Phytomediated biofabrication of silver nanoparticles derived from *Athyrium filix-femina* and their antibacterial potential against bacterial pathogens

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Abstract

The present study investigates the in vitro antibacterial potential of silver nanoparticles (AgNPs) synthesized through a green approach using acetone leaves extract of Athyrium filix-femina. The plant extract served both as a reducing and stabilizing agent in the eco-friendly synthesis of AgNPs. The antibacterial activity of the crude extract and biosynthesized AgNPs was evaluated against Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus aureus and Salmonella typhi using the agar well diffusion assay. Phytochemical profiling of the extract was performed and the synthesized AgNPs were characterized using UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The crude leaves extract exhibited moderate antibacterial activity with inhibition zones of B. subtilis (20.02 ± 0.421 mm), P. aeruginosa (17.9 ± 0.249 mm) and E. coli (12.2 ± 0.424 mm). In contrast, AgNPs showed enhanced activity, with inhibition zones of B. subtilis (21.06 ± 0.471 mm), P. aeruginosa (19.7 ± 0.295 mm) and S. aureus (14.6 ± 0.432 mm). The MIC values for AgNPs ranged from 0.009875 to 0.0395 mg/100 mL which were substantially lower than those of the crude extract 0.0781–0.3125 mg/100 mL, indicating superior antibacterial potency. These findings highlight the potential of Athyrium filix-femina-mediated AgNPs as promising candidates for the development of novel plant-based antimicrobial agents through an environmentally sustainable synthesis approach.

Keywords: Antimicrobial drugs, MIC, Nanoparticles, XRD, FTIR.

Introduction

For centuries, medicinal herbs have functioned as a fundamental source of pharmaceuticals. India boasts a long-standing tradition of utilizing plant-derived therapeutics for preventive and curative purposes, as evidenced in ancient practices such as Ayurveda, Siddha and Homeopathy. An estimated 88% of the global population relies on plant-based therapeutics to maintain good health and to treat various ailments[18].

Graphical Abstract

The increasing ineffectiveness of chemotherapeutics and the rise in bacterial resistance towards existing antibiotics have emphasized the immediate need to identify novel and potent antimicrobial molecules derived from environmental sources[25]. In recent years, plant-derived medications have experienced considerable attention owing to their potential effectiveness and minimal adverse effects[16]. Plants possess a diverse array of valuable secondary metabolites like tannins, alkaloids, flavonoids, glycosides, terpenoids, steroids, coumarins, saponins, polysaccharides, phenols and gums serving as a defense mechanism against various microorganisms, herbivores and insects[1]. Extracts from multiple medicinal plants demonstrate significant antibacterial, antifungal and antioxidant qualities which can be improved by employing contemporary scientific approaches such as nanotechnology[2].

Nanotechnology is a rapidly evolving subject with numerous applications in pharmacology, therapeutics, microelectronics, sensing devices and drug delivery[8]. Green synthesized silver nanoparticles (AgNPs) are one of the most significant and intriguing forms of nanoparticles owing to their diverse medicinal benefits as well as their well-known anti-inflammatory, antiviral, antibacterial, antifungal and anti-angiogenic characteristics[12]. The sustainable manufacturing of nanoparticles harnessing several plant elements has drawn remarkable interest for being environmentally friendly, simple, safe and cost-effective[17]. Numerous research studies have demonstrated the green production of AgNPs using plant extracts.

Plant extracts serve a dual purpose in nanoparticle fabrication, simultaneously serving as reducing and stabilizing elements[4]. Previous research has successfully synthesized AgNPs fabricated using various plant components including fruits from *Acacia concinna*, *Taraxacum mongolicum*, *Solanum melongena*, *Syzygium cumini*, *Lagerstroemia speciosa*, *Stachys lavandulifolia*, *Arctium lappa*, *Dillenia indica*, *Mimusops elengi* and *Emblica officinalis*, as well as leaves from *Arbutus unedo*, *Scutellaria barbata*, *Stevia rebaudiana*, *Nicotiana tabacum*, *Ocimum sanctum*, *Eucalyptus globulus* and *Ficus benghalensis*.

The current research investigation generated AgNPs using acetone leaf extract sourced from *Athyrium filix-femina*, commonly referred to as lady fern. *A. filix-femina* is a resilient, deciduous fern native to temperate regions of the Northern Hemisphere, particularly in the parts of Asia, Europe and North America[26,28]. The fern is recognized for its antioxidant, anti-inflammatory, antimicrobial and antifungal properties[24]. *A. filix-femina* contains various phytochemicals including flavonoids, terpenoids, phenolic compounds and tannins[21,27].

Material and Methods

Collection of plant material: *Athyrium filix-femina* leaves were gathered from the Sarahan region in Shimla district, Himachal Pradesh. After cleaning, the leaves were air-dried under the shade, coarsely ground and stored in well-labeled, airtight plastic containers for future applications.

Test organisms: The pathogenic bacteria, predominantly Gram-positive species, *Staphylococcus aureus, Bacillus subtilis* and Gram-negative species, *Salmonella typhi, Proteus vulgaris, Escherichia coli and Pseudomonas aeruginosa*, were obtained from IMTECH, Chandigarh and cultured on the nutrient agar medium. The selected test organisms and the antibiotics used as positive controls are tabulated in table 1.

Table 1
List of bacterial pathogens and corresponding antibiotics

Bacterial Pathogens	Antibiotics used (Positive control)
Staphylococcus aureus	Gentamycin
Proteus vulgaris	Gentamycin
Bacillus subtilis	Tetracycline
Pseudomonas aeruginosa	Gentamycin
Salmonella typhi	Chloramphenicol
Escherichia coli	Tetracycline

Preparation of *Athyrium-filix-femina* extract: The extracts from the plant were prepared using the cold percolation technique as outlined by Rosenthaler[19]. Dried and powdered plant materials were immersed in various solvents (1:10) and kept at 37°C for 24 hrs with continuous stirring at 100 rpm. After incubation, the samples were subsequently filtered using Whatmann filter paper and the resulting filtrates were carefully dried (evaporation). The dry extract was finely powdered and solubilized in 10% DMSO (Dimethyl sulfoxide), attaining a final concentration of 100 mg/ml[13,15].

