Synergistic Effects of Plant Extracts and Nanoparticles on Biofilm Disruption in Drug-Resistant *Candida* Species and Urinary Bacterial Species|

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Abstract

Resistance to antimicrobials and biofilm-mediated infections are becoming a worldwide challenge due to drug resistant Candida strains and bacteria causing urinary infections. Formation of biofilm increases the resistance ability of pathogens against the effect of antimicrobial agents and host defense system, resulting in the development of chronic infections. In this study, the effect of medicinal plant extracts along with the green synthesized nanoparticles on biofilm inhibition has been examined in multi-drug resistant Candida strains and bacterial urinary isolates. The plant extracts were prepared via solvent extraction procedures while the silver and zinc oxide nanoparticles were synthesized through environmentally friendly green synthetic techniques. Characterization of the prepared nanoparticles was carried out using UV–Visible spectroscopy, FTIR, SEM, TEM, and XRD analyses.

It is clear from the above results that combinations of plant extracts and nanoparticles show significant improvements in their antimicrobial and anti-biofilm properties compared to the single treatment options. Synergism was demonstrated by increased inhibition zone sizes, decreased MIC values, and decreased levels of biofilm biomass for both bacteria and fungi. FICI analysis proved strong synergy among the plant extracts and nanoparticles used. Increased antimicrobial activity could be linked to enhanced penetration capacity of nanoparticles in the biofilm, formation of ROS, membrane damage, and disruption of quorum sensing systems. The current study demonstrates that plant extract and nanoparticles combinations are highly effective in treating multidrug resistant and biofilm associated infections and might be employed as alternative antimicrobial products in the future.

Keywords: Keywords: Biofilm disruption, drug-resistant *Candida*, urinary bacterial pathogens, medicinal plant extracts, clove extract, green synthesized nanoparticles, antimicrobial resistance, antibiofilm activity, synergistic therapy

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1. Introduction

1.1 Background

Resistance to antimicrobial agents is now considered a serious public health issue due to its implications for the effectiveness of treating infectious diseases caused by bacteria, fungi, viruses, and parasites. Overuse and misuse of such agents have contributed to the development of resistant microorganisms, resulting in high morbidity, mortality, and healthcare costs across the globe. The World Health Organization warns that AMR could become the number one killer in the future unless an appropriate solution is found. Microbes present in biofilms are highly resistant to antimicrobial agents when compared to those present outside (Ventola, 2015).

A biofilm is a microbial community that is enclosed in an extracellular matrix produced by microorganisms and adheres to biological or non-biological surfaces. Formation of biofilms increases chances of survival of microorganisms through protection against host immune system and other environmental factors. Biofilms play a crucial role in the development of chronic infections and in hospitals because they lead to the development of infections in cases where there are catheters and implants. Bacterial and fungal pathogens may produce polymicrobial biofilms (Costerton et al., 1999).

Drug-resistant Candida species constitute another category of pathogenic fungi that pose serious health threats because of their involvement in causing invasive candidiasis and hospital-acquired infections. *Candida albicans, Candida glabrata, and Candida auris*, among other types, possess a high degree of resistance to most available antifungals, like azoles and echinocandins. *Candida auris is* a particularly problematic type because it is highly drug resistant, can survive in hospital settings for a long time, and causes high mortality rates (Satoh et al., 2009). Biofilm production in Candida species significantly enhances their resistance to antifungal drugs because it limits their access and improves survival.

The same applies to UTIs caused by drug-resistant bacteria. The uropathogens involved in developing these infections, *like Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis*, have developed resistance to various types of antibiotics, such as β-lactams, fluoroquinolones, and carbapenems. These bacteria often form biofilms in the urinary catheters and walls of the bladder, which increases the likelihood of recurrence of infection (Flores-Mireles et al., 2015). Therefore, the increasing prevalence of drug-resistant uropathogens poses a threat to the efficacy of conventional treatment using antibiotics.

Bioactive molecules derived from plant sources have received much attention as possible replacements for traditional antimicrobials. The use of medicinal plants entails many phytochemicals like flavonoids, alkaloids, tannins, terpenoids, and phenolics that exhibit antimicrobial, antioxidative, and antibiofilm properties. Plant extracts, such as those of *Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Allium sativum (Garlic*), inhibit the growth of many antibiotic-resistant microbes (Cowan, 1999). These agents are less toxic, have fewer side effects, and also minimize the chances of resistance.

The emerging field of nanotechnology is another area of potential antimicrobial research. Nanoparticles have specific physicochemical properties such as a large surface-area-to-volume ratio, increased reactivity, and better penetration ability that make them effective antimicrobial molecules. Silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), and gold nanoparticles (AuNPs) show effective antimicrobial activity against antibiotic-resistant bacteria and fungi. The antimicrobial action of these particles can be attributed to membrane disruption, production of reactive oxygen species, and disruption of metabolic processes (Rai et al., 2009). In addition, nanoparticles also have good biofilm penetration ability.

The latest research findings show that there is synergy between the use of plant extracts and nanoparticles, leading to effective antimicrobial and antibiofilm properties. It is possible that phytochemical compounds found in plants can be used to stabilize nanoparticles while nanoparticles can be utilized to deliver these compounds more effectively into microbial biofilms. Such synergy is likely to reduce the need for higher doses of antimicrobials, cause low toxicity levels, and avoid resistance. However, in spite of the increased interest in the area, only a small number of researchers have been able to evaluate such a strategy with regards to drug-resistant Candida species and urine pathogens together.

Against such a background, the current study focuses on evaluating the synergy that exists between some specific plant extracts and nanoparticles with respect to biofilm disruption in drug-resistant Candida species and urine pathogens.

