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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles of Hydroalcoholic Extract of *Lactuca runcinata* DC and its Antimicrobial Activity Evaluation by Molecular Docking Study

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Abstract

Background: Nanotechnology, particularly the use of silver nanoparticles (AgNPs), has gained significant interest due to its wide-ranging applications in healthcare, industry, and environmental sectors. However, traditional chemical methods for silver nanoparticles (AgNPs) synthesis raise concerns regarding their environmental and biocompatibility. Green synthesis approaches, such as using plant extracts, offer an eco-friendly alternative. **Aim:** This study investigates the synthesis of silver nanoparticles (AgNPs) using *Lactuca runcinata* DC extract to evaluate their antimicrobial properties, supported by an *in-silico* molecular docking study. **Methods:** Silver nanoparticles were synthesized by mixing an aqueous extract of *L. runcinata* DC with silver nitrate ($AgNO_3$) solution. The synthesis process was monitored visually and confirmed using UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Fourier-Transform Infrared Spectroscopy (FTIR). These techniques helped verify the formation, size, morphology, and structural integrity of the nanoparticles. **Results:** Characterization revealed that the synthesized silver nanoparticles (AgNPs), were spherical and crystalline. UV-Vis spectra showed a distinct peak, confirming the presence of silver nanoparticles. SEM images supported their spherical shape, while XRD confirmed their crystalline structure. FTIR analysis indicated that plant phytochemicals stabilized the nanoparticles. The silver nanoparticles (AgNPs), exhibited significant antimicrobial activity, especially against *Escherichia coli*. Molecular docking studies showed strong interactions between phytochemicals from the extract and Cyclin-dependent kinase 8 (CDK8), suggesting potential for antimicrobial and anticancer applications. **Conclusion:** The green synthesis of silver nanoparticles (AgNPs), using *L. runcinata* DC extract, produced nanoparticles with promising antimicrobial activities.

Major Findings: Silver nanoparticles synthesized from *L. runcinata* DC extract were spherical, crystalline, and phytochemically stabilized, exhibiting strong antimicrobial activity against *E. coli* and notable CDK8 binding, indicating potential antimicrobial and anticancer properties.

Keywords: Antimicrobial Activity, Green Synthesis, Molecular Docking, *L. runcinata* DC, Silver Nanoparticles (AgNPs).

Abbreviations: AgNPs - Silver nanoparticles, $AgNO_3$- Silver Nitrate, CDK8 - Cyclin-dependent kinase 8, FTIR - Fourier Transform Infrared Spectroscopy. MDR - Multidrug-Resistant, ROS- Reactive Oxygen Species, SEM - Scanning Electron Microscope, TEM- Transmission Electron Microscopy, XRD- X-Ray Diffraction

1. Introduction

Nanotechnology has surfaced as a revolutionary discipline with extensive applications across healthcare, industrial sectors, and ecological preservation. Among diverse nanomaterials, silver nanoparticles (AgNPs) have attracted immense interest due to their distinctive physicochemical characteristics, encompassing strong antimicrobial, anti-inflammatory, and anticancer properties. Nonetheless, conventional chemical