Assessment of concentration dependent bactericidal potency of *Athyrium-filix-femina* extract

Agar well diffusion assay: The antimicrobial effectiveness of acetone leaves extract against bacterial pathogens was determined through the agar well diffusion technique, with the inhibition zones (measured in mm) noted after 24 hrs of incubation time. The minimal growth-inhibitory concentration (MIC) of leaf-derived crude extract needed to suppress microbial proliferation was determined by the Resazurin dye method, employing 2-fold serial dilutions of the extract[7,9].

Minimum inhibitory concentration assay: The minimum inhibitory concentration (MIC) is a key microbiological metric for determining the lowest concentration of a substance such as a plant extract required to inhibit the visible growth of a specific microorganism under controlled conditions. Plant extracts, rich in bioactive compounds like alkaloids, flavonoids and phenolics, exert antimicrobial effects by disrupting cellular structures, inhibiting enzymatic activity, or altering membrane integrity[29].

Phytochemical screening of acetone leaf extract of *Athyrium filix-femina*: Phytochemical analysis of the acetone extract derived from the leaves of *Athyrium filix-femina* was conducted to identify the existence of various phytoconstituents including flavonoids, alkaloids, terpenoids, carbohydrates, tannins, saponins, soluble starch and steroids[14]. The analysis was carried out employing the methodology outlined by Harborne[10].

Synthesis and characterization of silver nanoparticles derived from *Athyrium filix-femina*: An aqueous silver nitrate ($AgNO_3$) solution with a concentration of 1 millimolar (1mM) was formulated for the synthesis of silver nanoparticles (AgNPs)[11]. The silver nanoparticles (AgNPs) were synthesized via co-precipitation, utilizing acetone leaves extract from *Athyrium filix-femina*, simultaneously serving as a reducing and capping agent. UV-visible spectroscopy, X-ray diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy were employed to characterize the biologically synthesized silver nanoparticles (AgNPs). UV-visible spectroscopy was employed to analyze the surface plasmon resonance (SPR) behavior, providing insights into the optical properties and size distribution of nanoparticles.

XRD was applied to determine the crystalline structure and phase purity, while FTIR spectroscopy elucidated the surface functional groups and biomolecular interactions involved in the synthesis and stabilization of AgNPs. Collectively, these techniques offer a comprehensive understanding of the structural, optical and surface characteristics of AgNPs, ensuring their optimized design and functionality for advanced applications in catalysis, biomedicine and antimicrobial activity.

Results

This study assessed the bactericidal potential of acetone extracts from *Athyrium filix-femina* leaves against both the Gram-positive bacterial pathogens (e.g. *Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative pathogens (e.g. *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*) by employing the agar well diffusion approach. The findings derived from the antimicrobial examination of *Athyrium filix-femina* leaves are illustrated in figure 1.

Antimicrobial activity evaluation using acetone leaf extract *of Athyrium filix-femina*: The acetone leaf extract demonstrated a broad-spectrum antimicrobial efficacy against all tested pathogens except *Salmonella typhi*, which exhibited no susceptibility (Table 1, Supplementary). As shown in figures 1 and 2, with 20µl of the acetone extract, the maximum inhibition zone was observed against *Bacillus subtilis* (20.02±0.421 mm) and *Pseudomonas aeruginosa* (17.9±0.249 mm). Moderate inhibitory effects were noted against *Escherichia coli* (14.2±0.424 mm) and *Proteus vulgaris* (12.27±0.225 mm). The minimal activity level was recorded targeting *Staphylococcus aureus* (10.7±0.244 mm).

Minimum inhibitory concentration of the acetone leaf extract of *Athyrium filix-femina*: The minimum inhibitory concentration (MIC) of the acetone leaves extract from *Athyrium filix-femina* against *Salmonella typhi* was found to be 0.3125 mg/100µl.

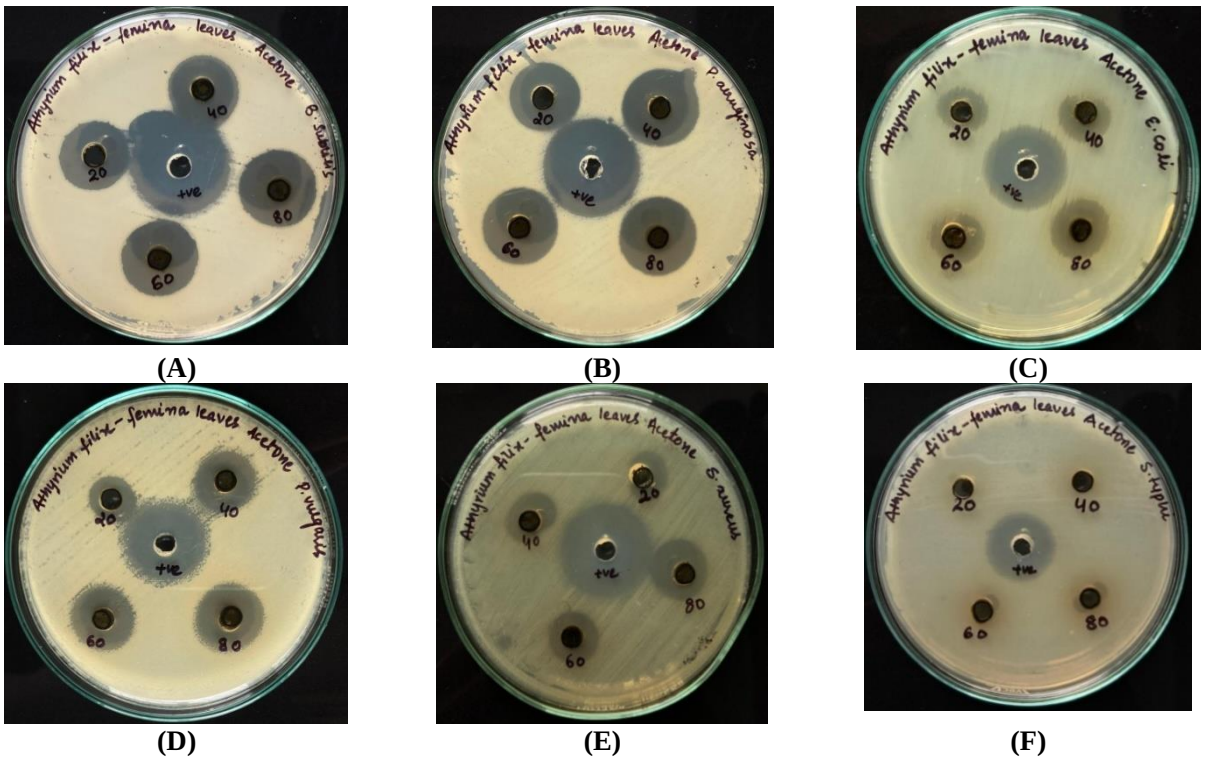


Figure 1: Antimicrobial action of *Athyrium filix-femina* acetone leaves extract on pathogenic microbes (A) *B. subtilis*, (B) *P. aeruginosa*, (C) *P. vulgaris* (D) *E. coli*, (E) *S. aureus*, (F) *S. typhi*