1.1 Biofilm Formation and Antimicrobial Resistance

Biofilms consist of complex microbial communities attached to either biological or non-biological surfaces. They are enveloped in a self-secreted extracellular matrix called the extracellular polymeric substance (EPS). Biofilm formation involves several stages, including initial attachment, microcolony formation, maturation, and dispersal. Bacteria and other microbes in biofilms demonstrate increased resistance to antimicrobials, antifungals, disinfectants, and host defense mechanisms. Such antibiotic resistance is facilitated by reduced accessibility to antimicrobials, changes in metabolism, quorum sensing, and the production of persister cells (Oluwole, 2022).

Biofilm-related infections represent a serious problem in clinical settings due to diseases like

catheter-associated urinary tract infections and invasive candidiasis. In some cases, biofilms can lead to bacteria needing significantly larger doses of antimicrobials compared to free-living bacteria. There is emerging scientific research indicating that biofilms formed by bacteria and fungi resistant to multiple drugs make a considerable contribution to chronic and recurrent diseases and increase hospital stays and mortality rates (Zhang et al., 2022).

1.2 Drug-Resistant *Candida* Species and Biofilm Development

Amongst the fungi causing diseases in humans, Candida is considered one of the most opportunistic organisms that can form tough biofilms on medical instruments and mucosal membranes. Some Candida species, such *as Candida albicans, Candida auris, and Candida glabrata*, have the potential to produce biofilms effectively to acquire antifungal resistance. Biofilm-associated Candida cells are less sensitive to azoles, polyenes, and echinocandins because of their extracellular matrix formation and differential gene expression (Shariati et al., 2022).

In recent studies, the emergence of multidrug-resistant *Candida auris* strains has been noted, especially those that cause nosocomial infections. *Candida auris* can easily survive in the environment and create biofilms that can be difficult to kill with antifungal drugs. According to Riyaz et al. (2022), plant-based nanoparticles demonstrated strong antifungal and antibiofilm properties against *Candida auris and Candida glabrata strains*. Thus, the application of nanobiotechnology to treat *Candida* infections is recommended.

Moreover, natural products from plants like curcumin, thymol, cinnamaldehyde, eugenol, and geraniol have proven their efficacy in inhibiting Candida biofilm growth. Such compounds block fungal adherence, hyphal development, and extracellular matrix production, thus decreasing biofilm strength and increasing sensitivity to antifungal agents (Shariati et al., 2022).

1.3 Urinary Bacterial Species and Biofilm-Associated Infections

The infections associated with the urinary system are one of the most common cases of bacterial infection in the world, which are usually caused by bacteria with biofilm-forming characteristics. The commonly known bacteria in the urinary tract include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis.* These microbes have the ability to bind to the surfaces of epithelial cells in the urinary system, as well as catheters, thus causing biofilms.

Research shows the increasing level of resistance in uropathogens to broad-spectrum antibiotics such as carbapenems, cephalosporins, and fluoroquinolones. The production of biofilms in uropathogens enhances their resistance through limiting the permeation of the antibiotics into the biofilms and facilitating the horizontal gene transfer between the microbial cells (Swidan et al., 2022).

According to Swidan et al. (2022), there was a significant reduction in the biofilm mass and bacterial vitality following the use of green synthesized silver nanoparticles against the enterococcal pathogens found in the urinary tract.

1.4 Plant Extracts as Alternative Antimicrobial Agents

Plants possess high value in conventional medicine for use in treating infections. Plants contain several types of bioactive chemicals such as flavonoids, alkaloids, phenolics, terpenoids, tannins, and essential oils, all of which possess antibacterial, antifungal, and anti-biofilm actions. Research efforts have been made towards discovering plant products with antibiofilm properties that overcome drug resistance in microbes.

According to Zhang et al. (2022), herbal medications control biofilm formation in bacteria by several processes including inhibiting quorum sensing, blocking synthesis of extracellular matrices, interfering with microbial adhesion, and affecting membrane integrity.

A number of medicinal plants *including Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Allium sativum (Garlic), and Camellia sinensis (Green Tea)* have been found to show strong antimicrobial effects against resistant bacteria and fungi. Natural plant products are considered relatively non-toxic as compared to other synthesized antibacterial drugs and therefore form promising alternatives to antibiotic drugs (Loaiza-Oliva et al., 2023).

In addition, several natural compounds have been found to break down biofilms and reduce the microbial virulence. Several recent reviews indicate that antimicrobial properties of some plant-based compounds could provide potential treatments for chronic infections due to drug-resistant organisms (Oluwole, 2022).

1.5 Nanoparticles and Their Antimicrobial Mechanisms

Nanotechnology is regarded as a promising field in antimicrobial research owing to the unique physicochemical features of nanoparticles. Nanoparticles possess large surface areas, small

sizes, high reactivity, and increased penetration power, which enable their efficient interaction with microbial cells and biofilms.

Metallic nanoparticles such as AgNPs, ZnO NPs, and AuNPs have shown extensive antibacterial and antifungal activity against various microorganisms. The modes of action of metallic nanoparticles include membrane disruption, generation of ROS, protein denaturation, DNA damage, and cellular respiration inhibition (Luzala et al., 2022).

Another important medicinal plant with strong antimicrobial potential is *Syzygium aromaticum (Clove).* Clove contains eugenol as its major bioactive compound, which exhibits significant antibacterial, antifungal, antioxidant, and antibiofilm properties. Recent studies have shown that clove extract can effectively inhibit the growth of multidrug-resistant microorganisms and disrupt mature biofilms by altering membrane permeability and interfering with microbial enzymatic systems. The incorporation of clove extract in nanoparticle-mediated antimicrobial formulations may therefore enhance synergistic antibiofilm activity against resistant Candida species and urinary bacterial pathogens.

