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Time kill kinetic analysis of Acorus calamus leaves mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus sp

Ishwarya. S[1], Dr. S Rajeshkumar[2], Dr. Anitha Roy[3]

[1]graduate student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai 77, Tamil Nadu, India. Email: 152101088.sdc@saveetha.com
[2]Professor, Nanobiomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai 77, Tamil Nadu, India. rajeshkumars.sdc@saveetha.com,
[3]Professor: Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai 77, Tamil Nadu, India.
Email:anitharoy@saveetha.com

ABSTRACT

INTRODUCTION
Dental caries, commonly known as tooth decay, is a prevalent oral health problem caused by the colonization and proliferation of cariogenic bacteria in the oral cavity. Streptococcus mutans and certain Lactobacillus species are recognized as the primary etiological agents responsible for dental caries initiation and progression. Traditional antimicrobial agents used for oral hygiene maintenance and caries prevention have encountered challenges, including the development of bacterial resistance and adverse side effects. Nanotechnology has emerged as a promising approach in dentistry, offering novel strategies for combating dental caries and enhancing oral health. Selenium nanoparticles have gained considerable attention due to their unique physicochemical properties and broad-spectrum antimicrobial activity.
AIM AND OBJECTIVE
To analyze the Time kill kinetic analysis of Acorus calamus leaves-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus sp
Assessing the antimicrobial activity of Acorus calamus leaf-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus sp.
Determining the kinetics of microbial growth inhibition by measuring the changes in microbial populations over time.
Evaluating the concentration-dependent effects of Acorus calamus leaf-mediated selenium nanoparticles on the targeted microorganisms.
MATERIALS AND METHODS
Obtain fresh Acorus calamus leaves and wash them thoroughly to remove any dirt or debris. Chop the leaves into small pieces and grind them in a blender or mortar and pestle.Add a suitable solvent, such as ethanol or methanol, to the ground leaves and mix well. Allow the mixture to stand for a specific period (e.g., 24 hours) at room temperature to facilitate extraction.Filter the mixture using a filter paper or a fine mesh to obtain the Acorus calamus leaf extract. Store the extract in a sterile container for further use.
CONCLUSION
Time-kill kinetic analysis revealed that the Acorus calamus leaf-mediated selenium nanoparticles exhibited significant antimicrobial activity against both Streptococcus mutans and Lactobacillus species. The results demonstrated a dose-dependent reduction in bacterial viability over time, highlighting the nanoparticles' ability to inhibit the growth and proliferation of these cariogenic bacteria.

KEYWORD: time kill kinetic analysis, Acorus calamus, selenium nanoparticles, Streptococcus mutans, Lactobacillus, oral diseases, dental caries, antimicrobial agents, nanotechnology, plant extracts, biocompatibility, Acorus calamus leaf, antimicrobial activity, bactericidal, bacteriostatic, exposure time, oral pathogens, therapeutic agents,

INTRODUCTION
Dental caries, commonly known as tooth decay, is a prevalent oral health problem caused by the colonization and proliferation of cariogenic bacteria in the oral cavity(‘Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line’, 2020).Streptococcus mutans and certain Lactobacillus species are recognized as the primary etiological agents responsible for dental caries initiation and progression.(Singh and Singh, 2021)These pathogens not only erode the dental enamel but also lead to tooth pain, infections, and potential systemic complications if left untreated.As the conventional antimicrobial agents are becoming less effective due to the emergence of drug-resistant strains, there is a pressing need to explore alternative strategies for combating these oral pathogens. (‘Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity’, 2017)Traditional antimicrobial agents used for oral

hygiene maintenance and caries prevention have encountered challenges, including the development of bacterial resistance and adverse side effectsNanotechnology has emerged as a promising approach in dentistry, offering novel strategies for combating dental caries and enhancing oral health.(Alamgir, 2018)This analysis involves measuring the survival or growth of bacteria over a specific time period in the presence of the nanoparticles.(Paul-MDPhDFAMSFNAScFAScFNA and Fisnfams, 2019) By monitoring the microbial population at regular intervals, we can determine the bactericidal or bacteriostatic activity of the nanoparticles and evaluate their long-term effectiveness.('Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica', 2021; Alrashidi and Gomaa, 2023)

