

Anti-cancerous and anti-bacterial potential of silver nanoparticles synthesized using leaf extract of fern- *Dryopteris barbigera*

F. Jan^a, S. Pareek^a, R. P. Srivastava^b, I. Zahoor^a, A. Sharma^a, D. Shrivastava^{a,*}

^a*School of Life Science, Jaipur National University, Jaipur 302025, Rajasthan, India*

^b*Department of Materials Science and Engineering, Yonsei University, Seoul, 03722 Republic of Korea*

Green synthesized nanoparticles are easily bio-available and eco-friendly in nature. Leaves of *Dryopteris barbigera* have medicinal properties. In this study, biogenic synthesis of silver nanoparticles (AgNPs) using *D. barbigera* leaf extract and their characterizations have been carried out. Surface plasmon spectra for AgNPs displayed absorbance peak with dark brown color at 418 nm. The synthesized AgNPs, were spherical with size ranging between 10.7-27.6 nm, lattice constant of 0.408 nm and crystallite size of 11.9 ± 2.9 nm. These AgNPs were biocompatible towards MCF-7 and A549 cancer cell lines and showed bactericidal effect against *Staphylococcus aureus* and *Escherichia coli*.

(Received December 28, 2021; Accepted February 26, 2022)

Keywords: Green synthesis, Silver nanoparticles *Dryopteris barbigera*, Antibacterial activity, Anti cancerous activity

1. Introduction

Nanoparticles exhibit a distinctive property of large surface area-to-volume ratio due to physical, chemical and biological properties, making them better as compared to their mass metals [1-4]. Unlike other metal NPs, silver nanoparticles (AgNPs) are unique as they can be incorporated into biosensor materials, composite fibres, cryogenic superconducting materials, cosmetic products and electronic components [5]. They have antibacterial [6, 7], antioxidant [8, 9], anti-viral [10,11], antiparasitic [12, 13], larvicidal [14, 15] and catalytic properties [16,17] besides being successfully used in the cancer diagnosis and treatment as well [18-20]. Existing physical and chemical methods have effectively delivered well characterized AgNPs, but these procedures are costly and include the utilization of toxic chemicals which further increase the toxicity issue due to the binding of hazardous chemicals on the surfaces of NPs [21-23]. Similarly, NPs synthesized using aqua-chemical methods show aggregation on storage [24] biomass has attracted the attention of researchers for nanoparticle production.

Synthesis of NPs using biological material is simple, cost effective and ecofriendly alternative as compared to physical and chemical methods as it do not involve use of toxic chemicals [25, 26]. The synthesis of NPs using plant extract of various plant parts is advantageous over use of microorganisms. It does not require costly media and intricate procedure of maintaining cell cultures [27]. Also, the reduction of silver ions to AgNPs is much faster using plant extract as compared to microbial culture filtrate [28]. Biological approaches of green synthesis of NPs have been accounted by utilizing different plant extracts [29–33].

The genus *Dryopteris* belongs to family *Dryopteridaceae* and its species can be found worldwide in temperate and mountain tropical regions, with a primary center of diversity in eastern Asia, and secondary centers in eastern North America, Mexico, South Africa, and Western Europe [34]. Modern pharmacological research shows that the plants in *Dryopteris* genus have not only anti-helminthic activities as the traditional use, but also antiviral, antitumor, antimicrobial, anti-inflammatory, and antioxidative activities [35]. *Dryopteris barbigera* (Moore) is a characteristic alpine fern, rich in chemical constituents like Filicine [36]; Oleoresin (7-9%) [37],

* Corresponding author: shrivastavdivyajnu@gmail.com
<https://doi.org/10.15251/DJNB.2022.171.285>

and Filicin (2.2 %) [38]. The root contains about 2.1% 'filicin' [39], a substance that paralyzes tapeworms and other internal parasites and has been used as a worm expellent [40]. It is one of the most effective treatments known for tapeworms.

Over the past decade, there are only two reports on the synthesis of NPs using genus *Dryopteris*, i.e., *Dryopteris crassirhizoma* and *Dryopteris cochleata* [6,36]. As part of our contributions to the growing interest of bio-mediated synthesis of AgNPs, we report for the first time an inexpensive one-pot synthesis of AgNPs by green route at room temperature, stabilized in situ using leaf extract of *Dryopteris barbigera* and evaluated its anticancerous and antibacterial activities.

2. Experimental

2.1. Sample Collection

Leaves of *Dryopteris barbigera* were collected from tribal area of Srinagar city, Jammu and Kashmir, India in the month of May 2016. The plant species was identified by Dr. Akhtar, Associate Professor, Centre for Biodiversity and Taxonomy, University of Kashmir, Jammu and Kashmir. The fresh young leaves were brought to the research laboratory in aseptic conditions in sterile polythene bags within 24 hrs.

2.2. Preparation of leaf extract

D. barbigera aqueous leaf extract was used to prepare AgNPs. Leaves were thoroughly washed in running tap water to remove the surface contaminants. This was followed by rinsing with double distilled water. The leaves were air dried at room temperature. Twenty grams of finely cut leaves were boiled in 200 ml of de-ionized water for 20 min. The extract was cooled to room temperature, filtered through Whatman filter paper No.1 and stored at 4° C for further use [41].

2.3. Green synthesis of AgNPs

For synthesis of AgNPs 20 ml of leaf extract was mixed with 180 ml of 1 mM aqueous silver nitrate with constant stirring in 500 ml flask. The mixed solution was kept in dark to avoid photo-activation of silver nitrate at room temperature for 24 hrs. Change in color from colorless to brown indicated formation of AgNPs [42].

