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Green Synthesis and Antibacterial activity of Silver Nanoparticles using *Oryza Sativa* Husk Extract

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Abstract

Green synthesis of nanoparticles is an important and beneficial way of controlling pathogens infection in humans without side effects caused by synthetic antibiotic and chemicals. Hence the present study is focused on the synthesis of silver nanoparticles using Oryza sativa husk extract. The formation of husk extract based nanoparticles was confirmed at 485nm by UV-spectral analysis and its antibacterial activity was evaluated against two harmful human pathogens Pseudomonas aeruginosa and Staphylococcus aureus. The best inhibition zone was observed in Pseudomonas aeruginosa than Staphylococcus aureus. The maximum zone was obtained in positive control Amikacin.

Keywords: Green systhesis, nanoparticles, antibacterial activity, UV -spectral analysis.

Introduction

Nanoparticles have been widely applied in the fields of investigation and regulation of cellular stage, drug delivery and diagnostic imaging of cancer detection, artificial implants, tissue engineering, HIV inhibition, sensing, artificial implants, tissue engineering, photonics, optoelectronics, biological tagging, environmental pollution control, material chemistry, and water filtration using nanotechnology[1-3]. Human beings are infected because of multiple antibiotic resistant microorganisms such as bacteria, molds, yeast, virus, etc. This research is focused on developing new and cost effective antimicrobial agents to control antibiotic resistant microorganisms using silver based antiseptics that possess broad spectrum antimicrobial activity and considerably decreasing the susceptibility to induce microbial resistance than the synthesized chemical antibiotics[4,5]. Green synthesis of silver nanoparticle is an eco- friendly method for avoiding harmful effect in medical application of silver nanoparticles synthesized by physical, chemical, photochemical such as reverse micelles, thermal decomposition of silver compounds, and radiation assisted process.

Bio activity of DNA conjugated metal nanoparticles and their application in detecting, manipulating and delivering genes has been observed[6,7]. Because of extraordinary chemical, physical and antimicrobial properties of silver the nano silver particles are used as a biocidal agents[8,9]. For example microbial infection of burn wound has been controlled using sputtered nano crystalline silver wound dressings[10,11]. Bactericidal effect of Ag- NPs on E- coli is due to reaction of Ag- NPs with cell wal land cytoplasmic membrane[12]. Ag-NPs antimicrobial activity investigation have increased due to highest bacterial resistance of silver ion, silver compounds Ag-NPs against microbes, virus [13-16] and classic antibiotics[17].

Medicinal plants such as *Basellaalba, Helianthus annus, Saccharum officinarum, Oryza sativa, Sorghum bicolour, Zea mays*[18], *Aloe vera*[19], *Medicago sativa* (Alfalfa)[20], *Capsicum annuum*[17], *Magnolia kobus*[21], *Cinnamomum camphora* leaf[22], *Geranium* Sp[23], *Azadirachta indica* (Neam)[24], *Emblica officinalis* (Amla, Indian Gooseberry)[25], methanolic extract of *Eucalyptus hybrida* leaves[26], *Vitex negundo* L. leaf water extract with heat treatment[27] and microorganism have been used in synthesis of Ag-NPs for pharmaceutical and biological application. Potent natural antioxidants such as phenolic compounds and flavonoids have been reported in *Vitex negundo*[28,29].

During the milling process the outermost layer of the paddy grain is separated from the rice grains is called rice husk (or hull). Around 20% of paddy weight is husk. In Asia about 770 million tons of husk was produced annually and often burned in open or dumped on landfills as a waste[30] (IRRI). Disposing of husk in huge amount is causing atmospheric and water pollution. Similar to the chemical composition of many common organic fibers, the husk contains 40-50 percent of cellulose, 15-20 percent of ash, 25-30 percent lignin, and moisture 8- 15 percent [31]. Hence synthesis of nanoparticles from paddy husk is an effective way of utilization of the waste husk in proper way and to reduce the environmental pollution. In the present study, green synthesis of Ag-NPs using *Oryza sativa* husk extract as reducing against and stabilizer and silver nitrate as silver precursor is attempted. The antibacterial activity of *Oryza sativa* husk extract Ag-NPs against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was evaluated.