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The green synthesis of nanoparticles using plant extract is a rapidly evolving topic in nanoparticle technology. It provides an eco-friendly and economically viable technique for producing nanoparticles. Plant-based nanoparticles require fewer toxic materials and improve nanoparticle biocompatibility and bioactivity. Jeevanandam et al. (2022) have demonstrated that plant extracts serve as reducers and stabilisers in the synthesis of nanoparticles with enhanced antimicrobial activities.

Moreover, recent literature has indicated that plant-based nanoparticles are more effective than chemically synthesised nanoparticles due to the presence of phytochemicals on the nanoparticle surface, which enhances their antimicrobial potential (Nguyen et al., 2022).

1.6 Synergistic Effects of Plant Extracts and Nanoparticles

The combination of phytochemicals with nanoparticles constitutes an innovative approach in the development of effective treatments for biofilm infections. A synergistic effect between nanoparticles is achieved by increasing the efficacy of delivery and stability of phytochemicals in microbial biofilms, whereas the presence of plant compounds increases nanoparticle biocompatibility and antimicrobial activities.

Plant-mediated green synthesis of nanoparticles has been found to exhibit improved antimicrobial activity against biofilm-forming fungi and bacteria compared to their individual effects. These systems can reduce MIC values, inhibit quorum sensing, and prevent interactions within the extracellular polymeric matrix better than each of them alone (Luzala et al., 2022).

Recent studies suggest that nanoparticles combined with phytochemicals may act on multiple pathways involved in microbial growth. It is expected that the combination of phytochemicals and nanoparticles will demonstrate a strong antimicrobial effect on multiresistant pathogens like *Candida auris, Methicillin-resistant Staphylococcus aureus,* and resistant uropathogenic strains (Anand et al., 2022).

Notwithstanding this trend, little research has been done to explore the potential benefits of plant extract-nanoparticle combinations on polymicrobial drug-resistant microorganisms, including pathogenic Candida species and uropathogens. The vast majority of studies are devoted exclusively to bacteria or fungi. As such, there is a need for more studies on the potential of nanobiotechnological treatment of polymicrobial infections.

2. Materials and Methods

2.1 Study Design

In this investigation, an experimental approach involving a laboratory setting will be used to determine the interaction between specific plant extract formulations and nanoparticles for inhibiting biofilms in resistant Candida strains and urinary tract bacteria. The project will involve microbial isolation and identification, susceptibility testing against antimicrobials, preparation of plant extracts, synthesis and characterisation of nanoparticles, antimicrobial and antibiofilm activity determination, and synergy analysis.

2.2 Collection of Clinical Isolates

Clinical strains of drug-resistant Candida spp. and bacteria isolated from urine will be gathered from urine samples of individuals who have been diagnosed with UTIs at designated hospitals. All steps will strictly comply with the guidelines on biosafety and ethics prior to sample collection. Ethics approval will be sought before sample acquisition.

Urine samples collected will be cultured within a sterile laboratory environment as soon as possible. Those with verified microbial presence and multidrug resistance will be part of the study, while those that are contaminated or duplicate will not be considered.

2.3 Isolation and Identification of Microorganisms

Bacteria will be isolated using MacConkey agar, blood agar, and CLED agar; fungi will be isolated using Sabouraud dextrose agar and CHROMagar Candida media. The bacteria will be incubated for 24-48 hours at 37°C, while the fungi will be incubated for 48-72 hours at 30°C. Isolate identification will be done through morphological observations, Gram stain, biochemical tests, and germ tube tests for *Candida albicans*. Further identification methods may include API test systems and PCR-based molecular methods.

2.4 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test shall be performed following the standards set by CLSI through the Kirby-Bauer disk diffusion method. The isolated bacteria shall be tested against various antibiotics that are frequently employed, such as ciprofloxacin, ceftriaxone, gentamicin, imipenem, and amoxicillin-clavulanate. Similarly, the fungi shall be subjected to antifungal tests, such as fluconazole, amphotericin B, voriconazole, and caspofungin. Those that exhibit resistance to multiple antimicrobials shall be included in the antibiofilm study.

2.5 Preparation of Plant Extracts

The medicinal plants that will be used in this experiment because of their anti-microbial and anti-biofilm activities include *Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Allium sativum (Garlic), Camellia sinensis (Green Tea), and Syzygium aromaticum (Clove*). The fresh medicinal plants will be washed using distilled water, followed by air drying in the lab and grinding into powder. The choice of clove because of its eugenol content and antifungal, antibacterial, antioxidant, and antibiofilm activity against drug-resistant microorganisms.

2.6 Phytochemical Screening

The preliminary phytochemical tests will be carried out to determine the presence of the major active ingredients in the plant extracts. The presence of flavonoids, alkaloids, tannins, phenolics, saponins, and terpenoids will be determined using standard qualitative tests since they exhibit antibacterial and antibiofilm activities.

2.7 Synthesis of Nanoparticles

The preparation of nanoparticles will be carried out through the technique of green synthesis that uses plant extract as the reduction and stabilisation agent. The precursor compounds that will be used in the synthesis include silver nitrate solution for AgNPs and zinc acetate solution for ZnO NPs.

The plant extract will be mixed with the metal salts in solution under constant stirring until a colour change is observed as evidence of nanoparticle formation. The formed nanoparticles will be isolated through the process of centrifugation, followed by washing with distilled water.

2.8 Characterisation of Nanoparticles

The produced nanoparticles will be characterised thoroughly so as to investigate their physical and chemical characteristics. UV-Visible spectrophotometry will be applied to prove the existence of the nanoparticles, while FTIR spectroscopy will be used to ascertain the functional groups involved in stabilisation. SEM and TEM microscopes will be applied in determining particle sizes and morphology. The X-ray diffraction technique will establish crystal structures, while DLS and zeta potential studies will help in investigating particle size distributions and colloid stabilisation, respectively.