Acorus calamus, a perennial herbaceous plant rich in bioactive phytochemicals, has garnered attention for its traditional medicinal applications. Previous studies have highlighted its diverse pharmacological properties, including antimicrobial potential.(Singh and Singh, 2021; Alrashidi and Gomaa, 2023) Additionally, selenium nanoparticles have emerged as a promising avenue in the field of nanotechnology-based therapeutics, owing to their unique physicochemical properties and biocompatibility.(Nandhini, Ezhilarasan and Rajeshkumar, 2021)('Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica', 2021)

Selenium nanoparticles have gained considerable attention due to their unique physicochemical properties and broad-spectrum antimicrobial activity.Nanotechnology has emerged as a promising field with immense potential for developing novel antimicrobial agents.(*View of In vitro Anti-inflammatory activity of Silymarin/Hydroxyapatite/Chitosan Nanocomposites and its cytotoxic effect using Brine shrimp lethality assay*, no date) Among the various nanoparticles investigated for their antimicrobial properties, selenium nanoparticles have garnered significant attention due to their unique physicochemical properties and inherent biocompatibility.('Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica', 2021) This study focuses on harnessing the potential of Acorus calamus leaves mediated selenium nanoparticles as a novel antimicrobial agent against Streptococcus mutans and Lactobacillus species.(Rajeshkumar *et al.*, 2019) The utilization of green synthesis methods for nanoparticle production holds ecological significance and ensures the biocompatibility of the resulting nanoparticles.

Selenium nanoparticles have demonstrated broad-spectrum antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses. Additionally, the use of plant extracts for synthesizing nanoparticles offers several advantages, such as eco-friendliness, cost-effectiveness, and the potential to enhance the therapeutic efficacy of the nanoparticles(Fedlheim and Foss, 2001).Moreover, natural products, such as Acorus calamus leaves, have shown significant antimicrobial potential attributed to their bioactive constituents.Understanding the time-dependent antimicrobial activity of Acorus calamus leaf-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus species is crucial for evaluating their potential as alternative therapeutic agents for oral health management.(), 1996; Fedlheim and Foss, 2001) This research contributes to the growing body of knowledge on nanotechnology-based approaches for combating oral infections and may pave the way for the development of innovative antimicrobial strategies with improved efficacy and reduced adverse effects.(Paul-MDPhDFAMSFNAScFAScFNA and Fisnfams, 2019)

MATERIALS AND METHODS

Preparation of Acorus calamus leaf extract:

Obtain fresh Acorus calamus leaves and wash them thoroughly to remove any dirt or debris. Chop the leaves into small pieces and grind them in a blender or mortar and pestle.Add a suitable solvent, such as ethanol or methanol, to the ground leaves and mix well. Allow the mixture to stand for a specific period (e.g., 24 hours) at room temperature to facilitate extraction.Filter the mixture using a filter paper or a fine mesh to obtain the Acorus calamus leaf extract. Store the extract in a sterile container for further use.

Synthesis of Acorus calamus leaf-mediated selenium nanoparticles:

In a reaction vessel, combine a specific volume of the Acorus calamus leaf extract with a selenium precursor solution (e.g., selenium chloride or selenous acid). Stir the mixture at a controlled temperature (e.g., 60-80°C) for a predetermined time period to facilitate the reduction of selenium ions and the formation of nanoparticles. Monitor the reaction progress visually or using suitable analytical techniques to confirm the synthesis of selenium nanoparticles.Once the synthesis is complete, centrifuge the reaction mixture to separate the nanoparticles from any residual plant extract or impurities.Wash the obtained nanoparticles several times with a suitable solvent (e.g., distilled water or ethanol) to remove any residual reactants or byproducts. Characterize the synthesized Acorus calamus leaf-mediated selenium nanoparticles using techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), X-ray diffraction (XRD), and zeta potential analysis to determine their size, shape, stability, and surface charge.

