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Green synthesis of silver nanoparticles using medicinal Fern *Blenchnum Orientale* leaves and its application as an antimicrobial agent against food borne pathogens

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***Abstract*---**Nanotechnology is the most active research area in modern materials science. Though there are numerous chemical and physical methods, green synthesis of nanomaterials is the most recent. In the present study, silver nanoparticles were synthesized by plant mediated green approach method using leaf extracts of medicinal fern *Blechnum orientale* Linn, provides necessary bioactive for reduction of silver ions to silver nanoparticles. *Blechnum orientale* was commonly known as 'Centipede fern', which is an important edible medicinal fern. Synthesized nanoparticles were elucidated using UV-visible spectrophotometer, Fourier transform infrared spectroscopy (FT-IR), surface emission microscopy analysis (SEM) and its antioxidant activity was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as well as antimicrobial properties were studied against common food-borne pathogens. The highest absorbance of the

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produced silver nano particles (AgNPs) was 433 nm and the conversion of Ag+ ions to AgNPs was validated using Fourier transform infrared spectroscopy. FTIR result revealed the existence of O–H stretching and N–H amide group peaks. The as-synthesized AgNPs exhibited effective antibacterial action against food borne pathogens *E. coli, Salmonella typhi, Pseudomonas aeruginosa* and *Shigella dysenteriae.* Thus, *Blechnum orientale* AgNPs could be used as antibacterial and therapeutic agents against foodborne pathogens, as well as in the food industry for large-scale synthesis for food preservation or packaging.

***Keywords*---**Green synthesis, Blechnum orientale leaves, AgNPs, Antimicrobial.

Introduction

Nanotechnology, due to its wide range of applications in domains such as adsorption, optical sensors, catalysis, water treatment, drug delivery, and nanomedicine, it has arisen as an attractive subject of study [1]. Nanobiotechnology, an emerging discipline, is expected to be one of the most fruitful fields of research in modern science. Due to its critical uses in biology, bioengineering, textile engineering, water treatment, metal-based consumer products, and other sectors, the synthesis and characterization of noble metal nanoparticles such as gold, silver, and platinum is an emerging subject of research [2]. The size and shape of nanoparticles determine their properties and functions. As a result, precise control over the shape and size of the nanoparticles is required for enhanced antibacterial and catalytic activity, which can be accomplished through the use of a variety of synthesis techniques, stabilisers, and reducing agents [3]. Nanoparticles are organic or inorganic compounds synthesized through a variety of physical, chemical, and biological processes. It is worth noting that both physical and biological methods involve the use of costly, toxic, and non-biodegradable materials that endanger humans, animals, and the environment. However, those which involve the use of plant extracts (green synthesis) are eco-friendly, cost effective, sustainable, easy to synthesize [1].

In the present study we are using *Blechnum orientale Linn.* a medicinal fern belonging to the Family Blechnaceae, commonly known as the ‘Centipede fern’ and ‘Oriental Blechnum’. Used as a source for bio reducing agents and capping agents in the synthesis of silver nanoparticles. According to our research, this is the first time this fern has been used to produce silver nanoparticles. *Blechnum orientale* has traditionally been used to cure female sterilization. Externally, the leaves are used to cure blisters, boils and ulcers. The leaves have also been utilized to treat stomach pain and urinary bladder issues. The rhizomes are thought to be anthelmintic and are used to treat typhoid fever. To relieve pain and stop bleeding, a paste of young frond and rhizome combined with dried ginger powder is applied to fresh cuts and wounds. The indigenous boil the young fronds and eat them as a vegetable. Fresh fronds are also used as a diaphoretic and to treat intestinal worm infections. The fronds of this fern include a few phytochemicals such as Blechnic acid, epiblechnic acid, and brainic acid. The fern has been shown to contain chlorogenic acid and tannins. *B. orientale* has been scientifically evaluated for a variety of pharmacological activities and has been discovered to have strong antioxidant, cytotoxic, antidiabetic, antibacterial, and wound healing properties. This fern is mostly used for biomedical applications due to its valuable therapeutic characteristics [4].

