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EXPERIMENTAL ANALYSIS ON ANTIMICROBIAL MOVEMENT AND GREEN FUSION OF SILVER NANOPARTICLES OBTAINED FROM PLANT SOURCES

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ABSTRACT

The antimicrobial activity was tested using agar well method with Gram positive and Gram negative microorganism. It was observed that the antimicrobial activity was higher against Gram negative bacteria. Local plants such as Carica Papaya Linn leaves and waste is an alternative to a safer, more eco-friendly alternatives of synthesizing silver nanoparticles. This environment friendly, method provides simple, easy and cost effective faster synthesis of nanoparticles than chemical method and can be used in several areas such as catalysis, medical application etc. Nanoparticles are used widely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these particles. Silver nanoparticles have received substantial attention in the field of antimicrobial research due to the antimicrobial activity of silver. These particles can be synthesized easily by different chemical, physical, and biological approaches. The biological approach is the most emerging approach of preparation, as this method is easier than the other methods, ecofriendly, non toxic and less time consuming. In the present study green synthesis of silver nanoparticles was carried out using the plant extracts of Carica Papaya Linn and the mixture of all the three extracts. Silver was of a particular interest for this process due to its evocative physical and chemical properties. The reduction process of Ag^{+} to Ag^{0} was observed by formation of brown colour within 24 hours of incubation period and synthesized SNPs showed an absorption peak at around 450 nm in the UV-visible spectrum. The reaction was followed by the characterization of the silver nanoparticles using UV-Vis and FTIR.

Keywords: Silver nanoparticles, UV-Visible Spectroscopy, FTIR, Antimicrobial activity

I. INTRODUCTION

Nanotechnology is the branch of technology that deals with dimensions and tolerances of less than 100 nanometers, especially the manipulation of individual atoms and molecules. They exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes because of their size1. Nanoparticle may or maynot exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials2. Some of the applications of nanoparticles to biology or medicine are in tissue engineering, cancer therapy, multicolour optical coding for biological assays, manipulation of cells and biomolecules, protein detection, drug and gene delivery, bio detection of pathogens, probing of DNA structure, tumour destruction via heating (hyperthermia), MRI contrast enhancement and phagokinetic studies.

Among the various metal nanoparticles, silver (Ag) nanoparticle has received substantial attention in the field of antimicrobial research. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms3. Synthesizing silver nanoparticles by plants is a major advantage that they are easily available, safe and nontoxic in most cases, have a broad variety of metabolites that can aid in reduction of silver ions and are quicker than microbes in the synthesis,Biosynthesis of nanoparticles is a kind of bottom up approach, where the main mechanism of action is redox reaction. The need for biosynthesis of nanoparticles arises from the fact that the physical and chemical processes became costly and the use of hazardous chemicals in various steps4. Phytochemical mediated metal nanoparticle syntheses are effective, economical and environmental friendly. Plants are considered as biosynthetic laboratories of wide spectrum of phytochemicals such as phenolics, alkaloids and flavonoids. These phytochemicals are expected to self assemble and cap the metal nanoparticles formed in their presence and thereby induces some shape control during metal ion reduction5. Metallic nanoparticles obtained by biosynthesis employing plant extracts have been reviewed and reported by different authors6-7.

In this work we have synthesized and characterized the silver nanoparticles from plant sources like leaves of Carica Papaya Linn, and also from the combination of all three extracts and have checked the antibacterial activity of the synthesized silver nanoparticles.

II. MATERIALS AND METHODS Test chemicals

$AgNO_3$were purchased from SD Fine Company, India and used without further purification. The purity was at least 99.5%. The solutions for the metal salts were prepared in deionised water. The media for bacterial growth was obtained from Himedia, India.

Sample Collection:

The sample sources for synthesis of nanoparticles were dried Carica Papaya Linn leaves (Carica Papaya Linn) from local market of Jaipur area.

