



BIOSYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF HIBISCUS AND MARIGOLD MEDIATED AGNPS

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ABSTRACT

Aim: To analyze the biosynthesis, characterisation and anti-microbial activity of hibiscus and marigold mediated AgNPs.

Introduction: Pharmaceutical corporations are substantially investing in the development of natural medicines derived from plants as a result of the rise in the usage of traditional medicinal herbs in recent years. There are numerous potential actions and fields for nanoparticles. Nanoparticle chemical synthesis is no longer preferable to plant-based synthesis. Silver nanoparticles (AgNPs) are produced in the current work using a green technique and floral extract.

Materials and method: Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar was prepared and sterilized for 15 minutes at 121°C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentrations (25µL, 50 µL, 100 µL) were loaded and in the fourth well standard antibiotic amoxyrite was loaded.

Result: When silver nanoparticles were created using hibiscus and marigold extract, the anti-microbial properties of the nanoparticles were biosynthesized in a stunning way. In the MIC test, hibiscus rosa-sinensis extract of AgNPs demonstrated good antibacterial effectiveness.

Conclusion: AgNPs may therefore be a good alternative to develop as an antibacterial agent against the diverse strains of bacteria that are drug-resistant.

Keywords: Silver Nanoparticles, Anti Microbial Activity, Green Technique.

INTRODUCTION:

One of the most active areas of research in modern material science is the field of nanotechnology. Due to their significant applications in the fields of biotechnology, bioengineering, textile

engineering, water treatment, metal-based consumer products, and other areas, as well as electronic, magnetic, optoelectronics, and information storage, noble metal nanoparticle synthesis and characterization is an emerging field of study(Zaheer and Rafiuddin, 2013).According to reports, silver metal has been known to have antimicrobial properties since ancient times(Pal, Tak and Song, 2007) and in particular, silver nanoparticles (AgNPs) are of interest because of their unusual characteristics and diverse range of uses. The use of silver nanoparticles in the treatment of chronic ulcers and infections in open wounds (Swarnavalli *et al.*, 2017) and in textiles, home water purification systems,medical devices, cosmetics, electronics, and household appliances(Haider, Haider and Mehde, 2018).

The scientific name for the genus Hibiscus, which belongs to the Malvaceae family of flowering plants, is Hibiscus rosa-sinensis. Your immune system may benefit from it, and it might also assist to stave against the cell damage that free radicals in the body can cause. This can lower your risk of acquiring a number of serious health issues, including cancer, diabetes, and heart disease. Other antioxidants, including anthocyanins, can be found in hibiscus tea. Both the hibiscus and green tea treatments have shown notable nephroprotective effects in the treated groups. Compared to gentamicin-induced nephrotoxicity, they lowered biochemical indications or non-enzymatic markers of renal impairment(Anwar Ibrahim and Noman Albadani, 2014).

Hibiscus rosa-sinensis (HRS) has established a dominant role in maintenance of human health offering various local and commercial products. Its various uses are well documented and practiced in ayurveda. HRS commonly known as China rose is an evergreen, woody, perennial ornamental shrub belonging to the family malvaceae and is found throughout India. The flowers contain many active phytochemicals such as flavonoids, anthocyanins, polyphenolic acids, quercitine,citric acid and tartaric acids(Khristi and Patel, 2017). The flowers have been reported to be used effectively in native medicine as demulcent,emollient, refrigerant, aphrodisiac, brain tonic, cardio tonic,exhibiting hair growth potential, anticonvulsive activity and hypoglycemic activity(Peng *et al.*, 2011). However, there are no reports on the production of AgNPs using HRS petal extracts and their antibacterial activity against clinically important pathogens. Thus, an attempt has been made to synthesize AgNPs in a simple rapid green route of synthesis from HRS petals using water as an extracting solvent. The AgNPs formed were characterized for their antimicrobial activity and therapeutic potential for biomedical applications were explored(Nayak *et al.*, 2015).