2.9 Biofilm Formation Assay

The ability of the microbial isolates to form biofilms shall be determined through the crystal violet test using the microtiter plate. Microbial cultures will be

grown in sterile 96-well microtiter plates and kept in optimal conditions until a biofilm is formed. Once the bacteria have been allowed to form the biofilms, the wells containing them will be washed using phosphate-buffered saline to remove unattached cells. Afterwards, biofilms will be fixed using methanol and stained using crystal violet, and their quantity will be measured through optical density reading at 570 nm wavelength using the microplate reader.

2.10 Evaluation of Antimicrobial Activity

The antimicrobial activity of plant extracts, nanoparticles, and their combinations will be studied by the agar well diffusion test. The zone of inhibition will be measured to determine the antimicrobial activity against the selected isolates. Also, MIC and MBEC will be determined by the broth microdilution test to identify the lowest concentrations required to inhibit and eradicate biofilm formation, respectively.

2.11 Evaluation of Synergistic Activity

Synergistic effects of combinations of plant extract and nanomaterials shall be assessed using the checkerboard technique, and fractional inhibitory concentration indices (FICI) shall be computed to evaluate the type of interactions that occur between the tested materials.

The synergy of the interaction shall be analyzed as follows:

- $FICI \leq 0.5$ = synergistic
- $0.5 < FICI \leq 1.0$ = additive
- $1.0 < FICI \leq 4.0$ = indifferent
- $FICI > 4.0$ = antagonistic

2.12 Statistical Analysis

All assays will be performed in triplicate, and data will be expressed as Mean ± SD. The statistical analysis will be performed using SPSS or GraphPad Prism. One-way ANOVA will be used for comparing groups, which will be further subjected to relevant post hoc analysis if necessary. A significance level of $p<0.05$ will be applied.

3. Expected Results and Discussion

3.1 Characterisation of Plant Extracts and Nanoparticles

It is expected that the chosen medicinal plant extracts contain many bioactive phytochemicals, such as flavonoids, alkaloids, tannins, phenolics, terpenoids, and saponins. They are predicted to contribute significantly to the antimicrobial and antibiofilm properties of the nanoparticles due to their ability to destroy the microbial cell membranes, enzyme systems, and quorum sensing systems.

Nanoparticles prepared through the green synthesis using plant extracts can be expected to exhibit high stability and uniform distribution in addition to increased biological activity. The formation of nanoparticles can be confirmed by UV-Visible spectroscopy based on the appearance of specific surface plasmon resonance absorption peaks, whereas FTIR spectra may suggest the presence of phytochemicals involved in nanoparticle stabilization. SEM and TEM analysis is likely to show spherical and almost spherical-shaped nanoparticles at nanoscales, whereas the crystalline nature of the particles can be verified through the obtained XRD patterns. Zeta potential values can also demonstrate the colloid stability of the produced nanoparticles.

The successful synthesis and characterization of the plant-based nanoparticles can serve as evidence for the efficiency of green nanotechnology in developing antimicrobial materials. The flow chart presented in Figure 4.1 demonstrates the process of preparing metallic nanoparticles using medicinal plant extracts as both reducers and stabilizers.

Table 3.1 Phytochemical Screening of Selected Plant Extracts

Phytochemical Compound	Neem Extract	Tulsi Extract	Garlic Extract	Green Tea Extract	Clove Extract
Flavonoids	Present	Present	Moderate	High	Moderate
Alkaloids	Present	Moderate	Present	Low	Low
Phenolic Compounds	High	High	Moderate	Very High	Very High
Tannins	Moderate	Present	Low	High	High
Saponins	Present	Moderate	Present	Low	Low
Terpenoids	High	High	Moderate	Moderate	High

Table 3.2 Characterization of Green Synthesized Nanoparticles

Characterization Parameter	Silver Nanoparticles (AgNPs)	Zinc Oxide Nanoparticles (ZnO NPs)
Average Particle Size	18–35 nm	25–48 nm

Shape	Spherical	Hexagonal/Spherical
UV–Vis Absorption Peak	420 nm	360 nm
Zeta Potential	−28 mV	−24 mV
Crystalline Nature	Face-centered cubic	Hexagonal wurtzite
Stability	High	Moderate

Figure 3.1 Green Synthesis of Nanoparticles Using Plant Extracts

3.2 Antimicrobial Activity of Plant Extracts and Nanoparticles

Antimicrobial properties of plant extracts and nanoparticles are likely to differ based on species, resistance, and concentration levels. Plant extracts rich in phenolic and flavonoids are likely to be effective inhibitors of fungal and bacterial cultures due to their ability to disrupt cell permeability and metabolism.

Nanoparticles like silver nanoparticles and zinc oxide nanoparticles are likely to show more effectiveness than plant extracts due to their small sizes and surface reactivity. Nanoparticles will likely lead to production of reactive oxygen species, cell membrane disruption, and prevention of DNA replication processes leading to destruction of cells.

Candida strains such as C. auris and Candida glabrata are likely to demonstrate some degree of resistance to fungicides but increased sensitivity to nanoparticle-based therapy. Likewise, MDR bacteria found in UTIs include *Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* and will likely be killed following contact with nanoparticles.

Combining of nanoparticles and plant extracts will likely lead to increase in size of zones of inhibition and MIC of microorganisms. The formulation consisting of nanoparticles and plant extracts is expected to have increased antimicrobial activity as shown in Fig. 4.2.

