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Synthesis of silver nanoparticles from wheatgrass extract and its biological applications

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Abstract
Nanoparticles are microscopic particles smaller than 100µm. Nanoparticles was synthesized using various metals. The nanoparticles synthesized with silver nitrate was preferred for numerous applications. In the present study wheatgrass was used as herbal medium. Wheatgrass has pharmacological and medicinal properties. The nanoparticles was synthesized by using incubation process. After addition of 1mM $AgNO_3$ in wheatgrass extract, the extract becomes brown indicated the synthesis of nanoparticles. The synthesized silver nanoparticles showed maximum absorption at 420nm indicated the formation of nanoparticles. The synthesized nanoparticles was showed considerable effect on seed germination rate and nodule formation. As well as the synthesized nanoparticles was showed countable antimicrobial activity against gram positive, gram negative and fungus.

Keywords: wheatgrass, biological, silver nanoparticles, microscopic

Introduction
Nanoparticles are the micro paricles synthesised using various sourses like plant, microrganism, and other medium with metal. Discoveries showed that once materials are prepared in the form of small particles their physical and chemical properties are changed, to the extent that their mechanism are changed. (Shah and Tokeer 2010) [12, 13]. Reducing the size of material increases the biocompatiblity of material. Many researchers are now engaged in developing new effective antimicrobial reagents with the emergence and increase of microbial organisms resistant to multiple antibiotics, which will increase the cost of health care. Therefore, there is an urgent need to develop new bactericides. Silver has been used for years in the medical field for antimicrobial applications such as burn treatment. (Parikh *et al.* 2005) [9]. Silver ions have long been known to exert strong inhibitory and bactericidal effect as well as to possess antimicrobial activity. (Berger *et al.* 1996) [3] There are some intresting reports that showed the encouraging activity of different drugs and antimicrobial agents in form of nanoparticles (Shah 2010) [12, 13]. The toxicity of nanoparticles depend on both method and size of nanoparticles like that of alumina, copper, gold, magnesium, silver and zinc. Nanotechnology is one of the growing area in the World today. It is known that living cells are the best example.

Material and Methods
Materials
Seeds of local variety of wheat was collected from local market of Nanded District. Chemicals like silver nitrate, nutrient agar was procured from Sigma Company. Cultures of *Escherichia coli*, *Styphlococcus aureus*, and *Aspergillus Niger* was collected from School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. Cultures were freshly inoculated and used.

Sample collection
The present study included with synthesis of nanoparticles and its uses in different aspect. For synthesis, we used 5-8 days grown wheatgrass. Wheat was sown in plastic tray for synthesis in M. G. Agri-Biotech College Pokharni, Nanded, and collected after full growth.

Nanoparticles synthesis
In the present work, the synthesis of silver nanoparticles has been carried out using the aqueous extract of wheatgrass. 1mM Silver nitrate solution was prepared and stored in amber

coloured bottle. (Azam, *et al*. 2009) [1].

Preparation of leaf extract

The wheatgrass leaves were washed several times with deionised water. 100gm of finely cut wheatgrass leaves was taken and boiled in 300ml of double distilled water for 3mins and filtered. After centrifugation at 10,000rpm for 15mins, the supernatant was collected and stored at 4 °C. (Krithiga *et al.* 2015) [8].

Synthesis of silver nanoparticles

Typical synthesis process of silver nanoparticles, 10 ml of leaf extract was added into 90 ml of 1 mM silver nitrate. When we add 90 ml of 1 mM silver nitrate solution into the 10 ml of wheatgrass leaf extract then immediately colour change to brown. Aqueous solution and incubated at room temperature. Formation of brown colour was indicates synthesis of silver nanoparticles. After the 24 hrs incubation period bottom of the flask observe silver nanoparticles (Banerjee *et al*. 2014) [10].

Analysis of nanoparticles synthesis

Spectroscopic analysis of synthesized nanoparticles was carried. The solution of AgNPs were checked at different nanometer from 300 to 700 nm at visible range. The AgNPs showed maximum absorbance at 420 nm.

