Synthesis of Magnesium Senna Auriculata Nanoparticles with its Anti-Oxidant and Antimicrobial Activity- A Green Synthesis

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green synthesis, magnesium oxide, nanoparticles, natural product, Senna ariculata.

ABSTRACT
Background: Green synthesis of nanoparticles refers to the synthesis of various metallic nanoparticles using various natural products. With the rise in the demand for successful treatment, all the criteria to be fulfilled are important. This demand will lead to the innovation of the antibacterial gels which are used post-operatively for healing the tissue and which have an antibacterial activity.
Aim: The study aims to synthesize magnesium senna auriculata nanoparticles.
Materials and Methods: Senna auriculata was used to synthesize magnesium oxide nanoparticles. Ethanol was used for extract preparation. After preparation of the nanoparticles, it was subjected to SEM analysis, EDX, FTIR, particle size analyzer cytotoxicity test, UV-B, antimicrobial test, and antioxidant test
Results: The obtained particles were highly antioxidant, and moderate antibacterial activity was observed(50 conc 93.15)
Conclusion: Senna auriculata synthesized magnesium nanoparticles were found to have more antioxidant activity so they can be used in an ointment for intra-oral wound healing.

1. Introduction

The field of nanotechnology has experienced remarkable advancements over the past decades, revolutionizing various scientific domains, particularly in medicine, environmental science, and material engineering(Abdulkhaleq *et al.*, 2017). Among the broad spectrum of nanomaterials, magnesium nanoparticles (Mg NPs) have garnered significant attention due to their biocompatibility, degradability, and wide-ranging applications. Mg NPs exhibit unique physicochemical properties such as a high surface-area-to-volume ratio, enhanced catalytic efficiency, and the ability to generate reactive oxygen species (ROS), making them suitable for diverse applications, particularly in antimicrobial and antioxidant fields(Ahmed *et al.*, 2010). However, the traditional nanoparticle synthesis methods involve using hazardous chemicals and energy-intensive processes, raising concerns about their environmental impact and biocompatibility(Amalan *et al.*, 2016). Consequently, green synthesis methods have emerged as sustainable and eco-friendly alternatives, utilising plant-based resources to achieve safer and cost-effective production of nanoparticles(Annie, Rajagopal and Malini, 2005).

Green synthesis, or plant-mediated nanoparticle synthesis, leverages the bioactive compounds present in plant extracts to act as reducing, stabilising, and capping agents during nanoparticle formation(S. Deshpande, Kewatkar and Paithankar, 2013). This approach is sustainable, scalable, and eliminates the need for toxic reagents, aligning with the principles of green chemistry. In this context, Senna auriculata, commonly referred to as Tanner's Cassia or "Avaram" in traditional medicine, emerges as an ideal candidate for green synthesis(S. S. Deshpande, Kewatkar and Paithankar, 2013). This plant, belonging to the Fabaceae family, is widely recognized for its therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities. Rich in secondary metabolites such as flavonoids, tannins, alkaloids, and phenolic acids, Senna auriculata offers a dual advantage: it serves as a natural reducing agent for nanoparticle synthesis while imparting bioactive properties to the

synthesized material(Duraipandiyan, Ayyanar and Ignacimuthu, 2006).

Magnesium, as a core component, is an essential mineral involved in numerous physiological processes, including enzyme activation, energy metabolism, and oxidative stress regulation. Its incorporation into nanoscale dimensions amplifies its biological activity, making it highly reactive and bioavailable(Elango, Ponnusankar and Sundaram, 2015). Magnesium nanoparticles synthesized through green synthesis methods exhibit enhanced antibacterial and antioxidant properties, offering potential solutions to current challenges such as antibiotic resistance and oxidative stress-related disorders. The synergistic combination of magnesium's intrinsic properties with the bioactive compounds of Senna auriculata presents a promising avenue for biomedical and environmental applications(Gupta *et al.*, 2016).

Senna auriculata is an extensively studied medicinal plant known for its pharmacological efficacy in treating a variety of ailments. Its leaves, flowers, and seeds are rich in phytochemicals that exhibit antibacterial, antioxidant, and hepatoprotective activities(Gupta *et al.*, 2016). The plant's phytochemical profile includes a wide range of secondary metabolites, such as catechins, quercetin, gallic acid, and saponins, which contribute to its therapeutic potential. These bioactive molecules not only stabilize the magnesium nanoparticles but also enhance their functionality by imparting additional biological activities(Singh and Sharma, 2020).