Lai *et al.* 2010 concluded that the methanolic extract of leaves of *B. orientale* possessed the strongest potential as broad spectrum bactericidal and his findings suggest that this fern could be employed as an antioxidant, for colon cancer treatment, and for the treatment of methicillin-resistant *Staphylococcus aureus* MRSA infections and other methicillin-susceptible *Staphylococcus aureus* MSSA/Gram-positive bacterial infectious illnesses [5]. Lai *et al.* 2016 investigated the ethnopharmacological effects of utilizing *B. orientale* as a topical wound treatment and validated. *B. orientale* was also found to be useful in the treatment of diabetic ulcer wounds [6]. The current research focuses on the green synthesis of silver nanoparticles utilizing *Blechnum orientale* leaf aqueous extract at room temperature. The synthesis, characterization, and biocidal action of AgNPs against food-borne microbes have all been demonstrated.

Materials and Methods

The fern species and its habitat

Blechnum orientale is an evergreen fern forming a clump of arching fronds from 20 - 200cm tall growing from a thick rhizome that rises to a short, erect trunk [8]. The fronds are pinnate and rarely simple. Pinnae are linear, entire or dentate. Sori are on the underside of the pinnae, linear and aligned to the midrib. The rhizome is densely covered with narrow, dark brown scales. It is terrestrial at middle to high elevations, usually found in exposed, drier areas, often in colonies [6].

Distribution

The plant is common along road sides in rainforest or dry areas. The fern is sometimes cultivated as an ornamental plant. It is known as the Centipede fern or 'paku ikan' (by the Malays) or 'Kuan Chung' (by the Chinese). It is Herb-Terrestrial natively distributed in Malaysia, in many Asia countries, Australia and Pacific islands. This fern can grow to a height of 2.5 m and is commonly found on exposed low hillsides up to 1500 m in elevation. A diverse range in the Anaikatty Hills, Coimbatore Forest Division, Southern Western Ghats. Anaikatty Hills is part of the Nilgiri Biosphere Reserve and lies between latitudes 11° 01'N to 11° 09'N and longitudes 76° 44'E to 76° 55'E, covering 180 square kilometers [7].

Systematic Position

Kingdom: Plantae
Phylum/Division: Filicophyta
Class: Pteridopsida
Order: Polypodiales
Family: Blechnaceae

Subfamily: Blechnoideae
Genus: *Blechnum*
Species: *Blechnum orientale*

Uses of Blechnum orientale

Blechnum orientale leaf is traditionally used as poultice to treat boils, blisters or abscesses and sores, as a diaphoretic, for stomach pain, urinary bladder complaints and sterilization of women. Leaves are also reported to be used in treating stomach pain and urinary bladder complaints. The rhizomes are believed to be anthelmintic and also recommended in the treatment of typhoid. In India, the paste of young frond and rhizome together with dried ginger powder is applied over fresh cuts and wounds to reduce pain and stop bleeding. Young fronds are boiled and eaten as vegetable by the natives. The fresh fronds are also used to cure intestinal worm infestations and as diaphoretic [4].

B. orientale methanol leaf extract had the greatest potential as a broad spectrum bactericidal. The fern leaf extract has cytotoxic activity against the human colon cancer cell HT-29 as well as bactericidal activity against methicillin-resistant Staphylococcus aureus (MRSA) [5]. The fresh plant *Blechnum orientale* was chosen for this investigation for the following reasons.

- It can be found on very common in fully exposed localities along hill roadsides.
- It is easily available in fertile condition during month of July to March.

Green synthesis of silver nanoparticles

Materials used

Blechnum orientale leaves, blotting sheet, silver nitrate, conical flask, screw cap bottle and de-ionized water.

Utilized Plant material
Medicinal Fern, leaves of *Blechnum orientale* in Figure:1 were collected from, Anaikatty hills, Coimbatore Forest Division, Southern Western Ghats area, Tamilnadu.

Instruments
Centrifuge, Magnetic Stirrer, Orbital shaker, Hot Air Oven and Heating Mantle.

Methods
Silver nitrate ($AgNO3$) was purchased from Sigma Aldrich Company, USA.

Preparation of aqueous extract
20 gm of fresh leaves of *Blechnum orientale* were washed thoroughly with double-distilled water and were then cut into small pieces. These finely cut pieces were then mixed with 100 mL doubled-distilled water, and this mixture was kept for boiling for a period of 5 minutes. It was filtered through Whatman Filter paper no. 1 after cooling [9].