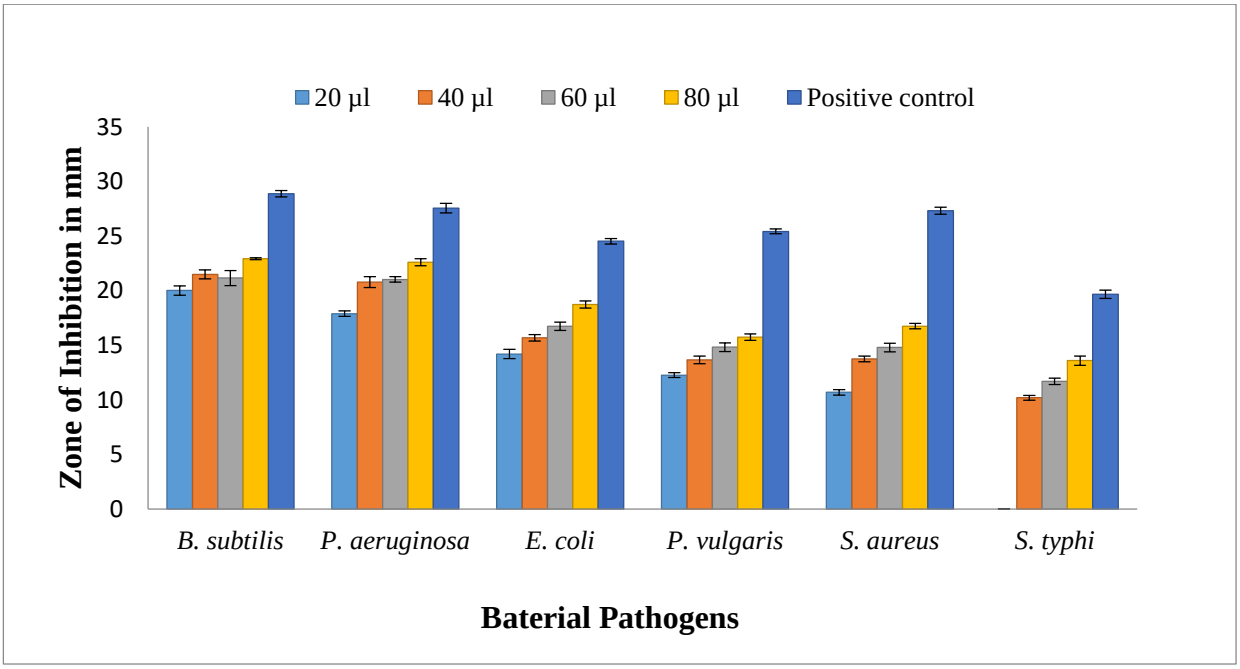


Figure 2: Antimicrobial potential of acetone extract of *Athyrium filix-femina* leaves

Supplementary Table 1
Antimicrobial activity of acetone extract from *Athyrium filix-femina* leaf on various pathogenic strains

Plant extracts	Microorganisms	20 µl	40 µl	60 µl	80 µl	Positive control
Acetone	*B. subtilis*	20.02 ± 0.421	21.5±0.408	21.16±0.694	22.93±0.094	28.89±0.294
	P. aeruginosa	17.9±0.249	20.8±.0.492	21.03±0.249	22.61±0.326	27.56±0.432
	E. coli	14.2±0.424	15.7±0.294	16.74±0.377	18.74±0.309	24.54±0.249
	P. vulgaris	12.27±0.225	13.67±0.339	14.83±0.385	15.76±0.294	25.43±0.224
	S. aureus	10.7±0.244	13.76±0.262	14.8±0.384	16.76±0.262	27.32±0.326
	S. typhi	0	10.2±0.224	11.7±0.294	13.6±0.432	19.67±0.377

Supplementary Table 2
Antimicrobial efficacy of silver nanoparticles synthesized from *Athyrium filix-femina* leaf

Plant extracts	Microorganisms	20 µl	40 µl	60 µl	80 µl	Positive control
Acetone	*B. subtilis*	21.31 ± 0.471	22.86±0.094	24.08±0.076	26.67±0.347	28.07±0.765
	P. aeruginosa	19.72±0.295	21.83±0.169	23.76±0.265	26.43±0.987	27±0.094
	E. coli	16.03±0.432	18.88±0.163	24.80±0.234	25.46±0.768	24±0.124
	P. vulgaris	14.27±0.205	17.86±0.124	19.65±0.765	21.34±0.643	25±0.234
	S. aureus	12.7±0.294	16.03±0.124	18.87±0.234	20.23±0.786	27±0.294
	S. typhi	10.2±0.224	12.05±0.297	13.98±0.098	14.87±0.236	19±0.169

Similarly, the MIC for *Pseudomonas aeruginosa* and *Escherichia coli* was recorded at 0.1562 mg/100µl. For *Bacillus subtilis*, a lower concentration of 0.0781mg/100µl was required to inhibit growth. In the case of *Staphylococcus aureus*, the MIC was determined to be 0.3125mg/100µl as exhibited in figure 3.

Phytochemical analysis of *Athyrium filix-femina* (Acetone leaf extract): The findings of a preliminary (qualitative) phytochemical investigation of the crude leaves extract of *Athyrium filix-femina* revealed the presence of different plant chemicals such as glycosides, flavonoids, alkaloids, tannins, steroids and saponins as indicated in table 2.