The use of Senna auriculata in the synthesis of Mg NPs aligns with the principles of green chemistry, as it eliminates the requirement for synthetic reducing agents like sodium borohydride or hydrazine. (Arnason, Mata and Romeo, 2013) Furthermore, the utilization of plant extracts offers scalability and cost-effectiveness, making it suitable for large-scale production. Recent studies have highlighted the efficacy of Senna auriculata-mediated nanoparticles in exhibiting potent antimicrobial and antioxidant activities, underscoring its potential as a viable alternative to conventional synthesis methods(Shukla and Iravani, 2018).

Conventional methods for nanoparticle synthesis, such as chemical reduction, sol-gel techniques, and physical methods like laser ablation, have been widely used to produce Mg NPs(Shukla and Iravani, 2018; Patra *et al.*, 2020). While these methods allow precise control over particle size and morphology, they are often associated with significant limitations, including the use of hazardous chemicals, high energy consumption, and generation of toxic byproducts. Additionally, these approaches lack biocompatibility, which restricts their application in sensitive domains like biomedicine(Shukla, 2021).

For example, chemical reduction methods typically employ strong reducing agents such as hydrazine or sodium borohydride, which pose risks to both human health and the environment. Similarly, physical methods demand high-energy inputs and specialized equipment, increasing the overall cost and carbon footprint. These limitations have necessitated the exploration of alternative synthesis methods that are not only environmentally sustainable but also produce biocompatible and multifunctional nanoparticles(Akshaykranth *et al.*, 2021).

Green synthesis methods have emerged as a sustainable alternative to conventional techniques, offering multiple advantages such as environmental safety, cost-effectiveness, and the incorporation of bioactive properties. In the case of magnesium nanoparticles, the green synthesis approach involves the reduction of magnesium salts, such as magnesium chloride or magnesium nitrate, using plant extracts rich in bioactive compounds(Humaira *et al.*, 2024). The process typically proceeds through a simple reaction mechanism where the phytochemicals present in the plant extract act as reducing agents, converting magnesium ions into their nanoscale form. Concurrently, these phytochemicals stabilize the nanoparticles, preventing aggregation and ensuring uniform morphology(Khandel *et al.*, 2018).

The synthesis of Mg NPs using Senna auriculata extract offers several advantages over conventional methods. The plant's phytochemicals not only facilitate the reduction of magnesium salts but also

impart additional functionality, such as enhanced antioxidant and antimicrobial properties. The process is carried out under mild conditions, often at room temperature or with minimal heating, reducing energy consumption and simplifying scalability(Pugazhendhi *et al.*, 2019).

Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, is a major contributor to various chronic diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases(Hasan *et al.*, 2018). Antioxidants play a crucial role in mitigating oxidative stress by scavenging free radicals and preventing cellular damage. Magnesium nanoparticles, particularly those synthesized through green methods, exhibit significant antioxidant activity due to their ability to generate ROS in controlled amounts, triggering cellular defense mechanisms without causing damage(Kanjana, 2020).

The antioxidant activity of Senna auriculata-mediated Mg NPs is further enhanced by the bioactive compounds present in the plant extract. These nanoparticles have been evaluated using standard assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and ferric reducing antioxidant power (FRAP) assay, demonstrating their efficacy in neutralizing free radicals and protecting against oxidative damage. Such properties make these nanoparticles promising candidates for applications in pharmaceuticals, cosmetics, and food preservation(Adil *et al.*, 2015).

The increasing prevalence of antibiotic-resistant pathogens poses a significant challenge to global healthcare. Nanoparticles, with their unique physicochemical properties, offer a novel approach to combating microbial infections(Humaira *et al.*, 2024). Magnesium nanoparticles, in particular, exhibit potent antimicrobial activity against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi. The mechanism of antimicrobial action involves multiple pathways, such as disruption of the microbial cell membrane, generation of ROS, and interference with intracellular processes, leading to microbial death(Periakaruppan, Naveen and Danaraj, 2022).

