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Anticariogenic Activity of Vitis vinifera-Assisted Carbon Nanoparticles and Its Incorporated Chitosan Nanocomposite

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Carbon nanoparticles (CNPs) are said to have various biological properties like antimicrobial activity, anti-inflammatoryeffect, and anticoagulant properties and it is also biocompatible with less toxicity. Dental caries is one of the common oral infectious diseases. The objective of this study is to investigatethe anticariogenic activity of Vitis vinifera-assisted carbon nanoparticles and their chitosan nanocomposite. The antimicrobial assay agar well diffusion and time-kill curve assay are used to check its anticariogenic effect including C.albicans, S.mutans, E.faecalis, and Lactobacillus sp. The clot-forming unit (CFU) and zone of inhibition are measured. The result showed antimicrobial properties vary between different species of bacteria and their concentrations. A maximum zone of inhibition of 15mm is obtained by carbon nanoparticles at 100µg/ml against Lactobacillussp.followed by a zone of inhibition of 14mm for other pathogens at 100µg/ml concentration. The lowest zone of inhibition by carbon CNPs is obtained at 25µg/ml against Lactobacillus sp and E.faecalis.From the above study, we can conclude that carbon nanoparticles have potent antimicrobial properties against dental pathogens. So, these can be used to prevent tooth decay (dental caries) caused by bacteria. Further studies can be done to check and improve the effectiveness of CNPs.

Keywords: Anticariogenic,agar well diffusion, carbon nanoparticles,green synthesis, mueller hinton agar plate, Vitis vinifera.

1. Introduction

Nanomaterials are materials between 1 to 100 nm in size, which is recentlyemerging field. Nanoparticles have many exciting properties that make them appealing to research in every biological application(Alimet al., 2018). These nanoparticles exist in different forms. These nanoparticles have various applications in computer industries, cosmetics, electronics, and other products. Now they are also used in medicine. The term "nanomedicine" is introduced for using nanotechnology for the diagnosis, prognosis, and treatment of diseases in humans (Radomskiet al., 2005). The word "nanotechnology"was first coined by Nobel laureate Richard P. Feynman in 1959. These nanoparticles are classified based on their size, shape, and properties. Their sizes, shape, and structure are responsible for their different properties.They have different and unique properties because of their nano size(Khanet al., 2019). These nanoparticles have large surface area and high charge density that give them their antibacterial properties by interacting with negatively charged bacterial cell walls (Song et al.,2019).

Carbon nanoparticles (CNPs) play one of the major roles in nanotechnology. Theseparticles attracted many researchers over the decade. They have great applications in medicine because of properties unique to them. They have gotten much attention because of their chemical and physical properties that are exclusive to them and for their biosafety. Many kinds of carbon nanoparticles have been synthesized like carbon nanotubes, and fullerenes (Xin et al., 2018). These CNPs have many desirable properties like low cytotoxicity and high biocompatibility. CNPs are known to have various biological applications like imaging, surface coating, etc. They can also be readily suspended in solution. In green synthesis, biomasses like leaves, wood, and husks are used to synthesize carbon nanoparticles. This biomass waste can be obtained from agricultural waste, industrial waste, and other animal and plant products. These biomasses undergo various processes to synthesize nanoparticles (Qasim et al., 2023). These nanoparticles are synthesized by green synthesis from grape (Vitis vinifera) plants. Green synthesis is preferred because it does not involve toxic and costly chemicals for the synthesis of nanoparticles. Several bioproducts are extracted from plants and microorganisms already. Biomaterials are also abundant in nature (Roy et al., 2019).

