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Green Synthesis, Characterization, and Antifungal Activity of *Ziziphus spina-christi*–Mediated Silver Nanostructures Against *Malassezia spp*

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ABSTRACT

Production of nanostructured antifungal agents through green synthesis has gained ground as the environmentally friendly approach to combat drug-resistant fungal pathogens. The nanoparticles (ZSC-AgNPs) were synthesized using aqueous leaf extract of *Ziziphus* spina-christi as reducing and stabilizing agent. Successful formation of Ag NPs was finally confirmed by the color change (pale yellow to dark brown), SEM, and XRD, which showed spherical-to-quasi-spherical shape with mean size 30-44 nm and face-centered cubic crystalline structure. The antifungal activity of ZSC-AgNPs was tested against clinical isolates of *Malassezia* spp (M. furfur, M. *pachydermatis* and M. globosa) by broth microdilution, agar well diffusion and qRT-PCR techniques. ZSC-AgNPs were much more active than the crude extract (mean MIC and MFC values 0.093 mg/mL and 0.187 mg/mL, respectively; ~134-fold improvement in potency). By comparison, the itraconazole and fluconazole MICs were 20 µg/mL and 32 µg/mL, respectively. Agar well diffusion test showed a concentration-dependent inhibition (mean zone of 17.56 mm) with ZSC-AgNps (2 mg/mL). In addition, qRT-PCR results demonstrated that the gene expression of lipase was decreased by fold 3.93 resulting in anti-virulence action rather than fungicidal property. These results indicate the potential of plant-fabricated silver nanostructures as potent and environmental friendly antifungal agents with two modes of action. This work will pave the way for the application of green nanotechnology in creating emerging antimicrobial materials for biomedical purposes.

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INTRODUCTION

Cutaneous mycoses due to *Malassezia spp*. are a major problem in the skin conditions of hot and humid places like Iraq [1,2]. These lipophilic yeasts also represent natural inhabitants of the skin, but can turn into pathogens when their host produces excessive sebum, is immunocompromised after organ transplantation or due to hormonal changes, developing diseases like pityriasis versicolor, seborrheic dermatitis and *Malassezia folliculiti*. With over 18 described species, including M. furfur, *M. pachydermatis*, and *M.*

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globosa, accurate identification and successful treatment are still clinically important features of this organism given increasing reports of relapse with therapy and antifungal resistance [3,4].

Current mode of treatment is dependent temporally on azole antifungals such as ketoconazole, itraconazole, fluconazole and allylamines -terbinafine. However these drugs are commonly plagued with limitations such as hepatotoxicity, high cost, variable efficacy and the problem of resistance particularly in resource-limited settings [5]. The latter has led to an increase in the search for alternative, low-cost and green methods for antifungal treatment.

And in this aspect, green nanotechnology has risen as a hopeful method leader new antimicrobial agents. The synthesis of silver nanostructures through plant extracts is a sustainable process involving no toxic chemical reductants and the phytochemicals work synergistically in this mechanism. In addition, *Ziziphus spina-christi* (L.) Desf., a medicinal plant known as Christ's thorn jujube has been used in the traditional medicine of the Middle East to treat skin infections, wounds and inflammatory diseases [6,7]. Its leaves are abundant sources of bioactive compounds like quercetin, rutin gallic acid and saponins, having anti-oxidant, anti-inflammatory and antimicrobial potentials [8].

There are some studies that have showed the possible ways in which nano-formulation can increase the bioactivity of plant extracts by improved solubility, stability and cellular uptake. These phytochemicals not only act as reducing as well as capping agents in the production of nanoparticles but also might be involved in its antimicrobial activity [7]. Despite this potential, there is an obvious scarcity in the literature reviewing the use of Z. spina-christi-associated AgNPs against *Malassezia* species especially with respect to their antifungal activity and level of molecular mechanism behind it.

Due to a high numbers of papers published on the synthesis and characterization of nanostructures, In the present study we have reported green synthesis, physicochemical characterisation and antifungal activity of aqueous leaf extract of *Ziziphus* spina-christi mediated biosynthesized silver nanostructures against clinical isolates of *Malassezia spp*. Herein, the concept of finding a possible alternative means for overcoming widespread fungal infection is pursued using botanical resources inclusive of nanotechnology to construct a locally available, cost-effective and environmental friendly antifungal remedy that may be potential for dermatological topical treatment.

