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Assessment of the characterization, antimicrobial and green synthesis of magnesium oxide nanoparticles using Psydium guajava - in-vitro analysis

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ABSTRACT

Aim: To assess the characterization, antimicrobial activity of green synthesised magnesium oxide nanoparticles using Psydium guajava leaves.

Materials And Methods: Psydium guajava leaves were dried and ground, of which 2 g of the powder was used for the study.the powder was then dissolved in 100ml of distilled water. 1 mg of magnesium chloride salt was dissolved in 20 ml of distilled water. Both the solutions were mixed and subjected to centrifuge. After the preparation of the nanoparticle, it was subjected for further investigation like SEM analysis, EDAX, UV-VIs, cytotoxicity and anti microbial tests. The cytotoxicity was investigated using a brine shrimps bioassay.The number of surviving naupli cells were manually counted under dissection microscope. The antibacterial activity was examined against four bacterial strains- E.coli, E.fecalis,S.aureus,P.aurogenosa by agar well diffusion method .The inoculated plates were incubated overnight (for about 24 hours at 37 degree celsius) and the inhibition zone around the nanoparticle solution was calculated. The antimicrobial tests were carried against standard antibiotic and the results were tabulated.

Results: SEM analysis showed a particle size of 70 nm and the UV -Visible spectroscopy showed a peak at 320nm confirming the presence of magnesium oxide nanoparticles that is prepared using Psydium guajava. Cytotoxicty tests revealed that 83% of the brine shrimp cells survived .The anti microbial tests revealed that the nanoparticle was effective against two micro-organisms -S.aureus and E.fecalis and their zone of inhibition was nearly equal to that of standard antibiotic against which the organisms were incubated.

Conclusion: Psydium guajava synthesized magnesium nanoparticles were found to have good cytotoxicity and acceptable anti-microbial activity especially against two most commonly harbouring micro-organism(S.aureus and E.faecalis) .Hence they can be extended for use in soft tissue healing.

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INTRODUCTION

Nanoscience and nano-technology is emerging with multitudinous nanoparticles and its versatility has gained immense attention in recent times .This can be attributed to their extremely small size bringing about remarkable enhancement in their physical,biological,chemical, thermal and electrical properties [(1)]. The nanoparticles are widely used in the medical field especially for the treatments of cancer, diabetes, infection and inflammatory conditions. cosmetics, renewable energies, environmental remediation and biomedical devices [(2)]. MgO nanoparticles are proven to be an excellent antibacterial agent and heterogenous inexpensive nontoxic catalyst. Since nano-synthetic procedures may lead to environmental toxic waste accumulation, green synthesis methodology was performed using nontoxic constituents to reduce the ecological problems [(3)]

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Magnesium oxide (MgO) is known as a extensively used nanoparticles that possess excellent optical, electronic, thermal, mechanical and chemical properties [(4)]. It is versatile functional metal oxide used in catalysis, refractory materials, paints, and superconductors . Several physical and chemical methods such as vapourisation by laser, deposition by chemical gas phase, aerosol combustion [(5)] have been adopted to synthesize the magnesium oxide nanoparticles .However, the biological constituents acts as a reducing agent in the green synthesis of nanoparticle as it avoids the necessity for increased energy, potentially waste chemicals, etc. Nanoparticles can be produced on a vast scale using biological synthesis in a safe manner.

Many literature stated that MgO NPs also grown by the green synthetic method[(6)] In this study, a safe ,natural and nontoxic green precursors, such as leaf extract of Psidium guajava (family Myrtaceae) that is widely cultivated in the tropical countries are used [(7)]. They are used as anti-diabetic agents to decrease blood glucose levels by increased insulin secretion and exhibits excellent anti- microbial property [(8)]. An added advantage is that cultivation of guava is relatively easy as it thrives in a variety of soils and adapts to different climatic conditions; the fruits are also borne fairly in a short period.[(9)] . Our team has extensive knowledge and research experience that has translate into high quality publications (10–19)

Thus the aim of the study is to assess and analyze the characterisation of magnesium oxide nanoparticles and to evaluate its cytotoxity and anti-microbial effects to extend the usage in soft-tissue healing in implant therapy.