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methods for AgNPs, frequently involve hazardous chemicals, which present environmental risks and raise apprehensions regarding biocompatibility. To address these challenges, green synthesis has been introduced as an environmentally friendly and sustainable option that utilizes biological substances, such as plant extracts, for nanoparticle production[1,2]. The plant-assisted synthesis of AgNPs provides numerous benefits, including affordability, scalability, and ecological security. Moreover, plant extracts are abundant in bioactive compounds like flavonoids, terpenoids, and phenolic acids, which function as reducing and stabilizing agents in nanoparticle formation. In this regard, *L. runcinata* DC[3], a medicinal herb renowned for its antioxidant, hepatoprotective, and antimicrobial qualities, emerges as a favorable option for the green synthesis of AgNPs. Utilizing the hydroalcoholic extract of *L. runcinata* DC in nanoparticle production could potentially amplify the bioactivity of the synthesized AgNPs, resulting in enhanced antimicrobial synergy[4]. The antimicrobial potency of AgNPs, synthesized from plant extracts, has been extensively documented, especially against multidrug-resistant (MDR) pathogens, which pose a significant global health threat. Silver nanoparticles exert their antimicrobial effects through various mechanisms, such as disrupting bacterial cell membranes, inducing the production of ROS, and impairing cellular metabolism[5]. Integrating molecular docking studies into antimicrobial research enhances the understanding of the interactions between AgNPs, and microbial proteins at the molecular scale, shedding light on possible binding sites and mechanisms of action. By estimating the binding affinity of AgNPs to crucial bacterial proteins, molecular docking offers valuable insights that can inform the development of more potent and specifically targeted antimicrobial agents[6]. In this investigation, our objective was to synthesize AgNPs utilizing the hydroalcoholic extract of *L. runcinata* DC via a green synthesis method. The synthesized AgNPs, were subsequently characterized through several analytical techniques, including UV-Vis spectroscopy, XRD, and TEM, to analyze their dimensions, morphology, and structural attributes[7]. The antimicrobial potential of the produced AgNPs, was tested against a variety of pathogenic bacteria and fungi, including *E. coli*, *Staphylococcus aureus*, and *Candida albicans*. Moreover, molecular docking studies were performed to examine the interactions between the AgNPs, and microbial proteins, offering insights into the underlying mechanisms of action and possible therapeutic applications[8].This study adds to the expanding body of research that endorses the use of green-synthesized AgNPs, as potent antimicrobial agents. By merging the inherent bioactivity of *L. runcinata* DC with the strong antimicrobial capabilities of silver nanoparticles, this research paves the way for the development of innovative antimicrobial treatments, particularly in combating MDR pathogens[9,10]. For molecular docking study CDK8 plays a key role in controlling the cell cycle and gene expression, with recent studies pointing to its antimicrobial potential. CDK8, as part of the mediator complex, affects immune responses by modulating transcription. Inhibition of CDK8 boosted antimicrobial peptide production in *Drosophila melanogaster* and strengthened immune responses in mammalian cells, suggesting its use in combating infections. However, the precise mechanisms underlying its antimicrobial effects remain uncertain[11].

2. Materials and Methods

2.1 Plant Materials and Hydroalcoholic Extract Preparation

Lactuca runcinata DC was meticulously collected from the Palayamkottai region in Tamil Nadu, and its botanical identity was verified by Dr. S. Mutheeswaran, a scientist at the Xavier Research Foundation, located at Xavier College, Palayamkottai, Tamil Nadu. The plant's authentication was confirmed with a registration certificate bearing the number XCH-40372, ensuring its accurate classification.

The extraction process employed was maceration, a method chosen for its effectiveness in preserving the plant's bioactive compounds. Initially, a hydroalcoholic solvent was prepared by combining alcohol and water in a 70:30 ratio to yield 500 ml of the solvent. For the extraction, 200 grams of the dried whole plant parts of *L. runcinata* DC was separately immersed in the hydroalcoholic solvent. The plant materials were allowed to soak in the solvent for five days, facilitating the release of bioactive constituents into the liquid. After the maceration period, the crude extracts from the two plants were collected separately, resulting in

distinct extracts for further analysis and application. This extraction process ensured the efficient isolation of the plants' active compounds, setting the foundation for subsequent investigations into their potential biological activities[12].

2.2 Green Synthesis of Silver Nano Particle with Plant Extract

The eco-friendly synthesis of silver nanoparticle (AgNPs) using *L. runcinata* DC extract was carried out by dissolving 1 mM of silver nitrate ($AgNO_3$) in deionized water, followed by adding the plant extract in a 1:1 proportion with continuous agitation. The mixture was then left to react at ambient temperature for 24 hours, resulting in a visible color shift, signaling the formation of nanoparticles. The synthesized silver nanoparticles (AgNPs) were characterized through UV-Vis spectroscopy, XRD, and SEM to evaluate their optical, structural, and morphological features. This approach offers a sustainable and economical method of synthesis[13].

