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AGRO - ENGINEERING OF GREEN SYNTHESIZED MAGNESIUM NANOPARTICLES USING *Datura stramonium* LEAF EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The experiment was conducted during 2019, at Department of Microbiology, Miraj Mahavidyalaya, Miraj, affiliated to Shivaji University, Kolhapur, Dist. Sangli, Maharashtra, India. *Datura stramonium* leaf extract was fused with magnesium nitrate hexahydrate, $Mg(NO_3)_2$Å•$6H_2O$ to form nanoparticles of leaf extract and $Mg(NO_3)_2$ Å•$6H_2O$ named as Ds-Mg Nanos using green synthesis method. FusedDs-Mg Nanos were studied for their optical, structural, surface morphological characteristics using TG/DTA and XRD and antibacterial properties. Optical study showed an appearance of SPR peak at 505 nm in the absorption spectrum, insisting the formation of Ds-Mg Nanos. The TG analysis showed a minor weight loss step (19%) between 28°C and 275°C, as well as two major weight loss steps (63%) in between 275°C and 452°C. No further weight loss was observed beyond 452°C and up to 1000°C. On the DTA curve, main exothermic peaks were observed at ~ 291°C and ~ 448°C. X-ray line broadening reflections of the Ds-Mg Nano's sample were (219), (310), and (435). Particle size distribution studies were carried out using dynamic light scattering techniques. It was observed that Ds-Mg Nanos were narrow in size and were distributed within the range of 16–26 nm. The synthesized Ds-Mg Nanos showed antibacterial activity against bacteria *E. coli and Bacillus subtilis* bacteria; nevertheless, sound marked activity was observed against *E. coli*. Now a days the pathogens are became resistant to antibiotic and minimized their wide inhibition range, in that case as the results recorded here, said study is more applicable with their antibacterial potentials, furthermore it is need to focus on synthesis of nanoparticles using the crops and their antagonisms.

(Keywords: Magnesium nitrate hexahydrate; *Datura stramonium* ;Ds-Mg Nanos; antibacterial)

INTRODUCTION

Datura stramonium is a commonly available plant, traditionally used for treatment of diseases. It has ample pharmaceutical potential. It has been reported to contain alkaloids, tannins, carbohydrates and proteins. Henceforth the plant leaves were selected for the study. *Datura stramonium* plant leaves and magnesium nitrate haven't shown any anti-bacterial activity neither are too effective individually, hence focus was kept on using them toprepare nanoparticles.

Recently, nanostructures of magnetic materials are receiving more attention because of their novel properties; that are significantly different from those of their bulk counterparts. Some biologically synthesized nanoparticles are used for gas sensing property; microorganisms along with metal oxides are used during the synthesis of such nanoparticles (Wu *et al.,* 2008; Chaugule *et al.,* 2011; Bangale *et al.,* 2013). Now a day's novel antibiotics have been reported being used against different pathogens to cure different microbial diseases but microbial pathogens are known to become resistant against novel antibiotics. Perhaps green biosynthesis of nanoparticles may be considered for treating infections caused by all those resistant pathogens. A study on post harvesting of *Penicillium* mold of sweet orange was done, which causes green mold disease (Patil *et al.,* 2017). This study focuses on biochemical green synthesis of magnesium nanoparticles using *Datura stramonium* leaf extract and their antimicrobial activity against selected pathogens. Nanoparticles are widely applicable and have become an alternative to antibiotic therapy. Nanoparticles are showing broad mechanisms with their target sites showed in Fig. 1 (Linlin *et al.,* 2017). In the study on fungicides hexagonasol (0.1%) was effective for disease severity to control brown leaf spot of tobacco caused by *Alternaria alternata* (Fr.) Kiessler (Dip *et al.,* 2020).