Characterization of biologically synthesized silver nanoparticles (AgNPs)

Standardization of silver nanoparticles (AgNPs): The characteristics of green synthesized silver nanoparticles (AgNPs) were determined by variables such as their morphology (shape), dimensions (size) and spatial arrangement (distribution). In the current research work, silver nanoparticles were developed by utilizing *Athyrium filix-femina* leaves extract as a reducing compound, with aqueous silver nitrate ($AgNO_3$) as the precursor component. The generated AgNPs were centrifuged for separation at 15,000 rpm for 20 mins. The procedure was performed again and the resulting pellets were redispersed in water yielding a clear supernatant. The formation of AgNPs was validated by

a significant color change from colorless to dark brown. The green-synthesized AgNPs were characterized by UV-visible spectroscopy, X-ray diffraction (XRD) and Fourier-transform infrared (FTIR) Spectroscopy, as depicted in figure 4.

UV-visible spectrophotometry: The reduction of silver ions (Ag^{+}) ions into metallic silver (Ag^{0}) was monitored with acetone extract of *Athyrium filix-femina* leaves through UV-visible spectroscopy. The absorption spectrum for measuring the wavelength range was between 300 and 700 nm. The analysis exhibited absorbance maxima at 421 nm, validating the formation of silver-based nanoparticles (AgNPs) within the solution mixture as demonstrated in figure 5A.

X-ray diffraction (XRD): The X-ray diffractogram of the synthesized AgNPs has been illustrated in figure 5B. XRD analysis was conducted on powdered AgNPs from *Athyrium filix-femina* leaves to affirm the presence of silver and to examine its crystallographic properties. The XRD pattern verified the crystalline structure of AgNPs, displaying distinctive diffraction peaks at 38.130°, 44.353°, 64.429° and 77.550°, aligning with the 111, 200, 220 and 311 Bragg peaks of silver. The size of the largest AgNPs was estimated to be 181.36 nm.

In comparison, the smallest was approximately 159.76 nm, determined by the full width at half maximum (FWHM) of the diffraction peaks calculated using the Scherrer equation. Along with the Bragg peaks, confirming the presence of a face-centered cubic structure, unidentified peaks imply the presence of bioorganic phase crystalline onto the nanoparticle surface, as depicted in figure 5B.

FT-IR analysis (Fourier-transform infrared spectroscopy): The synthesized silver nanoparticles of *Athyrium filix-femina* leaves were characterized using the FT-IR technique.

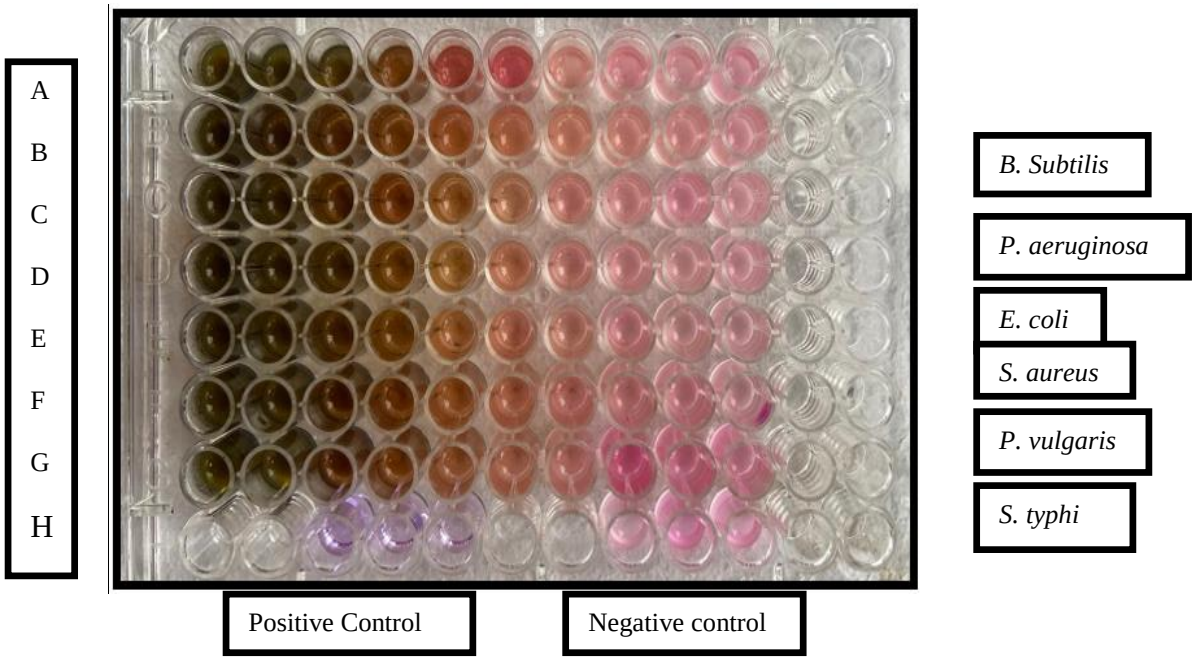


Figure 3: MIC of acetone leaves extract of *Athyrium filix-femina*

Table 2
Qualitative test for acetone extract of *Athyrium filix-femina leaves*

Phytochemical constituents	Results
Tannins	++
Saponins	+
Flavonoids	+
Quinones	++
Glycosides	+
Terpenoids	-
Phenol	++
Steroid	-

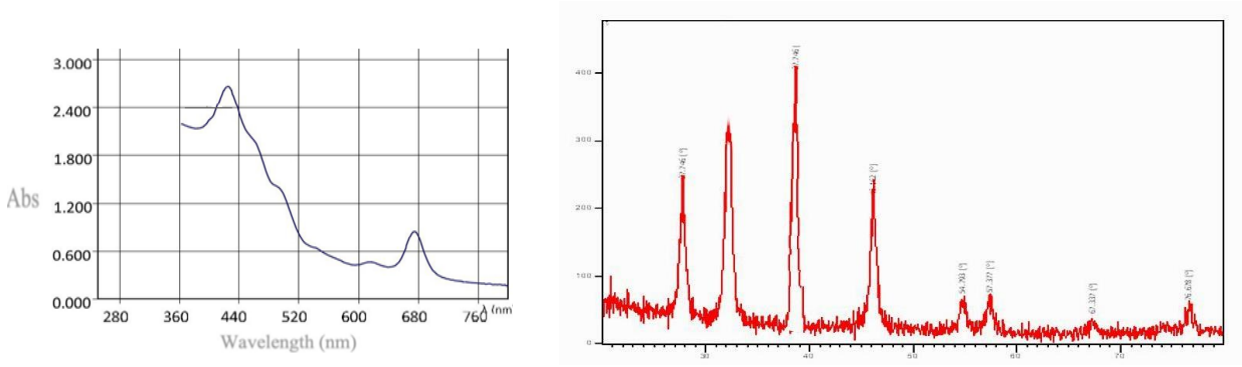
++: Strong Positive; +: Positive; -: Negative

The FT-IR spectra of *Athyrium filix-femina* synthesized AgNPs, as depicted in figure 5C, revealed distinct peaks at 3901.7 cm^{-1}, 3782.3 cm^{-1}, 3399.4 cm^{-1} and 573.5 cm^{-1}. The absorption bands at 3901.7 cm^{-1}, 3782.3 and 3399.4 cm^{-1} represent O-H symmetry stretching of alcohol groups, possibly associated with hydrogen bonding. Meanwhile, the vibration peak at 573.5 cm^{-1} represents the C-I stretching characteristic of alkyl and aryl halides exhibited in figure 5C.