Grapes belong to the genus Vitis and family vinifera. They have been used for free eating, juice production, and wine production for many years (Hassan et al., 2019). Grapes (Vitis vinifera) are rich in various phenolic compounds, stilbenes, and flavonoids Its seed, skin, fruit, and stem are known and studied to have many pharmacological properties. Dental caries (tooth decay) is one of the common oral infectious diseases that affect a large population. The main pathogen that is considered for causing this is Streptococcusmutans. The use of antibacterials like penicillin, and erythromycin are effective for treatment but they have unwanted side effects and they are also costly (Aslanimehr et al., 2020). Dental caries is caused because acids secreted by the bacteria cause pH fluctuation resulting in demineralization of teeth. Fluoride has been considered as one of the major anti-cariogenic agents for many years but using excess fluoride could result in dental fluorosis. So,in the last decade using natural products as anticariogenic agents hasbecome popular due to their biocompatibility, low cost, and availability(Khoriba et al., 2022)

The objective of this is to assess the anticariogenic activity of CNPs assisted by Vitis vinifera and its nanocomposite. In this carbon nanoparticles are synthesized by green synthesis from the plant Vitis vinifera. Its anticariogenic property can be assessed by agar well diffusion technique and time-kill curve analysis assay. These properties are tested on bacteria species like C.albicans, S.mutans, E.faecalis and Lactobacillus sp. From this study, we could find whether carbon nanoparticles can be used instead of traditional treatments which are costly and have unwanted side effects for dental caries.

2. Materials and Methods:

Preparation of carbon nanoparticles:

Vitis vinifera seeds are cleaned thoroughly using distilled water then dried, and then powdered. 5g of Vitis vinifera seeds powder is then mixed with 100 mL of distilled water and then boiled well using a heating mantle. The synthesis of carbon nanoparticles using 1 using a crucible using a microwave oven at 700 volts. The settled residues in the crucible were crushed well and made into a fine powder. The fine powder was mixed with distilled water. The powder was put in the sonicator for 10 minutes. The CNPs were dried in a hot air oven and then they were used for further studies. 500 mg of chitosan was added with 49 mL distilled water along with 1 mL glacial acetic acid. Till the solution became clear solution was kept in a magnetic stirrer. 0.2g in 3 ml of chitosan is mixed and kept in a sonicator for 15 minutes.

Antimicrobial Activity:

Agar well diffusion technique:

The antimicrobial property of carbon nanoparticles synthesized using green synthesis was assessed by the agar well diffusion method. Mueller Hinton plates are prepared and then sterilized using an autoclave at 121 degreesCelsius for 15- 20 minutes (Martínez-Castañón et al., 2008). After that sterilization medium was poured into the surface of Petri plates that were sterile and allowed for cooling at room temperature. The bacterial suspension of (C.albicans, S.mutans, E.faecalis, and Lactobacillus sp.) is then spread in the agar plates with the help of sterile cotton swabs. Wells with 9mm diameter were created using sterile polystyrene tips. Then wells are filled with different concentrations (25, 50, 100 µg/mL) of carbon nanoparticles (Rifaath et al., 2023). Antibiotics (for example amoxyrite and fluconazole) were used as standard. Then plates are incubated for 24 hours at 37 degreesCelsius for bacterial culture and fungal cultures for 48 hours. The antimicrobial property was evaluated by checking the diameter of the zone of inhibition surrounding the wells. The inhibition zone is measured using a ruler and recorded in millimeters and the zone of inhibition was calculated(Yazhlini et al., 2021).

Time kill curve analysis:

The time-kill curve analysis assay is used to assess bactericidal properties and the concentration-dependent relationship between Vitis vinifera-mediated carbon nanoparticles and the growth rate of C.albicans, S.mutans, E.faecalis, and Lactobacillus sp over time intervals (Tharani et al., 2023). The assay involves the culture of four organisms in the Mueller Hinton Broth supplemented with the different concentrations(25, 50, 100µg/mL) of CNPsand then doing the time-kill curve analysis. Antibiotics (amoxyrite, fluconazole) were used as standard. After 4 hours of pre-incubation period in a medium without any antimicrobial substances, then growth curves were carried out to ensure all pathogens reached a stable early to mid-log phase (Sankar et al., 2024). An Inoculum having 0.5 McFarland of each organism is created in a sterile phosphate-buffered saline. The inoculum was collected from cultures of organisms cultivated on Mueller Hinton agar plates for 18 to 20 hours at 37 degreesCelsius. After that 30 microliter of inoculum is diluted in the 15 mL of antimicrobial-free Mueller Hinton Broth medium that was already preheated to 37 °C. Then a resultant mixture of 90µ L was distributed evenly over each well of 96-well ELISA plates. Then to each well that contains

90 µL of pathogens, 10 µL of Vitis vinifera-assistedCNPs was added along with untreated control (Munuswamy et al., 20203.