Bacterial cultures:

Obtain pure cultures of Streptococcus mutans and Lactobacillus species from a reliable microbial culture collection or clinical samples. Inoculate the bacteria into appropriate growth media (e.g., tryptic soy broth) and incubate them under controlled conditions (e.g., 37°C, aerobic or anaerobic) until reaching the mid-log phase.

Time-kill kinetic assay setup:

Prepare the working solutions of Acorus calamus leaf-mediated selenium nanoparticles by diluting the nanoparticle suspension in a suitable medium (e.g., sterile broth).Adjust the concentration of the bacterial cultures to achieve the desired initial inoculum concentration (e.g., 10^6 CFU/mL). Add the appropriate volume of the nanoparticle solution to the bacterial suspension to obtain the desired final nanoparticle concentration. Prepare control groups with bacteria only (no nanoparticles) and positive control groups with appropriate antimicrobial agents (e.g., antibiotics) to validate the assay. Incubate all the samples at a specified temperature (e.g., 37°C) and collect aliquots at regular time intervals (e.g., 0, 1, 2, 4, 6, 24 hours). Plate the aliquots onto suitable agar plates and incubate them under appropriate conditions for colony formation. After the incubation period, count the viable colonies on each plate and calculate the bacterial survival or growth rate. Repeat the experiment at least three times to ensure the reproducibility of the results.

Data analysis:

Plot the bacterial survival or growth curves over time for each sample.Calculate the log reduction in bacterial count compared to the initial inoculum for each time point.Perform statistical analysis to determine the significance of the differences between the control and experimental groups using appropriate tests (e.g., t-test, ANOVA).Present the results in graphical and tabular formats, highlighting the antimicrobial activity of Acorus calamus leaf-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus species at different time points.

By following these materials and methods, you can conduct a time kill kinetic analysis of Acorus calamus leaf-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus species, providing insights into their antimicrobial efficacy and potential for oral health applications.

RESULT

Organism	25µg/mL	50µg/mL	100µg/mL	Control
S. mutans	**26**	**32**	**40**	**22**
Lactobacillus sp	**26**	**30**	**36**	**20**

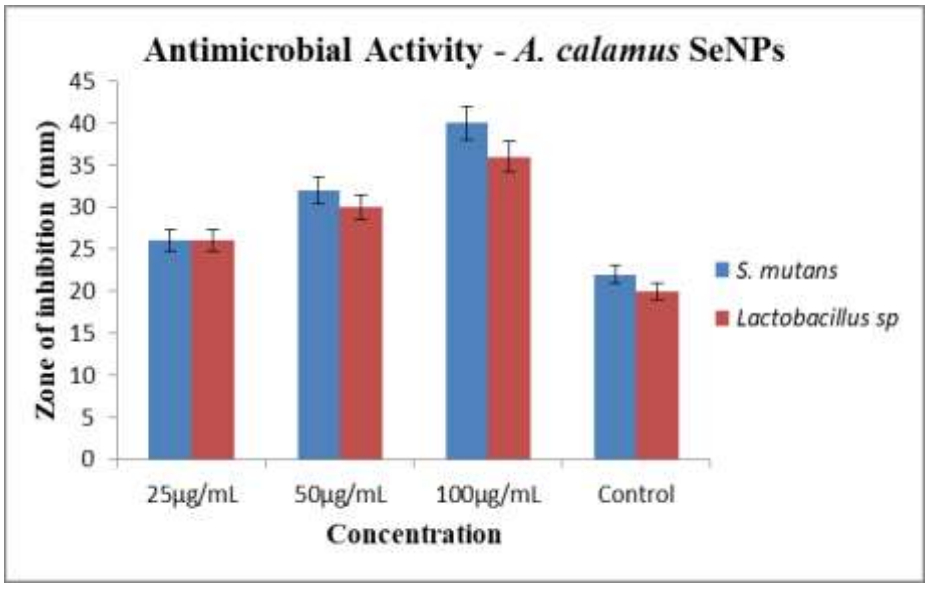

Figure 1: represents the anti microbial activity of *acorus calamus* selenium nano particles in *s.mutans.*The x - axis represents the concentrations of *s.mutans* and *lactobacillus species* .The y - axis represents the zone of inhibition.Figure 1 clearly depicts that both *s.mutans* and *lactobacillus species* have more zone of inhibition.

