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Green synthesis of Nanocomposite using silver nanoparticles, Sodium Alginate and Papaya leaf extract and its anticariogenic activity.

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KEYWORDS
Silver Nanoparticles, Papaya leaves, Antimicrobial activity, Oral Pathogens, Amoxicillin.

Abstract

Aim:

The current study is concerned with the production of AgNPs and silver sodium alginate nanocomposite employing Carica papaya extract as a stabilizing and reducing agent.Biosynthesized silver nanocomposite antibacterial effectiveness was investigated against oral pathogens such as Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans.

Materials and Methods:

In 90 mL of deionized water, 1 mM of precursor silver nitrate ($AgNO_3$) was added. 10 mL of filtered Carica papaya plant extract was added. For 48 hours, the mixture was stirred at 600-700 rpm using a magnetic stirrer. The synthesis of AgNPs was studied using a UV-Vis-double beam spectrophotometer at regular time intervals.The synthesised silver nanocomposite was centrifuged at 10,000 rpm for 10 minutes. The nanocomposite pellet was suspended in deionized water before being centrifuged and lyophilized. Antimicrobial activity against S. aureus, S. mutans, E. faecalis, and Candida albicans was checked.

Results:

The results show that the antibacterial activity of biosynthesized AgNPs, sodium alginate nanoparticles, and AgNP-based sodium alginate nanocomposite increases in a dose-dependent manner. The high inhibition zone was found in the gram-positive bacteria Staphylococcus aureus as well as gram-negative organism E. faecalis . In the opportunistic pathogenic yeast Candida albicans, the inhibitory zone was shown to be the smallest.

CONCLUSION:

Using Carica papaya plant extract, AgNPs and AgNP-based sodium alginate nanocomposite were

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synthesized, and the obtained AgNPs and nanocomposite showed outstanding stability.

Introduction

Nanotechnology has recently gained prominence due to its numerous uses in the biomedical realm (Govindarajan and Benelli 2017). This attracts a large number of researchers to develop various nanomaterials with specific tasks, such as curing diseases or improving and performing in equipment or items such as health care products, cosmetics, domestic products, and so on. The nanolevel need of these nanoparticles in live cells is important in combating disease-causing bacteria and organisms.(Goodsell 2004)

Pathogens that cause disease are becoming increasingly resistant to new antibiotics. To address this issue, researchers have identified metallic nanoparticles such as silver nanoparticles (AgNPs) as an efficient antibacterial agent in various investigations(Goodsell 2004; Pal, Tak, and Song 2007; Geetha et al. 2013). To avoid contamination from wound infections and other nosocomial pathogens, AgNPs are primarily employed in the treatment of burns and open injuries(Ip et al. 2006) . Because of their excellent physicochemical features, AgNPs play an important role in research and medicine. Antifungal, anti-inflammatory, antiviral, antibacterial, antiangiogenesis, and antiplatelet activities have been reported for AgNPs (Öztürk, Gürsu, and Dağ 2020; Dağlıoğlu and Öztürk 2019).

In several studies, sodium alginate, a natural biopolymer, has been reported to enhance the antibacterial efficacy of the metallic nanoparticles (Susilowati, Maryani, and Ashadi 2019; Królczyk et al. 2019). In this present study, sodium alginate was added to Carica papaya leaf extract-mediated AgNPs to attain as silver nanocomposite an increase in the potent antimicrobial efficacy of the AgNPs to a greater extent.

The papaya is the plant species **Carica papaya**, one of the 21 accepted species in the genus Carica of the family Caricaceae. It was first domesticated in Mesoamerica, within modern-day southern Mexico and Central America. Phytochemical screening of Carica papaya plant and fruit uncovered the presence of bioactive constituents, for example, tannins, phenols, terpenoids, flavonoids, glycosides, sugars, and saponins.

AgNPs incorporated with various polymers such as sodium alginate and its cross-linked polyvinylpyrrolidone in gelatin, and polygalacturonic with hyaluronic acid-based silver nanofibers demonstrate good wound healing activity; black berry-mediated silver, gold, and silver/gold bimetallic nanoparticles loaded with pectin demonstrate cardioprotective activity(Hussein et al. 2020). In animal model and cell line investigations, AgNPs manufactured using various chemical methods are more harmful, but AgNPs generated using biological agents such as bacteria, plant and its components, fungi, and algae have extremely good biomedical uses (Hussein et al. 2020; Roy et al. 2013).

The current study is concerned with the production of AgNPs and silver sodium alginate nanocomposite employing Carica papaya extract as a stabilizing and reducing agent. UV-double beam spectrophotometer, scanning electron microscopy, transmission electron microscope, atomic force microscope, and X-ray diffraction analysis were used to evaluate the produced silver nanocomposite. Biosynthesized silver nanocomposite antibacterial effectiveness was investigated against oral pathogens such as Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Candida albicans.