Synthesis of silver nanoparticles

An aqueous solution of silver nitrate (AgNO3) at a concentration of 1 mM was created to generate silver nanoparticles from aqueous extracts of *Blechnum orientale* leaf. A 200 mL aqueous *Blechnum orientale* extract was added to the 1800 mL aqueous 1 mM AgNO3 solution. During the reduction process, the solution was kept in the dark at normal temperature to avoid photochemical reactions. To confirm silver nanoparticle synthesis, place in a shaker (Remi) for an overnight incubation period. Changes in colour from pale yellow to dark brown indicated the production of silver nanoparticles. Transfer 46 mL of sample and 4 mL of deionized water into centrifuge tubes (50 mL) (repeat three times) 1000 rpm for 10 min using centrifuge (Remi) to isolate pure silver nanoparticles free of other bioorganic compounds present in the reaction mixture, and dry it at 50 C for 3 hours to obtain powder form of silver nanoparticles. The dried powder of AgNPs was used for additional testing [3].

Characterization of green synthesized silver nanoparticles

The following techniques were applied to characterize the synthesized AgNPs.

UV – visible spectra analysis

Regular sampling of the reaction mixture confirmed the presence of silver nanoparticles, and the absorption maximum was scanned in the UV spectrophotometer at a wavelength of 200 – 600 nm using Labman LMSPUV1900 at 1 nm resolution. A cuvette with a path length of 10 mm was used. The measurements were carried out as a function of reaction time at room temperature [10].

Fourier transform infrared spectrum (FT-IR)

FTIR (Fourier transform infrared spectroscopy) is used to analyze the biologically active components for silver reduction, formation and capping. FTIR was performed using Shimadzu FTIR 8000S with a scan range of 500−4000 cm−1 resolution. For further testing, the dried powder from AgNPs was used [11].

Field Emission Scanning Electron Microscope (FE-SEM)

Microstructure analysis of the AgNPs synthesized from the *Blechnum orientale* extract was examined using a scanning electron microscope (Nova Nano SEM 200, Netherlands) with an accelerating voltage from 10 to 15 kV. [3]

Antioxidant assay

DPPH analysis

Antioxidant activity was measured using the DPPH radical scavenging method described previously in Lai *et al* (2010). Methanol was used as a negative control and ascorbic acid as a positive control. In 2.5 ml centrifuge tubes, samples of 1ml in various dilutions (2-1000 g/ml) dissolved in methanol were added to 1ml of DPPH solution (200 M) and left in the dark for 30 minutes. At 517 nm, absorbance measurements were taken. Percentage scavenging activity was calculated using the formula:

Scavenging (%) = (Absorbance of control – Absorbance of sample)/Absorbance of control x 100.
The IC50 value is calculated by linear regression analysis by plotting between % inhibition and different concentrations of the sample or standard [12].

Antibacterial activity
Tested microorganisms
Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae.

The antibacterial activity of AgNPs was tested using the agar well diffusion method. described by[13] against common food borne pathogens *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysenteriae.* Muller-Hinton Agar plates that have been prepared, sterilised, and solidified (Himedia). Each bacterial strain (1 x 105 CFU/mL) was swabbed uniformly on the prepared individual petriplates with sterile cotton swabs. On prepared plates, four 6 mm wells are made with gel puncture. AgNPs at various concentrations (15, 30, 50, and 70 micro litre) were loaded into the wells of all plates. The inhibition zones' diameters were measured in millimetres (mm). After 24 hours of incubation at 37° C, the plates were processed for the incubation zone in mm [14].

Results and Discussion

Synthesis of silver nanoparticles -Visual observation & UV- Visible spectroscopy

The silver nanoparticles were synthesized by the green method of synthesis using *Blechnum orientale* leaf extracts as the source of bio reducing agents for reduction of silver ions to silver atoms.At room temperature, leaf extracts were combined in an aqueous solution of AgNO3. To confirm the production of silver nanoparticles, they were kept in a shaker for the night incubation. The creation of nanoparticles was confirmed by visual observation of a colour change from pale yellow to brown. The creation of AgNPs and the change in colour owing to Surface Plasmon Resonance excitation were confirmed by the change in brown colour in the reaction mixture. [15] as shown in figure1A. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 433 nm in case of *Blechnum orientale* leaf extracts as previously reported by [16] as shown in figure 1B.