MATERIALS AND METHODS

Study Design and Ethical Considerations

The present experimental study was conducted between January and August 2025 at the Microbiology Laboratory, College of Science, University of Wasit, Iraq. All participants were provided with written informed consent prior to sample collection. Confidentiality was strictly observed by the investigator throughout the study.

Clinical Sample Collection and Fungal Isolation

Sixty skin scrapings were collected from patients clinically diagnosed with pityriasis versicolor at dermatology outpatient clinics in Wasit Province. Samples were gently obtained from the active margins of lesions using sterile scalpels and placed in sterile containers. Each sample was cultured on Sabouraud Dextrose Agar (SDA) supplemented with 1% olive oil and chloramphenicol (0.05 g/L) to promote the growth of lipid-dependent *Malassezia* species and to prevent bacterial contamination. The plates were incubated aerobically at 32 °C for 5–7 days. Colonies showing the typical creamy or pasty appearance of *Malassezia* were sub-cultured for purification and maintained on SDA slants at 4 °C for further analysis [9]. Out of the sixty clinical samples, forty yielded *Malassezia* isolates that were included in this study.

Molecular Confirmation of Malassezia spp. (ITS-PCR)

PCR amplification of the ITS1–ITS4 region was performed to confirm the identity of the isolates at the genus level as *Malassezia* spp. The primers used were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [10]. Each reaction was prepared in a 25 µL volume using a commercial PCR Master Mix. The thermal cycling program consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 52 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Amplified products were electrophoresed on a 1.5% agarose gel and visualized under UV illumination. A distinct band of approximately 550 bp was observed in the representative isolates, confirming their affiliation

to the genus *Malassezia*.

Biochemical and Morphological Identification of Malassezia Isolates

Based on morphological and biochemical characteristics, the isolates were identified as *M. furfur* (50%), *M. pachydermatis* (30%), and *M. globosa* (20%). Identification was supported by lipid dependence, growth at different temperatures, and catalase and urease activities**.** Species differentiation followed standard taxonomic criteria [11]:

• *M. furfur*: lipid-dependent, catalase +, urease +, grows at 41 °C.

• *M. pachydermatis*: lipid-independent, catalase +, urease +, no growth at 41 °C.

• *M. globosa*: lipid-dependent, catalase +, urease –, no growth at 41 °C.

Although *M. pachydermatis* is mainly animal-associated, its occurrence in human samples may indicate transient colonization or indirect contact with domestic animals.Although *M. pachydermatis* is primarily an animal-associated, lipid-independent species, its detection in human samples may reflect transient colonization, indirect contact with domestic animals, or environmental adaptation. Similar findings have been reported in previous studies documenting occasional human isolation of this species [12].

Preparation of Ziziphus spina-christi Aqueous Extract

Fresh leaves of Ziziphus spina-christi were collected from Al-Hai district in Wasit Province. The leaves were thoroughly washed with distilled water, air-dried at room temperature for 72 hours, and then ground into a fine powder.. To prepare the aqueous extract, 10 g of the powdered leaves were boiled in 100 ml of distilled water for 30 minutes. The mixture was allowed to cool, filtered through Whatman No. 1 filter paper, and stored at 4°C. The stock solution was standardized to a concentration of 100 mg/ml (w/v) for antifungal assays [13].

HPLC Analysis of Bioactive Compounds

High-performance liquid chromatography (HPLC) was employed to identify the major phenolic and flavonoid compounds in the aqueous extract of *Ziziphus spina-christi* leaves. The analysis was performed on a SYKAM HPLC system (Germany) using a C18-ODS column (25 cm × 4.6 mm). The mobile phase consisted of methanol: distilled water: formic acid (70:25:5, v/v/v) at a flow rate of 1.0 mL/min with a 20-minute runtime, and detection was carried out at 280 nm.