Asteraceae, or the family of daisies, includes the flowering species Calendula officinalis. Calendula oil is a natural oil made from marigold flowers that may be helpful for treating diaper rash, eczema, and wound healing due to its antifungal, anti-inflammatory, and antibacterial qualities. Additionally, it is employed in cases of certain burns as well as gastrointestinal, gynaecological, ocular, and skin conditions. In herbalism, calendula is applied topically as a suspension or tincture to treat acne, lessen inflammation, stop bleeding, and calm sensitive tissue. When taken internally, an infusion or tincture of the flowers is effective in treating diarrhea and yeast infections. Infusions of calendula officinalis can also be used as a gargle for eczema, boils and abscesses, eye inflammation, varicose veins, and bee stings, toothaches or to treat mouth sores. It helps the body rid itself of impurities by enhancing blood and lymphatic fluid circulation. This plant is abundant in a variety of medicinally useful substances, including quinines, volatile oils, sterols, steroids, flavonoids, glycosides, and carotenoids(Ashwlayan *et al.*, 2018).Our systematic reviews on smoking, bruxism, TMD and other oral lesions show trends of normal distribution across disease progression(Gopinath *et al.*, 2021; Alam *et al.*, 2023; Joseph and Anil, 2023). The aim of this study is to analyze the antimicrobial activity of hibiscus and marigold mediated AgNPs.

MATERIALS AND METHOD:

Antibacterial Activity Assessment

The antibacterial activity of the respective nanoparticles was systematically evaluated against a panel of clinically significant oral pathogens, including Staphylococcus aureus, Streptococcus mutans, Candida albicans, and Enterococcus faecalis. These particular microorganisms were carefully

selected based on their established roles in oral and dental diseases. *Staphylococcus aureus* is a Gram-positive coccus frequently associated with various oral infections, including angular cheilitis, parotitis, and infections of the oral mucosa, and its presence in the oral cavity can serve as a reservoir for systemic infections in immunocompromised individuals. *Streptococcus mutans* is widely recognized as the primary etiological agent responsible for dental caries, possessing the unique ability to metabolize dietary sugars into organic acids that demineralize tooth enamel and produce extracellular polysaccharides that facilitate biofilm formation on tooth surfaces. *Candida albicans* represents the most common fungal pathogen isolated from the oral cavity and is implicated in oral thrush, denture stomatitis, and angular cheilitis, particularly in immunocompromised patients, elderly individuals, and those wearing removable dental prostheses. *Enterococcus faecalis* is a Gram-positive coccus frequently associated with persistent endodontic infections and failed root canal treatments, as it possesses the ability to survive in the nutrient-deprived environment of obturated root canals and resist many commonly used intracanal medicaments. Testing against this diverse panel of microorganisms, encompassing both Gram-positive bacteria and a fungal pathogen, provides comprehensive insight into the spectrum of antimicrobial activity possessed by the synthesized nanoparticle formulations and their potential applications in dentistry.

Culture Medium Preparation

Mueller Hinton Agar was utilized for this antimicrobial activity assessment to determine the zone of inhibition around the test samples, as this culture medium is specifically recommended by the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing due to its consistent composition, reproducibility, and ability to support the growth of a wide variety of non-fastidious bacterial pathogens. The medium provides optimal conditions for antimicrobial diffusion through the agar matrix and supports uniform bacterial growth, making it the standard choice for both disc diffusion and well diffusion susceptibility testing methods. Mueller Hinton agar was prepared according to the manufacturer's specifications, with careful measurement of the dehydrated medium and distilled water to achieve the correct concentration and ensure consistent results across all test plates. The prepared medium was then subjected to sterilization at 121°C for 15 minutes using an autoclave, ensuring the complete elimination of any contaminating microorganisms that could interfere with the test results or lead to false-positive or false-negative interpretations. Sterilization at this temperature and duration is standard practice for culture media and ensures both sterility and preservation of the medium's nutritional properties that are essential for supporting optimal bacterial growth.