2.3 Characterization of Silver Nano Particle by UV Spectroscopy

UV-Visible spectroscopy was utilized to assess nanoparticle formation, providing valuable information on their stability and aggregation. The process involved preparing solutions at concentrations of 1%, 2.5%, and 5%. These solutions were heated in water for 30 minutes at 100°C, and then filtered using Whatman No. 1 filter paper, with the resulting filtrate serving as a reducing agent in the nanoparticle synthesis. A 1 mM $AgNO_3$ stock solution was prepared[13]. *L. runcinata* DC leaf extracts (25 mL) were combined with an equal volume of $AgNO_3$ solution in a 1:1 ratio under dark conditions. Color changes and nanoparticle formation were confirmed using UV-Vis spectroscopy, with absorbance values at 270.5 nm recorded as 3.087, 2.079, and 1.716, respectively[14,15].

2.4 Characterization of Silver Nano Particle by SEM

SEM was utilized to investigate the size and morphology of the nanoparticles. To prepare for the analysis, a 5% solution was formulated along with a 1 mM silver nitrate ($AgNO_3$) solution. The two solutions were then blended in equal volumes, creating a homogeneous mixture. This mixture was further processed to yield a powder extract, which was then subjected to SEM analysis to accurately determine the size, shape, and surface characteristics of the nanoparticles. SEM provides a high-resolution imaging technique that enables detailed observation of the nanoparticle surface and structural features, offering crucial insights into their dimensions and distribution[16-18].

2.5 Characterization of Silver Nano Particle by XRD

The silver nanoparticles (AgNPs) were isolated by centrifugation at 10,000 rpm for 20 minutes, rinsed with purified water, and air-dried. Their crystalline structure was characterized by XRD analysis using a diffractometer with Cu Kα radiation (λ = 0.154 nm). Diffraction patterns were captured within a 2θ angle range of 20°–80°[19,20].

2.6 Characterization of Silver Nano Particle by FTIR

FTIR was applied to detect functional moieties within the extract contributing to nanoparticle synthesis, with spectra obtained within the 4000–400 cm^{-1} range. Significant peaks were evaluated to verify biomolecule involvement[21,22].

2.7 Anti-microbial Study

The antimicrobial activity of the synthesized silver nanoparticles (AgNPs) was assessed using the agar well diffusion technique. Overnight cultures of *E. coli*, *S. aureus*, and *P. aeruginosa* were evenly spread on nutrient agar plates. Wells were then created on the agar surface; each filled with 100 µL of the silver nanoparticle (AgNPs) solution. The plates were incubated at 37°C for 24 hours. The inhibition zones were measured in millimeters to determine the antimicrobial effectiveness. Standard antibiotics served as positive controls, while the solvent alone acted as the negative control. The experiment was conducted in triplicate, and the results were reported as the mean inhibition zone[23,24].

2.8 Molecular Docking Study for Anti-microbial Activity

Molecular docking is a computational technique commonly used in drug discovery and the examination

Table 1. Identified compounds of the hydroalcoholic extract of *L. runcinata* DC by GC-MS Study[26]