Chemical synthesis of nanoparticles may lead to adsorption of some toxic chemical species on the surface that can lead to adverse effects in its applications. Even though there are many means available for metal nanoparticles synthesis; low cost as well as non-toxic, high-yield and environmental friendly procedures are required to develop metal nanoparticles with desired sizes and shapes, thereby

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making biological synthesis process essential. In biological processes, microorganisms like bacteria, actinomycetes and fungi have been studied in metal nanoparticles preparation, while use of extracts of whole parts of plants in nanoparticles synthesis appears a facile alternative to chemical synthesis process. (Gomathi *et al.,* 2017).

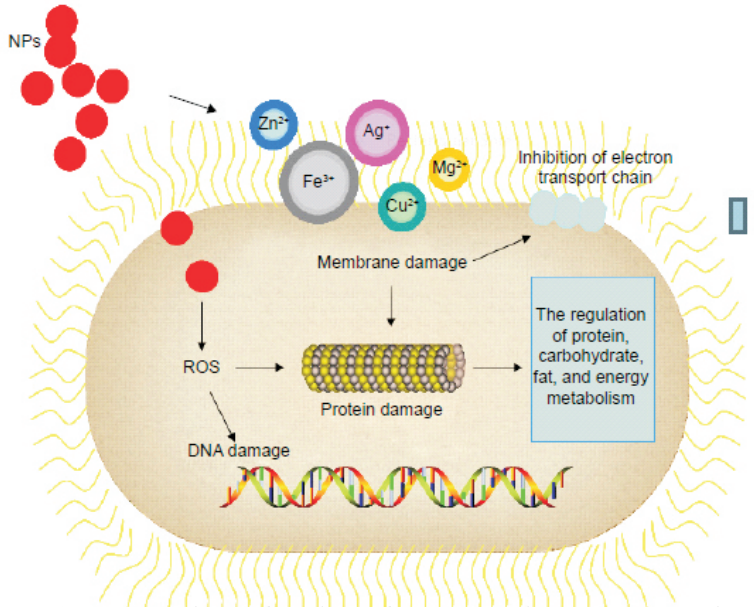

Figure 1. Mechanism of action of nanoparticles on bacterial cell

MATERIALS AND METHODS

Green synthesis method is used for preparation of magnesium nanoparticles using chemical agent magnesium nitrate hexahydrate, $Mg(NO_3)_2$Å"$6H_2O$and *Datura stramonium* plant.

The prepared nanoparticles were subjected to **TG/ DTA and XRD techniques** for their physical and morphological characteristics and their antagonistic property was reported by inspecting the material against selected pathogens *E. coli and Bacillus subtilis*. *E. coli* is used as an indicator of all coliforms.

Synthesis of Ds-Mg Nanos using magnesium nitrate and *Daturastramonium*.

Datura stramonium leaves were freshly collected from Sangli city, Maharashtra state, Indiafor the study. Green synthesis method was employed for preparation of present nanoparticles. Soil particles and other contaminants present on the surface of the fresh leaves were removed using flowing tap water followed by distilled water.

In a beaker containing 500 ml de-ionized water, 25 g of uniform leaves, previously cut down into number of small pieces, were added.

The beaker was heated at 60°C to 70°C for 15 min. with constant stirring; cooled and the mixture filtered. The filtrate was considered as leaf extract and was kept at - 4°C refrigeration till further use.

10 ml of filtrate was taken in a beaker and treated with drop wise addition of aqueous solution of magnesium nitrate hexahydrate, [Mg $(NO_3)_2 \cdot 6H_2O$], constantly stirring for 10 minutes to get colloidal particles.

The reaction was carried out till the colorless mixture turns to a dark color, indicating reductionof Mg^+ ions to free Mgwhich combines with the leaf extract components, leading to the formation of nanoparticles termed as Ds-Mg Nanos.

The dark material was filtered with filter paper or Whatman's paper and then the final filtrate was calcinated at 200°Cin a muffle furnace, leading to the formationof final product of nanoparticles (Fig. 2).