Standard solutions (5 ppm) of caffeic acid, apigenin, quercetin, rutin, ferulic acid, and cinnamic acid were used to determine retention times. The extract was filtered through a 0.22 µm membrane filter, and 20 µL were injected. Retention times and peak areas of the sample were compared with those of the standards to confirm compound identity [14]. The identified phenolic and flavonoid compounds.

Green Synthesis of ZSC-AgNPs

Silver nanoparticles (ZSC-AgNPs) were synthesized via green biosynthesis by mixing *Ziziphus spina-christi* leaf extract with 1 mM $AgNO_3$ at a 1:9 (v/v) ratio under continuous stirring. The mixture was incubated at 60 °C for 24 hours in the dark. A color change from pale yellow to brown confirmed Ag^+ reduction and nanoparticle formation. The particles were collected by centrifugation (10,000 rpm for 15 min), washed three times with distilled water, and re-dispersed to obtain a 2 mg/mL suspension for subsequent assays. Successful synthesis was verified using scanning electron microscopy (SEM) to assess particle morphology and size, and X-ray diffraction (XRD) to confirm crystalline structure [15].

Antifungal Susceptibility Testing

Broth Microdilution Assay (MIC and MFC)

Antifungal activity was evaluated using the broth microdilution method in 96-well microplates, following modified guidelines for lipid-dependent yeasts. *Malassezia* inocula were prepared from 7-day-old cultures on modified Dixon agar (mDA) and suspended in mDixon broth supplemented with 0.1% Tween-80 and 1% olive oil, adjusted to 0.5 McFarland (~$1–5 \times 10^6$ CFU/mL) [16].

Two-fold serial dilutions were prepared for Ziziphus spina-christi extract (100–0.78 mg/mL) and ZSC-AgNPs (2–0.0156 mg/mL), while itraconazole and fluconazole (1–512 µg/mL) served as reference antifungal agents. Each well received 100 µL of the test solution and 100 µL of inoculum, and plates were incubated at 32 °C for 72 hours.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration causing ≥50% growth inhibition, while the minimum

fungicidal concentration (MFC) was determined by subculturing 10 µL from wells showing no visible growth onto fresh mDixon agar. The absence of growth after 72 hours indicated fungicidal activity.

Agar Well Diffusion Assay

Antifungal activity was also assessed using the agar well diffusion method. Sabouraud Dextrose Agar (SDA) plates supplemented with 1% olive oil were evenly inoculated with a fungal suspension adjusted to 0.5 McFarland. Wells of 6 mm diameter were made in the agar and filled with 100 µl of each test solution. The concentrations tested were 100, 50, and 25 mg/ml for the *Z. spina-christi* extract and 2, 1, and 0.5 mg/ml for the ZSC-AgNPs. Distilled water served as the negative control [17].

Plates were incubated at 32°C for 72 hours, and the inhibition zones were measured in millimeters using a digital caliper (Mitutoyo, Japan). All assays were performed in duplicate, and mean values were recorded.

Assessment of Lipase Gene Expression in Malassezia spp. Following ZSC-AgNPs Treatment Using qRT-PCR

To evaluate the effect of ZSC-AgNPs on fungal virulence, the expression level of the lipase gene was quantified in selected *Malassezia* isolates using quantitative real-time PCR (qRT-PCR). Total RNA was extracted using TRIzol® reagent (Invitrogen, USA), and 1 µg of RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) [18].

The lipase gene, which plays a key role in lipid degradation and skin colonization, was chosen as the target gene, while ACTIN served as the internal control [24]. qPCR amplification was performed with SYBR® Green Master Mix on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) [18].

Oligonucleotide primers used in this study are listed in Table 1 and were synthesized by Macrogen (Korea).

Relative gene expression was analyzed according to the 2^−ΔΔCT method, with untreated samples serving as the reference group, using the following equations:

$$\Delta C_T = C_T^{(\text{Lipase})} - C_T^{(\text{ACTIN})}$$

$$\Delta\Delta C_T = \Delta C_T^{(\text{Treated})} - \text{Mean}\ \Delta C_T^{(\text{Untreated})}$$

$$\text{Fold Change} = 2^{-\Delta\Delta C_T}$$

"N1" means the sample of Malassezia strains after treatment with ZSC-AgNPs.