Senna auriculata-mediated Mg NPs have shown enhanced antimicrobial activity due to the synergistic effects of magnesium and the bioactive phytochemicals present in the plant extract. Studies have reported significant inhibitory effects against pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Additionally, the nanoparticles exhibit antifungal activity against species like Candida albicans, making them versatile agents for addressing various microbial infections.

The synthesis of magnesium nanoparticles using Senna auriculata extract offers immense potential for applications in various fields, including biomedicine, agriculture, and environmental remediation. The antioxidant and antimicrobial properties of these nanoparticles make them ideal for use in drug delivery systems, wound healing, and food packaging. Furthermore, their biocompatibility and eco-friendly synthesis process align with the growing demand for sustainable and green technologies.

Future research should focus on optimizing the synthesis process to achieve greater control over particle size and morphology. Additionally, in-depth studies on the toxicity and biocompatibility of these nanoparticles are essential to ensure their safe application in clinical settings. The integration of advanced characterization techniques, such as atomic force microscopy (AFM) and dynamic light scattering (DLS), can provide valuable insights into the structural and functional properties of the nanoparticles.

The synthesis of magnesium nanoparticles using Senna auriculata represents a significant advancement in green nanotechnology. By harnessing the bioactive properties of the plant extract, this approach not only provides a sustainable alternative to conventional synthesis methods but also enhances the biological functionality of the nanoparticles. The antioxidant and antimicrobial activities of these nanoparticles hold promise for addressing global challenges such as antibiotic resistance and oxidative stress-related disorders. Continued research and innovation in this field are expected to pave the way for the development of next-generation nanomaterials with diverse applications in healthcare,

agriculture, and beyond.

2. Materials and Methods

In the present study, green synthesis of the nanoparticles was aimed at accessing the properties of the prepared nanoparticles.

2.1 Collection of Senna auriculata flowers and powdering

Dried Senna auriculata flowers were bought from an organic shop. The leaves and the stock in the packed contents were then cleared and separated. The separated flowers were then put into a chopper where it was chopped into fine powder. The fine powder was then filtered using a fine metal mesh sieve and was separated into three components and stored in a falcon tube.

2.2 Preparation of Senna auriculata flower extract

250ml of 99% ethanol was measured in a measuring beaker and was transferred into a glass bottle of 400ml. The distilled water of 150ml was then added to the ethanol. This reduces the concentration to 70%. 50g of powdered senna auriculata was measured using a weighing pan and then added to the bottle containing 70% ethanol. The contents of the bottle were then mixed thoroughly and let be in the shade for about two days. After two days the solution was observed visually and filtration was done. A filter paper was taken and folded into a V-shape and placed in a funnel which was placed in the neck of another bottle. The mixture was then poured into the funnel containing filter paper and was let to filter. After the whole mixture is filtered the remaining solid contents are then carefully separated. To it, 60 ml of 99%ethanol was measured and poured. Then 150ml of distilled water was measured and poured into the solid remains and left in the shade for another day. The next day the same filtration was done and the filtered solution was stored in a refrigerator at 4^0C overnight.

2.3 Addition of magnesium

The extract was taken to a normal temperature to cool down. 5g of magnesium was weighed and taken in a petri dish. 200ml of distilled water was then taken in a conical flask. The magnesium chloride taken was then mixed well with the distilled water taken in the conical flask. 100ml of the extract was measured in a measuring beaker and was kept separately. A magnetic pellet was dropped into the conical flask and kept in a magnetic stirrer. During the stirring process, the extract was slowly added to the conical flask. The stirring process was done for about 1 hour. After one hour it was taken out and the pH of the solution was neutralised with 1N sodium hydroxide(NaOH).

2.4 Preparation of nanoparticles

The solution with extract was then transferred into 6 falcon tubes 10ml in each of the falcon tubes. The falcon tubes with the solution were then run in a centrifuge at 10000rpm for about 10 min. The solution was then carefully separated into a conical flask. The falcon tube was refilled with the neutralized extract and the centrifuge procedure was continued until fully finished with the neutralized extract solution. After full completion of the centrifuging of the neutralized extract, the particles that were collected in the base and the walls of the falcon tubes were allowed to dry in a hot air oven at 50 degrees Celsius overnight. After the particles were dried the particles in the falcon tubes were scraped with a spoon spatula into a motor and were grinned to fine particles with a pestle. The finely grounded particles were then collected in an Eppendorf tube.