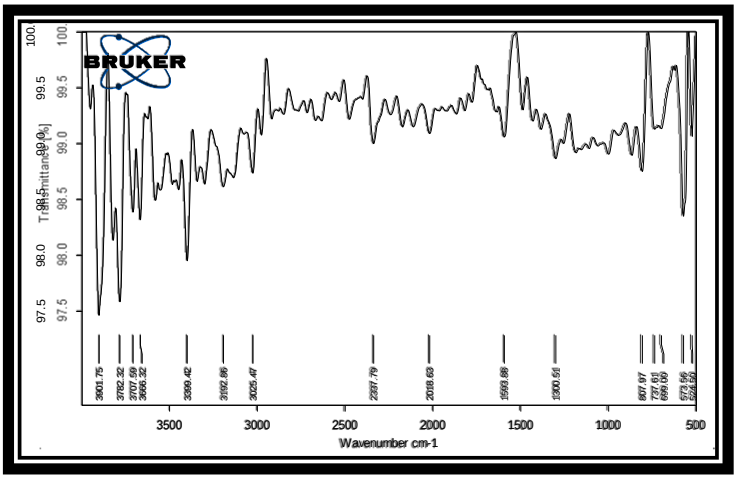
Antibacterial efficacy of silver nanoparticles: The microbicidal effect of silver-based nanoparticles (AgNPs) was assessed on multiple pathogenic bacterial strains. Maximum zone of inhibition was noted for *Bacillus subtilis* (21.06±0.471 mm), followed by *Pseudomonas aeruginosa* (19.7±0.295 mm), *Escherichia coli* (14.6±0.432 mm), *Proteus vulgaris* (13.97±0.205 mm) and *Staphylococcus aureus* (12.7±0.294 mm) with 20µl. The lowest inhibition effect was recorded against *Salmonella typhi* (10.2±0.224 mm) at the maximum concentration of plant extract, 80µl. Fig. 5 and fig. 6 demonstrate that the AgNPs exhibit greater effectiveness against all pathogenic microbes than the crude plant extract.

(A) **(B)** **(C)**

Figure 4: Biogenic synthesis of AgNPs using *Athyrium filix-femina* leaves
(A) $AgNO_3$ Solution; (B) $AgNO_3^+$ plant extract after 24 hrs; (C) Synthesized AgNPs



(A) **(B)**



(C)

Figure 5: Characterization of AgNPs (A) UV-VIS Spectrophotometry (B) X-Ray Diffraction (C) FT-IR spectrum of *Athyrium filix-femina* AgNPs

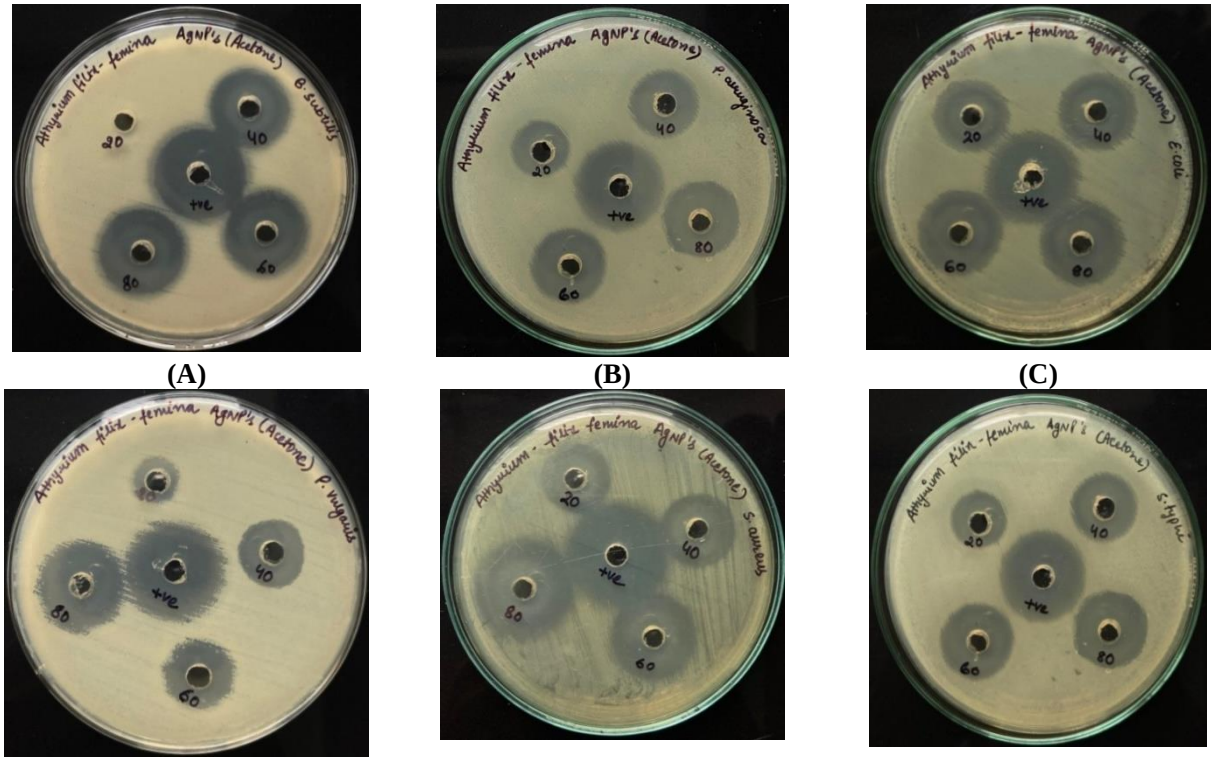


Figure 6: Effect of AgNPs from *Athyrium filix-femina* leaves extract against (A) *B. subtilis*, (B) *P. aeruginosa*, (C) *P. vulgaris* (D) *E. coli*, (E) *S. aureus*, (F) *S. typhi*