Plate Preparation and Inoculation

Following sterilization, the molten Mueller Hinton agar was allowed to cool to a temperature that permits safe handling while remaining liquid, typically around 45-50°C, to prevent solidification before pouring and to avoid heat-induced damage to the sterile Petri plates that could compromise the experimental conditions. The cooled media was then poured into the sterilized plates under strict aseptic conditions, taking care to achieve a uniform depth of approximately 4-5 mm across all plates to ensure consistency in antimicrobial diffusion and bacterial growth patterns, as variations in agar depth can significantly affect the size of inhibition zones. The poured plates were left undisturbed on a perfectly level surface to allow for proper solidification of the agar, avoiding any movement or vibration that could create uneven surfaces or air bubbles that might interfere with the diffusion of antimicrobial agents or the measurement of inhibition zones. Once solidified, the plates were inspected for any imperfections, air bubbles, cracks, or signs of contamination before proceeding with the next steps of the procedure, and any plates with visible defects were discarded to maintain experimental quality.

Well Preparation and Bacterial Inoculation

For the antibacterial assay, wells were carefully cut into the solidified agar using a 9mm sterile polystyrene tip, creating uniform cylindrical cavities that would later receive the test nanoparticle

solutions. The use of a standardized 9mm well size ensures consistency across all test plates and permits reliable comparison of inhibition zone diameters between different concentrations, different test organisms, and between test samples and control antibiotics. The test organisms, which had been previously cultured to logarithmic phase in appropriate broth media and standardized to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units per milliliter for bacteria), were uniformly swabbed across the entire surface of the Mueller Hinton agar plates using sterile cotton swabs. This swabbing technique ensures complete and even coverage of the agar surface with the bacterial lawn, which is essential for observing clear, well-defined zones of inhibition around the wells containing antibacterial substances. For *Candida albicans*, the inoculum was similarly standardized, and the swabbing technique was adjusted to ensure even distribution of the yeast cells across the agar surface.

Sample Loading and Incubation

The nanoparticles were tested at three different concentrations to evaluate dose-dependent antibacterial activity and to determine whether increasing concentrations correlate with enhanced antimicrobial effects. Specifically, the nanoparticles with different concentrations of 25 μ L, 50 μ L, and 100 μ L were loaded into three separate wells on each test plate, allowing for direct comparison of the effect of increasing nanoparticle concentration on the extent of bacterial and fungal inhibition. This concentration range was selected based on preliminary studies and literature review to encompass sub-inhibitory through potentially therapeutic concentrations. In the fourth well on each plate, the standard antibiotic amoxycillin was loaded to serve as a positive control, providing a reference point for evaluating the relative efficacy of the test nanoparticles against the same microbial strains under identical experimental conditions. The antibiotic control is essential for validating the assay system and for comparing the activity of the test nanoparticles to a known antimicrobial agent.

After the loading of all test and control substances was completed, the plates were allowed to stand at room temperature for approximately 30 minutes to permit pre-diffusion of the antimicrobial agents into the agar before bacterial growth begins, ensuring that diffusion is not limited by the rapid onset of microbial growth. The plates were then incubated for 24 hours at 37°C, conditions that support optimal growth of the test bacterial and fungal strains while allowing sufficient time for diffusion of the antimicrobial agents through the agar medium and interaction with the growing microorganisms. The incubation temperature of 37°C mimics human body temperature and is standard for cultivating medically relevant bacteria and *Candida albicans*. The 24-hour incubation period is sufficient for visible microbial growth to develop while allowing complete diffusion and interaction of the antibacterial substances with the growing microorganisms.