Statistical Analysis

The data were presented as the mean ± SD. Comparison of diameters of inhibition zone between treatments was done by One-way ANOVA followed by Tukey's post-hoc test. Correlations among variables were tested using Pearson correlation and Chi-square (χ^2) for contingency test. A two-sided p-value of < 0.05 was regarded as statistically significant. Analysis Data were analyzed with SPSS version 26.0 (IBM Corp., USA) [19].

RESULTS AND DISCUSSION

The recent increase in the incidence of superficial fungal infections due to *Malassezia* in its tropical climate, especially under hot and humid conditions like those found within Iraq, demands an alternative safe, low-cost and effective antifungal [1,2]. Belonging to the normal skin microbiota, these lipophilic-dependent yeasts may act as potential pathogens in particular conditions causing many dermatoses, including pityriasis versicolor and seborrheic dermatitis, *Malassezia folliculitis* [3].

A total of 40 clinical isolates were collected from patients diagnosed with PV in Wasit Province, during the period of this investigation. According to their morphological and biochemical characteristics, the isolates were identified as M.

Table 1. Oligonucleotide primers used in this study.

Gene	Primer	Sequence (5′ → 3′)
LIPASE	Lip-F	ACCCAACATTTGCTTCGTTC
	Lip-R	TCAATTATCAATGGTCGCGA
ACTIN	Act-F	CTCTCCTTGTACGCCTCTGG
	Act-R	TTGACAAGATGCTCCGTCAG

Table 2. Morphological and biochemical characteristics of Malassezia species (n = 40).

Characteristic	M. furfur	M. pachydermatis	M. globosa
Colonial appearance	Flat, smooth, slightly wrinkled	Flat to convex, occasionally umbonate	Elevated, coarse surface
Microscopic cell shape	Ovoid, cylindrical, spherical	Oval	Spherical
Growth on lipid-supplemented SDA	+	–	+
Growth on non-lipid SDA	–	+	–
Growth at 32°C (mDA)	+	+	+
Growth at 37°C (mDA)	+	+	+
Growth at 41°C (mDA)	+	–	–
Catalase activity	+	+	+
Urease activity	+	+	–
% of total isolates	50%	30%	20%

furfur (50%), M. *pachydermatis* (30%) and *M. globosa* (20%) (Table 2).

Even though *M. pachydermatis* is normally animal-associated, that its presence has been reported in humans might also imply transient colonization or indirect contact with domestic animals. All strains were catalase-positive and grew at 32 °C, 37 °C and only M. furfur grew at 41 °C, the urease test was positive in M. furfur and *M. pachydermatis* and negative in *M.globosa*. See (Fig. 1) for typical morphological and biochemical features of the organism, i.e., creamy colonies, oval budding cells, positive catalase reaction, and urease activity.

Molecular identification by ITS-PCR strategy with universal fungal primers (ITS1/ITS4) showed a specific ~550bp product in selected isolates (Fig. 2), which confirmed their identity to the genus *Malassezia* [10]. Phenotypic and molecular identification coupled gave confidence in verification for antifungal testing.

Ziziphus spina-christi Aqeous leaf Extract as both reducing and Stabilizing agent was employed for synthesizing the silver NPs (ZSC-AgNPs). The color change from pale yellow to brown was visibly observed because of the formation of nanoparticles with exhibiting surface plasmon resonance of metallic silver. SEM (Fig. 3) showed that the particles were spherical or quasi-spherical in shape with a particle size of about 30–44 nm, and the XRD (Fig. 3) confirmed an FCC structure. These results are in line with those found for green-generated AgNPs obtained from Moringa oleifera and Ziziphus spina-christi [7,8].

Antifungal assay results showed that ZSC-AgNPs had significantly higher activity when compared to the crude extract. The MIC of the crude extract had a mean value of 12.5 mg/mL (Table 3) while ZSC-AgNPs were efficient at 0.093 ± 0.02 mg/mL, which means that they are about ~134-fold more effective than the original antifungal product. Furthermore, the average MFC values in the extract and nanoparticles were ranged from 25 mg/mL to 0.187 ± 0.03 mg/mL respectively also indicated a fungicidal rather than fungistatic activity was developed by both types of samples [16]. This improvement can be ascribed to the nanosized dimensions of ZSC-AgNPs which enhance surface reactiveness and subsequent Ag^+ release, leading to injury on fungal membrane, enzyme inhibition and DNA breaks [20].

