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RESEARCH ARTICLE

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *THUJA OCCIDENTALIS* L. LEAF EXTRACT: A COMPREHENSIVE STUDY OF ANTIMICROBIAL POTENTIAL

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Abstract: The increasing prevalence of antimicrobial resistance has necessitated the exploration of novel therapeutic agents from natural sources. This study investigates the green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Thuja occidentalis* L. and evaluates their antimicrobial potential against pathogenic bacteria and fungi. The biosynthesis of AgNPs was confirmed by UV-Visible spectroscopy, revealing a characteristic surface plasmon resonance peak at 420 nm, with the rapid formation of stable nanoparticles observed within 20 minutes of reaction. The antibacterial activity, assessed using the agar well diffusion method, demonstrated significant concentration-dependent inhibition against *Pseudomonas aeruginosa*, with maximum zones of inhibition of 16 mm at 60 µg concentration, comparable to the standard antibiotic streptomycin (18 mm). However, *Staphylococcus aureus* exhibited resistance to all tested concentrations, indicating differential susceptibility between Gram-negative and Gram-positive bacteria. Antifungal evaluation revealed selective activity against *Candida albicans* with a zone of inhibition of 16 mm, while *Aspergillus niger* and *Phytophthora capsici* showed resistance. The study successfully demonstrates a simple, rapid, cost-effective, and environmentally friendly approach for synthesizing antimicrobial silver nanoparticles using *T. occidentalis* leaf extract. These findings validate the traditional medicinal use of *Thuja* and suggest its potential as a sustainable source for developing alternative antimicrobial agents. The green nanotechnology approach presented herein offers promising applications in biomedical fields, contributing to the global effort to combat antimicrobial resistance while promoting environmental sustainability.

Keywords: *Thuja occidentalis*, Silver nanoparticles, Green synthesis, Antimicrobial activity, UV-Vis spectroscopy

INTRODUCTION

The escalating global challenge of antimicrobial resistance has intensified the search for novel therapeutic agents from natural sources. Plant-derived bioactive substances have long served as repositories of medicinal compounds, offering significant potential for human health and combating various microbial diseases (Ateş *et al.*, 2003; Kumar *et al.*, 2006; Mathabe *et al.*, 2006). The bioactive constituents of plants, including tannins, flavonoids, saponins (Avato *et al.*, 2006), terpenoids (Funatogawa *et al.*, 2004), and alkaloids exhibit remarkable antimicrobial properties that have garnered increasing scientific attention in recent years. In the context of rising antibiotic resistance, medicinal plants represent promising resources for developing alternative antimicrobial agents capable of effectively controlling infectious diseases (Schulz *et al.*, 2001).

*Thuja occidentalis*L., commonly known as northern white-cedar and belonging to the family Cupressaceae, has been traditionally utilized for various medicinal applications. Its scaly leaves, growing from fan-like branches, are rich in vitamin C, and the essential oil derived from the plant has been employed in cleansers, disinfectants, hair preparations, insecticides, liniment, room sprays, and soft soaps (Chambers, 1993). Historically, in the 19th century, *T. occidentalis* was widely used as an externally applied tincture or ointment for treating warts, ringworm, and thrush (David Hoffmann, 2003). The plant is extensively utilized in homeopathy and evidence-based phytotherapy, with its immunopharmacological potential demonstrated through numerous *in vitro* and *in vivo* studies showing immunostimulating and antiviral activities. A critical factor for *Thuja*'s use as a medicinal herb is its content of thujone, which is reported in fresh leaves (David Hoffmann, 2003). From *Thuja* (Esberitox) is used for the treatment of the common cold and as a standard antibiotic treatment for severe bacterial infections.