Fig. 1A: Visual identification of silver nanoparticles A) 0.1 mmol silver nitrate in 90 ml of Mili Q Water B) silver nitrate solution added with plant extract C) Brown color change

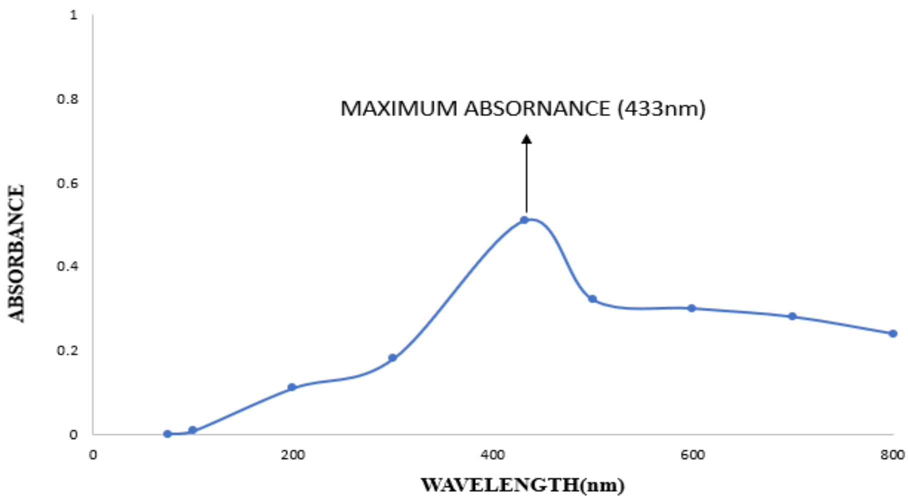

Fig. 1B: The UV-visible readings absorbance of silver nanoparticles

Fourier transform infra-red spectroscopy (FTIR) Analysis

FTIR measurements were carried out to identify the bio molecules for capping and efficient stabilization of the metal nanoparticles synthesized. The figure 2 demonstrates the possible functional groups involved in the reduction of silver ions, absorbance peak observed at 3362.56 cm $^{-1}$are predicted for alkynes, alcohols and phenols, 2859.23 cm^{-1} as nitriles group, 1622.78 cm^{-1}as aldehydes, ketones (C=O stretch), alkenes, primary amines (N-H bend) and amides (N-H bend and C=O stretch), 1322.33 cm^{-1} as nitro groups (N=O stretch), amines-secondary (N-H bend), 1022.27 cm-1as alcohols, esters, carboxylic acid or ether (C-O stretch),amines and 503.49 cm^{-1} alkyl halides. The peak at 1031 cm^{-1} characteristics to glycoside or ether (C–O–C) groups present in the extract. The functional groups such as –N–H, –O–H, C=O, and –C–O–C– groups present in sample might be responsible for bio reduction of Ag + to Ag nano particle Whereas the stretch for Ag-NPs were found around 400–450 cm^{-1}. The presence of organic compounds like flavonoids or terpenoids might be responsible for the synthesis of silver nanoparticles and the proteins in the leaf extract could be responsible for the stabilization of silver nanoparticles that is coated around it [9]. This suggests that biological molecules may perform both the formation and stabilisation of silver nanoparticles in aqueous media. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles as reported by [15].

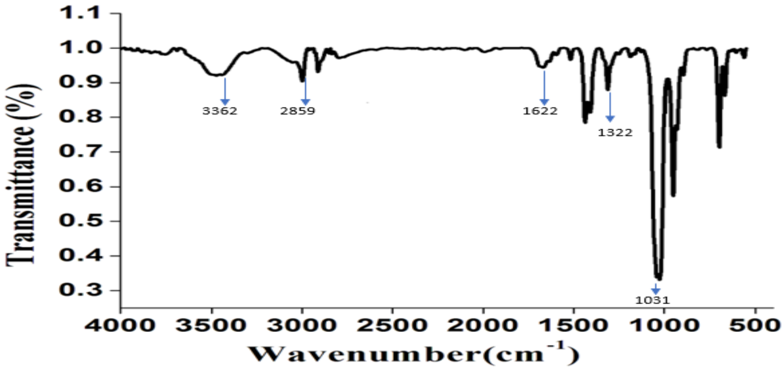

Fig. 2: FTIR analysis of silver nano particle synthesized by plant extract.