Methods and Materials

Synthesis of silver nanoparticles from *oryza sativa* husk extract: Preparation of husk extract[32,33]. The AR grade silver nitrate ($AgNO_3$) was purchased from J. A. College at Periyakulam in Theni (Dt) and fresh *Oryza sativa* husk were collected from surroundings of Periyakulam. The *Oryza sativa* husk extract used for the reduction of Ag^+ ions to Ag^0 was prepared by taking 20g of thoroughly washed husk in 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 5 min. then decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4^0 C and used for further experiments.

The husk extract (5 ml) was added to 90 ml of 1 mM $AgNO_3$ aqueous solution and kept at room temperature for 7 hours. The bioreduced aqueous component was used to measuring UV- Vis spectra of the solution. The particle suspension was diluted 10 times with distilled water to avoid the errors due to high optical density of the solution. The reduction of pure Ag++ ion monitored by the absorption maxima was scanned by UV-Spectral photometer at the wave length of 200- 800nm.

Antibacterials Test-Disc Diffusion Method[34]: The antibacterial assays against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were also performed by standard disc diffusion method. Nutrient agar (1g beef extract, 1g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water) was used to cultivate bacteria. The media was autoclaved and cooled. The media was poured in the petri discs and kept for 30 minutes for solidification. After 30 minutes the fresh overnight cultures of inoculums (100 µl) of two different cultures were spread on to solidified nutrient agar plates. Sterile paper discs made of Whatman filter paper, 5 mm diameter (dipped in 50 mg/ liter silver nanoparticles) along with a standard antibiotic containing disc were placed in each plate. The cultured agar plates were incubated at 37^0 C for 24 h. After 24 h of incubation the zone of inhibition was investigated.

Results and Discussion

The color changes was observed from pale white to yellowish brown color after 7 hours of incubation of rice husk extract and 1mM $AgNO_3$ aqueous solution mixture at room temperature due to the reduction of silver ions. This exhibited the formation of silver nanoparticles (plate-1). In this experiment the silver- ion and *Oryza sativa* husk extract interaction was confirmed. Aquoeus silver ions when mixed with *Oryza sativa* husk extract were reduced in solution. The husk extracts were pale white in color before adding of Ag ions and this change to yellowish brown color after 7 hours of incubation indicated the formation of silver nanoparticles.(plate 1) Due to excitation of surface plasmon vibration in silver nanoparticles produced yellowish – brown colour in aqueous solution [35,36,23,24]. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. There is always an essential need for reducing entities in the reaction medium to bring such conversions. To ensure the presence of reducing entities in the reaction medium, reducing assay was carried out. It showed in the photographs of sample solutions containing silver nanoparticles and silver nitrate in the presence of optimized amounts of *Oryza sativa* extract solutions after completion of the reaction (plate-1). Silver nitrate reduced the *Oryza sativa* husk extract and produced the silver hydrosol because Ag ions possessed good conductivity, catalytic and chemical stability, hence it was used as a reducing agent[37].