Itraconazole and fluconazole had MIC values of 20 µg/mL and 32 µg/mL, respectively; these cannot be directly compared with mg/ml formulation because of the differences in unit and molecular weight [21]. Thus, the findings represent relative biological enhancement and not equivalent potency. ZSC-AgNPs showed inhibition at a mere ~2% of the concentration of crude extract, highlighting their enhanced efficacy and suitability for topical application [22].

Agar-well diffusion test (Table 4) verified a dose-dependent inhibitory pattern. At 2 mg/mL, ZSC-AgNPs yielded a mean inhibition zone of 17.56 ± 0.42 mm, which is superior to that obtained with crude extract (14.91 ± 0.38 mm at a concentration of 100 mg/mL). While *itraconazole* produced bigger zones (26.68 ± 0.31 mm at 256 µg/mL), its hepatotoxicity and drug interactions constrain long-term use [13]. As such, ZSC-AgNPs

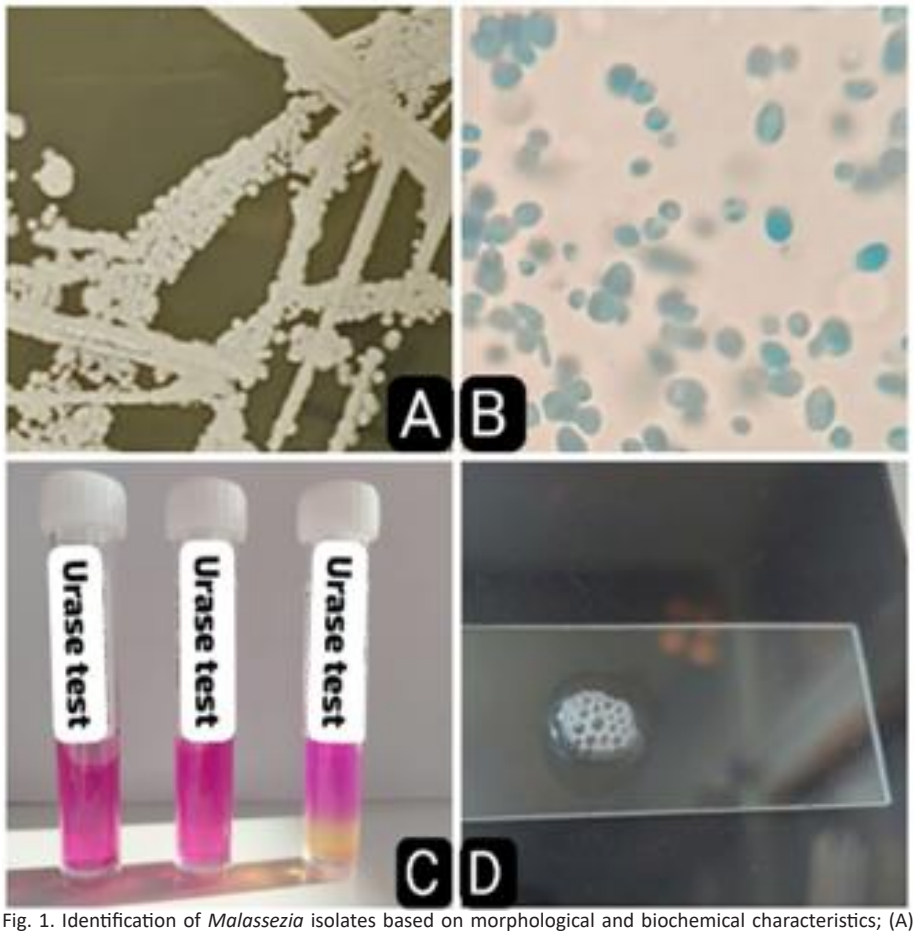

Fig. 1. Identification of *Malassezia* isolates based on morphological and biochemical characteristics; (A) colony growth on Sabouraud agar supplemented with olive oil showing smooth creamy yeast-like colonies, (B) microscopic observation of oval budding yeast cells stained with Lactophenol Cotton Blue, (C) urease test demonstrating positive enzyme activity indicated by a pink color change, and (D) catalase test showing bubble formation after addition of hydrogen peroxide (H_2O_2), confirming catalase activity in the isolates.

could potentially be a non-hazardous sustainable alternative for topical antifungal treatment.