MATERIALS AND METHODS

Preparation Of Nanoparticles

Drying Of Guava Leaves

Psydium guajava leaves were dried at room temperature for 14 days under sunlight. After the leaves dried completely, it was grinded to form a fine powder.(Fig1)

Fig 1: Represents the guava leaves that were dried for 2 week and grinded to form a fine powder

Filtrate Formation

2g of grinded powder as weighed in SAFFRON was dissolved in 100ml of distilled water and it was stirred continuously by REMI magnetic stirrer for 45 minutes. The resultant solution was filtered to form a filtrate. It was left overnight for it to undergo reduction.

Addition Of Mgcl2 Salt Solution

1 mg of magnesium chloride salt was dissolved in 20 ml of distilled water and the solution was added drop by drop to the above obtained filtrate . The complete mixture was now left to be stirred by a REMI 1MLH magnetic stirrer for about 6 hours.(Fig 2)

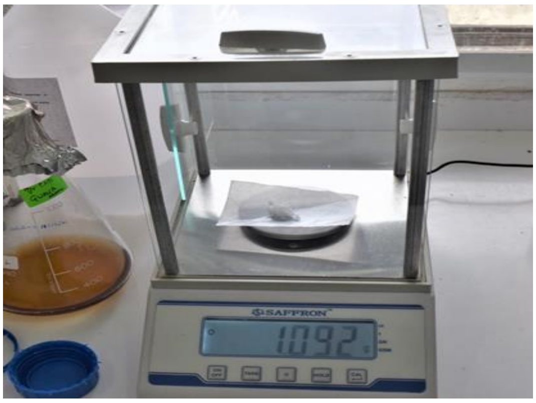


Fig 2: Represents the weighing of magnesium chloride salt which was further stirred by magnetic stirrer

Adjustments In Ph And Centrifugation

After 6 hours,the solution appeared to be turbid. The pH of the solution was adjusted towards alkalinity (7) by adding 1N OF Sodium hydroxide solution .The resultant solution of 100ml solution was equally distributed in 6-20 ml test tubes for centrifugation under 10000 rpm for 10 minutes(Fig 3). After centrifugation the test tubes revealed pellets of magnesium nanoparticles sedimented at the bottom . The test tubes were later dried under hot air oven at 50*C overnight to get dried nanoparticles.