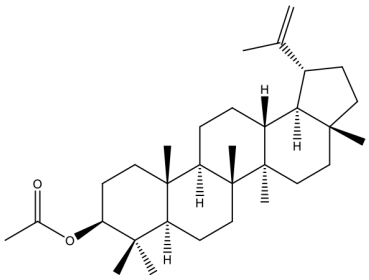
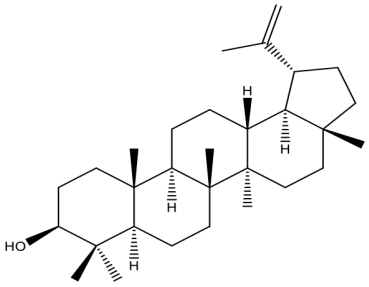
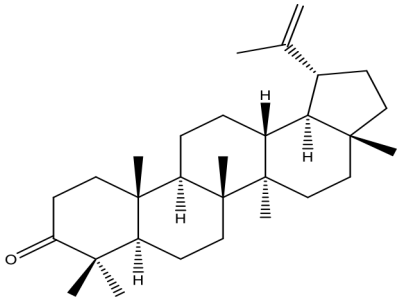
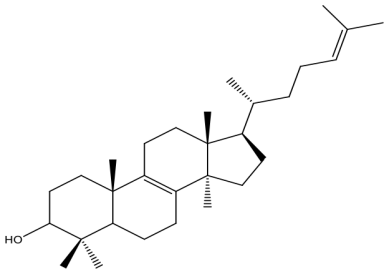
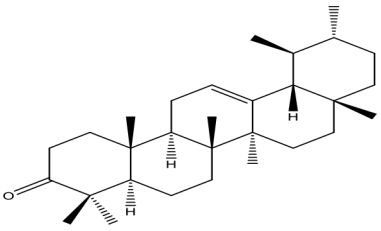
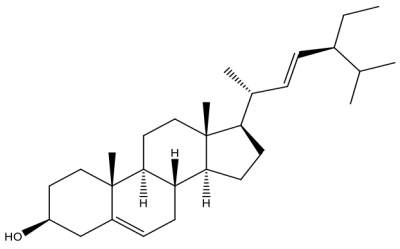
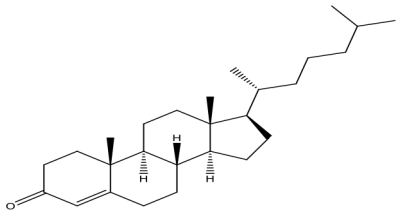
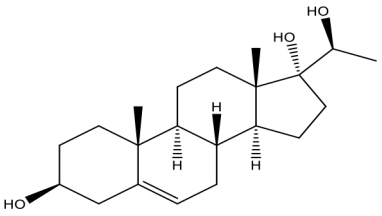
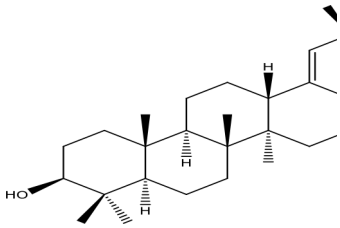
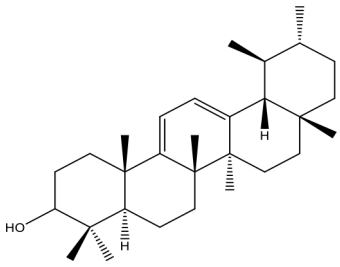
Sl. No.	Retention time	Percentage of peak area (%)	Compound Name	Structure
1	57.493	7.51	Lup-20(29)-en-3-ol	
2	59.533	6.31	Lupeol	
3	56.907	5.61	Lup-20(29)-en-3-one	
4	57.264	2.59	Lanosterol	
5	56.021	2.53	Alpha. -Amyrone	
6	54.753	1.91	Stigmasterol	

Table 1. Continued...

Sl. No.	Retention time	Percentage of peak area (%)	Compound Name	Structure
7	58.115	1.14	Cholest-4-en-3-one	
8	58.99	0.86	5-Pregnen-3. beta.,17. alpha.,20. beta. -triol	
9	56.146	0.7	Germanicol	
10	55.113	0.38	Ursa-9(11),12-dien-3-ol	

of molecular interactions. It facilitates the prediction of binding affinities and interactions between small molecules, referred to as ligands, and specific target proteins, known as receptors. In this study, two biologically significant proteins, Cyclin Dependent Kinase 8 (PDB ID- 5FGK), chosen for its antimicrobial properties, were analyzed. Employing Auto Dock Vina, widely utilized molecular docking software, the research aimed to investigate the binding affinities of phytochemical constituents (Table 1) extracted from *L. runcinata* DC with these target proteins[25] (Figure 1).

3. Result and Discussion

3.1 Characterization of Synthesized Silver Nanoparticles by UV Spectroscopy

The colloidal silver nanoparticle solutions were diluted tenfold using distilled water. The reduction of silver ions was monitored by recording UV-Vis spectra of the colloidal solutions at various time points, within the 200-700 nm visible spectrum range. Absorption peaks were consistently measured at moderate intervals, with the highest peak observed across all concentrations. The characteristic peaks for silver nanoparticles appeared at 270.5 nm for biologically synthesized nanoparticles at concentrations of 1.0%, 2.5%, and 5.0%, respectively (Figure 2).