Field Emission Scanning Electron Microscope (FE-SEM)

Field Emission Scanning Electron Microscopy was used to examine the morphological characteristics of synthetic silver nanoparticles made from *Blechnum orientale* leaf extracts. After 24 hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. To make the samples conductive, a thin layer of platinum was applied. The samples were then characterized in the SEM at a voltage of 20 KV. The FESEM images thus obtained are shown in figure 3. FE-SEM result shows silver nanoparticles are spherical and scatter well without agglomeration, as shown by micrograms. The silver nanoparticles appeared to be spherical in shape, with a length of 10 micrometres, as seen in the photos. Larger particles form a chain-like structure and are coordinated in their connections.

FESEM images give the clear picture of morphology and structure of AgNPs by using the scales of 30-35 nm. Images give the clear picture that AgNPs have size in the range between 30 and 35 nm and uniformly distributed in solution, average size is 30 nm and spherical in shape. These results are very close to the ones reported by Ajitha *et al.*, [17], who reported 25 nm to be the average size of the particles and a homogeneous mixture with nanoparticles spherical in shape.

Another study by Kuppusamy *et al.*, 2015[18] reported silver nano-particles produced from extract of broccoli. The results show average size of silver nano-particles thus formed to be around 35– 40 nm and spherical in shape.

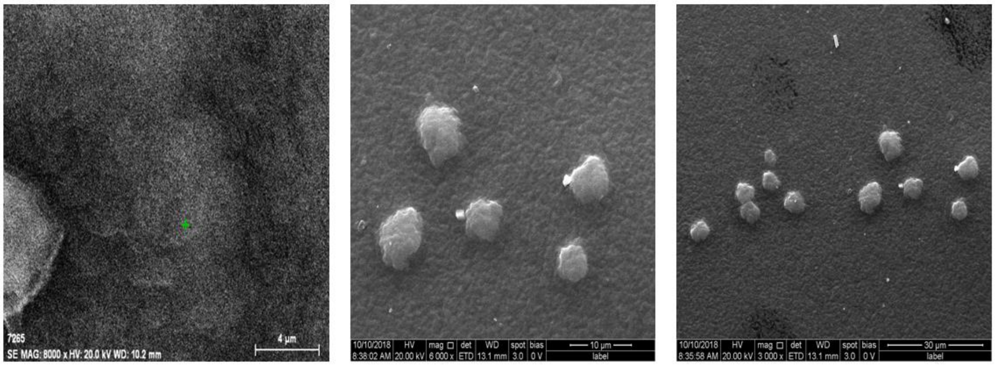

Fig. 3: FESEM image of the AgNP sample synthesized by plant extract

Antioxidant activity

The DPPH assay is the most acceptable, fastest and simplest method for the calculation of the free radical scavenging activity. As shown in the figure. 4 the Ag nanoparticles shows better antioxidant property than the standard ascorbic acid with an IC50 value at 5 mg/ml, while standard showed values of 7-8 mg/ml. When compared to normal ascorbic acid (IC50 value = 191.29 g/mL), the DPPH radical scavenging activity of the produced silver nanoparticles (AgNPs) revealed considerable results (IC50 values of 284.78 g/mL).

The DPPH, Hydrogen peroxide, hydroxyl radicals, superoxide scavenging methods confirmed the silver nanoparticles has antioxidant activity. The properties of silver nanoparticles occur due to the presence of functional groups on the surface of silver nanoparticles Abdel-Aziz, M. S *et al.*, 2014. Silver nanoparticles synthesized exhibit in vitro antioxidant activity by scavenging free radicals [19].