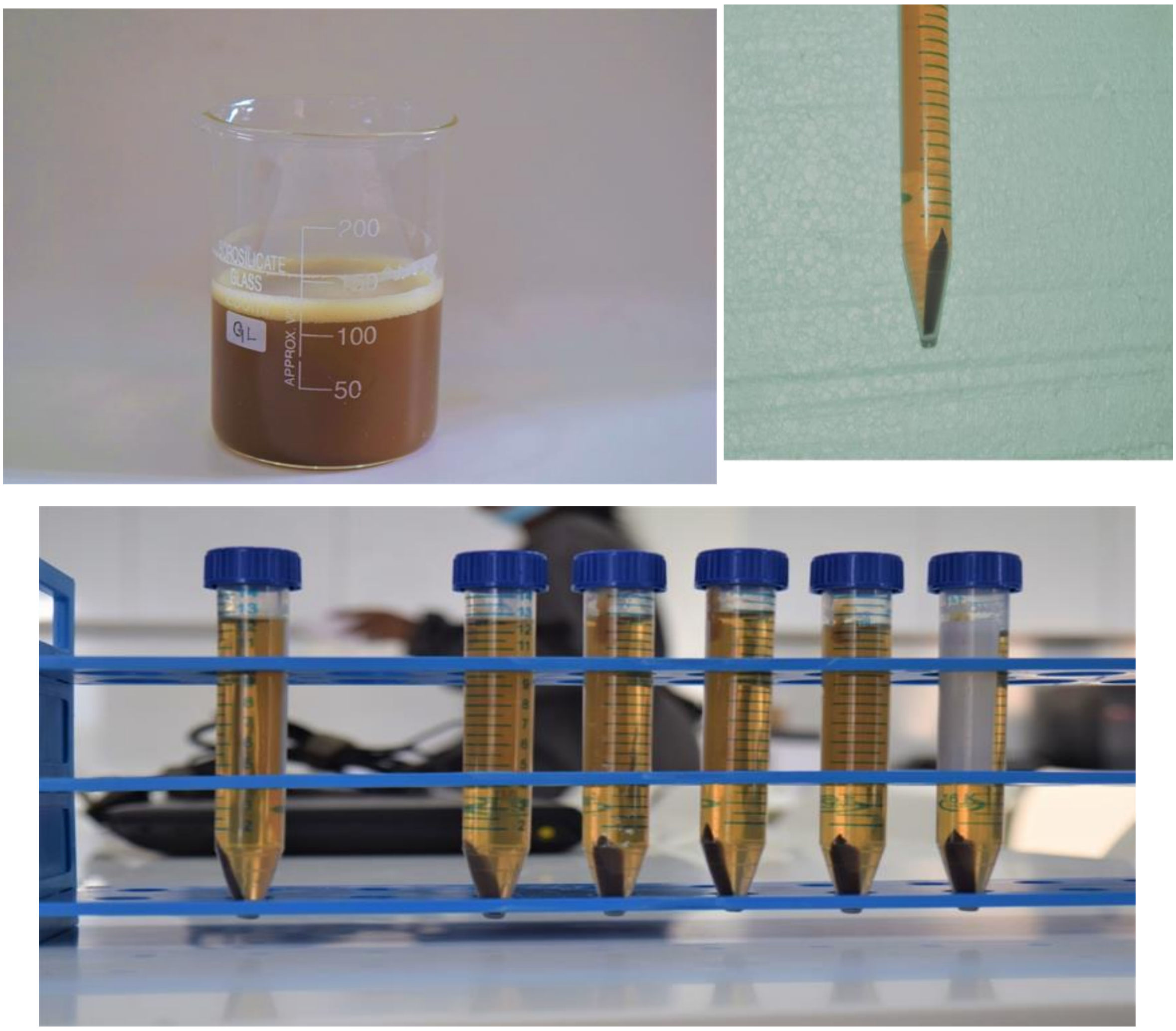


Fig3: Represents the pH modified solution and centrifugated product in the test tubes.

Characterization Of Magnesium Oxide Nanoparticles

Scanning electron microscope was used to analyse the size of the nanoparticles. The nanoparticles were dissolved in absolute alcohol and dried to analyse the morphology of the nanoparticles. The presence of MgO nanoparticles from Psydium guajava were confirmed by Double beamUV-Vis-spectrophotometry in which absorbance in 3ml cuvette solution was recorded every 2 hours between 250-600nm.

Cytotoxicity Analysis

Artificial seawater solution which was prepared by dissoluting 36g of sea salt in one litre of distilled water was used to culture the brain shrimps (napulli). The vials were left unattended for 24 hours. The results were recorded by counting the number of live brine shrimp directly under a dissecting microscope.It was observed that 83% of the cells were alive and results were recorded.

Antibacterial Analysis

The antibacterial activity was examined against four bacterial strains- E.coli, E.faecalis, S.aureus, P.aeruginosa by agar well diffusion method . Since the nanoparticle solution was polymer assisted, i.e

(suspended in the polymer solution of chitosan and gelatin), the polymer solution was also added separately in the same inoculation plate in order to appreciate the difference in the zone of inhibition due to nanoparticles. Gentamycin was the standard antibiotic used against S.aureus ,E.faecalis .Ciprofloxacin was used against P.aeruginosa and amikacin was used for E.coli .The inoculated plates were incubated overnight (for about 24 hours at 37 degree celsius)The inhibition zone around the incubated nanoparticle was calculated.

RESULTS AND DISCUSSION

Sem And Edax Analysis

SEM image confirms that synthesized MgO NPs shows dispersion in open, clustered fashion in quasi-linear superstructures phase .MgO NPs size ranged from 50 to 90 nm. The EDAX spectrum shows that the product was principally composed of Mg and O, and their respective atomic content was present(Fig 4).