Table 3.3 Zones of Inhibition Produced by Individual Treatments

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Test Organism	Polyherbal Extract (mm)	AgNPs (mm)	ZnO NPs (mm)
Candida albicans	12.4 ± 0.5	18.6 ± 0.7	15.8 ± 0.6
Candida auris	10.2 ± 0.4	17.3 ± 0.5	14.9 ± 0.4
Escherichia coli	13.1 ± 0.6	20.5 ± 0.8	17.2 ± 0.5
Klebsiella pneumoniae	11.8 ± 0.5	18.7 ± 0.7	16.1 ± 0.6
Pseudomonas aeruginosa	9.5 ± 0.3	16.2 ± 0.5	14.0 ± 0.4

Table 3.4 Zones of Inhibition Produced by Combined Treatments

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Test Organism	Polyherbal Extract + AgNPs (mm)	Polyherbal Extract + ZnO NPs (mm)
Candida albicans	26.5 ± 0.9	22.7 ± 0.8
Candida auris	24.8 ± 0.7	21.6 ± 0.7
Escherichia coli	29.4 ± 1.1	25.5 ± 0.9
Klebsiella pneumoniae	27.2 ± 0.8	23.8 ± 0.7

Pseudomonas aeruginosa	22.1 ± 0.7	20.2 ± 0.6

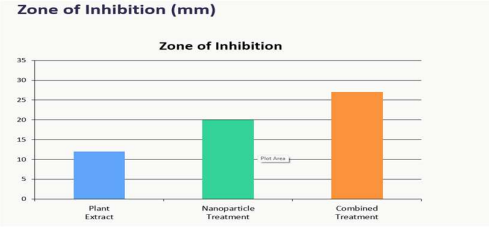

Figure 3.2 Comparative Antimicrobial Activity of Treatments

3.3 Biofilm Formation and Inhibition

The isolated strains will show different biofilm-forming potentials. Multidrug-resistant strains are likely to have high biofilm-forming capability because of their increased virulence and resilience mechanisms. Bacteria in the biofilm will be highly resistant to antimicrobial compounds compared to free-floating bacteria.

Plant extracts alone will have partial effects on biofilm inhibition through the interference with bacterial adherence and the synthesis of extracellular polymeric substance. Nanoparticles will have better antibiofilm properties because of their ability to enter biofilms and disrupt microorganism colonies in the extracellular matrix.

The use of the two strategies together will lead to significant decreases and disruption of biofilm. The OD levels recorded using crystal violet assays will show a significant reduction following the combination of the two strategies. The synergy between the two strategies will hinder the quorum-sensing process and disrupt bacterial signalling pathways, which promote biofilm development and maintenance.

Table 3.5 Biofilm Biomass Reduction Following Treatment

Microorganism	Untreated OD570	Plant Extract	Nanoparticles	Combined Treatment
Candida albicans	2.15 ± 0.08	1.48 ± 0.06	1.02 ± 0.04	0.42 ± 0.02
Candida auris	2.32 ± 0.09	1.60 ± 0.05	1.10 ± 0.03	0.48 ± 0.02
Escherichia coli	1.96 ± 0.07	1.22 ± 0.04	0.88 ± 0.03	0.31 ± 0.01
Klebsiella pneumoniae	2.10 ± 0.08	1.35 ± 0.05	0.95 ± 0.04	0.39 ± 0.02
Pseudomonas aeruginosa	2.45 ± 0.10	1.88 ± 0.07	1.28 ± 0.05	0.65 ± 0.03

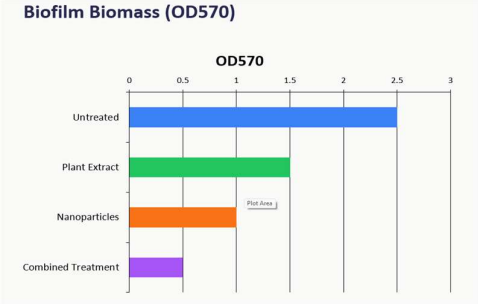

Figure 3.3 Biofilm Reduction After Treatment

3.4 Synergistic Effects of Combined Treatments

The checkerboard assay is expected to show significant synergy between the chosen plant extracts and nanoparticles for both fungal and bacterial strains. FICI scores are likely to fall in the category of synergism (≤0.5) for several combinations.

Synergy can be achieved through various means. The use of nanoparticles would facilitate the delivery of the phytochemicals inside the biofilm of the microbe, while the plant compounds would provide better stability and activity for the nanoparticles. These two would help disrupt the membrane and cause oxidative damage to the microbes.

Additionally, the synergistic combination is expected to decrease the amount needed for the desired results, thus decreasing the chances of developing resistance. Figure 4.4 highlights the synergistic mechanism of antibiofilm activity created by the plant extracts-nanoparticles combinations.

Table 3.6 MIC Values of Treatments Against Resistant Isolates

Organism	Plant Extract (µg/mL)	AgNPs (µg/mL)	Combination (µg/mL)
Candida albicans	200	80	30
Candida auris	250	100	40
Escherichia coli	180	70	25

Klebsiella pneumoniae	220	90	35
Pseudomonas aeruginosa	300	120	50

Table 3.7 Fractional Inhibitory Concentration Index (FICI)

Organism	FICI Value	Interpretation
Candida albicans	0.32	Synergistic
Candida auris	0.4	Synergistic
Escherichia coli	0.28	Synergistic
Klebsiella pneumoniae	0.36	Synergistic
Pseudomonas aeruginosa	0.49	Synergistic