2.5 SEM and EDX

From the collected nanoparticles 25 micrograms were weighed on a measuring balance scale. It was then submitted for SEM and EDX analysis. The results were then obtained and compiled.

2.6 Preparation of stock solution for antimicrobial assay

The stock solution is the master diluted solution that is gonna be used for different dilutions for antimicrobial assay. Here 2mg of nanoparticles were taken in an Eppendorf. 2ml of deionized water

was taken in a micropipette and poured into the nanoparticle's Eppendorf. It was then placed in an ultrasonic bath for about 10 min to obtain a uniform mix.

2.7 Anti-microbial Assay

The test used in this study is called a disk diffusion test agar diffusion test or Kirby-Bauer test. 30 sterile paper discs were first taken. Then 3 sterile paper discs were taken with dose-dependent magnesium Senna auriculata nanoparticle preparation of 25µl, 50µl, and 100µl each and transferred to another Petri dish. They were placed in the hot air oven at 50°C. These dry discs were placed on the surface of Muller Hinton agar coated with ESBL. The plates were incubated for 24 hours aerobically at 37 °C. After the incubation, the plates were checked for a zone of inhibition. Three other sterile discs were taken and dipped in chlorhexidine then transferred to a Petri dish. They were then placed in the hot air at 50°C. These discs were also later incubated and checked for results.

3. Results

The particles that were synthesized were subjected to various testing for analyzing multiple properties of the synthesized nanoparticles.

3.1 SEM and EDX

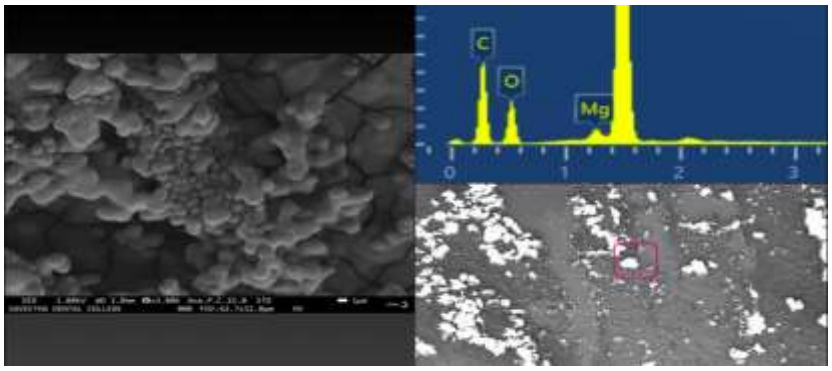

Figure 1: Showing the SEM and EDX results of the nanoparticles

The scanning electron microscope shows a well-defined structure of the nanoparticles. Further, the EDX electron microscopic image was obtained. The magnesium was present in the prepared nanoparticles with respect to other components.