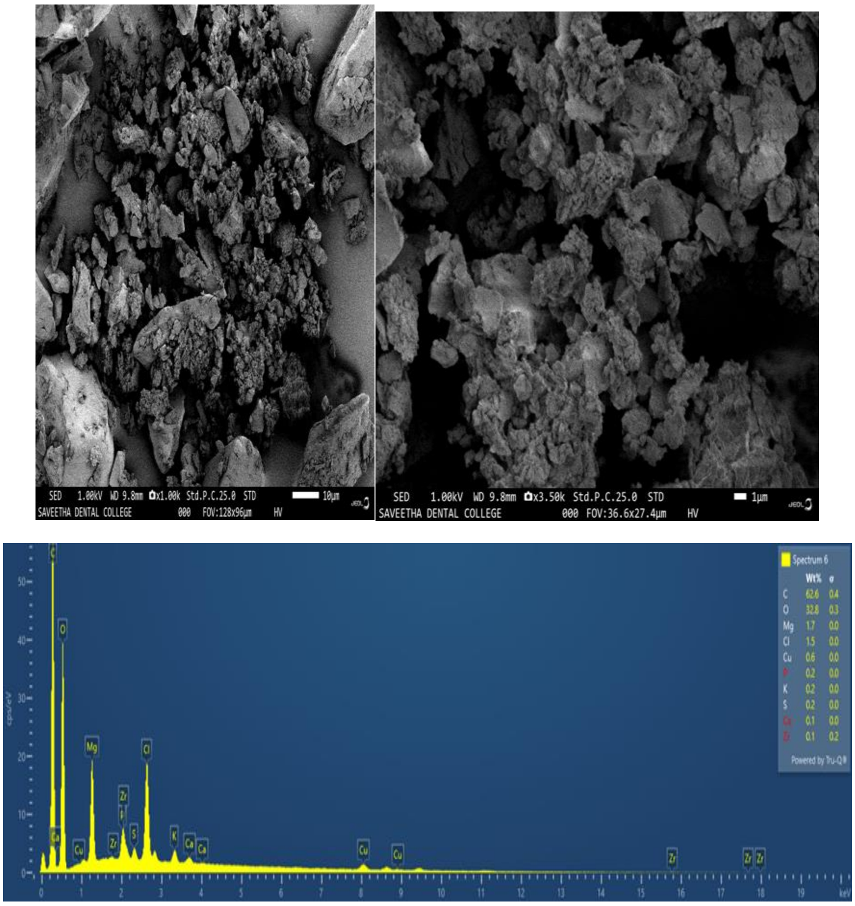


Fig 4:Represents the SEM and EDAX images of magnesium oxide nanoparticles

UV-VIS

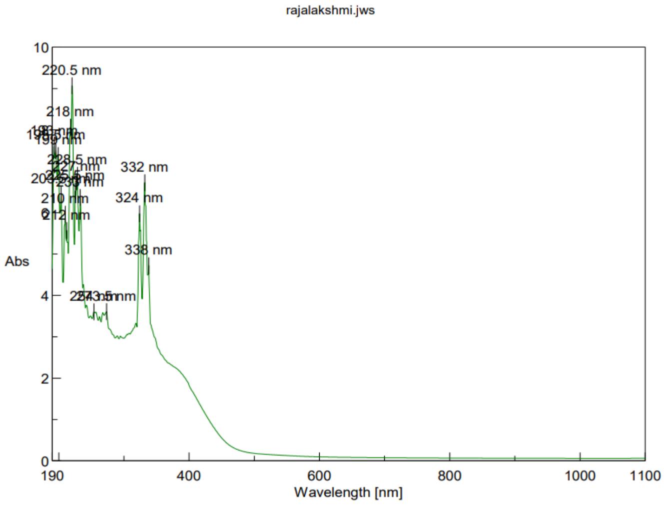


Fig5: Represents the UV-Visible Spectroscopy result of the nanoparticles. The peaks were recorded around 332nm which confirms the presence of metal oxide nanoparticles.