Nanotechnology has emerged as a transformative field, with silver nanoparticles (AgNPs) gaining particular prominence due to their unique physicochemical properties and broad-spectrum antimicrobial activities (Larue *et al.*, 2014; El-

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Chaghaby and Ahmad, 2011). The green synthesis of nanoparticles using plant extracts has become increasingly popular as an environmentally friendly alternative to conventional chemical methods, offering advantages of cost-effectiveness, biocompatibility, and reduced environmental toxicity (Thuesombat *et al.*, 2014; Mittal *et al.*, 2014). The biological synthesis of silver nanoparticles using plant extracts provides a convenient, extracellular, and environmentally safe approach that overcomes the limitations of conventional synthetic antimicrobial compounds (Ahmed *et al.*, 2014). Plants-mediated nanoparticles have demonstrated potential as remedies for various diseases, including malaria, cancer, HIV, hepatitis, and other acute conditions (Logeswari *et al.*, 2012).

The antimicrobial mechanism of silver nanoparticles involves disruption of cell membrane integrity, generation of reactive oxygen species, and interference with protein synthesis, leading to bacterial cell death (Sondi and Salopdek-Sondi, 2004; Kasthuri *et al.*, 2009). AgNPs can effectively disrupt the polymer subunits of cell membranes in pathogenic organisms, with the reciprocal action subsequently breaking the cell membrane and disturbing protein synthesis mechanisms in bacterial systems (Gong *et al.*, 2007; Li *et al.*, 2011). Studies have demonstrated that fungicidal mechanisms of biosynthesized metallic nanoparticles exhibit greater potential than commercial antibiotics such as fluconazole and amphotericin, with plant-derived AgNPs clearly showing membrane damage in *Candida* species and destruction of intercellular components (Logeswari *et al.*, 2012).

Various plant extracts have been explored for the synthesis of silver and gold nanoparticles, including *Azadirachta indica* (Shankar *et al.*, 2004), *Aloe vera* (Chandran *et al.*, 2006), *Bryophyllum* sp., *Cyperus* sp., *Hydrilla* sp., *Chenopodium*, *Cycas* (Jha and Prasad, 2010), *Acalypha indica* (Krishnaraj *et al.*, 2010), *Hibiscus rosa sinensis* (Philip *et al.*, 2010), *Ipomoea aquatica* (Roy and Barik, 2010), *Psidium guajava*, *Garcinia mangostana*, *Ocimum sanctum* (Philip and Unni, 2011), and *Cocos nucifera* (Roopan *et al.*, 2012). The presence of α-pinene, 3-carene, and α-cedrol in *Thuja* oil reveals that *Thuja* possesses potential for use in many medical applications, which is why the plant is also known as "The Tree of Life" (Tsiri *et al.*, 2009).

The present study aims to synthesize and characterize silver nanoparticles from the leaf extract of *Thuja occidentalis* and evaluate their antibacterial and antifungal potential using the agar well diffusion method. The antimicrobial activity is assessed against pathogenic bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and fungi *Aspergillus niger*, *Candida albicans*, and *Phytophthora capsici* (Tsiri *et al.*, 2009; Mishra *et al.*, 1992). This research contributes to the growing body of knowledge on green nanotechnology and the development of sustainable, eco-friendly antimicrobial agents from medicinal plant resources.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh leaves of *Thuja occidentalis* were collected, and taxonomic identification was performed by the Department of Botany, University of Kerala, Kariavattom. The plant specimens were authenticated, and voucher specimens were deposited for future reference, following standard herbarium procedures.

Preparation of Leaf Extract

Fresh leaves of *Thuja occidentalis* (10 g) were thoroughly washed with distilled water to remove surface contaminants and finely chopped to increase surface area for extraction. The chopped leaf material was placed in a 250 mL beaker containing 100 mL of distilled water and boiled at 80°C for 20 minutes. The resulting extract was filtered through Whatman No. 1 filter paper to remove particulate matter, and the filtrate was stored at 4°C for subsequent experiments (Mittal *et al.*, 2014; Krishnaraj *et al.*, 2010).

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich and used without further purification. Double distilled water was employed throughout all reactions. All glassware was thoroughly washed with aqua regia ($HCl:HNO_3$, 3:1), rinsed with double distilled water, and dried in a hot air oven.