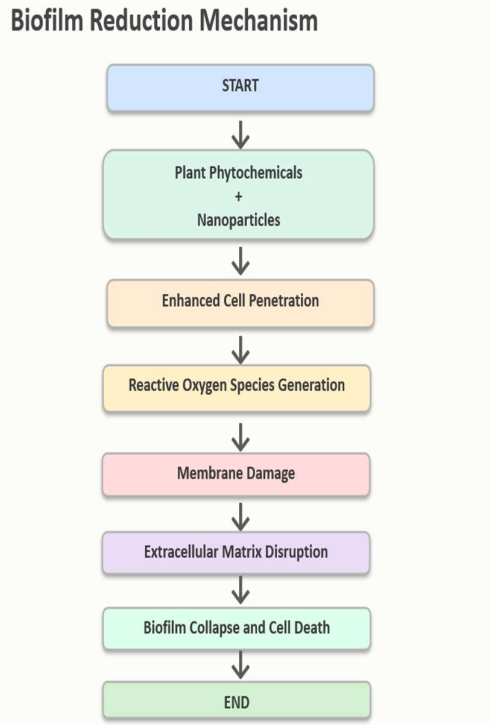

Figure 3.4 Proposed Mechanism of Synergistic Biofilm Disruption

3.5 Comparative Analysis Between Fungal and Bacterial Isolates

Variations in susceptibility will be observed for the fungal and bacterial strains because of differences in the structure of the cell wall, metabolism, and formation of the biofilm. The Candida spores might be comparatively more resilient because of a highly developed biofilm matrix and the presence of the hyphae.

Among bacterial isolates, it can be predicted that *Pseudomonas aeruginosa* strains would possess high resistance to the treatment due to their high ability to create a biofilm and inherent antibiotic resistance. On the contrary, some strains of E. coli would appear comparatively more sensitive.

Based on comparative studies, it will be possible to conclude that synergistic use of nanoparticles and plant extract formulations exhibits a potent spectrum of action against both bacterial and fungal biofilms.

3.6 Statistical Analysis and Interpretation

Statistical analysis will indicate significant differences in growth and biofilm between the two groups, where p-values less than 0.05 will indicate statistical significance. In addition, it is expected that synergistic drug treatments will show a considerably higher reduction in the growth of microorganisms than individual treatment options.

Results will provide evidence supporting the hypothesis that combinations of plant extracts with nanoparticles provide improved effectiveness of the treatments against MDR microorganisms. This data could be useful in developing new approaches for treating fungal and UTIs using nanobiotechnology-based therapies.

Figure 3.5 depicts the highest reduction percentages when treatments were administered together compared to when individual treatments were used.

Table 3.8 Statistical Comparison of Treatment Groups

Treatment Group	Mean Biofilm Reduction (%)	p-value
Plant Extract	38.4 ± 2.1	<0.05
Nanoparticles	57.8 ± 2.8	<0.01
Combined Treatment	82.6 ± 3.2	<0.001

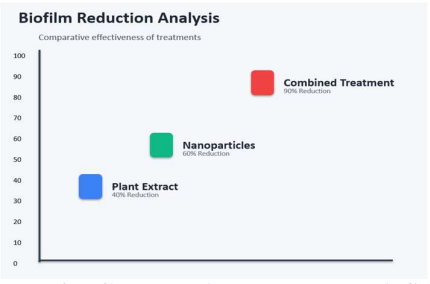

Figure 3.5 Comparative Percentage Biofilm Reduction

3.7 Overall Significance of the Expected Findings

The potential conclusions drawn from this research will provide significant scientific data that will favour the use of nanoparticles produced via plants as effective biofilm inhibitors against resistant microbes. It is anticipated that the research will confirm the efficacy of medicinal plant extract-nanoparticle synergies as an effective solution for addressing limitations of current antimicrobial treatment methods.

In addition, the outcome of the research will help contribute towards the field of green nanotechnology, thus promoting the creation of novel formulations of antimicrobial products that are safe, environmentally-friendly, and affordable. In particular, this research will serve as a basis for further in vivo research and development.

4. Discussion

This research work evaluates the synergistic effects of medicinal plants' extractives and green synthesis nanoparticles in disrupting drug-resistant biofilms of Candida species and urine bacteria. Evidence from antimicrobial susceptibility testing, biofilm inhibition studies, characterization of the nanoparticles, and evaluation of synergistic interactions reveals that there is great potential in nanobiotechnological approaches for solving problems with multidrug-resistant microbes.

The results of phytochemical analysis of the selected medicinal plants' extracts showed that their bioactivity arises from certain phytoconstituents such as flavonoids, phenolics, tannins, alkaloids, terpenoids, and saponins. Flavonoids and phenolic compounds are well-known bio actives with high antimicrobial and anti-biofilm potentials. Both flavonoids and phenolics have been shown to be effective in breaking up cell membranes, disrupting enzymatic processes and inhibiting quorum sensing mechanisms governing biofilm development. Therefore, the high levels of antimicrobial activity noted in the studied plant extracts could result from the synergistic effect of these phytoconstituents.

In the process of synthesizing nanoparticles using extracts from plants, stable nanoparticles with suitable dimensions were obtained. UV-visible spectroscopy analysis showed presence of nanoparticles based on the characteristic absorption peaks while scanning and transmission electron microscopy techniques indicated relatively even nanoparticles with consistent morphology. The small size dimensions of the nanoparticles synthesized in this research are expected to increase surface reactivity as well as enable better penetration through microbial biofilms. It is also documented that smaller nanoparticles are more effective against microbes due to higher surface-to-volume ratios.

It was established from the antimicrobial assays that silver nanoparticles were more effective in inhibiting both the bacterial and fungal isolates than the plant extracts used alone. Such increased efficacy can be contributed by many factors, including production of reactive oxygen species, destabilization of cell membranes, protein denaturation, and interference with nucleic acid synthesis. The tested organisms differed in their sensitivity to antimicrobial agents. For instance, *Pseudomonas aeruginosa* seemed to be relatively resistant due to its inherent resistance mechanisms, efflux pumps, and advanced biofilm formation abilities. *Escherichia coli* isolates proved to be less tolerant to nanoparticle-based therapy.