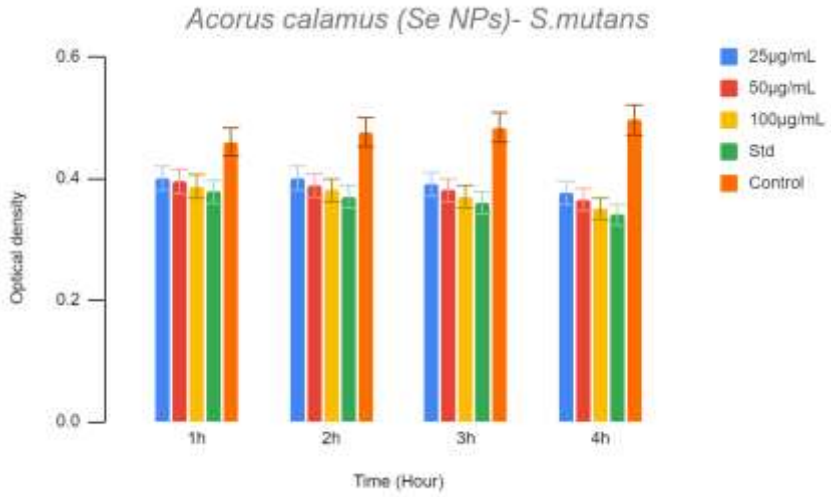

Figure 2: represents the anti microbial activity of *acorus calamus* selenium nano particles in *s.mutans.*The x - axis represents the antimicrobial activity time and control group of *s.mutans* in represented one hours, two hours, three and four hours . The y - axis represents the according optical density of the *acorus calamus* selenium nano particles in *s.mutans.* 25ug/mL, 50pg/mL,100pg/mL of *s.mutans* shows 0.4 optical density in every hour . The control group shows 0.5 optical density in every hour.

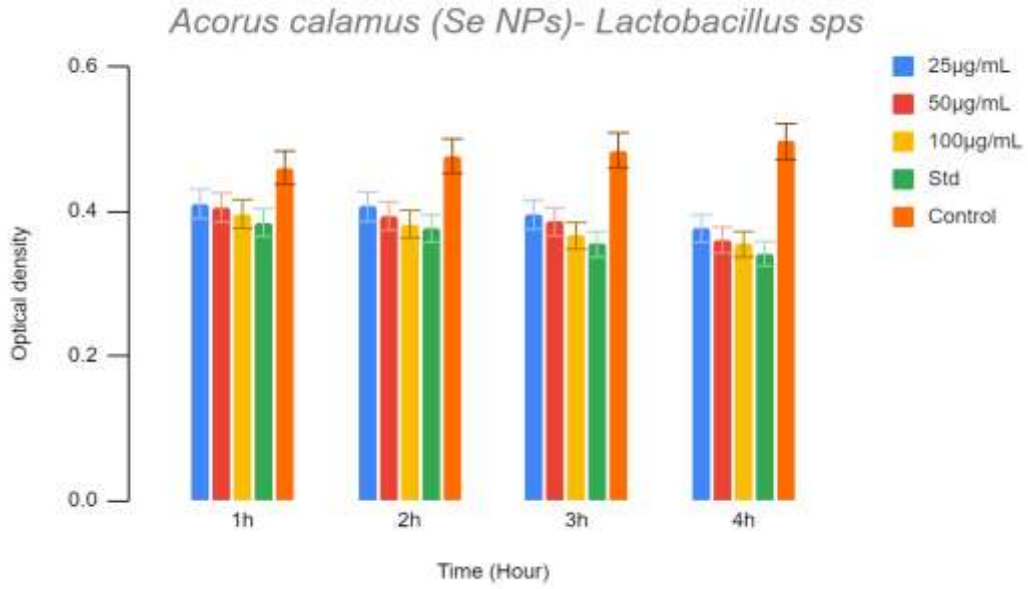

Figure 3: represents the anti microbial activity of *acorus calamus* selenium nano particles in *lactobacillus sp* .The x - axis represents the antimicrobial activity time and control group of *lactobacillus sp* in represented one hours, two hours, three and four hours . The y - axis represents the according optical density of the *acorus calamus* selenium nano particles in *lactobacillus sp* . 25ug/mL, 50pg/mL,100pg/mL of *lactobacillus sp* shows 0.4 optical density in every qhour . The control group shows 0.5 optical density in one hour.