For the synthesis of silver nanoparticles, 100 mg of $AgNO_3$ was dissolved in 300 mL of double distilled water with continuous stirring until complete dissolution. Subsequently, 10 mL of the filtered plant extract was treated with the aqueous $AgNO_3$ solution in an Erlenmeyer flask and boiled for 10 minutes (Ahmed *et al.*, 2014; Roopan *et al.*, 2012). The formation of silver nanoparticles was indicated by a color change from pale yellow to brown-yellow, suggesting the reduction of aqueous silver ions by the plant extract constituents (Gilaki, 2010). The biosynthesized nanoparticles were isolated by repeated centrifugation at 5000 rpm for 5-10 cycles, and the resulting pellets were dissolved in acetone and air-dried for further characterization (Kasthuri *et al.*, 2009).

Characterization of Silver Nanoparticles

UV-Visible spectroscopy was employed to monitor the formation and stability of silver nanoparticles. Approximately 2 mL of the nanoparticle solution was placed in a quartz cuvette, and UV-Vis spectra were recorded at 400-600 nm using a UV-Vis spectrophotometer. The absorbance of the leaf extract alone was measured to compare the spectral differences and confirm nanoparticle formation (Krishnaraj *et al.*, 2010; Philip and Unni, 2011).

Microbial Cultures

Pure cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained for antibacterial activity assessment. For antifungal studies, *Aspergillus niger*, *Candida albicans*, and the plant pathogen *Phytophthora capsici* were employed. All microbial cultures were maintained and subcultured according to standard microbiological protocols (Mishra *et al.*, 1992; Tsiri *et al.*, 2009). Antimicrobial studies were conducted at the Department of Biotechnology, University of Kerala, and the Department of Biotechnology, Mar Ivanios College.

Antibacterial Activity Assessment

The antibacterial activity of synthesized silver nanoparticles was evaluated using the agar well diffusion method (Alzoreky and Nakahara, 2003; Trombetta *et al.*, 2005). Mueller-Hinton Agar (MHA) medium was prepared by dissolving 38 g of commercially available nutrient medium (Hi Media) in 1000 mL of distilled water, followed by sterilization at 121°C for 15 minutes at 15 lbs pressure. The medium was dispensed into sterile petri plates and allowed to solidify.

Bacterial cultures were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and swabbed uniformly onto the surface of MHA plates. Wells of approximately 10 mm diameter were bored using a sterile cork borer, and varying concentrations (20-60 µL) of silver nanoparticles dissolved in dimethylsulfoxide (DMSO) were added to each well. Streptomycin (1 mg/mL) served as the positive control, while DMSO alone was used as the negative control (Nisha *et al.*, 2017; Tsiri *et al.*, 2009). The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters. All experiments were performed in triplicate.

Antifungal Activity Assessment

The antifungal activity of silver nanoparticles was evaluated using the agar well diffusion method against *Aspergillus niger*, *Candida albicans*, and *Phytophthora capsici* (Mishra *et al.*, 1992). Potato Dextrose Agar (PDA) was prepared and sterilized according to standard protocols. Fungal cultures were inoculated onto PDA plates, and wells of 10 mm diameter were bored. Silver nanoparticle suspension (100 µL) was added to each well, and mancozeb (ethylene-bis-dithiocarbamate) was used as the positive control. Plates were incubated at 28°C for 48-72 hours, and zones of inhibition were measured (Tsiri *et al.*, 2009).

RESULTS

Biosynthesis and Characterization of Silver Nanoparticles

The green synthesis of silver nanoparticles using *Thuja occidentalis* leaf extract was confirmed by the characteristic color change from pale yellow to dark brown upon addition of silver nitrate solution. This color transformation, observed within 20 minutes of reaction, resulted from the excitation of surface plasmon resonance vibrations in the silver nanoparticles. The biosynthesized nanoparticles were successfully recovered by centrifugation and stored for further characterization and antimicrobial studies.

UV-Visible spectroscopic analysis revealed a characteristic absorbance peak at 420 nm with an absorbance value of 1.061, confirming the formation of silver nanoparticles (Fig 1). The control solution containing silver nitrate without plant extract showed no color change and exhibited an absorbance maximum at 340 nm, specific for silver nitrate solution. The spectral difference between the leaf extract alone and the extract with silver nanoparticles was noted as 0.859, confirming the bioreduction of silver ions. The rapid reduction of silver ions and formation of stable nanoparticles occurred within 20 minutes, with no evidence of aggregation, indicating effective stabilization by phytochemicals present in the leaf extract.