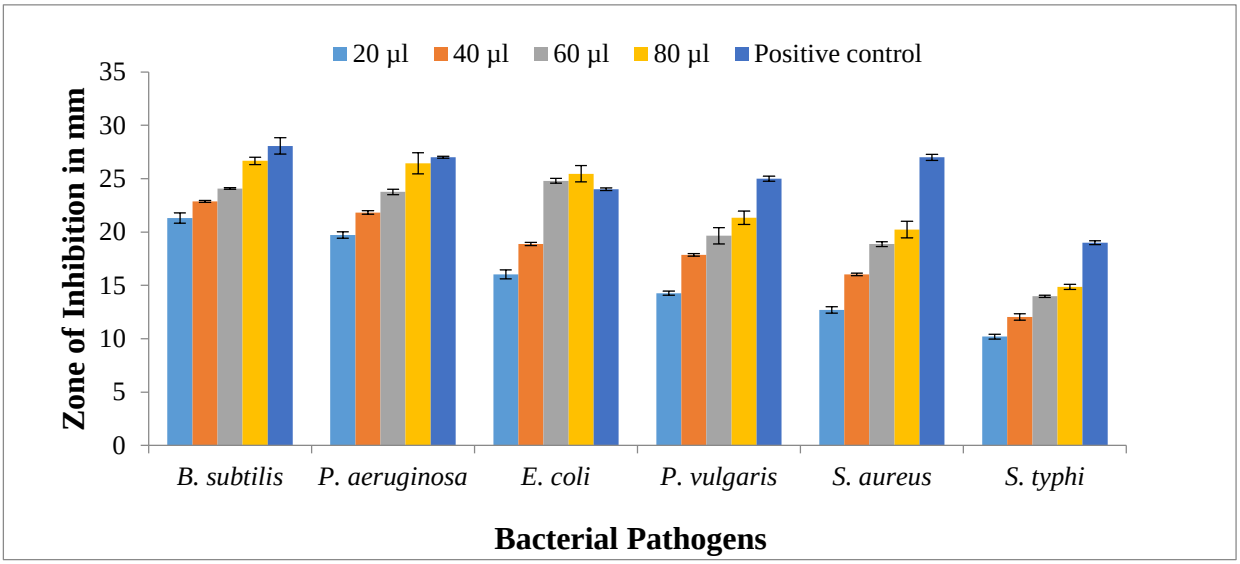


Figure 7: Antibacterial potency of silver nanoparticles (AgNPs) from *Athyrium filix-femina* acetone leaf extract

MIC of green-synthesized silver nanoparticles: The minimum concentration derived from *Athyrium filix-femina* silver nanoparticles (AgNPs) needed to suppress the growth of *S. typhi* was determined to be 0.1562 mg/100ml, significantly lower than that required for the crude extract. This was followed by *Staphylococcus aureus* and *P. aeruginosa* (0.0781 mg/100ml). *Bacillus subtilis, P. vulgaris* and *Escherichia coli* showed the least susceptibility compared to the other pathogens i.e. 0.1562 mg/100ml. Remarkably, the MIC of the biosynthesized AgNPs ranged from 0.009875 to 0.0395 mg/100ml, highlighting their efficacy compared to the MIC of the crude extract, which spanned from 0.0781 to 0.1562 mg/100ml as given in figure 8.

Discussion

In this study, *Athyrium filix-femina* acetone leaves extract was assessed for its antimicrobial efficacy against a range of pathogenic bacteria, comprising of Gram-positive pathogens like *Staphylococcus aureus* and *Bacillus subtilis* as well as Gram-negative microbial strains including *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Proteus vulgaris.*

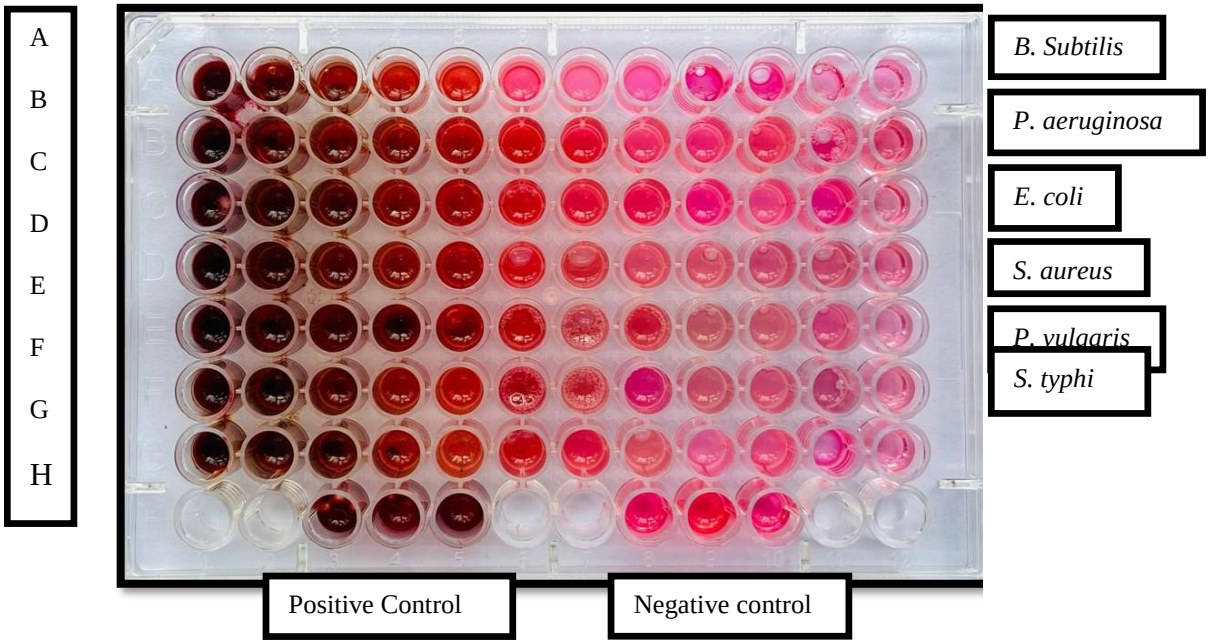


Figure 8: MIC of AgNPs of acetone leaves extract of *Athyrium filix-femina*

This observation aligns with earlier studies that have documented more substantial antimicrobial efficacy for different extracts than other solvents. This can be attributed to the organic nature of acetone and its superior competence in dissolving and releasing the bioactive antimicrobial components present in plants. The acetone extract demonstrated significant broad-spectrum antimicrobial activity in the present investigation, successfully inhibiting most tested pathogens. The highest inhibition zones were observed against *Bacillus subtilis* (20.02±0.421 mm) and *Pseudomonas aeruginosa* (17.9±0.249 mm). Moderate effects were seen against *Escherichia coli* (14.2±0.424 mm) and *Proteus vulgaris* (12.27±0.225 mm), while the lowest activity was against *Staphylococcus aureus* (10.7±0.244 mm).