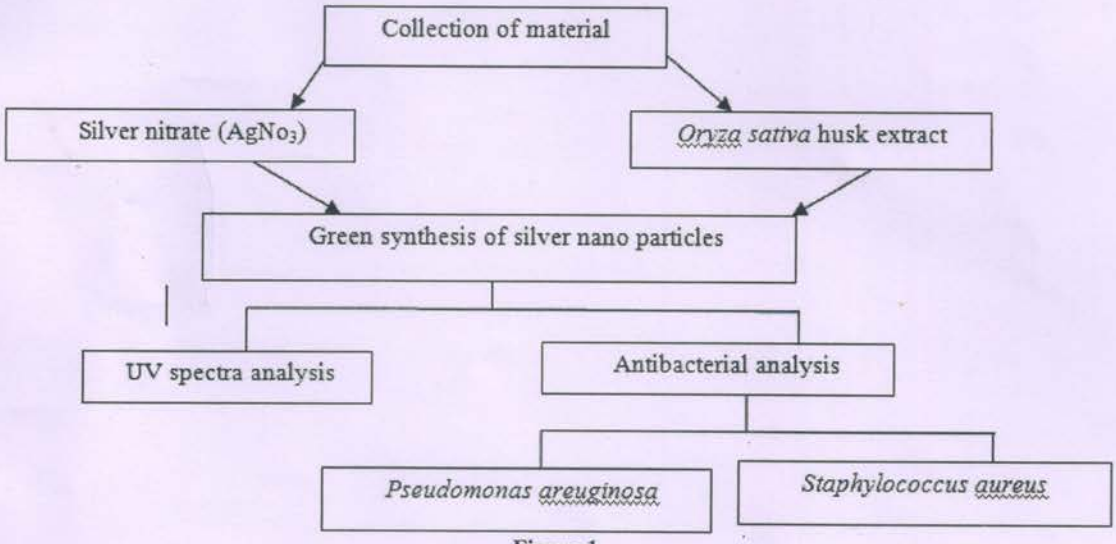

Figure-1
Experimental Design

Absorption spectrum at different wave lengths ranging from 200-800nm exhibited a peak at around 485nm. (figure- 2). This confirmed the formation of *Oriza sativa* husk nanoparticles. Similar phenomenon was demonstrated by the UV visible spectroscopy of the synthesized nano particles were in the range of 425- 519 nm. Out of 18 medicinal plants 15 were showed to synthesize the silver nano particles by the indication of suitable UV visible spectrum. The nanometer of various synthesized nano particles are Jeeragam- 466 nm, Kudagapalari – 455 nm, Dhaniya – 432 nm, Karpokarasi- 429.8 nm, Devadaru- 429.2 nm, Gugulu- 428 nm, Cherrupunneri- 425.2 nm, Athimadhuram- 437.8 nm, Veppampattai- 439.8 nm, Vasambu- 425 nm, Mahali- 444.6 nm, Pavu- 467.8 nm, Koduveli- 519.6 nm, Vettiver- 501 nm, Vilangam- 459 nm and chemical nano silver- 462 nm as a control. Shiva Shankar, *et al* [38] reported the faster bio- reducing methods to produce silver nanoparticle within an hour of reaction.

In our study, the AgNPs synthesized using *Oryza sativa* extract exhibited the antibacterial against two pathogens *Pseudomonas aeruginosa and Staphylococcus aureus.* The best inhibition zone (9mm) was observed in *Pseudomonas aeruginosa* than *Staphylococcus aureus* (6.2mm). The standard antibiotics Amikacin showed maximum zone of inhibition 15mm as compared to the nanoparticles treated discs and control. (Silver nitrate) (Table 1, plate 2 and plate 3). Silver nanoparticle has inhibitory and bactericidal effect hence it is used as antibacterial agents. They are highly toxic and hazardous to many microorganisms. Green synthesis silver based antiseptics that possesses broad spectrum antimicrobial activity and considerably decrease the susceptibility to induce microbial resistant than synthesized chemical antibiotics [4,5]. Coupling of inherent property of *Oryza sativa* extract with that of silver nanoparticles has really proved to be beneficial to minimize the dose that needs to be administered for total microbial reduction. Prabhu, *et al* [28] obtained the maximum inhibition at 150µl of plant leaf nanoparticle against *Staphylococcus aureus* using *Ocimum sanctum and Vitex negundo.* Pathogenic fungi namely *Candida albicans, C. kefyr* and *A. niger* effectively suppressed by *Euphorbia hirta* nanoparticles[39].

Conclusion

The present study confirmed that the green synthesis of nanoparticles from oriza sativa husk extract possesses antibacterial effect against human pathogens and suggest that it can be used to formulate new ecofriendly formulation of antibacterial agents for the treatment of bacterial infections in human beings for avoiding side effects caused by synthetic and semi synthetic chemical formulations and also proved that this is the better way of utilization of waste dumped *Oriza sativa* husk and reduce the environmental pollution to a significant extent.

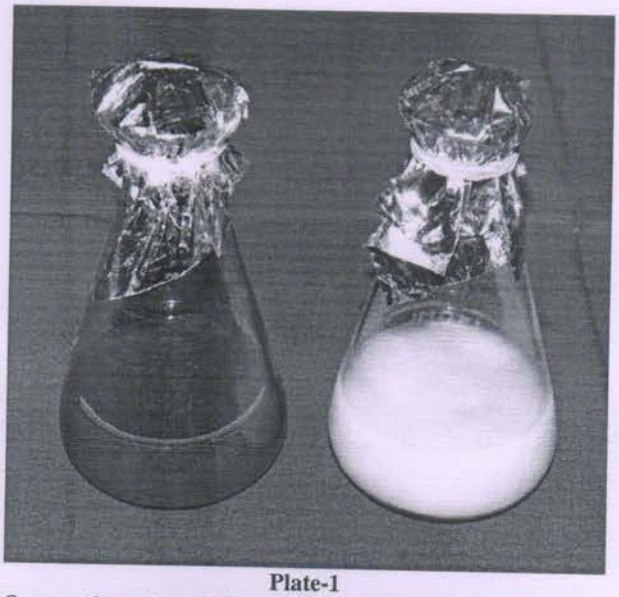

Plate-1
***Oryza sativa* extract before adding of $AgNO_3$ and The colour changes of plant extract after 6 hrs of incubation with $AgNO_3$**