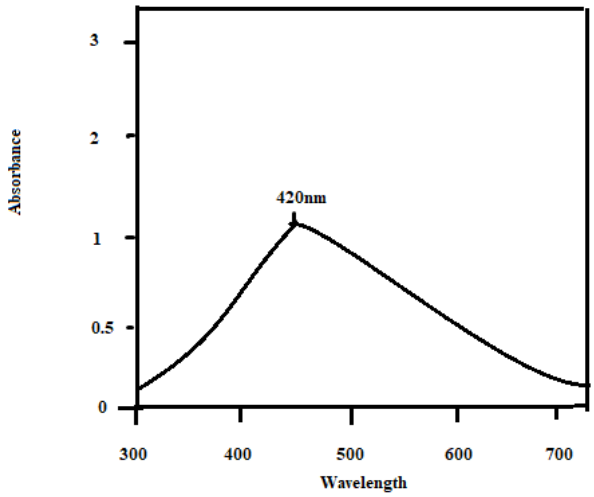


Fig 1: Absorbance peak at 420nm

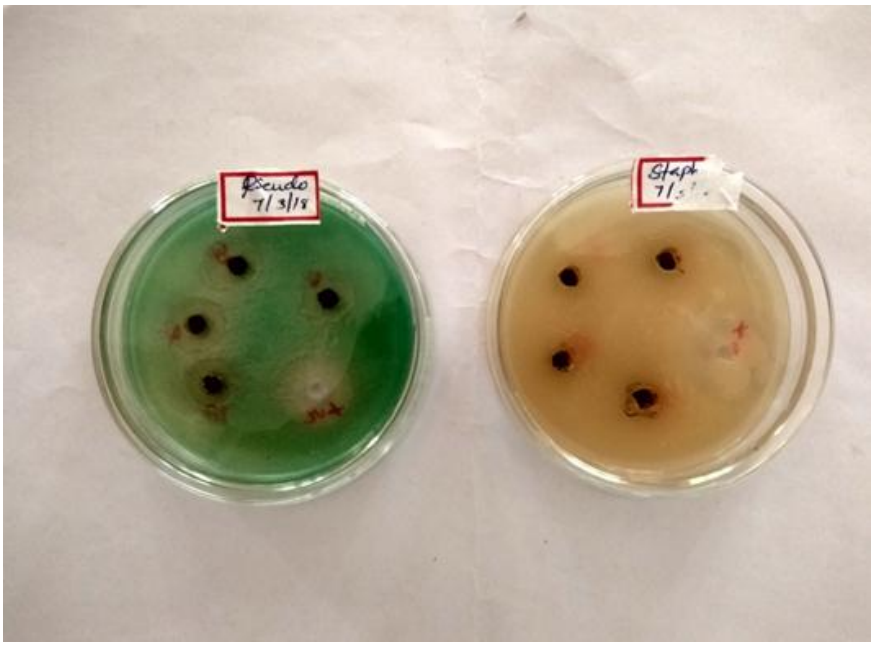
Microscopic examination of the synthesized nanoparticles revealed the presence of discrete particles, confirming successful biosynthesis. The stability and morphological characteristics of the nanoparticles suggest potential applications in various biomedical fields.

Antibacterial Activity

The antibacterial activity of biosynthesized silver nanoparticles was evaluated against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the agar well diffusion method. The results demonstrated concentration-dependent antibacterial activity, with increasing zones of inhibition corresponding to higher concentrations of silver nanoparticles (Table 1, Fig 2)

Table 1: Antibacterial Activity of Silver Nanoparticles from *Thuja occidentalis*

Concentration (µL)	***Pseudomonas aeruginosa* (Zone of Inhibition, mm)**	***Staphylococcus aureus* (Zone of Inhibition, mm)**
20	11	Resistant
40	13	Resistant
50	14	Resistant
60	16	Resistant
Streptomycin (1 mg/mL)	18	20