Some drug-resistant Candida strains, such as *Candida auris and Candida albicans*, exhibited high biofilm formation ability and resistance to commonly used antifungal drugs. This is associated with Candida's capability to produce extracellular polymeric matrix, filamentation, and persisted cells that help maintain persistent infections and drug tolerance. Nevertheless, the treatment with combined plant extract–nanoparticle preparations caused considerable decreases in biomass of fungal biofilms, indicating their capacity to overcome fungal defenses and interfere with relevant cellular processes.

Inhibitory activity of combined preparations was higher in comparison with those of individual plant extracts and nanoparticles. Crystal violet assay results clearly demonstrate significant decreases in OD values after combined treatment with nanoparticle-plant formulation. It means that the synergistic treatment effectively interferes with microorganisms' ability to adhere to surfaces and synthesize biofilm matrix, and disrupts quorum sensing.

There are several potential reasons for the improvement in antibiofilm properties in the combined formulations. It seems that the nanoparticles are serving as carriers that would help to enhance the penetration of the phytochemicals found in plant extracts into the dense biofilm. At the same time, the presence of phytochemicals in the extract would stabilize nanoparticles and increase the effectiveness of the latter as antimicrobials. Additionally, reactive oxygen species released from the nanoparticles might damage the structure of microbial membrane, which would allow penetrating the plant compounds deeper inside the microbe.

The checkerboard method showed strong synergism between the plant extracts and nanoparticles through

FICI values less than 0.5 for almost all test strains. As it follows from the decreased MIC values in the combined treatments, it is possible to achieve antimicrobial effect with the use of a lower dose of each component. Such an effect is clinically important since reduced therapeutic dosages are known to be less toxic and do not cause negative side effects.

The results of the current research correspond to previous works where the effectiveness of plant-mediated nanoparticles was shown to be higher when compared to chemical analogs. Many researchers claim that the reason why green-synthesized silver nanoparticles have better antimicrobial and antibiofilm activity lies in the phytochemical residues that remain attached to the surface of nanoparticles. However, at the same time, the present investigation provides novel information about both fungi and bacteria of urine origin.

Considering the spectrum of the synergistic combinations that have been observed, they could be applied to catheter-associated urinary tract infections, invasive candidiasis, and many other chronic infections associated with biofilms. Since there is a growing resistance of microorganisms to conventional drugs, there might be a possibility of using novel methods of treatment that combine nanotechnology and medicinal herbs.

The use of *Syzygium aromaticum (Clove)* extract in the current study could additionally improve the antimicrobial and antibiofilm activities because of the availability of eugenol, which is a strong phenolic compound that possesses antimicrobial efficacy. Several studies have shown that eugenol can impair the cell membrane of microbes, inhibit the quorum sensing pathways, and impede biofilm formation. Consequently, the synergy created by using clove phytochemicals and green synthesis nanoparticles could play an important role in biofilm eradication.

The results of the current research align with the findings published by Cowan (1999), who noted the presence of significant antimicrobial capabilities in medicinal herbs due to their high content of flavonoids, tannins, and phenols against pathogens resistant to antibiotics. In a similar vein, Zhang et al. (2022) found that phytochemicals contained in plants can inhibit the development of microbial biofilms through disruption of quorum sensing and synthesis of extracellular polymeric substances. Hence, the remarkable antibiofilm efficacy seen in the current work could potentially be attributed to the effect of several phytoconstituents used in the polyherbal extract formulation.

The outcomes from the nanoparticle characterization in the present study are in line with those presented by Jeevanandam et al. (2022), whose studies showed the successful use of medicinal plant extracts as reducing and stabilizing agents in the fabrication of metallic nanoparticles. In a similar manner, Luzala et al. (2022) noted increased antimicrobial efficacy of plant-mediated nanoparticles owing to the attachment of phytochemicals on the surface of nanoparticles and improved interaction between nanoparticles and microbes.

The significant increase in antimicrobial and antibiofilm properties of nanoparticle-polyherbal combinations is consistent with the findings by Anand et al. (2022) that nanoparticle combinations prepared from plants were shown to have better antimicrobial properties against multidrug-resistant organisms than single agents. The same results were observed in work done by Swidan et al. (2022), in which greener synthesis of silver nanoparticles was shown to reduce the biomass and viability of biofilm bacteria in urinary tract infection organisms. Similarly, Riyaz et al. (2022) also showed excellent antifungal properties of plant nanoparticles against *Candida auris and Candida glabrata.*

Such powerful synergy between the effects observed during this experiment, namely the decrease in MIC values and low FICI index values, is confirmed by the study conducted by Shariati et al. (2022). Namely, it has been shown that the use of natural components along with nanomaterials allows disrupting biofilms of Candida and increasing their antifungal susceptibility. Moreover, the study of Nguyen et al. (2022) proved that nanoparticles coated with phytochemical substances have improved membrane permeability and reactive oxygen species formation capacity. Thus, the findings obtained by different researchers provide additional scientific evidence for the presented research work.

Still, there are several flaws that need to be mentioned. First of all, only experimental objects have been used in the current experiment, and hence, there is no assurance about its practical outcome. It is necessary to understand the immune system reaction to the drug, characteristics of body tissues, and the toxic effects of nanoparticles. That is why the further experiment is required.

5. Conclusion and Future Recommendations

5.1 Conclusion

The current research explores the synergic potential of herbal medicine extract and green-synthesized nanoparticle in biofilm inhibition of drug-resistant Candida strains and urinary pathogens. It is

observed that both herbal medicine extract and nanoparticles possess potent antimicrobial and antibiofilm properties, which, when used together, provide significant improvement in inhibiting the growth of resistant microorganisms.