3. Results and Discussion:

Figure 1. Overall synthesis of Vitis vinifera-assisted carbon nanoparticles and its incorporated chitosan nanocomposite

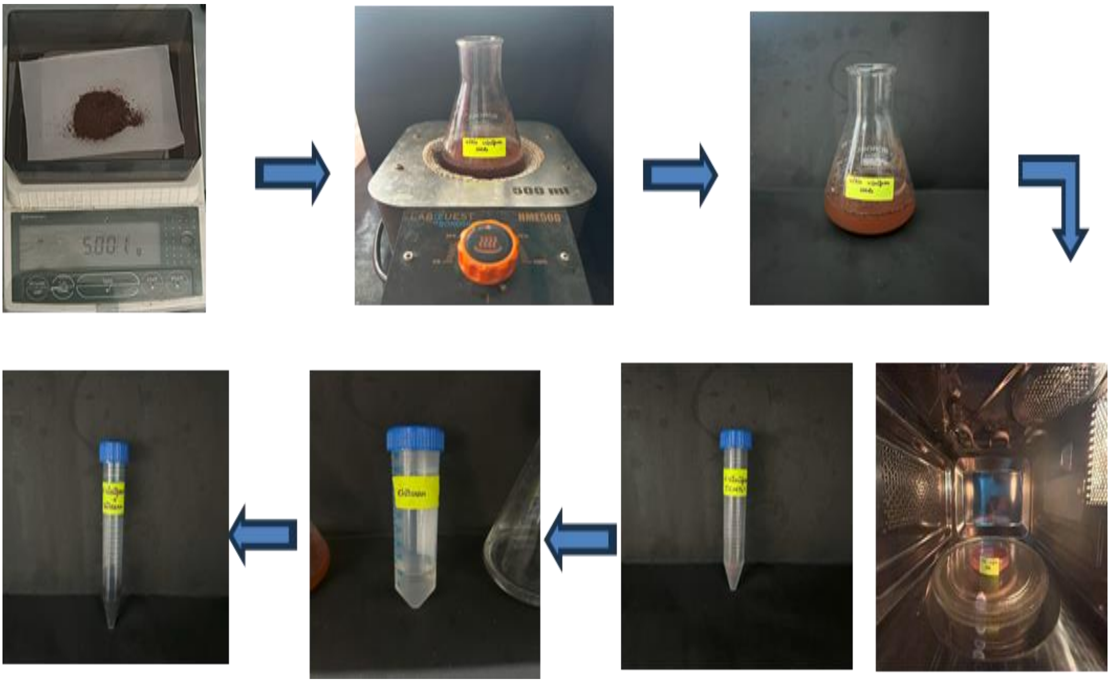


Figure 2. Antimicrobial activity of carbon nanoparticles against S. mutans, C. albicans, E. faecalis, Lactobacillus sp.