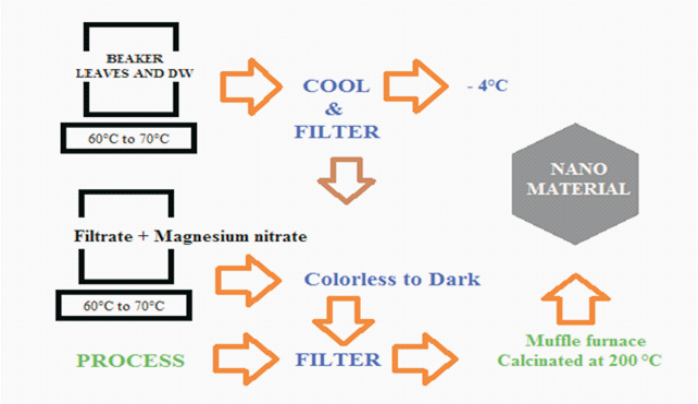

Figure 2. Synthesis of Ds-Mg Nanos

Characterization

Fused Ds-Mg Nanos were studied for their optical, structural, surface morphological characteristics using **TG/DTA, XRD and UV–vis–NIR spectrophotometer**

The prepared samples were characterized using TG/DTA thermal analyzer (SDT Q600 V 20.9 Build 20). The crystalline structures of the powder were analyzed with XRD Philips Analytic X-ray B.V. PW-3710 based model diffraction analysis using Cu-K_a radiation with a wavelength of 1.5418 Å, scanning electron microscope (JEOL JED 2300), transmission electron microscope operating at 200 kV.

UV–vis–NIR spectrophotometer was used for obtaining optical absorption spectrum of Ds-Mg Nanos.

Antagonism

One Gram positive *Bacillus subtilis* and one Gram negative *E. coli* bacteriawere used for the study. Pure cultures, 24 hrs. old, of both bacterial cells were taken were used during the study. Separate suspensions of both bacterial cells were added in two separate conical flasks containing molten and cooled nutrient agar media and then the media were poured in separate petriplates. After solidification of the media, in all 6 wells of 10 mmwere cut in the nutrient agar plates with sterilized cork borer. Each well was filled with different concentrations of Ds-Mg Nanos as 100, 200, 300, 400 ìl. with negative control as sterilized de ionized water. Positive control was standard penicillin antibiotic of 100 U ml^{-1}. Plates then were incubated at 37^0C for 24 hrs. The antibacterial activity of synthesized Ds-Mg Nanos was tested against Gram positive (*B.subtilis*) and Gram negative bacteria (*E. coli*) using well diffusion method, for their ability to prevent the bacterial growth. The formation of zones of inhibition around the well was observed and scaled in mm or cm. The zones of inhibition of Ds-Mg Nanos in various concentrations were matched with positive and negative control, which indicated the antimicrobial activity.

RESULTS AND DISCUSSION

UV-Vis Spectroscopy Studies

Colloidal dark brown suspension of reaction mixture of magnesium nitrate hexahydrate, $Mg(NO_3)_2 \cdot 6H_2O$ and leaf extract of *Datura stramonium* (previously wasvisually clear) confirmed the appearance of nanoparticles - Ds-Mg Nanos.

This dark brown color of reactionmixture might be due to resonance of externally applied UV-visspectrum and collective oscillation caused by the surface electrons of formed Ds-Mg Nanos ; this is a well known phenomenon of Surface Plasmon Resonance (SPR) (Ahamed *et al.,* 2011; Krishnaraj *et al*., 2010; Matos *et al .,* 2011).

The initial color of magnesium nitrate hexahydrate, $Mg(NO_3)_2 \cdot 6H_2O$ turned to dark brown with accumulation of leaf extract after a reactionperiod of 15 min. The changes in colour of the reactionmixture strongly specify the reduction of Mg+ ions to Mg. The formation of Mg crystals in reaction mixture was shown by appearance of sharp andnarrow SPR peak at 505 nm was shown (Table 1 and Fig. 3).