FTIR

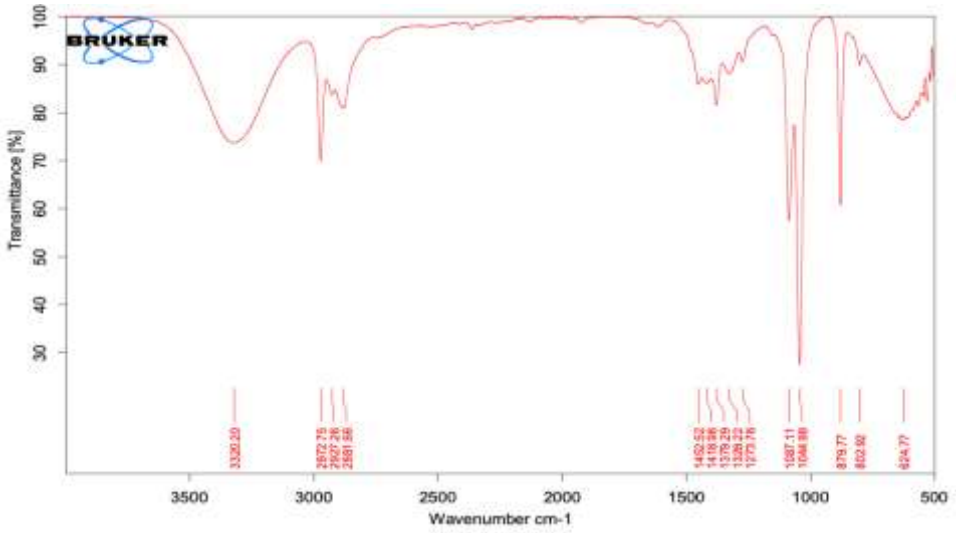

Figure 2: Shows the FTIR reading of the Magnesium Senna auriculata nanoparticles

PARTICLE SIZE ANALYSER

Table 1: Showing the Average Particle Size Diameter and the Standard Deviation

	Diameter(nm)	std . deviation
average	99.4	25.3

The following data show that the prepared magnesium senna auriculata nanoparticle is under the size of the nanoparticles.

UV-B

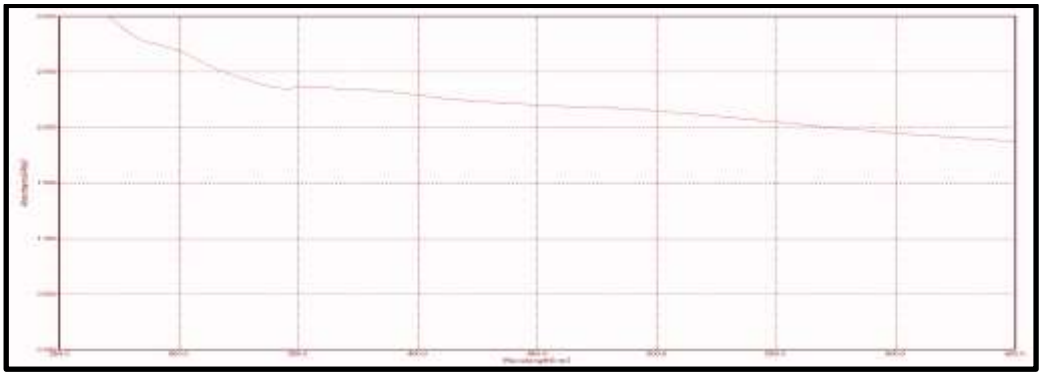

Figure 3: Shows the UV absorption chart

The UV absorption started at about 650nm with an absorption rate of 1.87. The rapid absorption of UV was observed at about 520-415nm absorption rate ranging from 2.11-2.52. The was the flat as UV absorption rate was equal to 270nm (rate-3).