Materials and Methods

Chemicals

Sigma Aldrich chemicals Pvt. Ltd. supplied the precursor silver nitrate (India). Hi-Media in India provided Mueller Hinton Agar. The leaves of the Carica papaya plant were discovered in rural Vellore, Tamil Nadu, India. S. aureus, S. mutans, E. faecalis, and Candida albicans were isolated and collected from Saveetha Dental College and Hospital, Chennai.

Carica papaya leaf extract preparation

The leaves of Carica papaya were carefully cleansed with tap water and Milli-Q water. The plant was then shade-dried for 3-4 days. The dried plant powder was crushed and stored in an airtight container. 100 mL of double-distilled water was mixed with 1 g of dried powdered plant. The mixture was then cooked for 15 minutes on a heating mantle at 70°C. All phytochemical components found in the Carica papaya plant are dispersed in the water solution using this technique. This final mixture was filtered using filter paper (Whatman No.1), and the filtered leaf extract was refrigerated for later use.

Synthesis of nanoparticles

In 90 mL of deionized water, 1 mM of precursor silver nitrate (AgNO3) was added. 10 mL of filtered Carica papaya plant extract was added. For 48 hours, the mixture was stirred at 600-700 rpm using a magnetic stirrer. The synthesis of AgNPs was studied using a UV-Vis-double beam spectrophotometer at regular time intervals from the initial wavelength of 360-500 rpm (revolutions per minute). Biosynthesized AgNPs were centrifuged at 8,000 rpm for 10 minutes to collect the pellet from the aqueous reaction mixture. The supernatant was removed, and the pellet was rinsed three times with ethanol before being placed in a hot air oven at 70°C for two hours. In addition, powdered AgNPs were maintained in an airtight Eppendorf tube for characterisation experiments.

Nanocomposites formation

1 mL of 1% glacial acetic acid and 49 mL of deionized water were used to dissolve 0.5 g of sodium alginate. The AgNPs were added to the sodium alginate solution and stirred for 3-4 hours in a magnetic stirrer. A brown nanocomposites gel was formed after adding Carica papaya-mediated AgNPs solution. The mixture was placed in a magnetic stirrer for another 48 hours. UV-Vis spectroscopy in the wavelength range of 360-500 nm was used to examine the formation of silver nanocomposites. The synthesised silver nanocomposite was centrifuged at 10,000 rpm for 10 minutes. The nanocomposite pellet was suspended in deionized water before being centrifuged and lyophilized. The lyophilized silver sodium alginate nanocomposites were dissolved in distilled water and employed in various characterisation experiments.

AgNPs and nanocomposites characterization

The UV-double beam spectrophotometer (UV-2450, Shimadzu) was used to characterise the Carica papaya-mediated AgNPs and nanocomposites in the wavelength range of 360-500 nm.

Antimicrobial activity against oral pathogens

Fresh bacterial cultures such as S. aureus, S. mutans, E. faecalis, and Candida albicans were inoculated in 10 L of sterile Hi-veg broth and maintained above an orbital shaker for 18 hours at 120-150 rpm. Mueller Hinton agar was produced, and 5 mm wells were drilled using a sterile polystyrene tip. Carica papaya-mediated AgNPs, biosynthesized AgNPs-mediated nanocomposite, and sodium alginate nanoparticles were tested for antibacterial activity. Various concentrations of three samples, such as 25, 50, and 100 L, were added to wells, and amoxyrite was added as a positive control (except for C. albicans, fluconazole is used as a standard drug).The inoculated sample petri plates were stored in a microbiological incubator at 37°C for 24 hours, and the zone of inhibition zone was determined in order to compare and evaluate the possible effect of Carica papaya-mediated AgNP and nanocomposites, as well as sodium alginate nanoparticle.

RESULTS AND DISCUSSION

Visual examination

The biosynthesis of nanoparticles using heterocyclic chemicals is gaining popularity due to its simplicity and environmental friendliness (Shankar et al. 2004). With increasing time, the colour intensity of the Carica papaya-mediated AgNPs solution mixture rose. In addition, the reduction of silver nitrate to Ag0 by the reducing agent (Carica papaya leaf extract) was shown by a colour change from light yellow to dark brown , which was validated by UV-Vis spectrophotometry analysis. The brown hue of the AgNPs synthesized from papaya leaves verifies the creation of nanoparticles , and the color formation of the AgNPs with sodium alginate confirms the formation of nanocomposite (Wei et al. 2009).

