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PREPARATION OF NANOANTIBIOTICS THROUGH GREEN SYNTHESIS OF MgO NANOPARTICLE USING LEAF EXTRACT OF *BRASSICA NIGRA*

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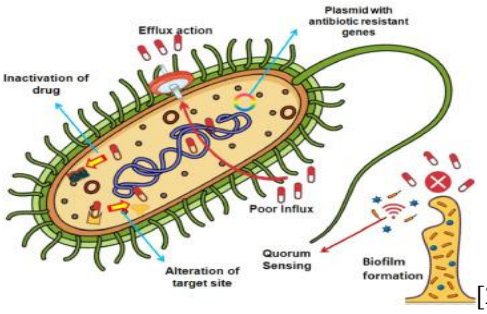
Abstract

Nanoparticle synthesis using plant extracts is an important method because it is non-toxic, biocompatible, and eco-friendly. Plant extracts contains diverse phytochemicals or metabolites such as carbohydrates, alkaloids, terpenoids, phenols, tannins, lipids, reductases, proteins, flavonoids, vitamins, etc. Antimicrobial resistance has become a cosmopolitan problem and it has been a challenge in the medical and pharmaceutical fields from 20th century. The biggest advantage of the research is that a medicine has been prepared from a non-medicinal plant, we conclude that *Brassica nigra* (Mustard) naturally is not a good source of antimicrobial activity but after synthesis of MgO nanoparticles, the antimicrobial activity increased. In this study, green synthesis of MgO nanoparticles using *Brassica nigra* leaf extract was conducted by mixing the extract with magnesium sulphate solution. The MgO nanoparticles have been successfully synthesized using *Brassica nigra* leaf extracts, with the help of co-precipitation method. The product was characterized using different techniques, scanning electron microscopy (SEM). The size of the synthesized MgO nanoparticles was found to be 100 nm. The future prospects of my project work would be to construct such plant based nano systems that can increase the duration of their presence in the bloodstream and can cause biomechanical damage by dissolving and releasing metal ions to disrupt the prokaryotic cellular structures thus inhibiting bacterial growth.

Keywords: Nanoparticles, plant extracts, magnesium oxide.

Introduction

Antimicrobial resistance has become a cosmopolitan problem and it has been a challenge in the medical and pharmaceutical fields from 20th century. With major advances in medicine, huge surgical procedures, such as heart surgery and kidney transplantation, are being victorious; but the infection after the surgeries is a major issue due to microbial resistance. Thus, our competitive medical world is deadlocked in the evolutionary arms of microbes. As we are developing newer approaches to treat microbial infections, microbes are using their own mechanisms to develop resistance [1].



[2]

Plant extract loaded NPs offers numerous advantages because of their size and unique physicochemical characteristics. Moreover, plant extract loaded NPs can be used to decrease plant extracts toxicity, to provide targeted drug delivery and to solve stability related problems [3]. Nanoparticle fabrication using plant extracts is an important alternative method because it is nontoxic, biocompatible, and environmentally friendly. Green synthesis techniques use reagents that are free of chemicals that are harmful to the environment such as using water solvents or plant extracts [4, 5]. The size and shape of magnetite particles are generally controlled by the synthesis method. A variety of methods have been reported in the literature on the synthesis of magnetite nanoparticles, such as the chemical co-precipitation [6], the hydrolysis [7], the thermal decomposition [8] and the sol–gel technique [9].

NPs have been widely used in many applications such as medications, electronics, manufacturing and materials, environment, mechanical industries, etc. [10]. Nanotechnology opens up many opportunities because of the enhancement in the characteristics of nanosized particles at their unique size and shape. Nanoscience and nanotechnology showcase prospective applications in almost every scientific field. Specifically, nanostructured metal oxide plays a vital role in many areas of chemistry, physics, materials science, and biotechnology [11].