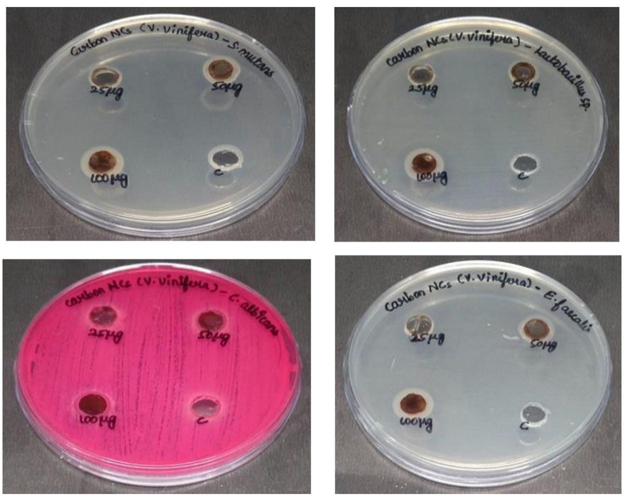
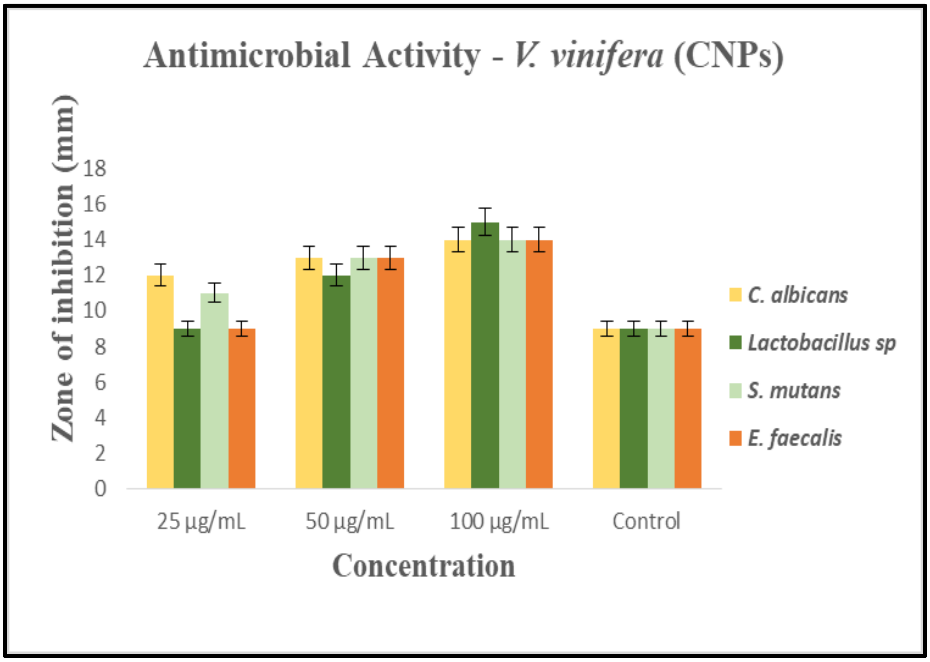
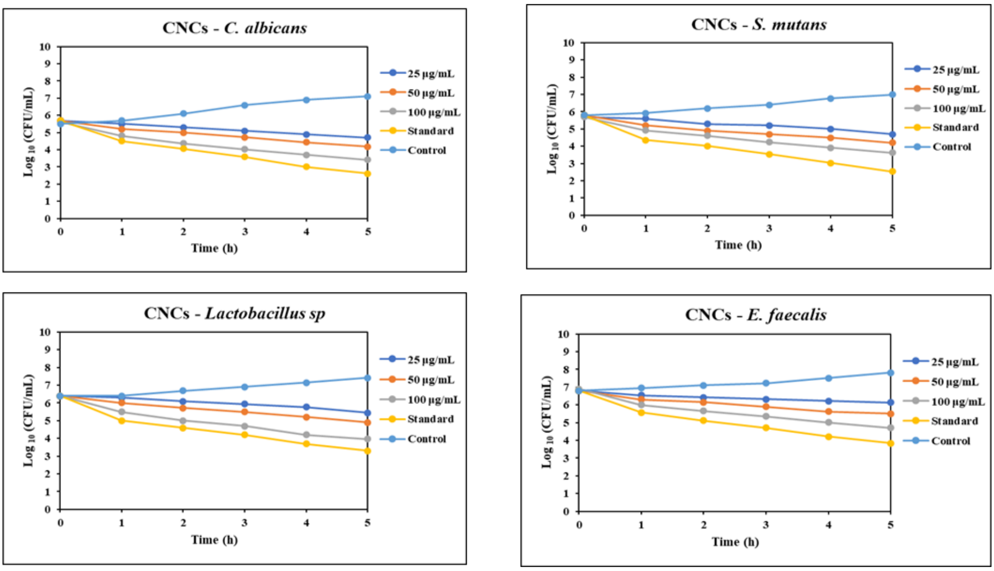


Figure 3. Displays the antibacterial effectiveness of carbon nanoparticles against oral pathogens.