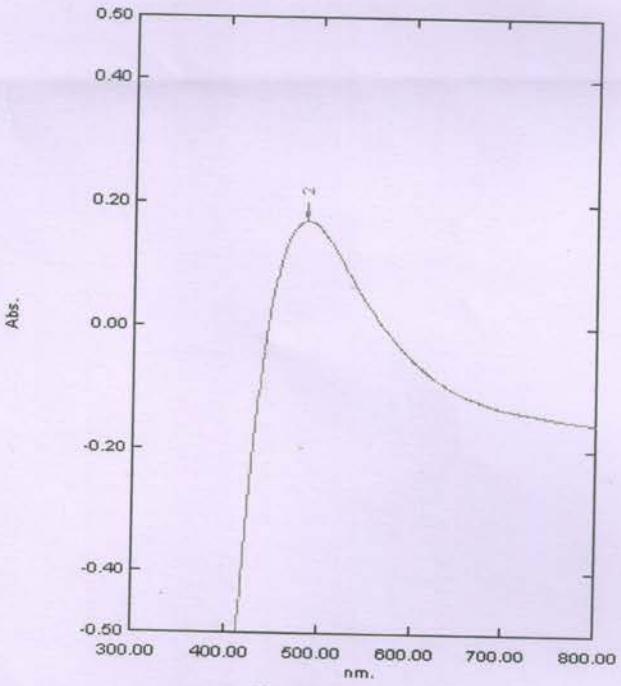

Figure-2
The UV spectrum of *Oryza sativa* silver nanoparticles recorded at room temperature

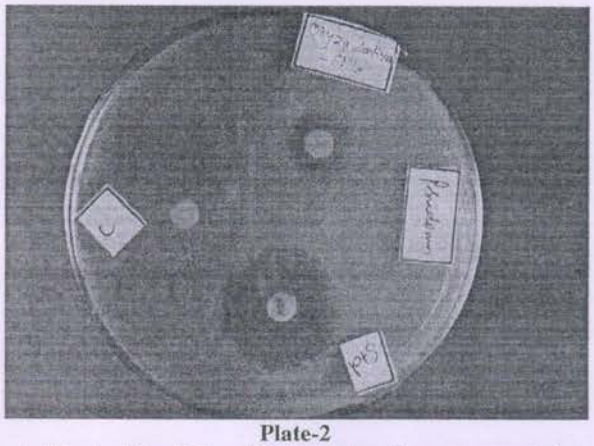

Plate-2
Antibacterial activity of *Oryza sativa* silver nanoparticles against *Pseudomonas aeruginosa* (Gram negative)
C: Control (silver nitrate), Std: Standard antibiotic (Amikacin), Sample: Silver nanoparticles of *Oryza sativa* husk extract, Pathogen: *Pseudomonas aeruginosa* (Gram negative)

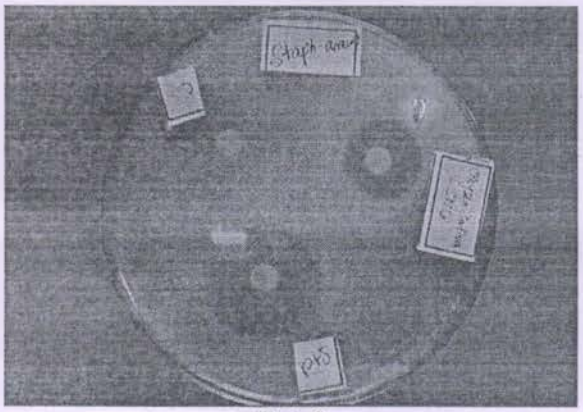

Plate-3
Antibacterial activity of *Oryza sativa* silver nanoparticles against *Staphylococcus aureus* (Gram positive)
C: Control (silver nitrate), Std: Standard antibiotic (Amikacin), Sample: Silver nanoparticles of *Oryza sativa* husk extract, Pathogen: *Staphylococcus aureus* (Gram positive)

Table-1
Antibacterial activity of *Oryza sativa* silver nanoparticles against two pathogens

Name of the organism	Zone of inhibition (diameter)
Pseudomonas aeruginosa	9
Staphylococcus aureus	6.2

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