A subset of nanotechnology is called bionanotechnology[12]. Nano-biotechnology is a unique combination of biotechnology and nanotechnology that can be used to truly integrate classical microtechnology into a molecular biological approach [13, 14]. Nanomaterials have unique properties and the inherent antimicrobial activity of some of them has been proven [15]. Furthermore, there has been considerable interest in employing novel nanostructured systems to enhance the delivery of antibiotics. This approach aims to improve the pharmacokinetics of the drug while minimizing its adverse effects. By encapsulating antibiotics within a polymer matrix, these nanosystems can prolong their circulation in the bloodstream, leading to a substantial reduction in both the required dosage and frequency of administration [16-17].

Material & methods

3.1 Sample collection

Table no. 1 Collection of sample:

S. No.	Sample name	Scientific name	Location
1.	Mustard	*Brassica nigra*	Vivek Khand 1,Gomti nagar,Lucknow(UP)

Fresh leaves and stems were collected:

Fig.2 Collection and cutting of leaf and stem of *Brassica nigra*(Mustard).

3.2 Plant extract preparation

Some of Leaves and stem of *Brassica nigra* were cut into pieces and dried under sunlight, while some of them were used in wet form to extract the phytoconstituents. Dried form of plants was grounded in mixer and filtered it. 5 gram powders were dissolved in 50 ml solvents i.e. D.W. and incubated at 60 ˚C in water bath. Similarly, 5 gram paste of leaves and stems were dissolved in different solvents i.e. 70% acetone, 50% ethanol separately and incubated at room temperature for over night.

Fig.3 Filtration of (*Brassica nigra)* Mustard leaf and stem sample.

Samples those were incubated in water bath were filtered through whatman filter paper on similar day while paste sample were filtered through whatman paper on next day. Filtered samples were stored at 4˚C in refrigerator for further use.

3.3 Green synthesis of MgO nanoparticles

10 ml of extraction and to 10 ml $MgSO_4$ solution of 10 mM were mixed in beaker with help of magnetic bead stirrer at 600 rpm with 90˚C. On reaching at 90˚C, 2M NaOH solution was added to the mixtures. Mixture was left for 3 hrs. aging process in order to optimize the formation of the $Mg(OH)_2$ precipitate. After precipitation, centrifugation were commenced at 12000 rpm for 10 min. for separation of precipitate. Collected precipitate was washed with 95% ethanol for 3 time. Washed material were dried over night using oven. Solubility was checked in different solvents.

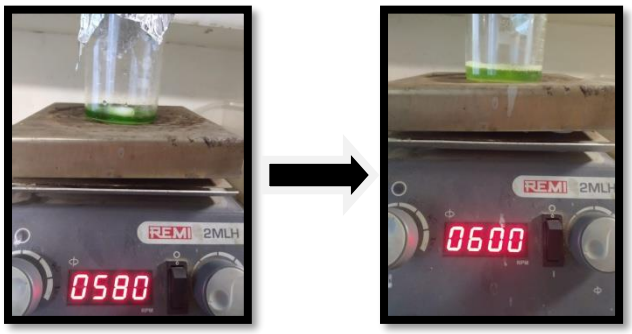


Fig.4 The color of leaf sample has changed due to oxidation reduction reaction.

3.4 Antimicrobial activity

Antimicrobial activities of *B.nigra* leaves extract, stem extract, MgO nanoparticles and Norfloxin were evaluated against gram negative *E.coli*, gram Postive *S.aureus* and *P. Aeruginosa* obtained from MRD Lifesciences. Pvt. Ltd., Uttar Pradesh Lucknow. Isolates were grown on nutrient broth at 37˚C for 24 hrs. Bacterial suspension spreaded on Nutrient agar media plates. Wells were prepared. Suspension (40µl) of above mention material were loaded in wells separately and incubated at 37˚C for 24 hrs to determine the zone of inhibition.

Fig.5 AST of Leaf and stem(ethanol) NPs of *Brassica nigra*.

Fig.6 AST of Leaf and stem(acetone) NPs of *Brassica nigra.*

3.5 Minnimum inhibitory test

Nutrient broth (0.26 gm) was prepared for 6 test tubes. 3 microliter nutrient broth was transfer in all test tubes. All the equipment was autoclaved at 121°C for 30 min. After autoclaving 100 microliter sample was loaded in 1st test tube then in second up to 6th test tube and discard 100 microliter from 6th test tube. Added 20µl bacterial sample in all test tubes except 6th test tube. All the test tubes were incubated in shaker incubator at 37° C for 24hrs except 6th test tube. Kept 6th test tube in refrigerator at 4°C after 24 hrs. Optical density was measured at 600 nm.