Notably, *Salmonella typhi* showed no susceptibility to the extract. These findings highlight the extract's varying efficacy depending on the pathogen. In the year 2012, Soare et al[23] reported that the methanolic extract from *Athyrium filix-femina* revealed potent bactericidal efficacy against different bacterial pathogens, including *Staphylococcus aureus*, *Salmonella abony*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*. The polyphenolic macromolecules found in *Athyrium filix-femina* are responsible for their antibacterial potency. Plant chemicals exhibit potent antibacterial activity in several ways including breaking down the bacterial cell wall, damaging DNA, blocking protein synthesis and stopping energy generation.

Furthermore, they degrade proteins and inhibit enzymes to disrupt bacterial activity, facilitating the generation of reactive oxygen species (ROS) and leading to oxidative stress. They additionally obstruct efflux pumps, preventing hazardous molecules from being released by bacteria. These processes work collectively to offer the solid antibacterial activity of these plant extracts[6]. The therapeutic applications of flavonoids, alkaloids, tannins and saponins have received considerable global interest, particularly for their antibacterial properties. These compounds are present in higher concentrations within the methanolic extract of *A. parasnathense*, suggesting that their presence may play an integral part in the plant's renowned antibacterial properties[20].

The bioactive potential of silver nanoparticles (AgNPs) was evaluated on a series of bacterial pathogens, revealing that these AgNPs displayed significantly greater potency than the crude extract of the plant and the positive control. The AgNPs displayed the most potent inhibitory activity among the tested organisms, showcasing their superior antibacterial potential with a maximum inhibition against *Bacillus subtilis* (21.06±0.471 mm) followed by *Pseudomonas aeruginosa* (19.7±0.295 mm).

Moderate inhibitory effects were observed for *Escherichia coli* (14.6±0.432 mm) and *Proteus vulgaris* (13.97±0.205 mm), while *Staphylococcus aureus* exhibited the lowest susceptibility (12.7±0.294 mm). These findings indicate that AgNPs have variable antibacterial potency based on the specific bacterial strain. Additionally, a dosage of 25 µg/ml of biosynthesized AgNPs from *Adiantum lunulatum* showed significant antibacterial action against *Listeria monocytogenes* by 72.64%, *Escherichia coli* by 88.7% and *Salmonella typhimurium* by 48.45%, highlighting their excellent antibacterial potential[5]. Gram-positive bacteria are distinguished by a thick peptidoglycan layer lacking an outer membrane, in contrast to Gram-negative bacterial species, such as *E. coli*, which possess a thin peptidoglycan layer separating two membranes. This structural difference may enable Gram-negative bacteria to have increased redox

activity, forming Ag-species aggregates with cell particles[22]. The minimum inhibitory concentration (MIC), which varied from 0.009875 to 0.0395 mg/100 ml, AgNPs significantly outperformed the crude extract, which had an MIC range of 0.0781 to 0.3125 mg/100ml.

It is evident that AgNPs showed greater antimicrobial potency than oil plant extracts and they hold significant potential for use in pharmaceutical, biotechnological and biomedical applications. Previously, AgNPs synthesized from *Pteris tripartite* leaves have shown the minimum inhibitory concentration for 5 mg/mL AgNPs treatment using *Pteris tripartite* ranged from 0.36 to 0.61 OD toward a variety of bacterial organisms including *S. typhi*, *P. vulgaris*, *S. sonnei* and *V. cholerae*. Increasing the AgNP concentration to 10 mg/mL reduced MIC values for all tested bacteria, with *P. aeruginosa* showing the lowest MIC of 0.29 OD[3].

Conclusion

The present study emphasizes that the crude acetone extract of *Athyrium filix-femina* leaves demonstrates notable antimicrobial activity against bacterial pathogens, underscoring its potential as an effective antimicrobial agent for contemporary medicinal use. Furthermore, the research presents an environmentally sustainable and cost-effective approach for synthesizing silver nanoparticle (AgNPs) formation via the leaf extract, demonstrating superior antimicrobial activity to the raw plant extracts. These findings suggest that *Athyrium filix-femina* can be utilized to develop value-added products for the biotechnological, biomedical, pharmaceutical and nanotechnology industries.

Moreover, this approach may contribute to developing new herbal formulations to combat drug-resistant bacterial infections using sustainable practices. Future investigations should emphasize isolating and identifying the plant-derived bioactive antimicrobial agents and conducting *in vitro* and *in vivo* research to understand better the toxicological risks associated with AgNPs.

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References

1. Al-Khayri J.M., Rashmi R., Toppo V., Chole P.B., Banadka A., Sudheer W.N., Nagella P., Shehata W.F., Al-Mssallem M.Q., Alessa F.M. and Almaghasla M.I., Plant secondary metabolites: The weapons for biotic stress management, *Metabolites*, **13(6)**, 716 **(2023)**

2. Antunes Filho S., Dos Santos M.S., Dos Santos O.A.L., Backx B.P., Soran M.L., Opriș O., Lung I., Stegarescu A. and Bououdina M., Biosynthesis of nanoparticles using plant extracts and essential oils, *Molecules*, **28(7)**, 3060 **(2023)**

3. Baskaran X., Geo Vigila A.V., Parimelazhagan T., Muralidhara-Rao D. and Zhang S., Biosynthesis, characterization and evaluation of bioactivities of leaf extract-mediated biocompatible silver nanoparticles from an early tracheophyte, *Pteris tripartita* Sw, *International Journal of Nanomedicine*, **11**, 5789-5806 **(2016)**