Molecularly, ΔΔCT values (Table 5) ranged from 1.09 to 3.49 among strains and averaged a mean of 2^{-}ΔΔCT of 0.255 ± (S.D.) = 0.12 that was indicative a lipase gene expression decrement of about a factor three ($p < 0.05$). Such substantial down regulations imply that ZSC-AgNPs not only inhibited growth of the fungus but also attenuated virulence by disrupting lipase dependent lipid metabolism and colonization [18].

The crude extract showed comparatively moderate antifungal activity might be attributed to the presence of phenolics and saponins however low solubility with poor skin penetration justifies for the restricted therapeutic application. The novelty and potential of nanoscale formulation presented here addresses the aforementioned limitations, through enhanced solubility, stability, and cellular uptake, signifying the impact of green nanotechnology on plant-based cures.

HPLC Study (Table 6 and Fig. 4) Most of the bioactive phenolic and flavonoid compounds were also detected in *Z. spina-christi* extract such as quercetin (112.6 ppm), rutin (166.8 ppm), and gallic acid (198.0 ppm). These polyphenols originated probably themselves in due to both a reduction and capping effect playing role as the NPs are acutely shaped [14].

One-way ANOVA of the treatments were found to be highly significant ($F = 24.8$, $p < 0.05$). In addition, Pearson correlation analysis

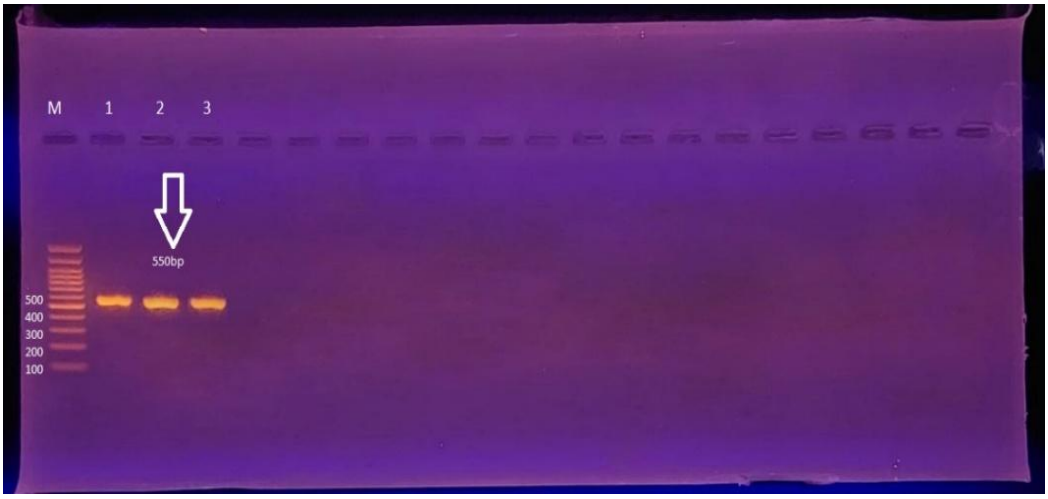

Fig. 2. Agarose gel electrophoresis of ITS-PCR products (~550 bp) from Malassezia isolates. Lane M: 100-bp DNA ladder; Lanes 1–3: positive Malassezia isolates.