The DPPH radical scavenging assay revealed that AgNPs have a significant antioxidant potential, with an IC50 of 30.04 g/mL. Butylhydroxytoluene was used as a control. Kharat and Mendhulkar (2016) [20] used the DPPH assay to examine the antioxidant activity of synthesised nanoparticles and the antioxidant potentials of photosynthesized nanoparticles. They proposed that photosynthesized NPs could be used as a free radical scavenger. Priya *et al.* (2016) [21] investigated the antioxidant activity of biosynthesized nanoparticles derived from *P. pinnata* extract *in vitro* and discovered significant free radical scavenging potential. Patra and Baek (2016) [22] found significant antioxidant activity in terms of DPPH radical scavenging (IC50 385.87 g/mL). The findings strongly support the use of AgNPs as natural antioxidants for health preservation against various oxidative stressors associated with degenerative diseases.

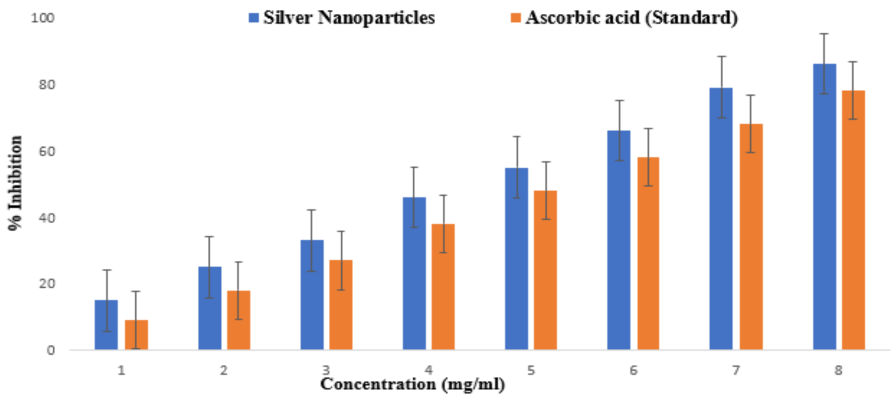

Fig. 4: DPPH scavenging activity of silver nanoparticles when compared with the standard ascorbic acid, measurements were done in triplicates (n=3, mean±SD)

Antimicrobial activity

The antibacterial activity of green synthesized AgNPs was tested by a well diffusion method against the common food pathogens like *E. coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae* as shown in the figure 5. The percentage inhibition was found to be maximum for *Shigella dysenteriae* with value of 27mm, and the maximum concentration for inhibition was at 50 µl of silver nanoparticles as shown figure 5. The high concentration of AgNPs (50 µl) demonstrated a clear inhibition zone.

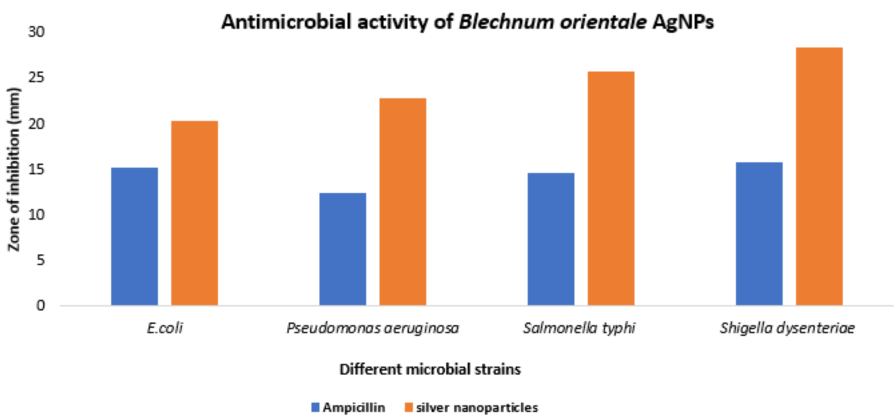

Fig. 5: Each value was inhibitory zones of leaf extracts of medicinal fern *Blechnum orientale* AgNPs on different microbial strains

The morphological characteristics and concentration of silver nanoparticles dominate their antimicrobial effect. Smaller nanoparticles have the advantage of higher percentages of interactions when compared to larger particles. The smallest nanoparticles displayed maximum activity towards the microbial pathogens, despite being more prone to diffusion than larger particles [23].