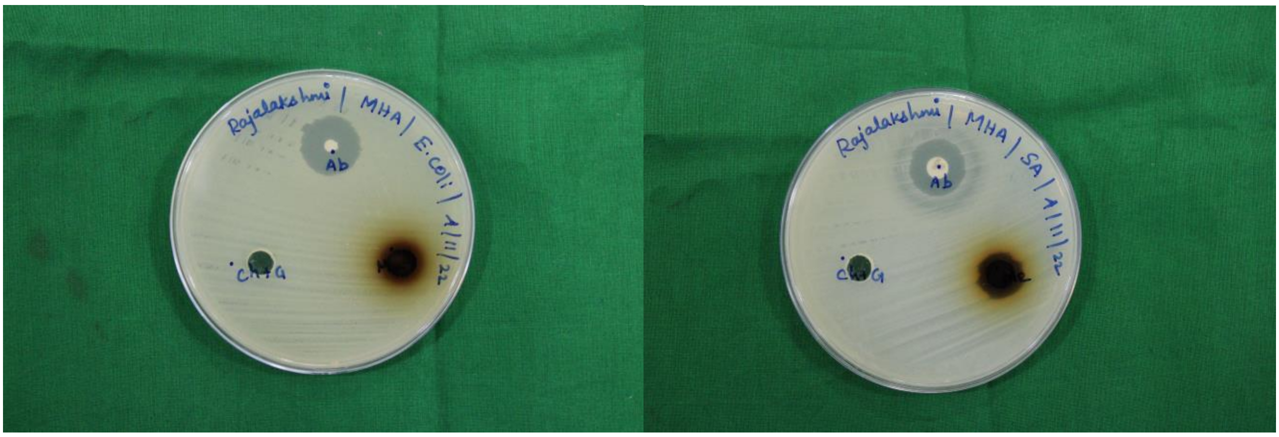
A peak was visible in the absorption spectra at 332 nm(Fig 5). The bio-reduction property of Psydium guajava is demonstrated in the UV-Visible spectroscopy by the formation of magnesium oxide nanoparticles. The MgO nanoparticle's band gap energy was discovered to be 5.6 eV. MgO's particle size and band gap values demonstrate that it can be utilized as an optical device fabricator, photo-catalytic activator, and antibacterial agent.

Cytotoxicity Analysis

Artificial sea water solution which was prepared by dissolving 36g of sea salt in one litre of distilled water was used to culture the brain shrimps(napulli). The vials were left unattended for 24 hours. The results were recorded by counting the number of live brine shrimp directly under a dissecting microscope.It was observed that 83% of the cells were alive and results were recorded.

Antibacterial Analysis

The incubated plates were analyzed for the zone of inhibition for all the three groups,i.e standard antibiotic , polymer(chitosan and gelatin) group and magnesium oxide nanoparticle suspended polymer solution.



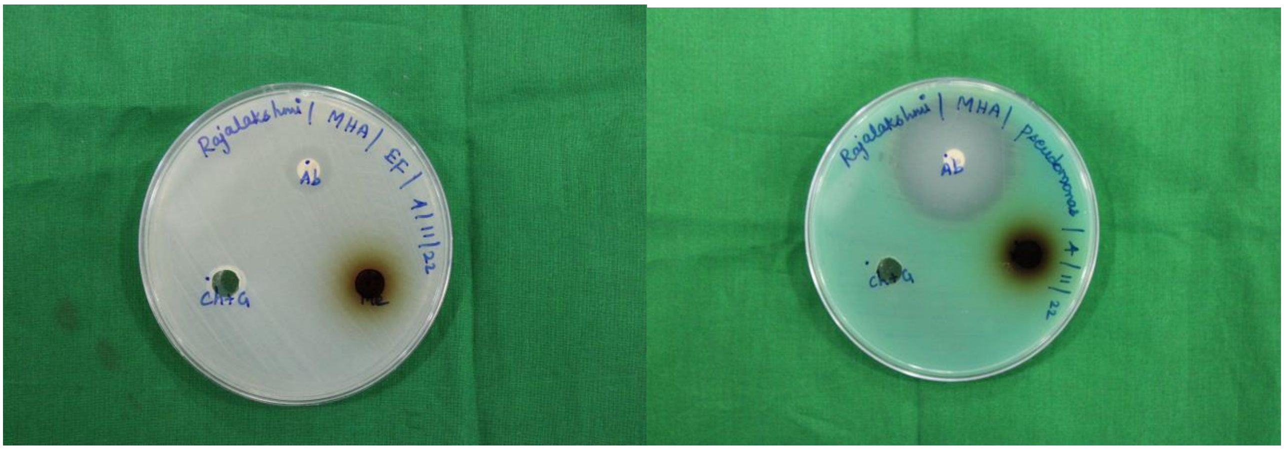


Fig6: Represents the zone of inhibition around the standard antibiotic, chitosan and gelatin(polymer) group and nanoparticle suspended polymer solution with respect to four micro organisms- E.coli,E-fecalis,S.aureus,P.aeruginosa and the results were tabulated.