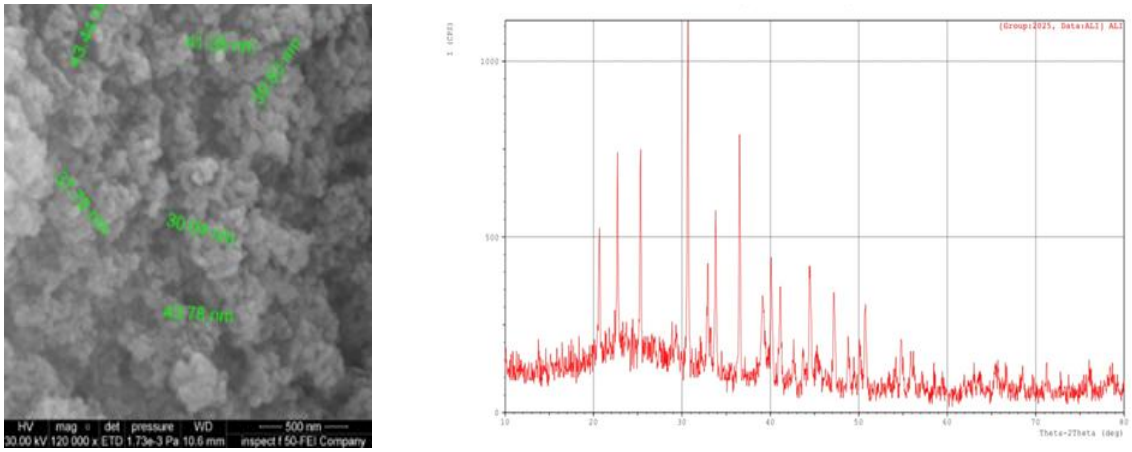

Fig. 3. SEM image showing spherical AgNPs (≈30–44 nm) and (Figure 4) XRD pattern confirming their crystalline FCC structure.

Table 3. MIC and MFC of test agents against Malassezia spp.

Agent	MIC Range	Mean MIC	MFC Range	Mean MFC
Z. spina-christi crude extract mg/ml	12.5	12.5	25	25
ZSC-AgNPs mg/ml	0.06 – 0.125	0.093	0.125 – 0.250	0.187
Itraconazole µg/ml	16 – 32	20	32 – 64	40
Fluconazole µg/ml	32	32	64	64

showed a significant positive correlation between nanoparticle concentration and inhibition-zone diameter ($r = 0.87$, $p < 0.01$), which confirms the dose-response pattern (Table 6).

Nevertheless, there are some limitations of this study. Limitations The sample is relatively small (n = 40) and collected from a single geographic origin (Wasit Province, Iraq), possibly limiting the generalisability of findings. More physicochemical (UV-Vis, FTIR, DLS, and zeta potential) characterization as well as assessment of cytotoxicity on human skin cells is necessary before clinical use. Future areas of study are safety profiling, topical formulation development (gels or creams), pre-clinical validation for effectiveness and biocompatibility.

In summary, *Ziziphus* spina-christi-silver nanoparticles caused multi-mechanistic impacts

Table 4. Mean inhibition zones (mm) against Malassezia.

Agent	Concentration	Trial 1	Trial 2	Mean
Z. spina-christi crude extract (mg/ml)	100	14.96	14.86	14.91
	50	12.44	12.62	12.53
	25	10.06	9.89	9.98
	Control (C)	8.22	8.44	8.33
ZSC-AgNPs (mg/ml)	2	17.97	17.14	17.56
	1	16.40	16.52	16.46
	0.5	14.80	13.86	14.33
	Control (C)	9.84	9.78	9.81
Itraconazole(µg/ml)	256	26.66	26.63	26.68
	128	17.25	17.22	17.27
	64	12.77	12.75	12.73
	Control	6.00	6.00	6.00
Fluconazole(µg/ml)	256	23.12	23.15	23.9
	128	14.88	14.86	14.84
	64	9.55	9.52	9.57
	Control	6.00	6.00	6.00

Table 5. qRT-PCR analysis of gene expression in Malassezia treated with ZSC-AgNPs.

Sample	CT(target)	CT(reference)	ΔCT	ΔΔCT	Relative Expression	Fold Change
N1-1	32.3	22.1	10.2	1.09	0.471	↓2.12
N1-2	33.9	21.3	12.6	3.49	0.089	↓11.2
N1-3	33.4	22.1	11.3	2.19	0.220	↓4.55
N1-4	32.0	21.5	10.5	1.39	0.383	↓2.61
N1-5	32.6	21.3	11.3	2.19	0.220	↓4.55
N1-6	32.7	20.8	11.9	2.79	0.145	↓6.90
Mean	—	—	—	—	0.255	↓3.93

Table 6. HPLC profile of *Ziziphus spina-christi* leaf extract.