The antimicrobial efficiency of nanoparticles is also based on their shape. In particular, triangular nanoparticles have shown more significant inhibitory activity than spherical and rod-shaped AgNPs. Furthermore, the concentration has a significant impact on the antimicrobial activities of silver nanoparticles. For example, a 50 to 100 mg of total silver content is required to reduce bacterial colony-forming units. [24-25]. The AgNPs were mainly extracellular, and the resulting nanoparticle size range was 5 to 100 nm, specifically 10–50 nm, with spherical shapes [26]. The antimicrobial and antitumor potential of Asafoetida Gum-AgNPs demonstrates the comprehensive development of new antimicrobial particles with an improved antimicrobial mechanism against human microbial pathogens [13].

Conclusion

Blechnum orientale leaf extract was used to demonstrate an environmentally acceptable synthesis of AgNPs. UV – Visible spectroscopy and FE-SEM were used to determine the morphology and size of the silver nanoparticles. The existence of silver nanoparticles with nitrate reductase enzymes on their surface was discovered using the FT-IR approach. A well diffusion approach was used to determine the diameter of the inhibition zone (ZoI), and the findings revealed visible and clear zones. The antibacterial activity of synthesized AgNPs was shown to be substantial against the majority of food-borne diseases. All of these findings point to AgNPs, as synthesized in this study, being a good biocidal agent against food pathogens.

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References

1. Femi-Adepoju, A. G., Dada, A. O., Otun, K. O., Adepoju, A. O., & Fatoba, O. P. (2019). Green synthesis of silver nanoparticles using terrestrial fern (Gleichenia Pectinata (Willd.) C. Presl.): characterization and antimicrobial studies. *Heliyon*, *5*(4), e01543
2. Sundar, J. S., Christobel, R. G. J., Selvi, N. K., Abirami, M. P., & Samuel, S. (2018). Efficient green synthesis of silver nanoparticles from Caesalpinia bonducella seeds and its antibacterial and cytotoxic effects: An in vitro study.
3. Lekeshmanaswamy, M., & Anusiyadevi, K. (2020). Biosynthesis of silver nanoparticles using Pergularia daemia (Hamilton, 1822) leaf extract and its enhanced antibacterial activity against gram negative bacteria (Escherichia coli). *Materials Today: Proceedings.*

4. Kumar, D. G., Syafiq, A. M., Ruhaiyem, Y., & Shahnaz, M. (2015). Blechnum orientale Linn: An important edible medicinal fern. *Intl J Pharmacogn Phytoch Res*, *7*(4), 723-726.
5. Lai, H. Y., Lim, Y. Y., & Kim, K. H. (2010). Blechnum orientale Linn-a fern with potential as antioxidant, anticancer and antibacterial agent. *BMC Complementary and Alternative Medicine*, *10*(1), 1-8.
6. Lai, J. C. Y., Lai, H. Y., Rao, N. K., & Ng, S. F. (2016). Treatment for diabetic ulcer wounds using a fern tannin optimized hydrogel formulation with antibacterial and antioxidative properties. *Journal of ethnopharmacology*, *189*, 277-289.
7. Prakash, L. A CONTRIBUTION TO FERN FLORA OF ANAIKATTY HILLS, WESTERN GHATS, TAMIL NADU, INDIA. *Indian Journal of Plant Sciences ISSN*, *2319*, 3824.
8. World Health Organization. (2009). *Medicinal plants in Papua New Guinea: information on 126 commonly used medicinal plants in Papua New Guinea*. Manila: WHO Regional Office for the Western Pacific.
9. Ramteke, C., Chakrabarti, T., Sarangi, B. K., & Pandey, R. A. (2013). Synthesis of silver nanoparticles from the aqueous extract of leaves of Ocimum sanctum for enhanced antibacterial activity. *Journal of chemistry*, *2013*.
10. Menon, S., Agarwal, H., Kumar, S. R., & Kumar, S. V. (2017). Green synthesis of silver nanoparticles using medicinal plant Acalypha indica leaf extracts and its application as an antioxidant and antimicrobial agent against foodborne pathogens. *International Journal of Applied Pharmaceutics*, *9*(5), 42-50.
11. Du, J., Hu, Z., Yu, Z., Li, H., Pan, J., Zhao, D., & Bai, Y. (2019). Antibacterial activity of a novel Forsythia suspensa fruit mediated green silver nanoparticles against food-borne pathogens and mechanisms investigation. *Materials Science and Engineering: C*, *102*, 247-253.
12. Abdel-Aziz, M. S., Shaheen, M. S., El-Nekeety, A. A., & Abdel-Wahhab, M. A. (2014). Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract. *Journal of Saudi Chemical Society*, *18*(4), 356-363.
13. Gudikandula, K., & Charya Maringanti, S. (2016). Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. *Journal of Experimental Nanoscience*, *11*(9), 714-721.
14. Devanesan, S., Ponmurugan, K., AlSalhi, M. S., & Al-Dhabi, N. A. (2020). Cytotoxic and antimicrobial efficacy of silver nanoparticles synthesized using a traditional phytoproduct, asafoetida gum. *International Journal of Nanomedicine*, *15*, 4351.
15. Song, J. Y., & Kim, B. S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and biosystems engineering*, *32*(1), 79-84.
16. Ghoshal, G., & Singh, M. (2022). Characterization of silver nano-particles synthesized using fenugreek leave extract and its antibacterial activity. *Materials Science for Energy Technologies*, *5*, 22-29.
17. Ajitha, B., Reddy, Y. A. K., & Reddy, P. S. (2014). Biogenic nano-scale silver particles by Tephrosia purpurea leaf extract and their inborn antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *121*, 164-172.