Pseudomonas *Staphylococcus*

Fig 2: Diameter of zone of inhibition against the different test bacteria

Against *Pseudomonas aeruginosa*, the synthesized silver nanoparticles exhibited significant antibacterial activity with inhibition zones of 11 mm at 20 µL, 13 mm at 40 µL, 14 mm at 50 µL, and 16 mm at 60 µL concentration. The maximum zone of inhibition (16 mm) was observed at the highest tested concentration (60 µL). This activity was comparable to the positive control streptomycin, which produced an inhibition zone of 18 mm against *P. aeruginosa*. The results indicate that *P. aeruginosa* was susceptible to the synthesized silver nanoparticles, with activity approaching that of the standard antibiotic.

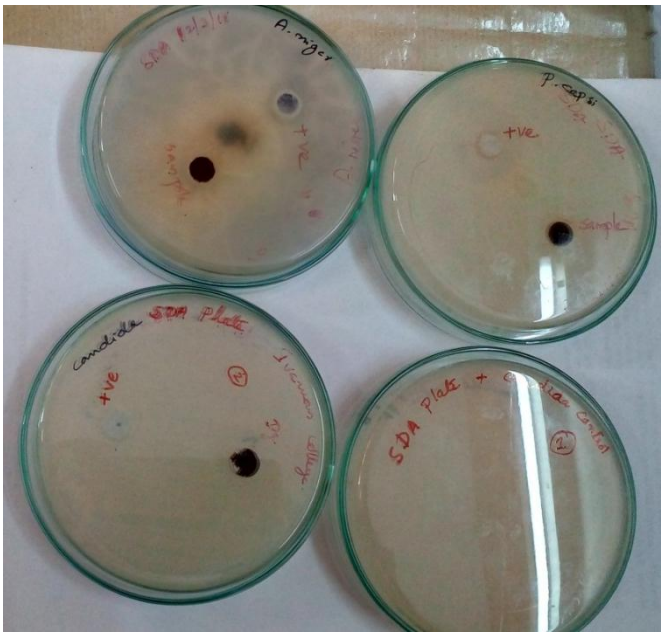
In contrast, *Staphylococcus aureus* demonstrated resistance to all tested concentrations of silver nanoparticles, showing no inhibition zones even at the highest concentration (60 µL). The positive control streptomycin exhibited a zone of inhibition of 20 mm against *S. aureus*, confirming the validity of the assay. This differential susceptibility suggests that *S. aureus* may possess inherent resistance mechanisms or cell wall characteristics that limit the penetration or efficacy of the biosynthesized silver nanoparticles.

Antifungal Activity

The antifungal activity of *T. occidentalis* silver nanoparticles was evaluated against *Aspergillus niger*, *Candida albicans*, and *Phytophthora capsici*. The results demonstrated selective antifungal activity, with significant inhibition observed against *Candida albicans* only (Table 2, Fig 3)

Table 2: Antifungal Activity of *Thuja occidentalis* Silver Nanoparticles

Extract/Concentration	*Aspergillus niger*	*Candida albicans*	*Phytophthora capsici*
AgNPs (100 µL)	Resistant	16 mm	Resistant
Mancozeb (Positive Control)	Resistant	20 mm	20 mm



A.niger *T. capsci* *C. albicans*

Fig 3: Diameter of zone of inhibition against the different test fungi

The biosynthesized silver nanoparticles exhibited a zone of inhibition of 16 mm against *Candida albicans*, while mancozeb (positive control) showed 20 mm inhibition against *C. albicans*. The nanoparticles showed no antifungal activity against *Aspergillus niger* or *Phytophthora capsici*, indicating selective antifungal efficacy. This differential activity may be attributed to variations in cell wall composition and susceptibility mechanisms among fungal species.

DISCUSSION

The present study successfully demonstrated the green synthesis of silver nanoparticles using aqueous leaf extract of *Thuja occidentalis* and evaluated their antimicrobial potential. The biosynthesis of metal nanoparticles using biological systems represents an expanding research area with significant implications for nanomedicine and antimicrobial therapy (Ahmed *et al.*, 2014; Mittal *et al.*, 2014). The green synthesis approach offers distinct advantages over conventional chemical methods, including environmental safety, cost-effectiveness, biocompatibility, and absence of toxic byproducts (Thuesombat *et al.*, 2014; El-Chaghaby and Ahmad, 2011).