Compound	Retention Time (min)	Area (mAU·s)	Height (mAU)	Area %	Peak Width W05 (min)	Concentration (ppm)
Gallic acid	2.17	2032.65	395.80	10.00	0.05	198.0
Caffeic acid	3.78	5011.45	645.98	15.00	0.08	95.0
Apigenin	4.19	4895.08	644.02	15.00	0.08	77.0
Quercetin	6.17	7412.65	895.09	21.00	0.15	112.6
Rutin	8.84	4012.32	597.48	15.00	0.08	166.8
Ferulic acid	9.94	1565.09	365.09	10.00	0.05	107.9
Cinnamic acid	11.74	6521.00	674.09	14.00	0.08	96.9

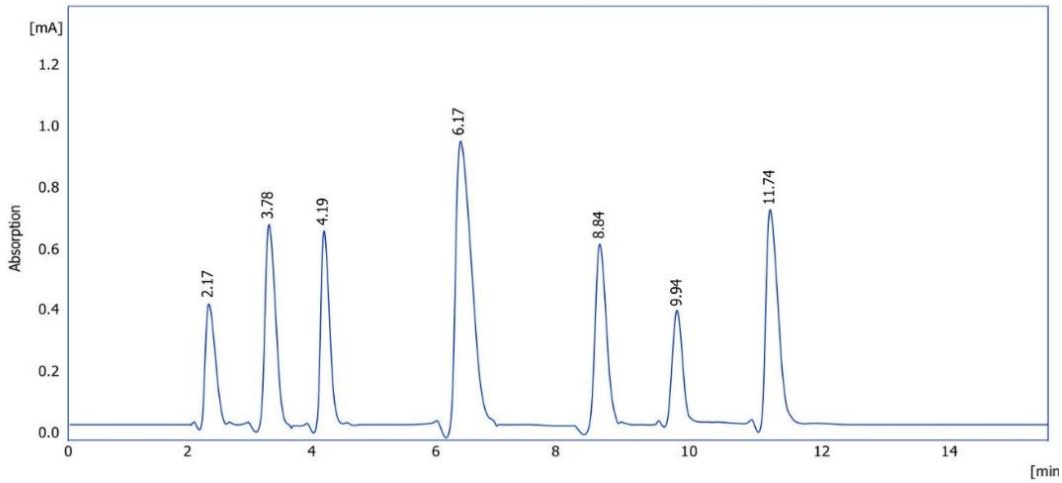

Fig. 4. HPLC chromatogram of ZSC leaf extract shows the main phenolic compounds.

on clinically relevant *Malassezia* species against which they exerted powerful antifungal activity. Demonstrating the green MMNPs synthesis process and biomedical relevance highlights the potential of this indigenous plant derived material and contributes to efforts aimed at generating safe, low cost, and eco-friendly anti-fungal treatments in resource-limited regions.

CONCLUSION

This study demonstrated the successful green synthesis of silver nanoparticles (ZSC-AgNPs) using *Ziziphus spina-christi* leaf extract as a natural reducing and stabilizing agent. The obtained nanostructures exhibited spherical morphology (30–44 nm), face-centered cubic (FCC) crystallinity, and strong antifungal activity against clinical isolates of *Malassezia* spp. The biosynthesized nanoparticles showed approximately a 134-fold enhancement in efficacy compared with the crude extract, with a mean MIC of 0.093 mg/mL and marked inhibition of fungal growth at low concentrations. Moreover, qRT-PCR analysis revealed a 3.9-fold downregulation of the *lipase* gene, indicating a dual antifungal mechanism involving both direct fungicidal activity and suppression of virulence factors. Overall, these findings highlight the promise of *Z. spina-christi*-mediated silver nanoparticles as sustainable and multifunctional bioagents. However, further studies including cytotoxicity assessment, topical formulation development, and in vivo testing are essential to validate their safety and clinical applicability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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