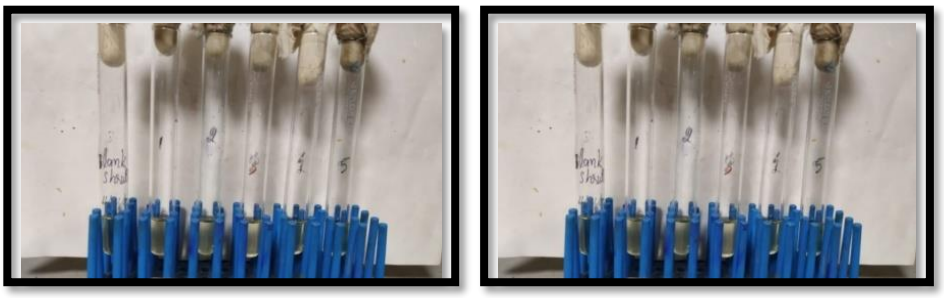


Fig.7 MIC of Leaf(ethanol) and Leaf(acetone).

3.6 Characterization of prepared Nanoparticles

Prepared NPs was followed by Uv-Visible spectrophotometer analysis. An aliquot of 1ml of reaction mixture was placed in glass cuvette and the absorbance of the sample was scanned from 200nm-800nm wavelength. Surface morphology was studied using SEM. Surface functional groups were characterized using FTIR.

3.7 Phytochemical test

Table no.2 Summarization of Phytochemical test of *Brassica nigra*

S.No.	Phytochemical Test	Sample	Reagent	Observation
1.	Flavanoid	Leaf extract(ethanol)	1ml of 10% lead nitrate in 1ml leaf extract	Yellow precipitate was observed
		Leaf extract(acetone)	1ml of 10% lead nitrate in 1ml leaf extract	Yellow precipitate was observed

2.	Saponin	Leaf extract(ethanol)	3ml D.W. in 1ml leaf extract	Froth was observed
		Leaf extract(acetone)	3ml D.W. in 1ml leaf extract	Froth was observed
3.	Tannin	Leaf extract(ethanol)	Few drops of Lead nitrate in 1ml Leaf extract	Precipitate was observed
		Leaf extract(acetone)	Few drops of Lead nitrate in 1ml Leaf extract	Precipitate was observed
4.	Steroid	Leaf extract(ethanol)	2ml chloroform + 2ml H2SO4 in 1 ml leaf extract	Reddish brown interface was observed
		Leaf extract(acetone)	2ml chloroform + 2ml H2SO4 in 1 ml leaf extract	Reddish brown interface was observed
5.	Terpenoid	Leaf extract(ethanol)	0.1ml chloroform + 0.1 ml H2SO4 in 0.1 leaf extract	Red color was observed
		Leaf extract(acetone)	0.1ml chloroform + 0.1 ml H2SO4 in 0.1 leaf extract	Red color was observed
6.	Carbohydrate	Leaf extract(ethanol)	0.1 ml Fehling A + 0.1ml Fehling B in 0.1 ml leaf extract	Red Precipitate was observed.
		Leaf extract(acetone)	0.1 ml Fehling A + 0.1ml Fehling B in 0.1 ml leaf extract	Red Precipitate was observed.

Results:

Table3: Antimicrobial sensitivity test of Nanoparticles against different pathogens.