The formation of silver nanoparticles was confirmed by the characteristic color change from pale yellow to dark brown upon addition of silver nitrate solution, attributed to the excitation of surface plasmon resonance vibrations (Savithramma *et al.*, 2011; Gilaki, 2010). The absorption spectra showed a maximum peak at 420 nm, consistent with previous reports for silver nanoparticles synthesized using plant extracts (Krishnaraj *et al.*, 2010; Chandran *et al.*, 2006; Philip and Unni, 2011). The rapid reduction of silver ions within 20 minutes, without evidence of aggregation, suggests that the phytochemicals present in *T. occidentalis* leaf extract act as effective reducing and capping agents, providing stability to the nanoparticles (Ahmed *et al.*, 2014; Roopan *et al.*, 2012). This is consistent with earlier studies that have reported the presence of various phytochemicals including phenolics, flavonoids, terpenoids, and alkaloids in *Thuja* species, which can facilitate metal ion reduction and nanoparticle stabilization (David Hoffmann, 2003; Tsiri *et al.*, 2009).

The antibacterial activity of biosynthesized silver nanoparticles demonstrated concentration-dependent inhibition against *Pseudomonas aeruginosa*, with the maximum zone of inhibition of 16 mm at 60 µg concentration. This activity was comparable to streptomycin (18 mm), indicating the potential of *T. occidentalis* AgNPs as effective antibacterial agents (Nisha *et al.*, 2017; Tsiri *et al.*, 2009). The antibacterial efficacy of green-synthesized AgNPs against *P. aeruginosa* has been previously reported for various plant extracts (Krishnaraj *et al.*, 2010; Shankar *et al.*, 2004; Roy and Barik, 2010). The mechanism of action likely involves the attachment of AgNPs to the bacterial cell membrane, penetration into the bacterial cell, and subsequent interaction

with phosphorous and sulfur-containing compounds such as DNA and proteins, leading to bacterial cell death (Sondi and Salopdek-Sondi, 2004; Gong *et al.*, 2007; Li *et al.*, 2011). Furthermore, AgNPs can induce the generation of reactive oxygen species, resulting in oxidative stress and cell damage (Kasthuri *et al.*, 2009).

Pseudomonas aeruginosa, a Gram-negative bacterium, is among the main agents in nosocomial infections, contributing to longer hospital stays and mortality. Among Gram-negative bacteria, *P. aeruginosa* is highly susceptible to genetic modifications leading to resistance to antimicrobials and consequent complications in impaired or immunocompromised patients. Due to its ability to survive in harsh environments, *P. aeruginosa* is known as one of the most important agents in hospital infections. The significant activity observed against *P. aeruginosa* in this study is particularly noteworthy and supports the potential application of *T. occidentalis* AgNPs in combating hospital-acquired infections.

Interestingly, *Staphylococcus aureus* showed resistance to all tested concentrations of the synthesized nanoparticles. This differential susceptibility between Gram-negative *P. aeruginosa* and Gram-positive *S. aureus* may be attributed to differences in cell wall structure and composition (Gong *et al.*, 2007; Li *et al.*, 2011). Gram-positive bacteria possess a thick peptidoglycan layer that may act as a barrier to nanoparticle penetration, whereas Gram-negative bacteria have an outer membrane that may be more susceptible to nanoparticle-induced damage (Sondi and Salopdek-Sondi, 2004). However, previous studies have reported variable susceptibility of *S. aureus* to AgNPs, suggesting that the efficacy may depend on nanoparticle size, shape, concentration, and the specific phytochemical composition of the plant extract used for synthesis (Krishnaraj *et al.*, 2010; Kasthuri *et al.*, 2009). It was reported that when streptomycin was supplemented with AgNPs, its antibacterial activity was increased up to 57.98%, suggesting potential synergistic effects (Gong *et al.*, 2007).

