listed by the International Organization for Standardization (ISO). Since, agriculture is the primary occupation of the country, these spices can be the ideal sources for the Green Synthesis of AgNP's and thus an exemplary route to empowerment of the farming community; eventually a boon to nation's economy. The traditionally used spices can be a good source for synthesis of silver nanoparticles (AgNP's). Also, these spices can serve as remarkable anti-microbial and/or anti-viral agents. Thus, it is important to determine whether they posses such properties or not; and if yes, then to what extent? Also, if they can help cure disorders such as cancer, Alzheimer's, HIV, infections caused by *Mycobacterium* family and many other such ailments which are not only threat to the mankind but also to other living organisms.

According to the recent studies, silver nanoparticles (AgNP's) can be synthesized from various plants like *Ananas cosmosus(*pineapple*), Ocimum tenuliforum(*tulsi*), Chrysenthemum indicum* (chrysanths),*Thevetia peruviana(*yellow oleander or lucky nut*), Allium sativum(*garlic*), Brassica oleracea var. Capitata(*cabbage*), Aloe Barbadensis Miller(*aloe vera*)* etc.

Amongst spices, little is known about the Black Stone Flower. Also, Cinnamon and Star Anise have not been checked for these properties so far. Probably these spices can serve as source(s) of synthesis of silver nanoparticles (AgNP's) and/or anti-microbial and/or anti-viral and/or anti-cancer agent. Thus, determination of all such properties of these spices is necessary.

Many natural and synthetic substances possess anti-microbial properties either anti-bacterial and/or anti-fungal and/or anti-viral. But an ideal anti-microbial agent is the one which has a broad spectrum, easily available and cheap, and has no class resistant

to it. Manufacturing of these agents synthetically, is expensive. Besides, certain processes and/or byproducts cause various hazards to the environment. Hence, an ecofriendly and cost-effective process of production is essential. Since, AgNP's are emerging as an ideal anti-microbial, anti-cancer agent, there production needs to be simplified. *Parmotrema perlatum*, *Cinnamomum cassia* and *Illicium verum* can be good source(s) of AgNP's.

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications [3]. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route [4]. So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

An inclination amongst scientists across the globe towards Green Synthesis has resulted in discovery of varied attributes of Silver nanoparticles. Hence, the quest to discover the most ideal local and global source for green synthesis of AgNP's is leading to enlightenment in comprehension of plants and plant materials less or unexplored.

MATERIALS AND METHODS

All the chemicals and reagents were purchased from SRL Limited, Mumbai. In this study, biosynthesis of the silver nanoparticles (AgNP's), was done in two step – preparation of extract and preparation of silver nanoparticles (AgNP's). For preparation of extract, the samples (i.e. *Parmotrema perlatum*, the bark of *Cinnamomum cassia* and fruit of *Illicium verum*) were crushed using mortar and pestle and 10g of the sample was placed inside the main body of the Soxhlet apparatus in a thimble prepared using Whatmann filter paper No.1. In the round bottom flask of the apparatus, 150 mL of the solvent (absolute ethanol and water) was added and the temperature of the apparatus was set at 60°C. After 5-6 cycles, the liquid was eluted and the remaining amount in the sample residue

was extracted using a muslin cloth; color of the extracts was recorded. For preparation of silver nanoparticles, 5mL of each sample extract was added to 50mL $AgNO_3$ solution in a beaker. The beakers covered with foil papers were then placed in dark for 24hrs and were then observed for color change. For characterization of the AgNP's, the optical absorbance was recorded on UV-Vis spectrophotometer in 200 - 800 nm wavelength range. Both, the aqueous and the solvent extract of all the three samples were then screened for their Anti-microbial activity against ***Staphylococcus aureus***, ***Escherichia coli***, ***Candida albicans*** using Agar cup method, wherein 0.1mL of solvent extract, aqueous extract of concentration 100µg/mL and10µg/mL of each sample was added to the wells and the result was recorded after 24hrs of incubation at 37℃. Each sample was analyzed similarly.

RESULT AND DISCUSSION

Biosynthesis of Silver nanoparticles

Formation of silver nanoparticles by the reduction of silver ions during the exposure to extract of Black Stone Flower, Cinnamon and Star anise was recorded by change in color of the reaction mixture from dark green to pale green, dark brown to light brownish orange and from dark brownish green to pale white, respectively, after 10 minutes of incubation which indicated the formation of silver nanoparticles.

Figure 1: Preparation of AgNP's

Plants contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. *Illicium verum* contains shikimic acid which renders it the anti-microbial, anti-oxidative properties and now it is also considered to evince significant anti-cancer potential. This antioxidative compounds delay or inhibit the oxidation of molecules by inhibiting the initiation or propagation of oxidative chain reaction. The antioxidative activity of phenolic compounds is mainly due to their redox property, which plays an important role in absorbing and neutralizing free radicals. It is thus possible that Shikimic acid acts as a reducing agent and is oxidized by $AgNO_3$, resulting in the formation of AgNP's. The reaction could be summarized as:

$AgNO_3 \longrightarrow Ag^+ + NO_3^-$

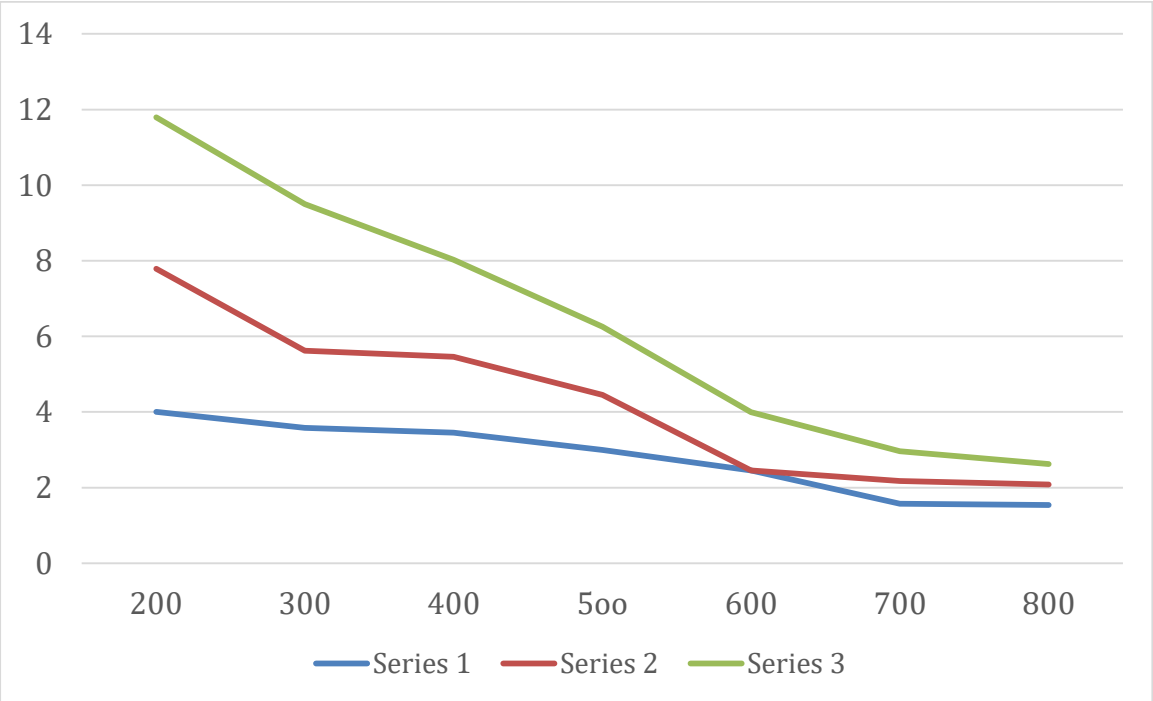
$e^- + Ag^+ \longrightarrow Ag$

The distinct smell and flavour of *Cinnamomum cassia* is due to a compound called cinnamaldehyde which constitutes 90% of the essential oil of cinnamon. Cinnamon is loaded with powerful anti-oxidants, such as polyphenols. Thus, it is possible that these polyphenols and/or other anti-oxidants act as a reducing agent, resulting in the formation of AgNP's.

Parmotrema parletum contains alkaloids, phytosterols, phenolic compounds and tannins. Henceforth, formation of AgNP's can be attributed to its phenolic compound.

Thus, the different types of antioxidants present in the extracts of these spices synergistically reduce the Ag metal ions as each antioxidant is unique in terms of its structure and antioxidant function of trapping the different free radicals.

Characterization of AgNP



Graph 1: UV-visible absorption spectra of silver nanoparticles [Absorption vs wavelength(nm)]

Anti - Microbial Screening

The solvent extracts of the samples i.e. *Parmotrema perlatum*, *Cinnamomum cassia* and *Illicium verum*, used for synthesis of AgNP's are very effective against micro-organisms. On contrary, aqueous extracts at concentration of 100µL/mL & 10µL/mL are poorly effective. Thus, perhaps the solvent used i.e. absolute ethanol, enhances the action or works synergistically with the phytocompounds in inhibiting the growth of the microbes. Anti- microbial activity of these solvent and aqueous extracts was studied through Agar cup method in present study. Positive and negative control set for the three organisms gave precise results. Two human, pathogenic bacteria and one fungal pathogen such as *Staphylococcus aureus, Escherichia coli* & *Candida albicans* were used for the screening of antimicrobial activity.

Table:1 Observation of anti- microbial activity

ORGANISM	CONC. µg/mL	ZONE OF INHIBITION (mm)			SENSITIVITY
		B.S.F.	CINN.	S.A.	
S.aureus	S.E.	9	7	8	+ve
	100	-	-	-	-ve
	10	-	-	-	-ve
E.coli	S.E.	15	16	20	+ve
	100	-	-	-	-ve
	10	-	-	-	-ve
C.albicans	S.E.	12	12	13	+ve
	100	-	-	-	-ve
	10	-	-	-	-ve

Figure 2: Anti – microbial Screening

In case of *Staphylococcus aureus*, highest inhibition was observed by *Permotrema parlatum*, while *Illicium verum* was observed to have most toxic effect on the other two.

Cinnamomum cassia was found to be most toxic for *Escherichia coli* among the three organisms tested.

SUMMARY & CONCLUSION

Further study required to use these nanoparticles for the treatment of disease using animal models. The biological synthesis of nanoparticles has paved revolutionary approaches in medical field. However, these are only the first results in this relatively new research area. Determination of efficacy and toxicity of commercially available silver nanomaterials is necessary for safe and efficient application, both in everyday life and medical practice.

The extracts have proved anti-microbial properties against fungi *Candida albicans* and Gram negative *Escherichia coli* with high efficacy and not much appreciable against Gram positive *Staphylococus aureus*. Thus, these plant materials and AgNP's synthesized from them can serve as a potent anti – fungal agent and also against Gram negative bacteria.

Further investigation is needed for both the aspects to get this plant materials into the market as sources of synthesis of AgNP's and anti – microbial agents.

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