S.No.	Sample	Solvent	*S.aureus*	*P.aeruginosa*	*E.coli*
1.	Leaf paste nanoparticles	50% ethanol	13.1mm	13.1mm	14.6mm
2.	Stem paste nanoparticles	50% ethanol	0	0	0
3.	Norfloxin	D.W.	69.3mm	72.3mm	62.6mm

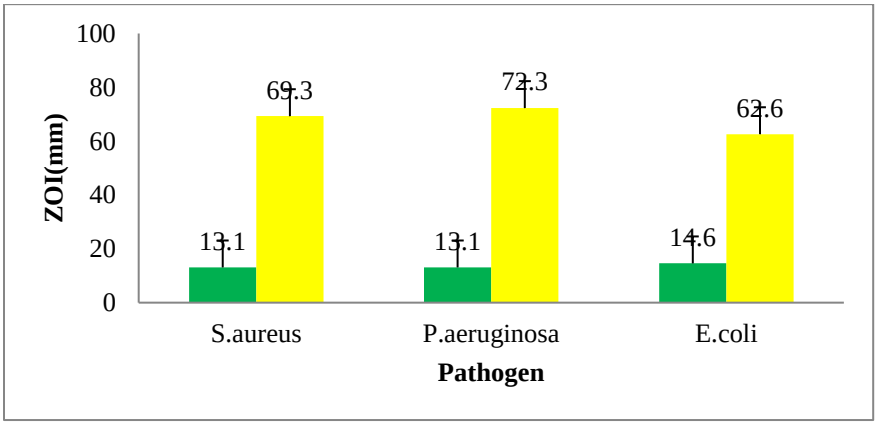


Fig.8 AST of *Brassica nigra*(50% ethanol) NPs against different pathogens.

Table 4:AST of *Brassica nigra*(mustard) leaf paste NPs -

S.No.	Sample	Solvent	*S.aureus*	*P.aeruginosa*	*E.coli*
1.	Leaf paste NPs	70% acetone	13.6mm	14.3mm	16mm
2.	Norfloxin	D.W.	72.3mm	77.4mm	69.3mm

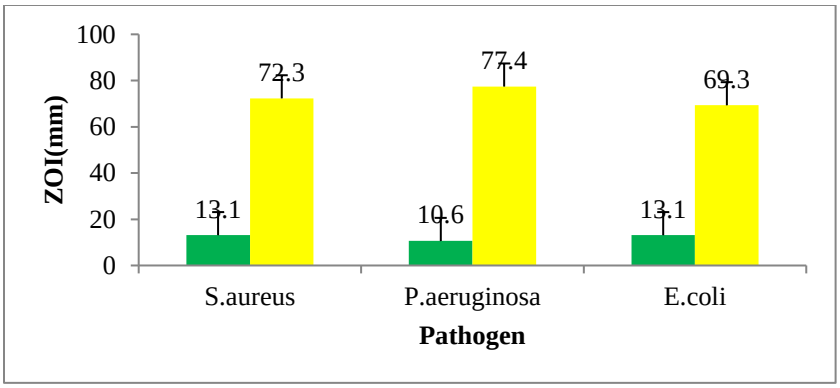


Fig.9 AST of *Brassica nigra*(70% acetone) leaf paste NPs

Table5. AST of *Brassica nigra*(mustard) stem paste NPs –

S.No.	Sample	Solvent	*S.aureus*	*P.aeruginosa*	*E.coli*
1.	Stem paste NPs	70% acetone	0	0	0
2.	Norfloxin	D.W.	82.7mm	105mm	93mm

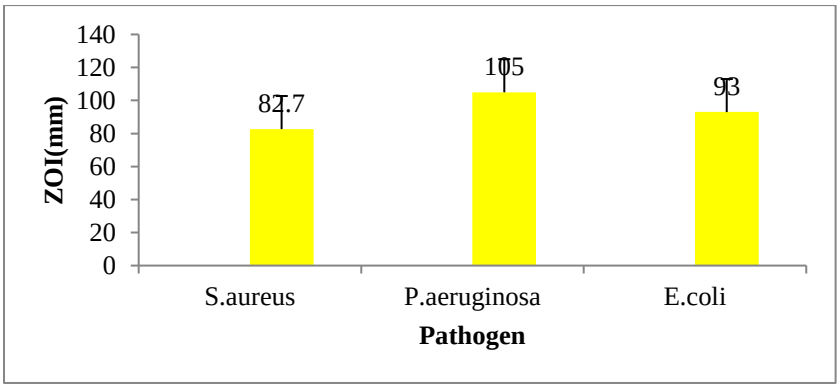


Fig.10 AST of *Brassica nigra*(mustard) stem paste NPs

Table no. 6. MIC of *Brassica nigra* leaf paste(ethanol) Nanoparticles -

S.No.	Conc. of Nanoparticles	Bacteria	O.D. Value at 600 nm
Blank	0	20µl	0.00
1.	66.6µl	20µl	0.07