The adequate reductionof bio-molecules within theleaf extract greatly reduced the $AgNO_3$ solution as silver crystaland wrapped around the Ag NPs. (Gomathi *et al*., 2017).

Structural studies

Spinal structure and formation analysis

The TG curve in Figure 4 shows a minor weight loss step (19%) between 28°C and 275°C, as well as two major weight loss steps (63%) in between 275°C and 452°C. No further weight loss was observed beyond 452°C and up to 1000°C. The minor weight loss in the as-spun Ds-Mg Nano's Nano powder was due to loss of moisture by trapped solvents like water and existing carbon dioxide during the combustion, whereas, major weight loss was due to the combustion of organic matrix. On the DTA curve, main exothermic peaks were observed at ~291°C and ~ 448°C, suggesting that the thermal events were related to the decomposition of Mg nitrates carried out by dehydration of the nano-powder, which was confirmed by a drastic weight loss in TG curve at the corresponding temperature range (275–452°C). The plateau formed between 452°C and 1000°C on the TG curve indicates the formation of crystalline Ds-Mg Nanos which acts as the decomposition product after combustion reaction. This was confirmed by XRD.

XRD study

The XRD patterns of the calcinated Ds-Mg Nanos are shown in Figure 5. All of the observed main peaks were indexed and matched with the spinel Ds-Mg Nano's in the standard data (JCPD No. 88-1935). The average crystallite sizes of Ds-Mg Nano's sample were calculated. X-ray line broadening reflections of the Ds-Mg Nanos sample were (219), (310), and (435), calculated using Scherer's equation (i.e. $D = 0.89k/(â \cos è)$, where k is the wavelength of the X-ray, K is a constant taken as 0.89, h the diffraction angle and b is the full width at half-maximum (Verma *et al.,* 2004 ; Bangale*et al.,*2011; Cullity *et al*., 2001 ;Wang *et al.,* 2005) and were found to be 16, 18, 25 and 26 nm for the Ds-Mg Nanos samples calcinated at 500°C, and 600°C respectively.

Antibacterial activity

Well diffusion technique was used for checking antagonistic activity of Ds-Mg Nanos which were tested against pathogens as Gram negative *Escherichia coli (E. coli)* and Gram positive *Bacillus subtilis (B. subtilis).* Zones of inhibition were found against both the pathogens. The observed zones of inhibition were noted in terms of diameter in mm. (Table 1). No zone of inhibition was found for negative control and prominent zone ofinhibition was seen for positive control. As the concentrations of Ds-Mg Nanos increases,they lead to increase in the zones of inhibition. Same results were noted for synthesized Ag NPs antibacterialactivity against the bacterial strains of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* using well diffusion technique (Nagaraju *et al.,*

2017). The zones of inhibition around the wells depicted that synthesized Ag NPs showed significant impact on the growth of bacteria around the well.The antibacterial activity of Ag NPs increased gradually as the concentrations increased from 50 to 200 ìl(Gomathi *et al.,* 2017) (Table 2 and Fig. 6).

Shape is an important factor related to antimicrobial activity of Nano particles. NPs with different shapes can cause varying degrees of bacterial cell damage through interactions with periplasmicenzymes(Romero *et al*.,2010). A study had shown that preparation of ZnO NPs under different stirring conditions can affect their antibacterial activity against Grampositive (*B. subtilis*) and Gram negative (*E. coli*) bacteria and a fungus (*C. albicans*). (Khan *et al*.,2016).