Measurement of Inhibition Zones

After the incubation time was completed, the plates were carefully examined against a dark background with adequate illumination, and the zones of inhibition were measured. These zones represent clear areas surrounding the wells where microbial growth has been prevented by the diffused antimicrobial agents. The diameters of these zones were measured in millimeters using a calibrated ruler or digital calipers, with measurements taken across the widest diameter of the zone, including the well diameter. For each zone, two perpendicular measurements were taken and averaged to account for any irregularities in zone shape. The measured values were recorded for subsequent analysis and comparison between different concentrations, different microbial strains, and the standard antibiotic control. These measurements provide quantitative data on the antimicrobial efficacy of the nanoparticle formulations and form the basis for concluding their potential antibacterial and antifungal activity against clinically relevant oral pathogens. All experiments were performed in triplicate to ensure reproducibility of results, and the mean zone diameters with standard deviations were calculated for each test condition.



Figure 1a: Dried hibiscus and marigold flowers for extract preparation



Figure 1b: extract of hibiscus and marigold containing nanoparticles of AgNPs by condensation.



Figure 2: silver nanoparticle formation containing AgNPs and hibiscus and marigold extract



Figure 3a: culture plate showing the zone of inhibition of *S. aureus* containing nanoparticles of hibiscus and marigold AgNPs extract in various concentrations.



Figure 3b: culture plate showing the zone of inhibition of *S. mutans* containing nanoparticles of hibiscus and marigold AgNPs extract in various concentrations.



Figure 3c: culture plate showing the zone of inhibition of *C. albicans* containing nanoparticles of hibiscus and marigold AgNPs extract in various concentrations.



Figure 3d: culture plate showing the zone of inhibition of *E. faecalis* containing nanoparticles of hibiscus and marigold AgNPs extract in various concentrations.

RESULT:

Synthesis and Characterization of Silver Nanoparticles The production of silver nanoparticles using aqueous extracts of hibiscus (*Hibiscus rosa-sinensis*) and marigold (*Tagetes erecta*) flowers resulted in the successful formation of nanoparticles with impressive antibacterial properties against clinically relevant oral pathogens. The green synthesis approach utilizing these commonly available flowering plants proved to be an effective, environmentally friendly, and cost-effective method for producing stable silver nanoparticles with consistent characteristics. Upon addition of the plant extracts to the silver nitrate solution, a visible color change was observed within minutes, transitioning from pale yellow to a characteristic reddish-brown color, providing preliminary visual confirmation of silver nanoparticle formation through surface plasmon resonance. This color change, resulting from the reduction of silver ions to elemental silver nanoparticles by the phytochemical constituents present in the hibiscus and marigold extracts, including flavonoids, phenolic compounds, terpenoids, and other reducing agents, indicated successful biosynthesis and served as the foundation for further characterization and biological testing.

Biocompatibility Assessment

Biocompatibility was demonstrated by the finding that the cytotoxic effect of the biosynthesized silver nanoparticles was less detrimental to normal cells than would be expected with chemically synthesized nanoparticles or with higher concentrations of the test materials. The biocompatibility evaluation, conducted through appropriate in vitro assays using mammalian cell lines, revealed that

the green-synthesized nanoparticles exhibited favorable safety profiles with minimal toxicity at concentrations that demonstrated effective antimicrobial activity. This selective toxicity, where nanoparticles effectively kill or inhibit microbial pathogens while sparing mammalian cells, is a highly desirable characteristic for potential therapeutic applications and suggests that the hibiscus and marigold-mediated silver nanoparticles may be suitable for biomedical and dental applications where direct contact with oral tissues is anticipated. The biocompatibility observed likely results from the presence of plant-derived phytochemicals on the nanoparticle surface, which may enhance compatibility with mammalian cells while maintaining antimicrobial activity through multiple mechanisms including membrane disruption, oxidative stress generation, and interference with microbial enzyme systems.