18. Kuppusamy, P., Ichwan, S. J., Parine, N. R., Yusoff, M. M., Maniam, G. P., & Govindan, N. (2015). Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of Brassica oleracea L. and studies on their physicochemical and biological properties. *Journal of Environmental Sciences*, *29*, 151-157.
19. Patil, M. P., Seong, Y. A., Kim, J. O., Seo, Y. B., & Kim, G. D. (2021). Synthesis of silver nanoparticles using aqueous extract of Cuscuta japonica seeds and their antibacterial and antioxidant activities. *Inorganic Chemistry Communications*, *134*, 109035.
20. Herliah, A., Baso, Y. S., Hidayanty, H., Syarif, S., Aminuddin, A., & Bahar, B. (2022). Effect of web-based she smart education models on adolescent girl's knowledge, attitudes, and practice about obesity. International Journal of Health & Medical Sciences, 5(1), 50-55. https://doi.org/10.21744/ijhms.v5n1.1832
21. Kharat, S. N., & Mendhulkar, V. D. (2016). Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using Elephantopus scaber leaf extract. *Materials Science and Engineering: C*, *62*, 719-724.
22. Patra, J. K., Kwon, Y., & Baek, K. H. (2016). Green biosynthesis of gold nanoparticles by onion peel extract: Synthesis, characterization and biological activities. *Advanced Powder Technology*, *27*(5), 2204-2213.
23. Priya, R. S., Geetha, D., & Ramesh, P. S. (2016). Antioxidant activity of chemically synthesized AgNPs and biosynthesized Pongamia pinnata leaf extract mediated AgNPs–A comparative study. *Ecotoxicology and environmental safety*, *134*, 308-318.
24. Sondi, I., & Salopek-Sondi, B. (2004). Silver nanopartiklar som antimikrobiellt medel: En fallstudie på E. coli som modell för gramnegativa bakterier. *J Colloid Interface Sci*, *275*(1), 177-182.
25. Arnawa, I.K., Sapanca, P.L.Y., Martini, L.K.B., Udayana, I.G.B., Suryasa, W. (2019). Food security program towards community food consumption. Journal of Advanced Research in Dynamical and Control Systems, 11(2), 1198-1210.
26. Vickers, N. J. (2017). Animal communication: when i'm calling you, will you answer too?. *Current biology*, *27*(14), R713-R715.
27. Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *Applied and environmental microbiology*, *73*(6), 1712-1720.
28. Alfuraydi, A. A., Devanesan, S., Al-Ansari, M., AlSalhi, M. S., & Ranjitsingh, A. J. (2019). Eco-friendly green synthesis of silver nanoparticles from the sesame oil cake and its potential anticancer and antimicrobial activities. *Journal of Photochemistry and Photobiology B: Biology*, *192*, 83-89.