Table 1: Represents the collective results of organisms, the corresponding antibiotic used and zone of inhibition around each individual group

ORGANISM	ANTIBIOTIC USED	ZOI-ANTIOBIOTIC	ZOI-CHITOSAN AND GELATIN	ZOI- MgO Np SUSPENDED POLYMER SOLN.
S.aureus	GENTAMYCIN	17mm	NEGATIVE	**16mm**
E.fecalis	GENTAMYCIN	13mm	NEGATIVE	**12mm**
P.aeurogenosa	CIPROFLOXACIN	35mm	NEGATIVE	NEGATIVE
E.coli	AMIKACIN	17mm	NEGATIVE	NEGATIVE

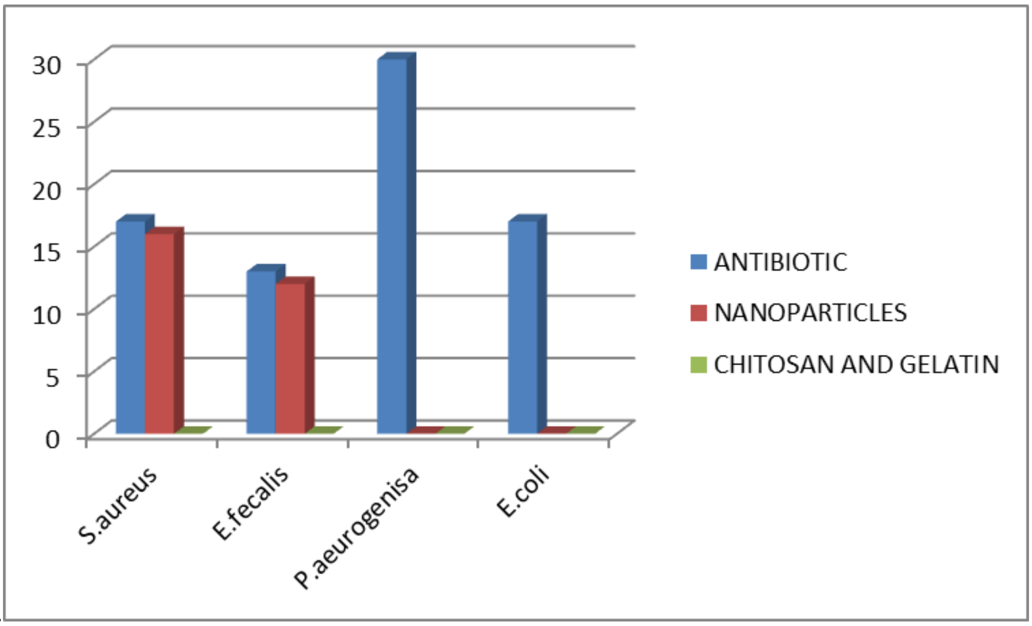


Fig 7: Represents the graphical interpretation of comparison of zone of inhibition around individual organism .

The nanoparticle solution was found to be effective against two most important oral commensals-S.aureus and E.fecalis. The nanoparticle solution had just diffused through the agar gel for the other organisms (P.aeruginosa and E.coli) without exhibiting any zone of inhibition.