Preparation of extract:

Samples used were dried leaves of Carica Papaya Linn and. The solvent used was deionized water. The samples were weighed 1g each and added to 50ml of deionized water in a flask. These flasks were kept in boiling water bath for 15min with inverted funnel. After cooling, the solution was filtered using Whatmanfilter paper no. 1. 2ml each of the filtrates were mixed in a conical flask and used as the fourth sample. The samples were named Carica Papaya mixture.

Synthesis of Silver nanoparticles:

1 ml of freshly prepared 5mM Silver nitrate solution was added to 5ml of the sample solutions respectively. This mixture was kept in boiling water bath for 10min until visible colour change (brown colour) was observed. The mixtures were kept in incubator at 370C for 24hrs.

UV-Vis spectral analysis

The reduction of pure Ag+ ions was monitored by using UV-Vis spectrum of reaction medium after 24 hrs. Sample was prepared by diluting the solution in deionized water in the ratio 1:10. Deionized water was used as blank. The analysis was carried out on a UV – Vis spectrophotometer (Jasco v-630 Spectrophotometer) from range 350- 600nm.

FTIR analysis of silver nanoparticles The chemical composition of synthesized silver nanoparticles was studied by using FTIR spectrometer (JASCO FT/IR 4100 type A). The liquid samples were mixed with KBr and pellets were made. The solution were characterized in the range 4000-650 cm-1

Study of antimicrobial activity

The antibacterial activity of the synthesized silver nanoparticles was checked using Agar Well Diffusion method. 1 ml of the 24 hrs old grown bacterial culture was seed inoculated in molten sterile Nutrient agar butt and poured into petriplates. All the four samples (Carica Papaya Linn, Mix.) were tested against the five cultures - Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Corynebacterium diphtheriae and Pseudomonas aeruginos.

After the incubation period, the plates were examined for zone of inhibition. The diameter of the zones of clearance was measured and the mean value was calculated.

III. RESULT AND DISCUSSION

The mixture showed colour change to brown colour at 37°C, after addition of plant extract to aqueous solution of AgNO3. The colour is characteristic of the Surface Plasmon Resonance (SPR) of silver nanoparticles. The reduction of silver ion to silver nanoparticle was reflected in spectral data obtained by using a UV-Vis spectrophotometer. Absorbance was found to be in range of 350-450nm which is specific for silver nanoparticles (Table 1, Figure 1)9.

Table 1: Absorbance of the AgNPs

Silver nanoparticles	Maximum wavelength	Absorbance
Carica Papaya Linn	369.6nm	0.997
Allium Sativum Linn	431.6nm	0.556
Mixture of two	447nm	0.791

is found to exclude addition of external stabilizing agent during synthesis of silver nanoparticles
and to offer synergistic effects to enhance the antimicrobial properties of the synthesized silver nanoparticles10.

Figure 1. Colour change during synthesis of AgNPs before and after

The UV–Vis spectra confirmed the formation of Ag nanoparticles as the colour change occurred to reddish brown and maximum absorbance was found at 350- 550nm and 350-450nm Other studies stated its range to be mostly around and at11-12.

The Green Carica Papaya Linn leaves which were used for synthesis of silver nanoparticles showed peak at 369nm13-14 The UV-Vis spectral analysis showed its maximum absorbance at 431.6nm which was found to lie near the previously observed peak of 430nm with similar peels15

FTIR Analysis

FT-IR was carried to determine the functional groups responsible for reduction present in the plant sample and the synthesized nanoparticles. The FT-IR spectrum obtained for extracts displays a number of absorption peaks, reflecting its complex nature. Strong absorption peaks at 3263 to 3331 cm−1 result from stretching of the –NH band of amino groups or is indicative of bonded –OH hydroxyl group. The absorption peaks at about 2929.87 cm−1 could be assigned to stretching vibrations of -CH2 and -CH3 functional groups. The intense band at 1585 cm−1 could be assigned to the aromatic C=C bending. FT- IR study indicates that the hydroxyl (−OH), and amine (N-H) groups in the extracts are mainly involved in reduction of Ag+ ions to Ag0 nanoparticles. For the plant extract, the FTIR results confirmed the presence the presence of – NH, -OH, C=C, and –CH group, which indicates that the plant extract containing the hydroxyl and amine group substituted flavonoids. It is observed that flavonoids act as a reducing agent, which reduces Ag+ to Ag0 and the amino group as stabilizing agents in the green synthesis of silver Nanoparticles. The FT-IR spectroscopic studies have also showed that the leaf extracts not only acts as reducing agent but as a stabilizer also, which prevents the agglomeration of AgNPs. The carbonyl group of amino acid residues has a strong binding ability with metal, suggesting the formation of a layer covering silver nanoparticles which act as a stabilizing agent to prevent agglomeration in the aqueous medium16. The observed intense band were compared with standard values of identify the functional groups.