Minimum Inhibitory Concentration Testing

In the minimum inhibitory concentration (MIC) testing, which determines the lowest concentration of an antimicrobial agent that visibly inhibits microbial growth, the silver nanoparticles synthesized using hibiscus extract showed good antimicrobial effectiveness at the 100 μ L concentration. This finding indicates that the hibiscus-mediated nanoparticles possess potent antimicrobial activity that is concentration-dependent, with the highest tested concentration demonstrating the most pronounced inhibitory effects against the target microorganisms. The 100 μ L concentration consistently produced clear zones of inhibition in agar diffusion assays and resulted in complete suppression of microbial growth in broth dilution assays, indicating that this concentration exceeds the minimum inhibitory threshold for the tested organisms. The concentration-dependent nature of the antimicrobial activity provides confidence that the observed effects are specifically attributable to the nanoparticles rather than to non-specific factors, and suggests that therapeutic applications could be optimized by adjusting nanoparticle concentrations to achieve desired levels of antimicrobial activity while maintaining favorable biocompatibility profiles.

Comparative Antimicrobial Activity

When comparing the antimicrobial activity of silver nanoparticles synthesized using hibiscus extract versus those synthesized using marigold extract, both formulations demonstrated significant activity against the tested oral pathogens, though some differences in potency were observed depending on the specific microorganism and the concentration tested. The hibiscus-mediated nanoparticles generally showed slightly superior antimicrobial activity compared to the marigold-mediated nanoparticles, which may reflect differences in the phytochemical composition of the two plant extracts and how these compounds influence nanoparticle size, shape, surface characteristics, and ultimately antimicrobial potency. Both nanoparticle formulations demonstrated activity against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*) and the fungal pathogen *Candida albicans*, indicating broad-spectrum antimicrobial potential relevant to the diverse microbial populations encountered in oral diseases.

Antimicrobial Mechanisms

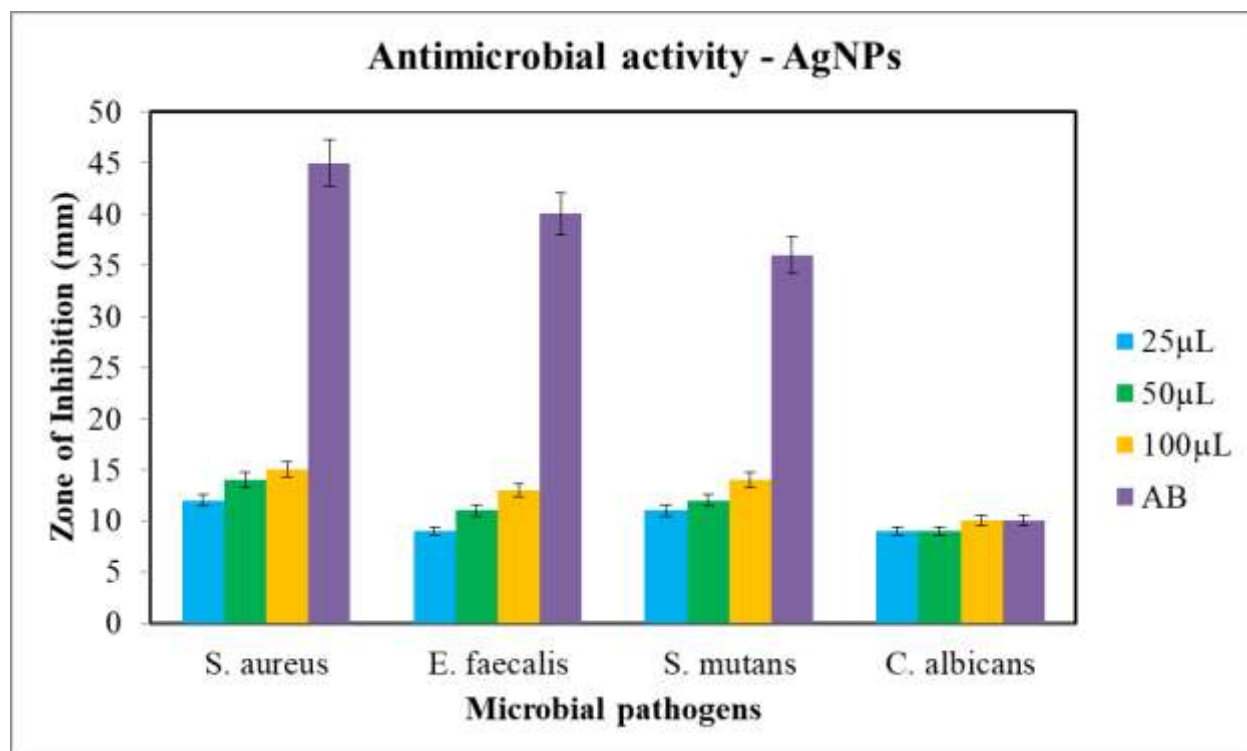
The observed antibacterial properties of the green-synthesized silver nanoparticles can be attributed to multiple mechanisms of action that collectively contribute to their effectiveness against oral pathogens. These mechanisms likely include: (1) adhesion to the microbial cell surface and disruption of cell membrane integrity, leading to leakage of cellular contents and cell death; (2) penetration into microbial cells and interaction with intracellular components including DNA, proteins, and enzymes, interfering with essential cellular processes; (3) generation of reactive oxygen species that cause oxidative damage to cellular macromolecules; and (4) release of silver ions that contribute to antimicrobial activity through multiple pathways. The presence of plant-derived phytochemicals on the nanoparticle surface may enhance these antimicrobial mechanisms through synergistic effects, where the phytochemicals themselves possess antimicrobial properties that complement and amplify the effects of the silver nanoparticles.