UV-Vis spectrophotometer optical analysis

UV-Vis spectroscopy is an important step in determining the growth and stability of nanoparticles(Palem et al. 2018). At 420 nm, the greatest absorption peak of AgNPs was detected. The wide peak observed between 380 and 460 nm verifies the silver sodium alginate nanocomposite (Figure 1). The modest peak variation in the UV-Vis spectroscopy absorbance validates the AgNPs' stability after microwave treatment. The signal at 400-440 nm indicates the synthesis of AgNPs using Clerodendrum inerme and Pedalium murex leaf extracts.(Palem et al. 2018; Anandalakshmi, Venugobal, and Ramasamy 2016)

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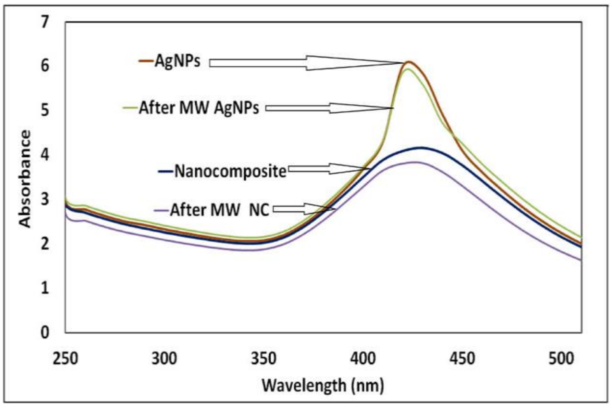

Figure 1:UV-Vis spectrophotometer optical analysis

Antimicrobial activity against oral pathogens

Agar well diffusion assay was used to evaluate the antibacterial activities of silver nanocomposite. Figure 2 depicts the antibacterial action of Carica papaya-mediated AgNPs, sodium alginate NP, and AgNPs-based nanocomposite against four oral pathogens: S. aureus, S. mutans, C. albicans, and E. faecalis. The standard control was amoxyrite. The results show that the antibacterial activity of biosynthesized AgNPs, sodium alginate nanoparticles, and AgNP-based sodium alginate nanocomposite increases in a dose-dependent manner. The high inhibition zone was found in the gram-positive bacteria Staphylococcus aureus as well as gram-negative organism E. faecalis . In the opportunistic pathogenic yeast Candida albicans, the inhibitory zone was shown to be the smallest.

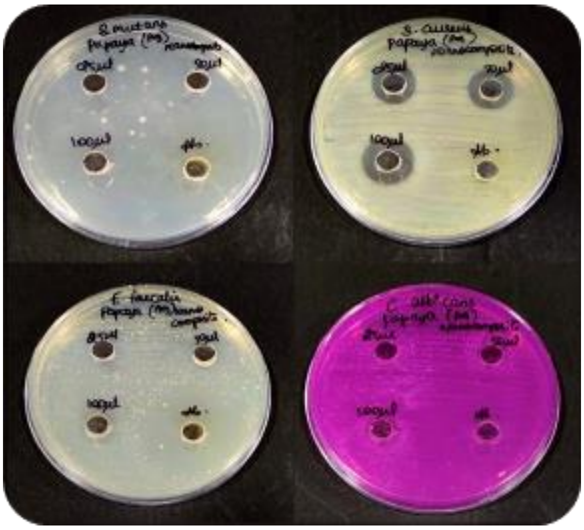

Figure 2: Antimicrobial activity

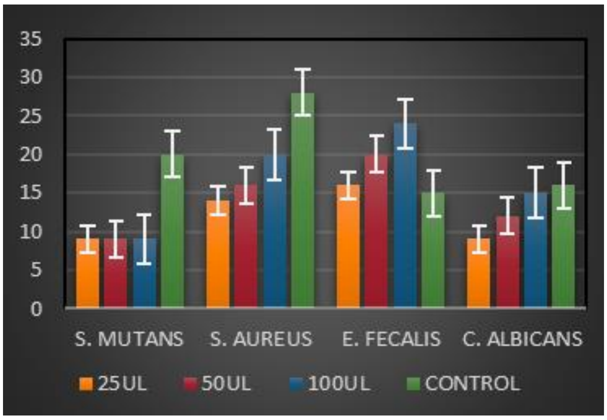

Figure 3: Antimicrobial activity of nanocomposite against oral pathogens

The biosynthesized AgNPs, sodium alginate nanoparticles, and AgNPs-based sodium alginate nanocomposite have a significant antibacterial effect, which must be used as a biomedical application in the future to achieve desired results.

It was also found that gram-positive organisms are more sensitive due to bacterial cell wall variations. AgNPs and their coated cellulose and zinc oxide nanoparticles are less toxic and are used in a variety of biomedical applications (Abdelsalam et al. 2019).

Conclusion

Using Carica papaya plant extract, AgNPs and AgNP-based sodium alginate nanocomposite were synthesized, and the obtained AgNPs and nanocomposite showed outstanding stability. Sodium alginate was used as a powerful stabilizing agent in conjunction with AgNPs to create a Carica papaya-mediated AgNPs-based sodium alginate nanocomposite. AgNPs and nanocomposite products were employed as an excellent antibacterial material. UV-Vis spectroscopy, SEM, and TEM investigation verified the presence of elemental silver in the nanocomposite, as well as its spherical shape and size of 30-40 nm. Carica papaya plant extract generated AgNPs and AgNPs-based sodium alginate nanocomposite with increased action against oral infections. The current study is a low-cost, environmentally friendly approach for producing silver nanocomposite. As a result, the produced AgNPs and AgNPs-based sodium alginate nanocomposite can be used in future biomedical applications as an effective antibacterial material.

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