CYTOTOXICITY

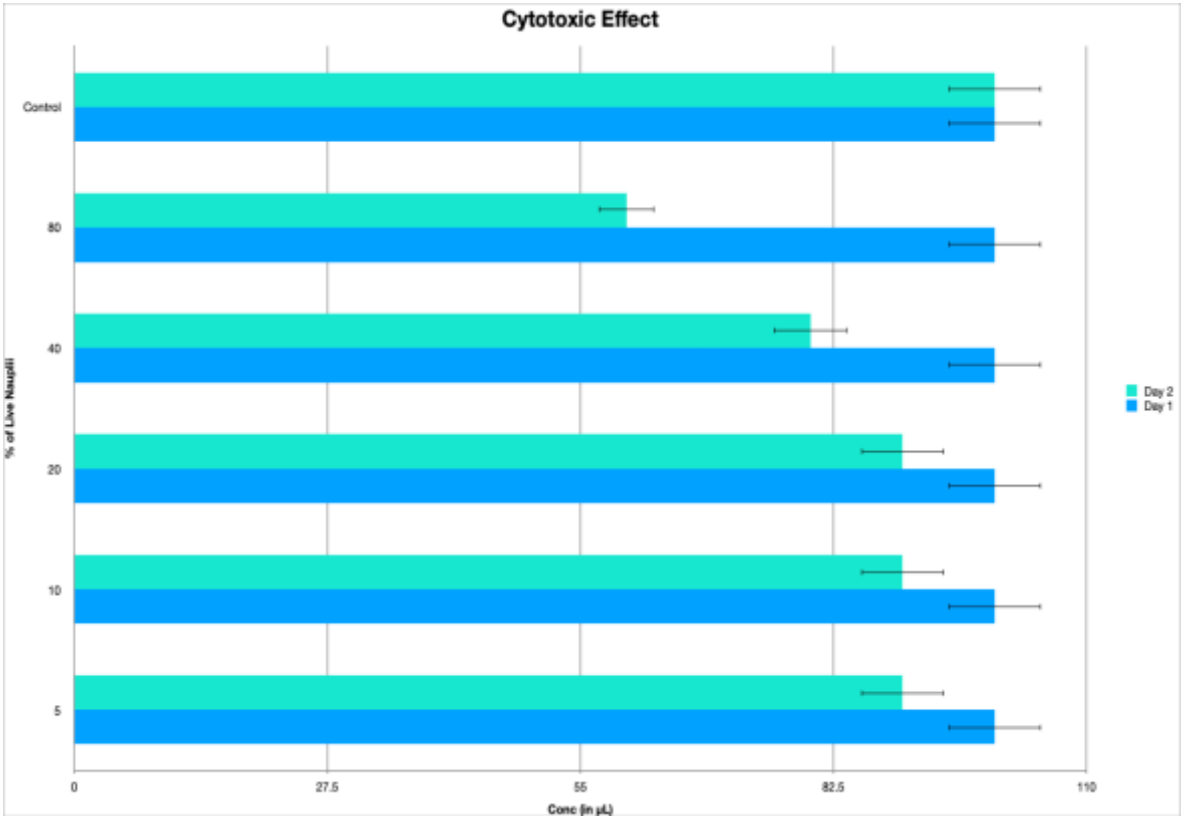

Chart 1 Showing the Cytotoxicity Effect of the Nanoparticle

Table 2: Showing the Cytotoxicity Values of the Nanoparticles in Day 1 and Day 2 in Comparison with Control

Conc (in µL)	Day 1	Day 2
5	100	90
10	100	90
20	100	90
40	100	80
80	100	60
Control	100	100

The cytotoxicity test was done using live nauplii cells. It is seen as up to the range of 40-60 microlitre of concentration the nanoparticle concentration is non-toxic and toxicity is seen in 80 microlitre concentration.

ANTIOXIDANT ACTIVITY

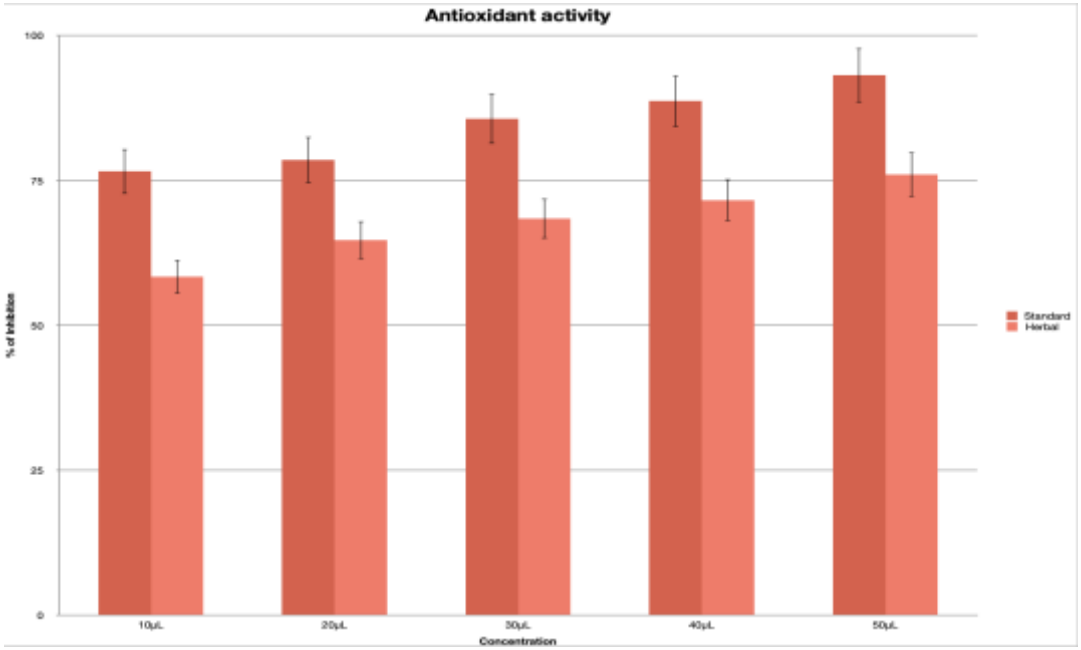

Chart 2: Showing the Antioxidant Test Chart of the Magnesium Senna Auriculata Nanoparticle