It has been shown that Mg NPs can adhere to and diffuse into the biofilms; this leads to disruption of the membrane potential and enhanced lipid peroxidation and DNA binding. Disorder in the normal functioning of these processes decreases the ability of bacteria to form biofilms(Lellouche *et al.,*2012). The structure of biofilms makes bacteria very resistant to foreign chemicals. Earlier reports demonstrated that NPs interfere with biofilm integrity by interacting with Bacterial exopolysaccharides (EPSs) (Su*et al., 20*09). Ag NPs inhibit the production of Bacterial exopolysaccharides (EPSs), which further leads to action against the biofilms of drug-resistant strains of *E. coli* and *Klebsiella pneumonia* (Ansari *et al.,* 2012).

It was seen that the prepared Ds-Mg Nanos nanoparticles were more effective against Gram negative *E. coli* than the Gram positive *B. subtilis* .

Through green synthesis method nanoparticles Ds-Mg Nanos were prepared using magnesium nitrate hexahydrate, $Mg(NO_3)_2$Å"$6H_2O$ and leaf extract of *Datura stramonium*. UV-vis spectroscopy studies indicated the formation of Mg crystals with leaf extract in the reaction mixture; shown by the appearance of sharp and narrow SPR peak at 505 nm indicating the formation of nanoparticles Ds-Mg Nanos.

The TG curve shows a minor weight loss step (19%) between 28°C and 275°C, as well as two major weight loss steps (63%) in between 275°C and 452°C. No further weight loss was observed beyond 452°C and up to 1000°C. The XRD patterns of the calcinated Ds-Mg Nanos were shown and were found to be 16, 18, 25 and 26 nm calcinated at 500°C, and 600°C respectively. The maximum antibacterial activity of Ds-Mg Nanos nanoparticles was observed against *E. coli*.Work can be continued with reference to antagonism mechanisms of newly synthesized nanoparticles against reported pathogens.