The overall synthesis of the CNPs is represented in Figure 1. The antibacterial property of carbon nanoparticles is tested by the agar well diffusion method against four pathogens. In Figure 2, we can see the visual representation of the result of the antibacterial activity of nanoparticles. In Figure 3, we can see the graphical representation of the antibacterial activity of carbon nanoparticles against dental pathogens. The controlexhibited a zone of inhibition of 9nm on all pathogens. CNPs at a concentration of 25µg/ml exhibited a zone of inhibition of 12 nm on C.albicans, 11nm on S.mutans, 9nm on Lactobacillus sp, and 9nm on E.faecalis. CNPs at a concentration of 50µg/ml exhibited a zone of inhibition of 12 nm on C.albicans, 13nm on S.mutans, 13 nm on Lactobacillus sp, and 9nm on E.faecalis. Carbon nanoparticles at a concentration of 100 µg/ml exhibited a zone of inhibition of 14 nm against C.albicans, 14nm on S.mutans, 15 nm on Lactobacillus sp, and 14 nm on E.faecalis. The zone of inhibition is high in higher concentrations. This indicates dose dose-dependent effect.

Figure 4. Time-kill study analysis of carbon nanoparticles mouthwash against S. mutans, C. albicans, E. faecalis, Lactobacillus sp.



The antimicrobial effect of carbon nanoparticles is assessed by time-kill curve assay on four pathogens C.albicans, S.mutans, Lactobacillus sp, and E.faecalis,and compared with control and standard. At all concentrations of carbon nanoparticles, a noticeable decrease in growth is seen. Control doesn't have any effect in reducing the growth of pathogens. CNPs effectiveness increased with an increase in concentrations. Against S. mutans 25µg/ml carbon nanoparticles exhibited the least effect followed by 50µg/ml and at 100µg/ml they exhibited the highest effect.Against E. faecalis 25µg/ml carbon nanoparticles exhibited the least effect followed by 50µg/ml and at 100µg/ml they exhibited the highest effect.Against Lactobacillussp. 25µg/ml carbon nanoparticles exhibited the least effect followed by 50µg/ml and at 100µg/ml they exhibited the highest effect.C.albicans 25µg/ml carbon nanoparticles exhibited the least effect followed by 50µg/ml and at 100µg/ml they exhibited the highest effect. The standard decreased the growth of pathogens more than CNPs at the highest concentrations as shown in Figure 4.

4. Discussion:

The synthesis of Vitis vinifera carbon nanoparticles was successful. The antimicrobial property of Vitis vinifera carbon nanoparticles is evident through agar well diffusion assay and time-kill curve assay techniques. They showed concentration-dependent antimicrobial activity. At higher concentrations of nanoparticles, there was more reduction in bacterial count. The Vitis vinifera carbon nanoparticle was more effective against Lactobacillussp followed by C.albicans, S.mutans, and E.faecalis.In the study conducted by (Gautam et al., 2017) carbon nanoparticles synthesized from mustard oil showed antimicrobial activity against bacteria causing dental caries and were able to inhibit the growth of bacteria that are resistant to antibiotics.In the study conducted by V.Pahal et al., the bactericidal activity of carbon dots and nanoparticles from soot of clarified butter and mustard oil was evaluated. The agar well diffusion assay was performed and it showed potent antimicrobial activity of carbon nanoparticles at higher concentrations (Chan et al., 2021).In the previous study conducted by Chitra et al., the antimicrobial properties of carbon nanoparticles were isolated from the chimney soot. They performed agar well diffusion against Staphylococcus aureus, Streptococcus pyrogenes, Proteus mirabilis, Klebsiella pneumonia, and E. coli. The study showed that carbon nanoparticles prevent bacteria from dividing and multiplying (Aloysius et al., 2019). In the study conducted by Lifeng et al., the antimicrobial property of single-walled carbon nanotubes suspended in different types of surfactants is assessed. They checked it against S.enterica, E. coli, and E.faecalis. Carbon nanotubes showed a great impact on wound pathogens.

5. Conclusion:

From the above study, we can conclude that wesuccessfully synthesized NCs from Vitis vinifera-incorporated chitosan. The nanoparticles synthesized had potent antimicrobial properties against the dental pathogens. Using medicinal plants is increasing worldwide. These plants are safe with minimal to no side effects. From the results of the study, we can use these carbon nanoparticles synthesized from Vitis vinifera as an anticariogenic agent. This study provides a scientific base. The nanoparticles' effectiveness can be tested further with other studies.

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