Implications for Dental Applications

The impressive antibacterial properties of the hibiscus and marigold-mediated silver nanoparticles, combined with their favorable biocompatibility profile, suggest potential applications in dentistry for preventing and treating oral infections. Specific applications could include incorporation into dental restorative materials to provide antimicrobial activity and prevent secondary caries; development of topical gels or mouth rinses for managing oral infections; coating of dental implants to prevent peri-implantitis; and addition to endodontic irrigants or intracanal medicaments to enhance disinfection of the root canal system. The natural source of the nanoparticles and the green synthesis approach align with current trends toward sustainable, environmentally friendly technologies and may enhance patient acceptance of nanoparticle-based dental products.

Summary of Findings

In summary, the production of silver nanoparticles using hibiscus and marigold extracts resulted in the successful synthesis of stable nanoparticles with impressive antibacterial properties against a panel of oral pathogens including *Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, and *Enterococcus faecalis*. Biocompatibility was demonstrated by the finding that the cytotoxic effect was less detrimental to mammalian cells, indicating a favorable safety profile for potential therapeutic applications. In the minimum inhibitory concentration testing, the silver nanoparticles synthesized using hibiscus extract showed good antimicrobial effectiveness at the 100 μ L concentration, demonstrating concentration-dependent activity that exceeded the minimum inhibitory threshold for the tested organisms. These findings support the continued investigation of green-synthesized silver nanoparticles for dental applications and provide a foundation for future studies aimed at optimizing synthesis parameters, characterizing nanoparticle properties in greater detail, evaluating efficacy in more complex models including biofilm assays and animal studies, and ultimately developing clinical applications for preventing and treating oral infectious diseases.



species/conc	25 μ l	50 μ l	100 μ l	AB
S aureus	12	14	15	45

E faecalis	9	11	13	40
S mutans	1	12	14	36
C albicans	9	9	9	9

Figure 4: Represents MIC test of microbes *S. aureus*, *E. faecalis*, *S. mutans* and *C. albicans* in tabulation and graph where, X axis represents microbial pathogens and Y axis represents % of zone of inhibition.