2.	4.44µl	20µl	0.08
3.	0.296µl	20µl	0.10
4.	0.019µl	20µl	0.11
5.	0.001µl	20µl	0.17

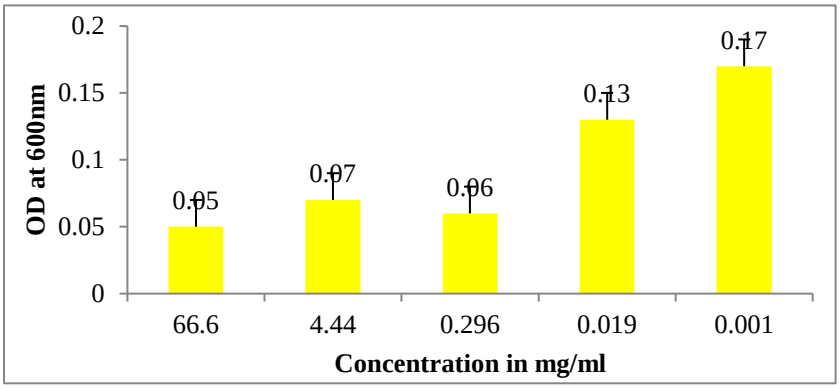


Fig.11 AST of *Brassica nigra*(mustard) stem paste NPs

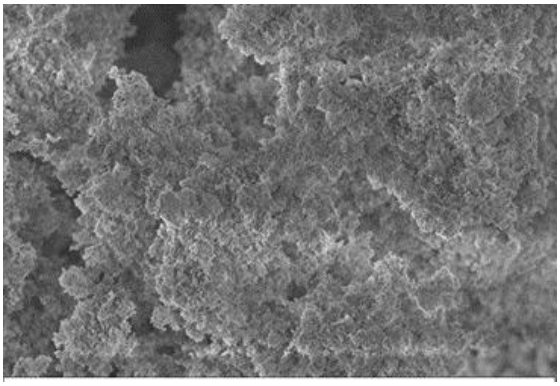


Fig.12 SEM images of MgO nanoparticles

Discussion & Conclusion

The use of nanotechnology-based systems has been grown rapidly. Plant extract loaded nanoparticles can bring many benefits which can be aimed to facilitate in crossing the biological barriers, to increase bioavailability of poorely water-soluble phytochemicals, to encapsulate mixture compounds of different phytochemicals, to provide targeted delivery of phytochemicals to specific organs resulting in low toxicity and to provide a more flexible control over the size and shape of the NPs.

I have prepared the nanoparticles with the metal ions and plant extract combination and obtained great results with respect to normal metal salts and crude plant extract.

The nanoparticles formed in the ratio 1:1, shows good antimicrobial properties so then the minimum concentration of that nanoparticle was calculated, which is responsible for the maximum inhibition growth. The product was characterized using different techniques, scanning electron microscopy (SEM). The size of the synthesized MgO nanoparticles was found to be 100 nm.

For finding the Phyto-compounds which are present in the plants are analyzed with phytochemical tests regarding the phytochemicals. Due to ethanolic property, leaf nanoparticles of *Brassica nigra* have been good results. Where ZOI of *S.aureus* is 13.1mm, *P.aeruginosa* is 13.1mm and *E.coli* is 14.6mm.

We come up to a point where we conclude that *Brassica nigra* (Mustard) naturally is not a good source of antimicrobial activity but after synthesis of MgO nanoparticles, the antimicrobial activity increased.

They yield of antimicrobial can be enhanced by using more sophisticated procedure and can be tested in various other solvent. It has proved to be effective against bacteria and used at elevated temperatures. So we can conclude that drugs made out of would not be dependent on what conditions that are stored, this gives an advantage in handling the drugs made out of this plant.