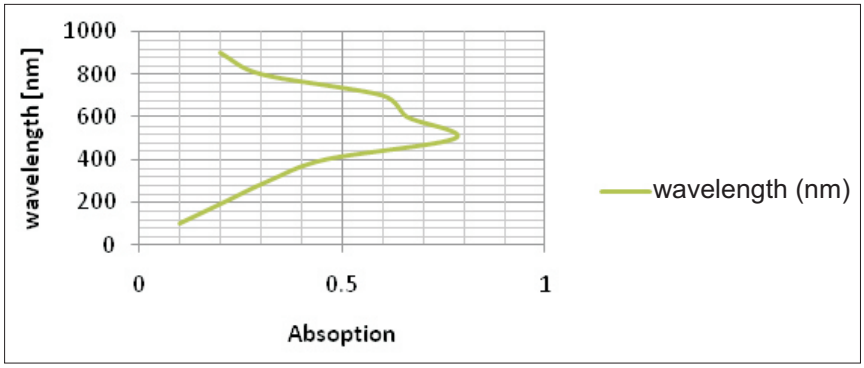

Figure 3.Absorption spectrum of Ds-Mg Nanos

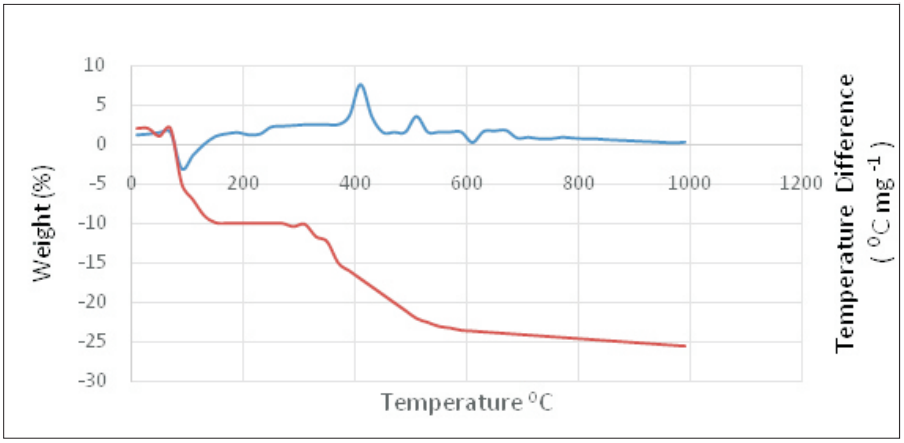

Figure 4.Thermo gravimetric differential analysis curve of Ds-Mg Nanos

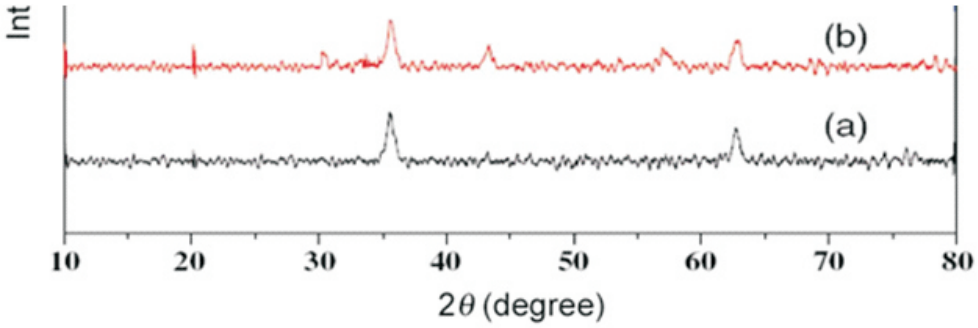

Figure 5. XRD pattern of calcinated mixed precursor Ds-Mg Nano's in air to: (a) 500°Cand, (b) 600°C

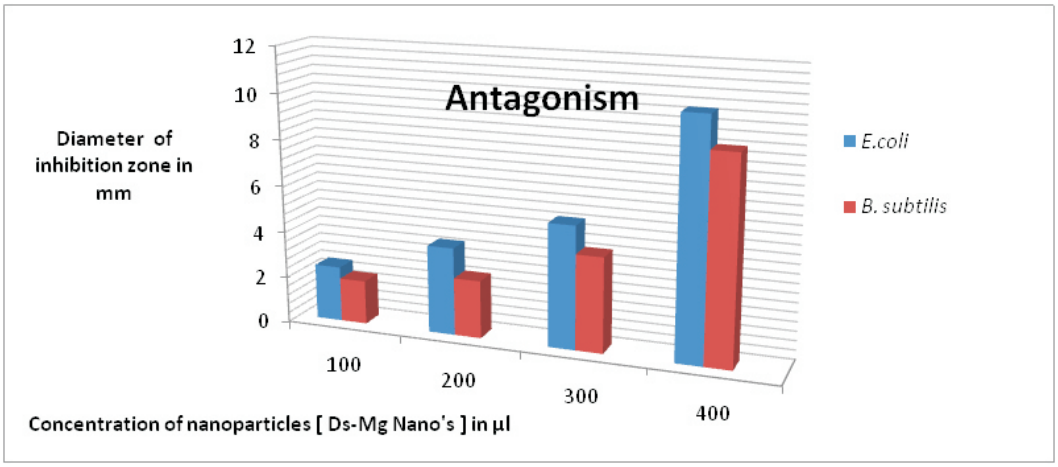

Figure 6. Antagonism of nanoparticles

Table 1. Absorption spectrum of Ds-Mg Nanos

Sr. No.	Absorption	wavelength (nm)
1	0.1	100
2	0.21	200
3	0.32	300
4	0.46	400
5	0.78	500
6	0.66	600
7	0.6	700
8	0.3	800
9	0.2	900

Table 2 Antagonism of nanoparticles [Ds-Mg Nano's] at different concentrations

Sr. No.	Concentration of nanoparticles [Ds-Mg Nano's] inìl	Diameter Zone of inhibition in Mm	
		E.coli	***B. subtilis***
1	100	2.4	1.9
2	200	3.8	2.5
3	300	5.3	4.1
4	400	10.2	8.8

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