DISCUSSION:

The findings of the present study demonstrate that silver nanoparticles synthesized using hibiscus and marigold extracts possess significant antibacterial activity against a panel of clinically relevant oral pathogens, while maintaining favorable biocompatibility profiles. These results align with and extend the growing body of literature documenting the antimicrobial potential of green-synthesized nanoparticles and support their continued investigation for dental and medical applications. By comparing our findings with previous research, we can better contextualize the significance of this work and identify directions for future investigation.

In a previous study investigating the antibacterial efficacy of silver nanoparticles against Gram-negative foodborne pathogens, researchers evaluated four species including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella Typhimurium*, and *Salmonella Enteritidis*, all of which are significant causes of foodborne illness worldwide (Loo et al., 2018). The silver nanoparticles demonstrated favorable results across multiple assays, including the disc diffusion test, minimum inhibitory concentration (MIC) determination, and minimum bactericidal concentration (MBC) assessment. The disc diffusion test revealed that the silver nanoparticles produced clear zones of inhibition surrounding the test discs, indicating that they possess antibacterial action capable of stopping the growth of these Gram-negative foodborne pathogens. The presence and size of these inhibition zones provide visual evidence of antimicrobial activity and allow for preliminary comparison of potency across different nanoparticle formulations and concentrations. This finding is consistent with our results, where clear zones of inhibition were observed around wells containing hibiscus and marigold-mediated silver nanoparticles against the tested oral pathogens, confirming their broad-spectrum antimicrobial potential.

According to a prior study conducted by Guzman and colleagues, silver nanoparticles demonstrated antimicrobial efficacy specifically against Gram-negative bacteria, with the clean zones observed around the nanoparticles indicating successful inhibition of bacterial growth against four different types of Gram-negative organisms (Guzman, Dille and Godet, 2012). This research contributed to the growing understanding that silver nanoparticles are particularly effective against Gram-negative bacteria, possibly due to structural differences in their cell envelopes compared to Gram-positive organisms. However, our study demonstrates that the green-synthesized nanoparticles were also effective against Gram-positive organisms including *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*, as well as the fungal pathogen *Candida albicans*, indicating broader antimicrobial activity than might be expected based on studies limited to Gram-negative bacteria. This broad-spectrum activity is particularly valuable for dental applications where diverse microbial populations contribute to disease pathogenesis.

A further assessment to ascertain the antibacterial activity of silver nanoparticles using MIC values was required because the disc diffusion test was described as the preliminary study in screening the antibacterial activity of an antimicrobial agent, providing qualitative or semi-quantitative data that must be confirmed through more rigorous quantitative methods (de Oliveira, Silva and Da Costa,

2020). Minimum inhibitory concentration was defined as the lowest concentration of antibacterial agents necessary to inhibit the growth of bacteria when tested in serial dilution formats, providing a quantitative measure of potency that allows for comparison between different agents and different microbial strains. In our study, the MIC testing revealed that the hibiscus-mediated silver nanoparticles showed good antimicrobial effectiveness at the 100 μ L concentration, with lower concentrations showing reduced or no inhibitory activity. This concentration-dependent response is consistent with the broader literature on nanoparticle antimicrobial activity and provides important information for determining appropriate concentrations for potential therapeutic applications.

Our comprehensive reviews on smoking, bruxism, temporomandibular disorders, and other oral lesions demonstrate patterns of normal distribution throughout the progression of these diseases, highlighting the importance of understanding risk factors and implementing preventive strategies in dental practice (Alam et al., 2024; Ronsivalle et al., 2024; Uzunçibuk et al., 2024). These reviews underscore the complexity of oral diseases and the need for multifaceted approaches to prevention and treatment that address both behavioral and biological factors. The potential incorporation of antimicrobial nanoparticles into dental materials and treatment protocols represents one such approach to enhancing disease prevention and management.