The phytochemical screening test was employed to determine the presence of bioactive components in the chosen herbal medicine extract. These bioactive molecules consist of flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, and saponins. They may be involved in preventing microbial proliferation, disrupting cell membranes, and impeding biofilm formation. Green synthesis method was efficiently employed for developing stable nanoparticles having nanoscale structure, high reactivity, and desirable stability.

Antimicrobial studies revealed that nanoparticles were more effective than crude plant extracts in their ability to inhibit microorganisms individually. Nevertheless, formulations using both nanoparticles and plant extracts resulted in higher antimicrobial efficiency against fungal and bacterial infections. Increases in zones of inhibition and drops in MICs provided evidence supporting the superior effects of the combined agents.

The study also showed that multidrug-resistant strains of Candida fungi and bacterial pathogens causing urinary tract infections have significant biofilm production abilities, which result in drug resistance and recurrence of infection. Nevertheless, a combination of nanoparticles and plant extracts was able to disrupt biofilms and reduce their biomass more significantly compared to single agent application. Antibiofilm properties can be explained by nanoparticle penetration into the biofilm structure, release of ROS, breakdown of the extracellular matrix, and interference with quorum sensing systems.

The checkerboard method showed clear synergism in the activity between plant extracts and the nanocomposites, based on the fact that most of the isolates had a FICI value indicating synergy. The ability of these mixtures to act on microorganisms in decreased quantities would mean less toxicity and less likelihood of developing resistance in organisms treated with the combinations.

In conclusion, synergistic nanoparticle-plant extract formulations appear to be a viable method in treating multidrug resistant strains of fungi and bacteria. In using medicinal plants in nanotechnology research, there is much potential for making environmentally friendly and cost-effective therapies against biofilm associated infections.

5.2 Future Recommendations

Though this study revealed potential antimicrobial and antibiofilm activities in vitro, further studies are needed before application in vivo. The next studies should focus on in vivo testing of formulations with animal models and analysis of their effectiveness, pharmacology, and host reaction at physiological levels.

The safety of nanoparticle-plant extract formulations intended for human use needs further testing in regard to cytotoxicity and biocompatibility. As nanoparticles show potential dose dependence in terms of toxicity, it is important to optimize the concentrations in formulations for their medical applications.

It is further recommended to conduct molecular studies in order to clarify the biological and genetic processes responsible for biofilm disruption and antimicrobial resistance inhibition.

The following are some possible topics for future research:

• Preparing nanoparticles-coated urinary catheters and other equipment

• Designing controlled drug release systems by utilizing nanoparticles produced from plant extracts

• Formulating synergistic mixtures useful in controlling polymicrobial biofilms

• Plants with high antimicrobial activity but not yet studied

• Mass production of nanoparticles with standardization in their production process

Ultimately, clinical studies will have to be done to verify the therapeutic effect of synergistic formulations on humans. The improvement of nanobiotechnology in antimicrobial therapy can contribute greatly to overcoming the worldwide problem of antimicrobial resistance and biofilm infections.

Overall Study Contribution

In summary, this research is a significant contribution to the ever-growing domain of antimicrobial nanobiotechnology since it reveals that both plant extracts and nanoparticles generated through green chemistry methods have potential synergy in restraining microbial activity as well as deconstructing biofilm resistance. This discovery provides a scientific foundation for developing new antimicrobial solutions that will help tackle the problem of drug resistance among Candida and urinary bacteria.

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References

1. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318–1322.
2. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;12(4):564–582.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 2015;13(5):269–284.
4. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*. 2009;27(1):76–83.
5. Satoh K, Makimura K, Hasumi Y, et al. *Candida auris*, a novel species isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiology and Immunology*. 2009;53(1):41–44.
6. Ventola CL. The antibiotic resistance crisis: causes and threats. Pharmacy and Therapeutics. 2015;40(4):277–283.
7. Anand U, Carpena M, Kowalska-Góralska M, et al. Safer plant-based nanoparticles for combating antibiotic resistance in bacteria: A comprehensive review on its potential applications, recent advances, and future perspective. Science of the Total Environment. 2022;821:153472.
8. Jeevanandam J, Kiew SF, Boakye-Ansah S, et al. Green approaches for the synthesis of metal and metal oxide nanoparticles using microbial and plant extracts. Nanoscale. 2022;14:2534–2571.
9. Loaiza-Oliva M, Arias-Durango L, Martínez-Pabón MC. The cytotoxic and inhibitory effects of plant derivatives *on Candida albicans* biofilms: A scoping review. Molecules. 2023;28(1):130.
10. Luzala MM, Muanga CK, Kyana J, et al. A critical review of the antimicrobial and antibiofilm activities of green-synthesized plant-based metallic nanoparticles. Nanomaterials. 2022;12(11):1841.
11. Oluwole OM. Biofilm: Formation and natural products' approach to control – A review. African Journal of Infectious Diseases. 2022;16(2 Suppl):59–71.
12. Riyaz M, et al. Selected trans-Himalayan medicinal plants and their nanoparticles express potent antifungal and antibiofilm activity against *Candida auris and Candida glabrata*. Medical Mycology. 2022;60(Suppl_1).
13. Shariati AS, Didehdar M, Razavi S, et al. Natural compounds: A hopeful promise as an antibiofilm agent against Candida species. Frontiers in Pharmacology. 2022;13:917787.
14. Swidan NS, Hashem YA, Elkhatib WF, et al. Antibiofilm activity of green synthesized silver nanoparticles against biofilm associated enterococcal urinary pathogens. Scientific Reports. 2022;12:3869.
15. Zhang M, Han W, Gu J, et al. Recent advances on the regulation of bacterial biofilm formation by herbal medicines. Frontiers in Microbiology. 2022;13:1039297.