The field of medicine is evolving with the introduction of nanotechnology. Their unique properties have attracted several healthcare fields including dentistry. Nano technology has been made to analyze the nature of chemical bonds between the atoms and molecules of innumerable compound(20). In the field of dentistry, several innovations have been made till date in which nanoparticles , also known as " zero-dimensional " nanoscale atoms, that range under 100 nm have been revolutionizing the field(21). The nanoparticles' mobility rate, absorption quotient , biocompatibility, reactivity rate and depth of penetration makes it highly exceptional and has provided remarkable results till date (22). The nanoparticles incorporated into the drugs have been used to deliver antimicrobial agents directly to the site of an infection, allowing for more targeted treatment and potentially reducing the need for systemic antibiotics(23). The drugs are dissoluted, linked and suspended in the nanomatrix to form nanocapsules,spheres that has the ability to overcome drug resistance in the targeted cells and can enhance the movement through blood brain barriers(24)(25). Due to multiple electrostatic and chemical interactions, the positively charged nanoparticles are able to bond with the negatively charged bacterial cell leading to distruption and increased permeability, ultimately resulting in pathological alteration of biological pathways in the microbial cells(26). The release of reactive oxygen species further spikes up the oxidative stress leading to glutathione oxidation ,thus decreasing the defence capacity of bacteria (26,27).).The exponential increase in the reactivity potential can be attributed to the large surface to volume ratio and the catalytic activity is proven to be inversely proportional to the size of the nanoparticles. Luo et al in 2021 have proved that the catalytic nature enables the nanoparticles to attach easily to the cell membrane ,thereby influencing and increasing the influx rate of the nanoparticles into the target cells (28,29). Mangalampalli et al have stated that high catalytic activity and reactivity can serve as a double edged sword that influences both availability and toxicity (30).Mukherjee and Acharya et al have proven that the quantity of Reactive Oxygen Species (ROS) can be increased by NADPH oxidase and mitochondrial imbalance.(29)

With the advent spike in demand and popularity of utilising extracts from the plant for deriving nanoparticles[(31)], this study aimed to biogenically synthesise magnesium oxide nanoparticles using the leaves of Psydium guajava. According to Rajeshwari et al and Rajiv et al the absorption peak range of 300–400 nm confirms the presence of metal oxide NPs[(32)].The results of UV-Vis absorption peak (320nm) in this study is in accordance with previous mentioned study. Psydium guajava's remarkable antioxidant properties are attributed to their number of methyl and hydroxyl group and their esterification level. (33)

Due to multiple interaction between the molecular elements in the body with the nanoparticles ,there is increased risk of toxicity[(34)].To assess the interactions, cytotoxicity bioassay test are used as preliminary test for investigations .The United States Food and Drug Administration (21CFR184.1431) has reccognised magnesium oxide nanoparticles as safe product . In the academic laboratories,the most feasible and reliable method to measure the toxicity is by exposing the experimental solution to certain live cells and co-relating the number of cells that remain after exposure for certain duration(35).In this study , live brine shrimps was experimented to analyse the cytotoxic activity. Brine shrimps possess numerous analogy to mammalian cells(36). Thus this research holds validity and significant reliability. In this study,the tests revealed that the cytotoxicity property was shown by the prepared nanoparticle solution on brine shrimps which is dependent on the dose and it was also proportional to the concentration.Thus when used in optimal concentration maximum benefits can be obtained.

The antibacterial properties of Psidium guajava was significantly proven against bacterial isolates (, E. coli, S. aureus, and Pseudomonas aeruginosaProteus mirabilis, Streptococcus pyogenes) from patients with trauma, soft tissue infections by Abubakar.(37)Doss et al had demonstrated the mechanism of MgO NPs against E. coli and concluded that the effect was because of increase oxygen content on the surface of the magnesium. Krishnamoorthy et al had also explained the action of magnesium NPs against infective agents Thus nanoparticles that are synthesized through the plant medium have been proven to be effective alternatives for treating the antimicrobial resistance. The antibiotic test conducted in this study compared the zone of inhibition among the control(standard antibiotic) and the magnesium oxide nanoparticle suspended in polymer solution group and the efficiency was proven to be nearly equal to that of standard antibiotic that was tested .This signifies the extension of its use in the field of nano-technology and soft tissue healing.

CONCLUSION

The MgO nanoparticles were synthesized using aqueous extracts of P. guajava. The nanoparticles were characterized by SEM , UV–visible, XRD, FTIR analyses. Due to their excellent purity, these MgO nanoparticles could be applied in biological settings. Both E. coli and S. aureus were found to be significantly impacted by the antibacterial action of MgONPs which are the most common microbes found in the oral cavity. The green synthesis described above used the plant precursors, making it an environmentally benign process. We indicate that this green MgO NPs may be advantageous for applications based on antimicrobials and may be extended for use in soft tissue healing,

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CONFLICTS OF INTEREST

NIL

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