The antifungal activity assessment revealed that *T. occidentalis* AgNPs exhibited selective activity against *Candida albicans* with a zone of inhibition of 16 mm, while mancozeb showed 20 mm inhibition. *Candida albicans* is responsible for a number of major diseases as well as recent cases of resistance to the main antifungals (Logeswari *et al.*, 2012). Therefore, new substances should be researched as an alternative to combat such resistance. Ezzat (2001) studied the inhibition of *Candida albicans* in the plant extract of *Thuja* leaves and male cones and showed considerable inhibition for *C. albicans*, supporting our findings. Mishra *et al.* (1992) studied antifungal activity of aqueous leaf extract of *T. orientalis* and reported similar results. The fungicidal mechanism of biosynthesized metallic nanoparticles has been shown to have more potential than commercial antibiotics such as fluconazole and amphotericin (Logeswari *et al.*, 2012). The plant-derived AgNPs have clearly shown membrane damage in *Candida* sp. and damage in fungal intercellular components, finally destroying cell functions (Logeswari *et al.*, 2012).

The resistance observed against *Aspergillus niger* and *Phytophthora capsici* in this study is consistent with previous reports. Studies of *Thuja* oil in control of biocontamination in libraries and archives storage areas showed that this oil had little inhibitory effect on the fungal combination (Tsiri *et al.*, 2009). However, Tsiri *et al.* (2009) tested the essential oils of four varieties of *Thuja*, including *T. occidentalis* 'globosa' and *T. occidentalis* 'aurea' against six Gram-positive and Gram-negative bacteria and three pathogenic fungi. They demonstrated significant antimicrobial activity, with this being especially powerful in the isolates of the mixture of α- and β-thujone and beierene. The presence of α-pinene, 3-carene, and α-cedrol in *Thuja* oil reveals that *Thuja* possesses potential for use in many medical applications (Tsiri *et al.*, 2009).

The findings of this study have significant implications for the development of alternative antimicrobial agents. The biosynthesis of silver nanoparticles using *T. occidentalis* leaf extract provides a simple, rapid, and economical route for nanoparticle synthesis (Ahmed *et al.*, 2014; Mittal *et al.*, 2014). The environmental sustainability of this approach, avoiding the use of hazardous and toxic solvents and waste, makes it particularly attractive for large-scale production and biomedical applications (Thuesombat *et al.*, 2014; El-Chaghaby and Ahmad, 2011). The antimicrobial potential demonstrated in this study supports the traditional use of *Thuja* as "The Tree of Life" and provides scientific validation for its therapeutic applications (David Hoffmann, 2003).

CONCLUSION

The present study successfully demonstrated the synthesis of silver nanoparticles using aqueous leaf extract of *Thuja occidentalis* through a green, cost-effective, and environmentally friendly approach. The formation of AgNPs was confirmed by UV-Vis spectroscopy, showing a characteristic absorption peak at 420 nm, and the nanoparticles exhibited excellent stability without aggregation. The biosynthesized silver nanoparticles demonstrated significant concentration-dependent antibacterial activity against *Pseudomonas aeruginosa*, with a maximum zone of inhibition of 16 mm at 60 µg concentration, comparable to the standard antibiotic streptomycin. However, *Staphylococcus aureus* showed resistance to all tested concentrations, indicating differential susceptibility between Gram-negative and Gram-positive bacteria. In antifungal

studies, the AgNPs exhibited selective activity against *Candida albicans* (16 mm zone of inhibition), while showing no activity against *Aspergillus niger* or *Phytophthora capsici*.This research validates the traditional medicinal use of *Thuja occidentalis* and demonstrates its potential as a source for green synthesis of antimicrobial silver nanoparticles. The eco-friendly synthesis approach, utilizing readily available plant material, presents a sustainable alternative to conventional chemical methods for nanoparticle production. Future studies should focus on characterizing the phytochemical constituents responsible for nanoparticle synthesis, evaluating the cytotoxicity and biocompatibility of the synthesized nanoparticles, exploring synergistic effects with conventional antibiotics, and investigating the molecular mechanisms of antimicrobial action. The development of such plant-based nanomedicines could contribute significantly to addressing the global challenge of antimicrobial resistance while promoting sustainable and environmentally conscious approaches in nanotechnology.

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