Future prospects of my project work would be to construct such plant based nano systems that can increase the duration of their presence in the bloodstream and can cause biomechanical damage by dissolving and releasing metal ions to disrupt the prokaryotic cellular structures thus inhibiting bacterial growth. Secondly to develop a more sophisticated protocol for the extraction of secondary metabolites from the plant materials.

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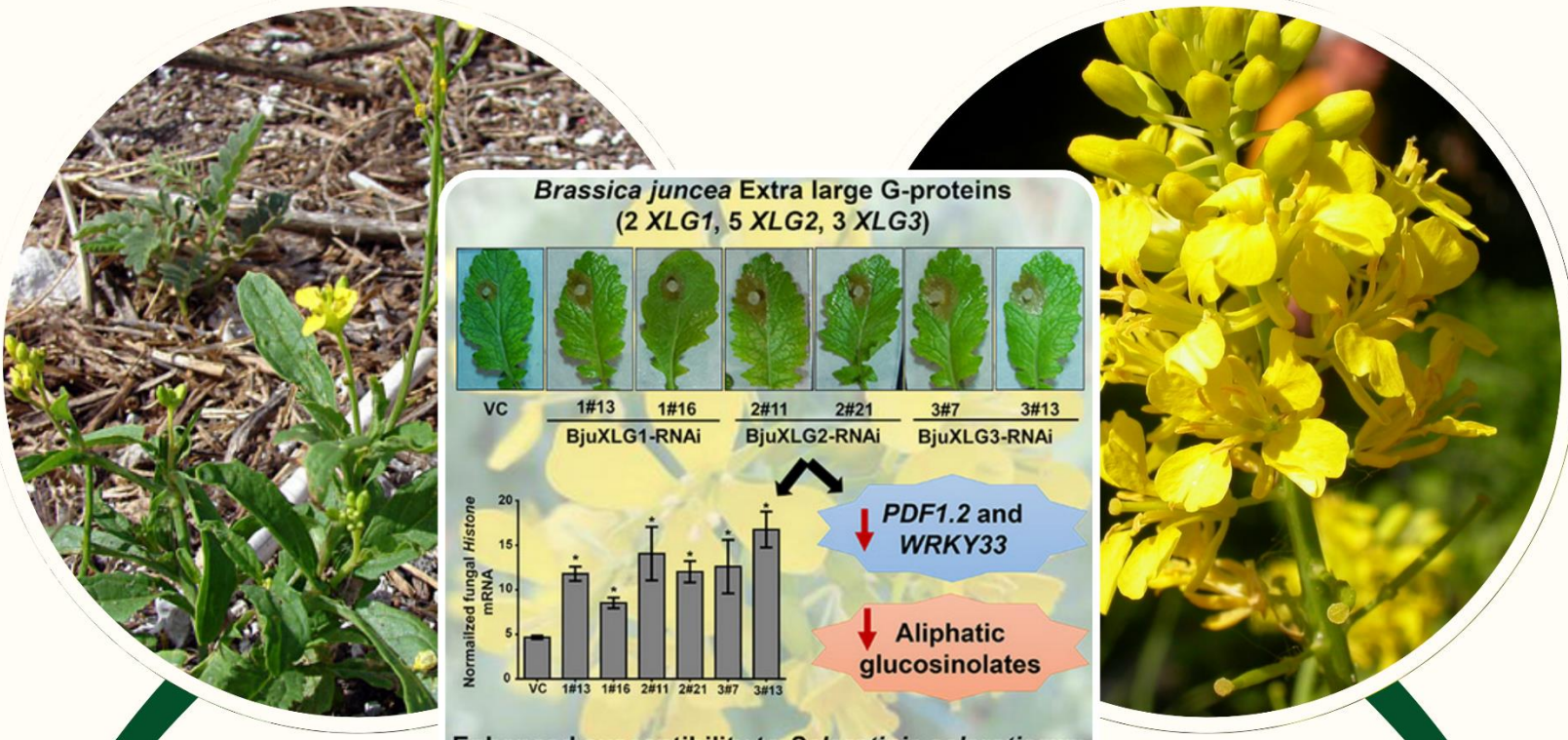

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