DISCUSSION

Antimicrobial activity: Discuss the results of the time-kill kinetic analysis, highlighting the antimicrobial activity of Acorus calamus leaf-mediated selenium nanoparticles against Streptococcus mutans and Lactobacillus sp. yPresent the microbial growth inhibition data over time and compare it with control groups or standard antimicrobial agents. Emphasize the effectiveness of the nanoparticles in reducing the microbial populations and inhibiting the growth of these oral pathogens(Fedlheim and Foss, 2001).Elucidating the precise mechanism of action of Acorus calamus leaf-mediated

selenium nanoparticles against Streptococcus mutans and Lactobacillus sp. would be an important focus for future research. Molecular and cellular studies can help uncover the specific targets or pathways affected by the nanoparticles, providing a deeper understanding of their antimicrobial activity(Alvarez-Munoz and Farre, 2020; Colin Campbell and Disla, 2020).(Blasco and Corsi, 2019) Techniques such as transcriptomics, proteomics, and metabolomics can be employed to explore the global gene expression, protein profiles, and metabolic changes induced by the nanoparticles.(Tao, 2014)(Alvarez-Munoz and Farre, 2020)

Compare the efficacy of Acorus calamus mediated selenium nanoparticles with traditional antimicrobial agents commonly used in dental care. Highlight any advantages or unique properties of the nanoparticles in terms of antimicrobial activity and potential reduced likelihood of bacterial resistance.(Rastogi *et al.*, 2012)

Address the biocompatibility of Acorus calamus mediated selenium nanoparticles, emphasizing their potential as a safe alternative to synthetic antimicrobials. Discuss any potential cytotoxic effects on human cells or adverse reactions.(Rastogi *et al.*, 2012; Paul-MDPhDFAMSFNAScFAScFNA and Fisnfams, 2019)

Discuss the potential applications of Acorus calamus mediated selenium nanoparticles in oral health maintenance and disease prevention(Hassan, 2016)(Koul, 2019). Consider scenarios such as toothpaste or mouthwash formulations, and their potential to target specific microbial species associated with dental cariesEmphasize the eco-friendly nature of the synthesis process, which utilizes Acorus calamus leaves as a natural source for nanoparticle production. (Fedlheim and Foss, 2001)This may have significant implications for sustainable biomedicine and environmental conservation.Acknowledge any limitations of the study, such as potential variations in nanoparticle synthesis or potential synergistic effects with other compounds present in Acorus calamus leaves.(Swamy and Akhtar, 2020)

Suggest avenues for future research, such as exploring the nanoparticle's efficacy against a broader spectrum of oral pathogens, conducting in vivo studies, or investigating potential synergistic effects with other natural compounds.(Singh, 2018)(Shabatina and Bochenkov, 2020)

CONCLUSION

Time-kill kinetic analysis revealed that the Acorus calamus leaf-mediated selenium nanoparticles exhibited significant antimicrobial activity against both Streptococcus mutans and Lactobacillus species.(Adkins, Shabbir and Dhileepan, 2018) The results demonstrated a dose-dependent reduction in bacterial viability over time, highlighting the nanoparticles' ability to inhibit the growth and proliferation of these cariogenic bacteria.(Singh and Singh, 2021)These findings hold promise for the potential application of Acorus calamus leaf-mediated selenium nanoparticles as an alternative antimicrobial agent in the prevention of dental caries.(Ahmed and Ali, 2020) By leveraging nanotechnology and natural plant resources, this approach offers a novel avenue for developing effective and sustainable strategies for oral health care.(Alamgir, 2017)

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