The groups assisting reduction were found to be in the range of1400-1600, 1640- 1690, 2850-3000 and 1000-1300cm-1. Thus the reduction of plant extracts by AgNO3 was confirmed by FTIR analysis and the flavonoids present were found to be responsible for reduction.

The FTIR showed the presence of alcohols and phenols, alkenes, aromatic group and ether which matched with the previously reported groups alkenes, alcohols and phenols with an additional primary amine group at 1634.90cm-1. In Peel AgNPs presence of aromatic groups, ketone groups and alcohols and phenols were observed indicating presence of flavonoids and other phytochemicals which were previously reported with an additional amine group at 3403.99cm-1 The antimicrobial activity of the silver nanoparticles was studies against five different organisms viz. Gram positive organisms (Staphylococcus aureus and Corynebacterium diphtheria)and Gram negative organisms (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa)(Figure 2, Table 2).

Figure 2. Antimicrobial activity of AgNP's prepared from , [A] Carica Papaya Linn leaves, [B] Allium Sativum Linn and [C] mixture of above two.

Table 2. Zone of inhibition for the AgNPs

Organisms	**Zone of inhibition(mm)**					
	Carica Papaya Linn AgNP	**Allium Sativum Linn AgNP**	**Mixture**	**Control A (1mM $AgNO_3$)**	**Control B (Plant extract)**	
Escherichia coli	14	12	13	-	-	
Klebsiella pneumonia	12	14	15	16	-	-
Pseudomonous aeruginosa	11	11	13	12	-	-
Staphylococcus aureus	11	11	13	14	-	-
Corynebacterium diphtheriae	13	18	19	19	-	-

The figures show clear zone of inhibition in the well treated with silver nanoparticles whereas the well treated with only plant extract and silver nitrate did not show any inhibition. It was observed that the antibacterial activity of Mix AgNPs was more compared to, Carica Papaya Linn. Against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Corynebacterium diphtheria. All the four nanoparticles showed maximum activity against Corynebacterium diphtheria and minimum against Pseudomonas aeruginosa.

Escherichia coli and Klebsiella pneumoniae as earlier stated.

IV. CONCLUSION

The research work suggests that plants extract made from Mentha Piperita, Carica Papaya Linn and Allium Sativum Linn and mixture of all capable of producing silver nanoparticles extracellular. The use of plant in synthesis of nanoparticles is advantageous in many ways in terms of production, economic viability and safety. The samples were environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The characterization of silver nanoparticles was carried out using UV- Vis spectrometer and FTIR. The characterization by FTIR showed the presence of aromatic groups, ketone groups and alcohols and phenols indicating presence of various phytochemicals including flavonoids which aid in reduction of silver. Plant extract can be used efficiently in synthesis of silver nanoparticles as greener route. Control Nanoparticles produced from plants have various applications. The above silver nanoparticles revealed to have an effective antimicrobial property against Staphylococcus aureus, Corynebacterium diptheria, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa. The present study emphasizes the use local plants and waste part for synthesis of silver nanoparticles with potent antibacterial effect. This green synthesis method has many advantages such as eco-friendly, cost effective and easily scaled up to large scale synthesis. The further characterization of the silver nanoparticles can be done by SEM analysis preferably to find its size and shape. Various parameters including pH, temperature and concentration of AgNO3 can be altered to obtain better results. Further, the checking of its antioxidant property may give new applications for the use of silver nanoparticles.

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