3.2 Characterization of Synthesized Silver Nanoparticles by SEM

Particle size analysis was conducted using SEM, revealing that the biosynthesized *L. runcunata* AgNPs were uniform and spherical, with an average size of 10 µm. At higher magnifications, the nanoparticle structure became more defined. The SEM images were influenced by electrostatic interactions between bioorganic capping molecules on the



Figure 1. 3D structure of cyclin-dependent kinase 8 (PDB ID-5FGK)[27].

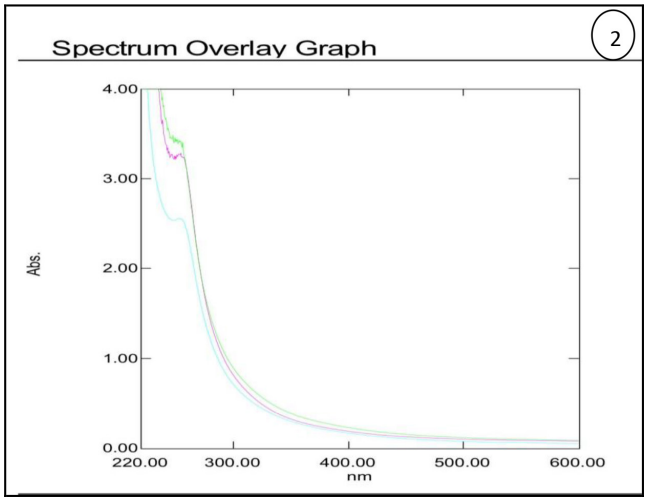


Figure 2. UV spectra of the synthesized silver nanoparticles.

AgNP surfaces. Factors such as nanoparticle aggregation and the SEM measurement process may have contributed to the formation of larger AgNPs (Figure 3).

3.3 Characterization of Synthesized Silver Nanoparticles by XRD

3.3.1 Characteristic Peaks

The XRD pattern displays distinct, sharp peaks, indicating a crystalline structure. Typical 2θ values for silver nanoparticles appear near 38°, 44°, 64°, and 77°, corresponding to the (111), (200), (220), and (311) crystallographic planes of face-centered cubic (FCC) silver (Figure 4).

3.3.2 Crystalline Structure

The strong peaks, particularly around 38° and 44°, indicate the presence of well-crystallized silver nanoparticles with an FCC configuration, characteristic of silver.

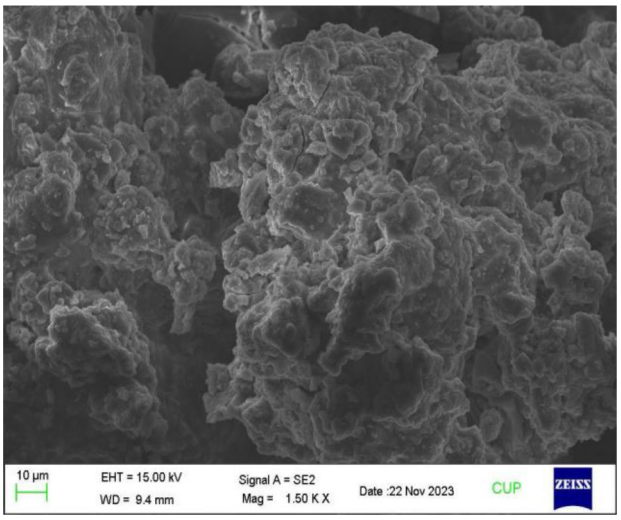


Figure 3. SEM image of synthesized silver nanoparticle.

3.3.3 FWHM (Full Width at Half Maximum)

Refers to the width of the most prominent peak, commonly around 38° for silver nanoparticles.

3.3.4 Bragg Angle (θ)

Represents half of the 2θ value for the most intense peak observed in the XRD pattern.