Effect of AgNPs treatment on germination rate and nodule formations

Synthesised wheatgrass silver anoparticles were used to check the germination rate and formation nodule to groundnut crop. of local varity. Seeds of groundnut were treated and incubated overnight. Treatment of synthesised Ag nanoparticles solution was in 10:20 (No. of seeds: AgNps solution) ratio (Shakeel and Saiqa 2015) [14].

AgNPs antimicrobial activity

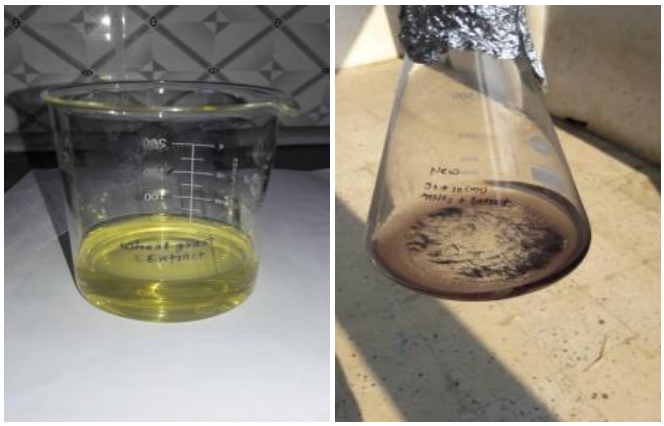
The antibacterial assay were done on two bacteria *Escherichia coli*, *Staphylococcus aureus* and one fungus species *Aspergillus niger* by using standard disc diffusion method. Fresh overnight cultures were taken and spread on the nutrient agar and potato dextrose plates to cultivate bacteria and fungus. Sterile paper discs of 5 mm diameter were saturated with plant extract, silver nanoparticles and double distilled water (as control) were placed in each plate and incubated at 37 ºC for 24 h and the antibacterial activity was measured based on the inhibition zone around the disc impregnated with plant extract synthesized silver nanoparticle. (Shakeel *et al*. 2016, Ratika and Vedpriya 2013) [15, 11].

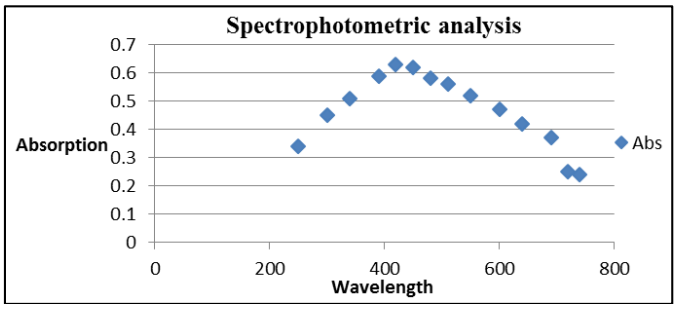
Results

UV-VIS spectral analysis

Wheatgrass were collected and extract were prepared with homogenisation method. Plant materials were collected and plant leaf extracts were prepared both by conventional and homogenization methods. Biosynthesis of silver nanoparticles by the filtrate of wheatgrass *was* confirmed by change in the colour of the filtrate to brown after addition of silver nitrate. The obtained nanoparticles were recovered and stored. This resulted due to excitation of surface plasmon vibrations in the silver nanoparticles.

The bioreduction of silver in the filtrate reaction solution was monitored by using UV-Vis spectroscopy. Control flasks maintained with silver nitrate solution (without plant filtrates) did not show any change of colour and its absorbance maximum was found to be at 420nm, which was specific for silver nitrate solution.





Effect of AgNPs treatment on germination rate and nodule formations

The biosynthesized nanoparticles showed considerable effect on germination rate and nodule formation. Compared between control and treated seeds of groundnut we found germination rate increased by 20% due to treatment of nanoparticles. Out of 100 seeds groundnut 50 % seeds were showed germination in control and 70% seeds were showed germination in treated seeds.