4. Begum S.R. and Jayawardana N.U., Green synthesized metal nanoparticles as an eco-friendly measure for plant growth stimulation and disease resistance, *Plant Nano Biology*, **3**, 100028 **(2023)**

5. Chatterjee A., Khatua S., Acharya K. and Sarkar J., A green approach for the synthesis of antimicrobial bio-surfactant silver nanoparticles by using a fern, *Digest Journal of Nanomaterials and Biostructures*, **14**, 479-490 **(2019)**

6. Chau T.P., Narayanan M., Chinnathambi A., Pimpimon T., Pikulkaew S. and Pugazhendhi A., Phytochemicals, Antibacterial and Larvicidal Activities Profile of Various Crude Extracts of *Athyrium Parasnathense* and Characterized Their Bioactive Groups, *SSRN*, http://dx.doi.org/10.2139/ssrn.4002339 **(2022)**

7. Chen S., Li Z., Chitrakar B., Gu Z., Ban X., Hong Y., Cheng L. and Li C., Minimum Inhibitory Concentration (MIC) of Essential Oils Against Microorganisms: Methods, Function, Accuracy Factors and Application, *Food Reviews International*, **40**, 1-26 **(2024)**

8. Ezike T.C., Okpala U.S., Onoja U.L., Nwike C.P., Ezeako E.C., Okpara O.J., Okoroafor C.C., Eze S.C., Kalu O.L., Odoh E.C. and Nwadike U.G., Advances in drug delivery systems, challenges and future directions, *Heliyon*, **9(6)**, e17488 **(2023)**

9. Gonzalez-Pastor R., Carrera-Pacheco S.E., Zúñiga-Miranda J., Rodríguez-Pólit C., Mayorga-Ramos A., Guamán L.P. and Barba-Ostria C., Current landscape of methods to evaluate antimicrobial activity of natural extracts, *Molecules*, **28(3)**, 1068 **(2023)**

10. Harborne J.B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd ed., London, Chapman and Hall **(1998)**

11. Iravani S., Korbekandi H., Mirmohammadi S.V. and Zolfaghari B., Synthesis of silver nanoparticles: chemical, physical and biological methods, *Research in Pharmaceutical Sciences*, **9(6)**, 385-406 **(2014)**

12. Jangid H., Singh S., Kashyap P., Singh A. and Kumar G., Advancing biomedical applications: an in-depth analysis of silver nanoparticles in antimicrobial, anticancer and wound healing roles, *Frontiers in Pharmacology*, **15**, 1438227 **(2024)**

13. Khoddami A., Wilkes M.A. and Roberts T.H., Techniques for the extraction of bioactive compounds from plant materials: A review, *Phytochemistry Reviews*, **12(1)**, 147-170 **(2013)**

14. Lewis N.G., Davin L.B., Munasinghe V.R.N. and Roberts A., Eds., The Lignan Handbook, CRC Press **(2022)**

15. Mitra A. and Routray W., Bioactive Compounds in Cumin, In Spice Bioactive Compounds, CRC Press, 165-186 **(2022)**

16. Nasim N., Sandeep I.S. and Mohanty S., Plant-derived natural products for drug discovery: current approaches and prospects, *The Nucleus*, **65(3)**, 399-411 **(2022)**

17. Osman A.I., Zhang Y., Farghali M., Rashwan A.K., Eltaweil A.S., Abd El-Monaem E.M., Mohamed I.M., Badr M.M., Ihara I., Rooney D.W. and Yap P.S., Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural and food applications: A review, *Environmental Chemistry Letters*, **22(2)**, 841-887 **(2024)**

18. Pandey M.M., Rastogi S. and Rawat A.K.S., Indian traditional Ayurvedic system of medicine and nutritional supplementation, *Evidence-Based Complementary and Alternative Medicine*, **2013(1)**, 376327 **(2013)**

19. Rosenthaler L., The principles of cold percolation in plant extraction, *Journal of Pharmaceutical Sciences*, **19(4)**, 288-298 **(1930)**

20. Roy A., Khan A., Ahmad I., Alghamdi S., Rajab B.S., Babalghith A.O., Alshahrani M.Y., Islam S. and Islam M.R., Flavonoids a bioactive compound from medicinal plants and its therapeutic applications, *BioMed Research International*, **1**, 5445291 **(2022)**

21. Salehi B., Ezzat S.M., Fokou P.V.T., Albayrak S., Vlaisavljevic S., Sharifi-Rad M., Bhatt I.D., Sharifi-Rad M., Belwal T., Ayatollahi S.A. and Kobarfard F., Athyrium plants-review on phytopharmacy properties, *Journal of Traditional and Complementary Medicine*, **9(3)**, 201-205 **(2019)**

22. Silhavy T.J., Kahne D. and Walker S., The bacterial cell envelope, *Cold Spring Harbor Perspectives in Biology*, **2(5)**, a000414 **(2010)**

23. Soare L.C., Ferdes M., Stefanov S., Denkova Z., Nicolova R., Denev P., Bejan C. and Paunescu A., Antioxidant activity, polyphenols content and antimicrobial activity of several native pteridophytes of Romania, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **40**, 53-57 **(2012)**

24. Valizadeh H., Sonboli A., Mahmoodi Kordi F., Dehghan H. and Bahadori M.B., Cytotoxicity, antioxidant activity and phenolic content of eight fern species from north of Iran, *Pharmaceutical Sciences*, **21(1)**, 18-24 **(2015)**

25. Vaou N., Stavropoulou E., Voidarou C., Tsigalou C. and Bezirtzoglou E., Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives, *Microorganisms*, **9(10)**, 2041 **(2021)**

26. Wei R. and Zhang X.C., Athyrium sessilipinnum: A new lady fern (Athyriaceae) from southern China, *Brittonia*, **68(4)**, 440-447 **(2016)**

27. Williams C. and Davis M., Phytochemical analysis of *Athyrium filix-femina*: Flavonoids, terpenoids, phenolic compounds and tannins, *International Journal of Plant Science*, **12(3)**, 145-160 **(2018)**

28. Wood K.R. and Wagner W.L., Athyrium haleakalae (Athyriaceae), a new rheophytic fern species from East Maui, Hawaiian Islands, with notes on its distribution, ecology and conservation status, *PhytoKeys*, **76**, 115 **(2017)**

29. Zouine N. et al, A comprehensive review on medicinal plant extracts as antibacterial agents: Factors, mechanism insights and future prospects, *Scientific African*, **26**, e02395 **(2024)**.

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