3.4 Characterization of Synthesized Silver Nanoparticles by FTIR

The FTIR spectrum analysis revealed a broad peak at 3297.86 cm^{-1}, attributed to O-H stretching vibrations, indicating the presence of hydroxyl groups likely derived from water or alcohol groups associated with the nanoparticle surface or bioorganic capping agents used in synthesis. The peak at 1638.84 cm^{-1} corresponds to C=O stretching, suggesting the presence of proteins or other bioorganic molecules in the capping layer of the silver nanoparticles.

The absorption at 1376.53 cm^{-1} is likely due to C-H bending, pointing to alkanes or methylene groups in the surrounding organic layer. At 1080.91 cm^{-1}, C-O stretching vibrations were observed, which may indicate the presence of alcohol, ether, or ester groups. Additionally, the peaks at 594.93 cm^{-1} and 816.70 cm^{-1} fall within the fingerprint region, potentially representing metal-oxygen interactions, such as Ag-O bonds, further confirming the interaction between silver nanoparticles and bioorganic molecules.

These findings from the FTIR spectrum highlight the presence of functional groups on the surface of silver nanoparticles, indicating the role of organic capping agents in stabilizing the nanoparticles and facilitating their formation (Figure 5).

3.5 Antimicrobial Activity

The antimicrobial effectiveness of *L. runcinata* DC silver nanoparticles was assessed against *E. coli, S. aureus*, and *P. aeruginosa* at different concentrations (50, 100, 150, and 200 µg/ml). At 50 µg/ml, the inhibition zones measured 2.26 cm^2 for *E. coli*, 3.4 cm^2 for *S. aureus*, and 6.16 cm^2 for *P. aeruginosa*, reflecting the lowest antimicrobial potency. As the concentration increased, there was a notable enhancement in inhibition across all bacterial strains. At 100 µg/ml, the inhibition zones were 3.14 cm^2 for *E. coli*, 15.90 cm^2 for *S. aureus*, and 4.15 cm^2 for *P. aeruginosa*, indicating a marked antimicrobial effect on *S. aureus* at this concentration.

At 150 µg/ml, the inhibition zones expanded to 4.15 cm^2 for *E. coli*, 7.068 cm^2 for *S. aureus*, and 4.15 cm^2 for *P. aeruginosa*. The most pronounced antimicrobial activity was observed at 200 µg/ml, with inhibition zones of 8.04 cm^2 for *E. coli*, 7.547 cm^2 for S. aureus, and 6.605 cm^2 for *P. aeruginosa*. These results underscore the strong antimicrobial potential of *L. runcinata* DC silver nanoparticles, which escalates with concentration. The highest activity was noted against *E. coli* at 200 µg/ml, supporting the nanoparticles' prospective application as an effective antimicrobial agent (Table 2).

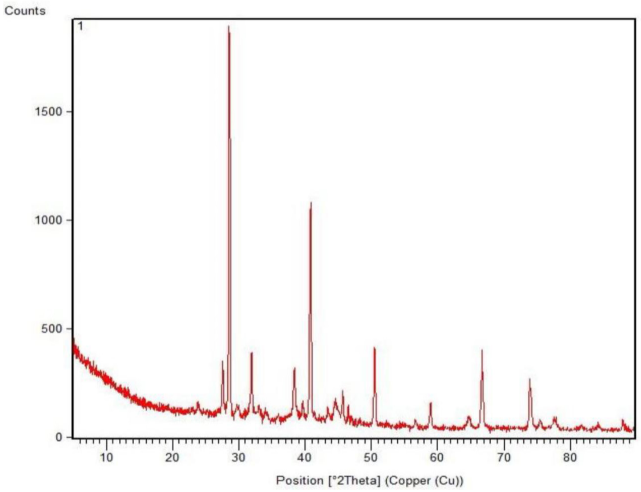


Figure 4. XRD analysis of the synthesized silver nanoparticle.

3.6 Molecular Docking Study for Antimicrobial Activity

The binding affinity of the top three identified compounds from *L. runcinata* DC with CDK8 was assessed. Alpha Amyrone demonstrated the highest binding affinity at -12.2 kJ/mol, closely followed by Lupeol at -12.1 kJ/mol, and Ursa-9(11),12-dien-3-ol at -11.8 kJ/mol (Table 3 and Figure 6). These compounds exhibited significant interactions with the CDK8 receptor, suggesting their potential as potent inhibitors.