Table 1: Effect of synthesized nanoparticles on germination rate

S. No	Groundnut crop (100 seeds)	No. of seeds Germinated
1	Without treated (Control)	50
3	Treated with wheatgrass NPs	70

While compared between control and treated nanoparticles to seeds of groundnut for nodule formation, treated seeds showed increased number of nodule count than control. Nodules play important role in nitrogen fixation. Control plant of groundnut showed maximum 9 number of nodules while treated plant of groundnut showed maximum 13 number of nodules.

Table 2: Effect of synthesized nanoparticles on nodule formation

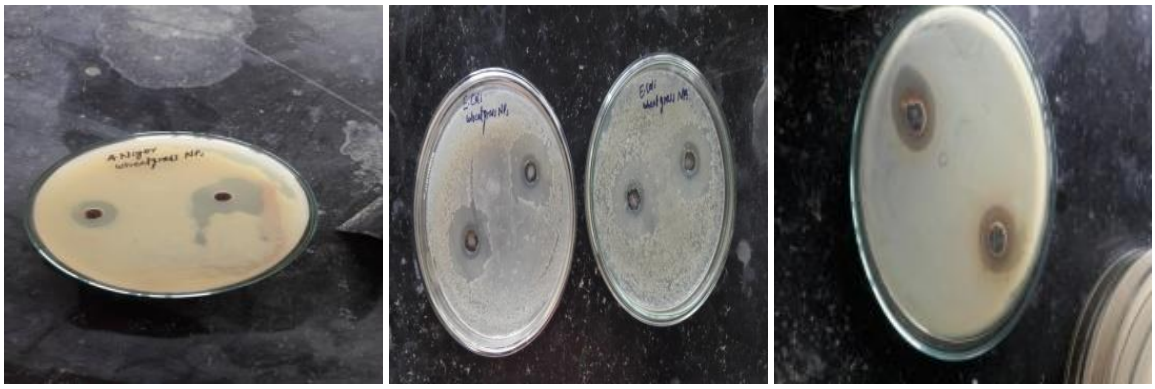
Sr. No.	Groundnut crop	Number of nodules
1	Without treated (Control)	7-9
3	Treated with wheatgrass NPs	10-13

Antimicrobial activity of AgNPs

The antibacterial activity of synthesized nanoparticles against *E. coli* (-ve), *S. aureus* (+ve) bacteria and fungus showed moderate antimicrobial activity. Compared in between gram negative and gram positive organism gram negative (-ve) bacteria showed significant zone of inhibition. While antifungal activity against *A. Niger* showed varied zone of inhibition from 5 to 9mm.

Table 3: Antimicrobial activity of synthesized nanoparticles

Sr. No.	Nanoparticles	Microorganism	Zone of inhibition (mm)
2	Biosynthesized from Wheatgrass	*Escherichia coli*	10
		Styphlococus aureus	5-8
		Aspergillus niger	5-9



Discussion

The absorbance of spectra of synthesized nanoparticles were analysed on spectrophotometer exhibit orahbe-yellow colour due to excitation of the locakised surface Plasmon vibrations of metal nanoparticles (Kelly *et al*. 2003, Stepanov 1997) [6, 16]. Previous studies showed that spherical AgNP contribute to the absorption bands at around 400nm in the UV-visible spectra (Maiti *et al*. 2013; Barman *et al*. 2014) [7, 2].

Chemical antibiotics are day by days becoming resistant. The substitute for antimicrobials are required the mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. It is reported that the positive charge on the silver ion is the reason for antimicrobial activity as it can attract the negatively charged cell membrane of microorganisms through the electrostatic interaction (Dibrov *et al*. 2002; Hamouda *et al*. 2000) [4, 5]. Due to their unique size and greater surface.

This study indicates that Ag-NPs can be used as effective antibacterial materials against various microorganisms which can endanger human beings. In conclusion, this study showed that Ag-NPs have potent antibacterial activities against E. *coli* and *A. Niger* cells. The growth and reproduction of Ag-NPs treated bacterial cells were inhibited.

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