Table 3: Showing the Antioxidant Absorption Rate of the Nanoparticles

CONC	STD
10	76.56
20	78.52
30	85.63
40	88.68
50	93.15

The antioxidant test chart reveals that as the concentration of the magnesium senna auriculata nanoparticles increase there was an increase in the antioxidant activity. This shows that the prepared nanoparticles are highly antioxidant.

ANTIMICROBIAL ACTIVITY

Figure 4: Shows the antibacterial activity of the Senna auriculata nanoparticles.

4. Discussion

Synthesis of nanoparticles is increased in this era due to its higher penetrability and superior mode of action. In the present study, green synthesis was preferred to chemical synthesis as natural antioxidants have more effect on the target tissue cells with low adverse effects. So even though it's a long process, in the present study nanoparticles were synthesized from senna auriculata. It was then put into a lot of testing. In the sem analysis, a clear structure of the nanoparticles was seen. It was well bonded with each other. The well-bonded nanoparticles have few branches from the main stem of the nanoparticles(figure 1). The XRD from the samples shows the crystalized magnesium oxide with the other components into it and a bit of oxygen and carbon molecule. This carbon molecule is the residue from the prepared nanoparticles and the natural agents tend to have more carbon molecules. In the FTIR analysis the peaks at 1044.98 and 1087.11 wavelengths (figure 2). This indicates the presence of the secondary metabolites of the senna auriculata as those components peak at the same wavelength in infrared spectroscopy. So in this study, the secondary metabolites of Senna auriculate are present in the prepared nanoparticles. Then the particle size analyzer was done on the nanoscale and was found to be under the nano size(table 1). The UV absorption started at about 650nm with an absorption rate of 1.87. The rapid absorption of UV was observed at about 520-415nm absorption rate ranging from 2.11-2.52. The was the flat as UV absorption rate was equal to 270nm (rate-3) (figure 3). Then the cytotoxicity test was done with live nauplii cells. A two-day test was performed to check the number of cells present in the two-day count at various concentrations. The concentration nanoparticles taken were 5,10,20,40,60 and 80 microlitres. There was the presence of all cells on day one as the cells have just been exposed to the prepared nanoparticles at various concentrations. On the second day, it was noted that reduction in cells past 40 and at 80 concentrations there were significantly more cells lost. This shows that the prepared magnesium senna auriculata nanoparticles can be used up to a concentration of 40 being the threshold safe limit. ORAC test was performed to check the anti-oxidant effect of the prepared nanoparticles. It was done in 5 different concentration(10,20,30,40 and 50). It was seen that with an increase in the concentration of the magnesium senna auriculata nanoparticles there was an increase in the antioxidant activity. This show that the nanoparticles are highly antioxidant. The antibacterial activity of Magnesium Senna auriculata nanoparticles was assessed against Streptococcus mutans at various concentrations (10 µg, 50 µg, and 25 µg) on the Mueller

Hinton Agar plate. In this study, amoxyrite was used as a positive control. Interestingly, the results show that the zone of inhibition was found against Streptococcus mutans: 10.5 mm, 13 mm, and 13 mm in diameter at 10 µg, 50 µg, and 25 µg concentrations of Magnesium Senna auriculata nanoparticles, respectively. The positive control drug amoxyrite exhibits 13 mm in diameter of zone of inhibition at a concentration of 1.0 µg. It confirms that the selected Magnesium Senna auriculata nanoparticles show effective antibacterial activity against Streptococcus mutans.

5. Conclusion

Thus this study concludes that the magnesium nanoparticles prepared using senna auriculata is highly antioxidant and have antimicrobial activity. So these nanoparticles can be used to coat implant surfaces. Due to its high antioxidant activity, it can be used to prepare gels which is used for tissue healing.

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