In terms of bonding characteristics (Table 4), Alpha Amyrone established notable Pi donor hydrogen bonds with TRP 105, along with Pi-Alkyl interactions with ALA 172, ALA 155, and Alkyl bonds with ILE 79 and PHE 97. Lupeol exhibited Pi-Sigma interactions with PHE 97, Alkyl interactions with ALA 50, LEU 158,

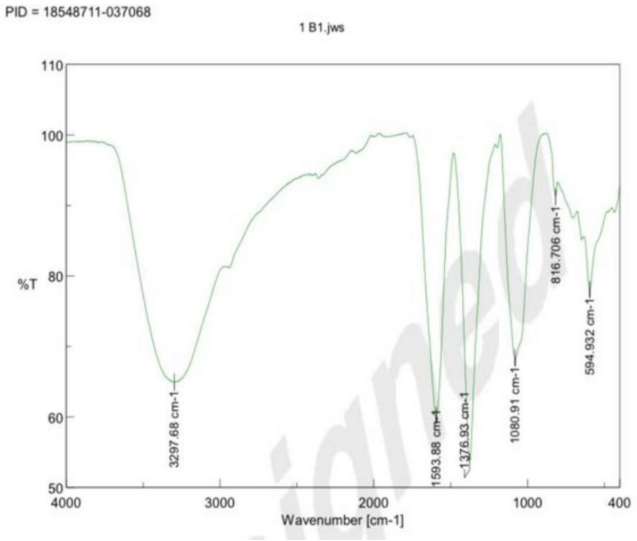


Figure 5. FTIR analysis of the synthesized silver nanoparticle.

Table 2. Zone of inhibition of *L. runcinata* DC. for Gram-positive and Gram- negative bacteria at 50,100,150,200µg/ml concentration

Concentration (µg/ml)	Zone of inhibition (cm^2) of *E. coli*	Zone of inhibition (cm^2) of *S. aureus*	Zone of inhibition (cm^2) of *P. aeruginosa*
50	2.26	3.4	6.16
100	3.14	15.90	4.154
150	4.15	7.068	4.15
200	8.04	7.547	6.605

Table 3. Binding affinity of three best fitted identified compounds of *L. runcinata* DC with cyclin-dependent kinase 8 (5FGK)

Sl. No.	Ligand	Binding Affinity (KJ/mol)
1	Alpha Amyrone	-12.2
2	Lupeol	-12.1
3	Ursa-9(11),12-dien-3-ol	-11.8