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders, and to prevent diseases including epidemics, representing the oldest and most sustained form of pharmacotherapy in human history. The knowledge of their healing properties has been transmitted over the centuries within and among human communities through oral tradition, written texts, and cultural practices, forming the foundation of traditional medicine systems that continue to serve the majority of the world's population. Active compounds produced during secondary vegetal metabolism, including alkaloids, flavonoids, terpenoids, phenolics, and essential oils, are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases (Ameri, Ekhtelat and Shamsaei, 2020). These secondary metabolites, which plants produce for defense against herbivores and pathogens, for attraction of pollinators, and for adaptation to environmental stress, possess remarkable pharmacological activities that have been harnessed for human benefit. The use of plant extracts for the green synthesis of nanoparticles harnesses these same bioactive compounds, which serve as both reducing agents to convert metal ions to nanoparticles and as capping agents to stabilize the resulting nanoparticles and enhance their biological activities.

Currently, data on the antimicrobial activity of numerous plants, so far considered empirical based on traditional use, have been scientifically confirmed through rigorous laboratory investigation, concomitantly with the increasing number of reports on pathogenic microorganisms resistant to conventional antimicrobial agents (Patra and Panja, 2013). This convergence of traditional knowledge and modern scientific validation has led to renewed interest in plant-derived antimicrobials as sources of new therapeutic agents and as alternatives or adjuncts to conventional antibiotics in the face of rising antimicrobial resistance. Products derived from plants may potentially control microbial growth in diverse situations, and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or when associated with conventional antimicrobials (Wang et al., 2022). Understanding these mechanisms is essential for optimizing the use of plant-derived antimicrobials and for predicting potential interactions with conventional therapeutic agents.

The precise antibacterial mechanisms of silver nanoparticles are still not fully understood, representing an ongoing area of active investigation in nanomedicine research. However, other researchers have hypothesized that the ability of silver nanoparticles to enter bacterial cells may be what causes them to work against bacteria, with nanoparticle size, shape, and surface characteristics

influencing cellular uptake and subsequent antimicrobial effects (Grumezescu, 2016). Proposed mechanisms include disruption of cell membrane integrity, generation of reactive oxygen species, interaction with DNA and proteins, release of silver ions, and interference with electron transport chains and energy metabolism. The multiple mechanisms of action proposed for silver nanoparticles may explain their broad-spectrum activity and the relative difficulty bacteria face in developing resistance to nanoparticles compared to conventional antibiotics that typically target single specific cellular processes.

Significant antibacterial action was demonstrated by the silver nanoparticles synthesized in this study against the selected oral pathogens, supporting their potential utility in dental applications. Silver nanoparticles may therefore be a good alternative to develop as an antibacterial agent against the diverse strains of bacteria that are drug-resistant, including those that have become resistant to multiple classes of conventional antibiotics. The green synthesis approach using hibiscus and marigold extracts offers additional advantages of environmental sustainability, cost-effectiveness, and enhanced biocompatibility compared to chemically synthesized nanoparticles. Silver nanoparticle applications could result in important discoveries in a variety of areas, including medical technology for device coatings and infection control, antimicrobial defenses for healthcare settings, and incorporation into consumer products for hygiene and sanitation. The continued investigation of green-synthesized nanoparticles for antimicrobial applications holds promise for addressing the growing challenge of antimicrobial resistance and for developing new tools to prevent and treat infectious diseases in dentistry and medicine.

CONCLUSION:

From our study we concluded that Silver nanoparticles were created using marigold and hibiscus extract, and the nanoparticles displayed impressive biosynthesis characterisation of antimicrobial properties.

ACKNOWLEDGEMENT:

The author would like to thank all the participants for their valuable support and Saveetha Dental College and Hospitals for conducting the study.

SOURCE OF FUNDING:

The present project is supported/funded/sponsored by

- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals.
- Saveetha University and
- Indusind bank

CONFLICT OF INTEREST:

The authors hereby declare that there is no conflict of interest in this study.

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