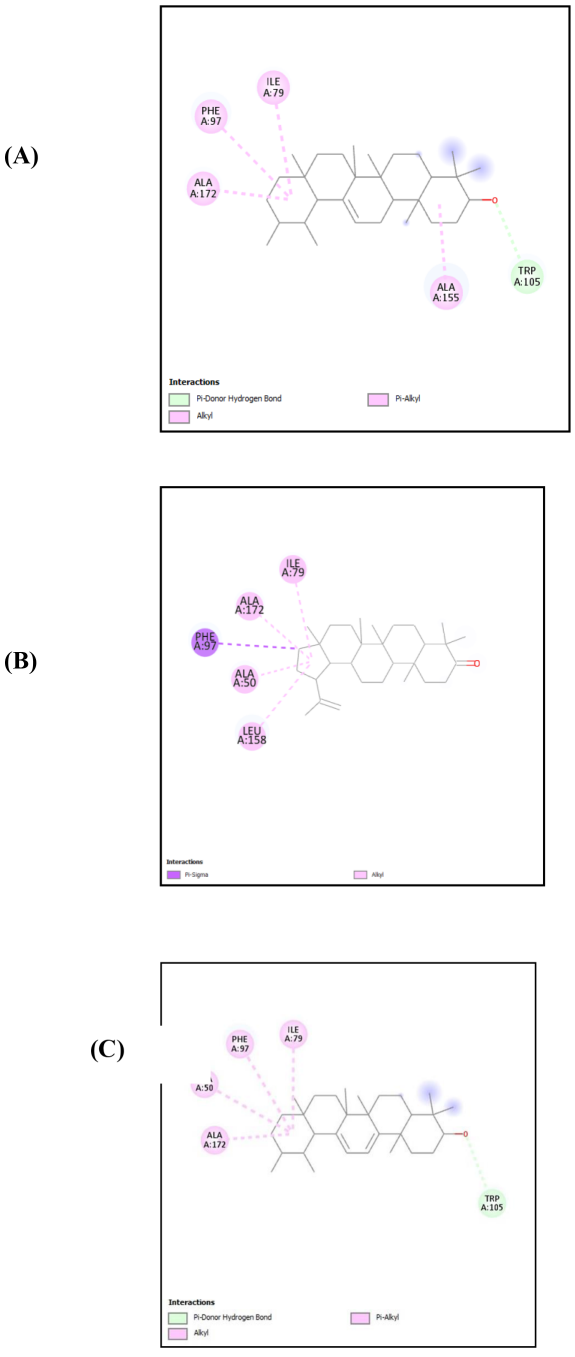


Figure 6. Interaction with 5FGk with (A)-alpha amyrone (B)-lupeol (C)-ursa-9(11),12-dien-3-ol.

Table 4. Different types of bonding formed of the three best-fitted identified compounds of *L. runcinata* DC with cyclin-dependent kinase 8 (5FGK)

Sl. No.	Ligand	Bonding with the Receptor CDK8 Kinase
1	Alpha Amyrone	**Pi Donor Hydrogen Bond**- TRP 105, **Pi Alkyl-** ALA 172, ALA 155, **Alkyl**- ILE 79, PHE 97
2	Lupeol	**Pi Sigma**- PHE 97, **Alkyl**- ALA 50, LEU 158, ILE 79, ALA 172,
3	Ursa-9(11),12-dien-3-ol	**Pi Donor Hydrogen Bond**-TRP 105, **Pi Alkyl**- ALA 172, ALA 50, **Alkyl**- PHE 97, ILE 79

ILE 79, and ALA 172. Ursa-9(11),12-dien-3-ol formed Pi donor hydrogen bonds with TRP 105, Pi-Alkyl interactions with ALA 172 and ALA 50, and Alkyl bonding with PHE 97 and ILE 79.

These interactions indicate that these compounds could play a crucial role in inhibiting CDK8, an essential enzyme in numerous cancer-associated pathways. The high binding affinities and favorable bonding interactions of these compounds with CDK8 position *L. runcinata* DC silver nanoparticles as potential candidates for further exploration in antimicrobial drug development.

4. Conclusion

This research effectively illustrates the eco-friendly synthesis of silver nanoparticles (AgNPs) utilizing *L. runcinata* DC extract, emphasizing their notable antimicrobial characteristics. Characterization methods such as UV-Vis spectroscopy, SEM, XRD, and FTIR validated the successful formation and structural stability of the AgNPs. Antimicrobial assays demonstrated robust activity against both Gram-positive and Gram-negative bacteria, with the greatest effectiveness seen against *E. coli*. Molecular docking analyses further revealed strong binding affinities between key phytochemicals from the extract and Cyclin-dependent kinase 8 (CDK8), hinting at their potential role in suppressing microbial proliferation and possibly contributing to antimicrobial effects. These findings highlight the promise of *L. runcinata* DC silver nanoparticles as an antimicrobial agent. This study opens avenues for further investigations into the therapeutic applications of these nanoparticles in

addressing multidrug-resistant infections and antimicrobial-related mechanisms.

5. Author Contribution

Surajit Maity designed the research framework, carried out the synthesis of nanoparticles, and drafted the manuscript. Suman Pattanayak and Lakshmi Kanta Kanthal supported the antimicrobial assays and material characterization. Soumyadeep Manna and Anirudha Das performed molecular docking studies and helped analyze the results. All authors have read and agreed to the published version of the manuscript.

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