The biosynthesized AgNPs solution was centrifuged at 10000 rpm for 20 min. The supernatant was discarded and the pellet obtained was again dispersed in de-ionized water for repeated centrifugation to separate free entities from AgNPs [43]. The resulting pellet was collected and used for further characterization. The experiment was run in triplicates.

2.4. Optimization of different parameters

(a) Effect of quantity of leaf extract on AgNP synthesis

Effect of leaf extract concentration on synthesis of NPs was studied by taking 1- 5 ml of leaf extract. To each concentration 9 ml of 1 mM AgNO₃ solution was added separately. UV-visible absorbance spectra of the resulting solutions were monitored. Stable NPs were obtained when 1 ml of leaf extract was added to 1 mM AgNO₃ solution and therefore was chosen for further studies.

(b) Effect of concentration of AgNO₃ on synthesis of AgNPs

Effect of silver nitrate concentration on NPs synthesis was studied by using different concentration of silver nitrate 1- 5 mM. UV-visible absorbance spectra of the resulting solutions were monitored. Stable NPs were obtained at concentration 1 mM and therefore were chosen for further studies.

(c) Effect of temperature on synthesis of AgNPs

Effect of temperature on nanoparticles synthesis was studied at various temperatures 37°C, 50°C, 60°C, 80°C and 100°C using water bath. UV-visible absorbance spectra of the resulting solutions were monitored. Stable NPs were obtained at temperature 37 °C. Further studies were therefore, carried out at room temperature.

2.5. Characterization of synthesized AgNPs

UV–Vis absorbance of synthesized AgNPs was measured with dual beam spectroscope (Lambda 25, Perkin Elmer) in the wavelength range of 300–700 nm. FTIR spectra of the samples were collected on FTIRTwo-Spectrum (PerkinElmer) in 4100–400 cm^{-1} range. Structural characterization of synthesized nanoparticles was carried out using PANalytical, (X'PertPRO) X-ray diffractometer with Cu-K α monochromatic radiation ($\lambda = 0.154$ nm). The X-ray source was set at 45 kV and 40 mA. A scan rate of 2°/min was used in 20 - 70° (2 θ) range. Morphology of AgNPs was visualized with Nova Nano FE- SEM 450 (FEI) and the elemental composition was determined using Energy Dispersive X-ray Analysis (EDX). The size of AgNPs was further confirmed using transmission electron microscope [Tecnai G² 20 (FEI) S-Twin TEM] operating at a voltage of 200 kV. The selected area electron diffraction (SAED) patterns were collected to analyze the phase and crystalline nature of biosynthesized AgNPs.

2.6. Cell viability by MTT assay

The cell viability assay of green synthesized AgNPs was carried out in cancer cell lines (MCF-7 and A549) with the conventional MTT reduction assay as per the method given by Lovitt *et al.* [44] with slight modifications.

2.7. MTT Assay

A549, MCF-7 cells and test compounds were prepared in 96-well plates containing a final volume of 100 μl /well. It was incubated for desired period of exposure. Ten μl MTT solution was added per well to achieve a final concentration of 0.45 mg/ml. It was then incubated at 37 °C for 4 hrs and 100 μl solubilization solution was added to each well to dissolve formazan crystals and ensure complete solubilization. Viable cells were determined by the absorbance at 540 nm. The effect of the samples on the proliferation of A549 and MCF-7 cells was expressed as the % cell viability, using the following formula:

$$\% \text{ Cell viability} = \text{OD value of sample} / \text{OD value of control} \times 10$$

2.8. Antimicrobial studies of silver AgNPs

The synthesized AgNPs were analysed for antibacterial activity as per Threlfall *et al.* [45] and Walker [46] against Gram positive bacterial strain *Staphylococcus aureus* and Gram negative bacterial strain *Escherichia coli* by agar well diffusion method. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The nutrient agar plates were prepared and wells of 6 mm were made in the plates. Each plate was inoculated with 18 hrs old cultures (100 μl , 10⁴ cfu/ml) and spread evenly on the plate. After 20 min, the wells were filled with sample (10 mg/ml of solvent) and antibiotic ciprofloxacin (10 mg/ml) at different concentrations 25, 50, 100, 250, 500 and 1000 μg . The plates thus prepared were left at room temperature for 10 min for allowing the diffusion of the sample and antibiotic into the agar bacterial lawn. The inhibition zones were observed after incubating all the plates at 37°C for 24 hrs.

3. Results and discussion

3.1. Visual observation

Addition of aqueous leaf extract of *D.barbigerrato* AgNO₃ solution resulted in colour change from colourless to brown (Fig. 1). The color change was due to reduction of silver ion demonstrating the synthesis of AgNPs. It is well known that AgNPs display yellowish brown color in aqueous solution because of excitation of Surface Plasmon Resonance (SPR) in AgNPs[47,48].

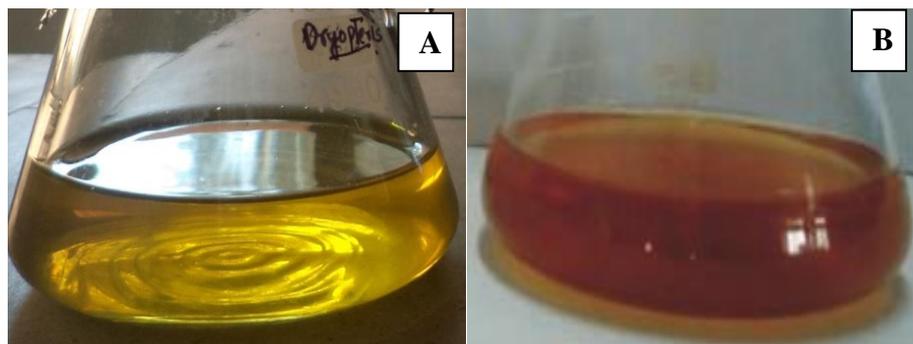


Fig. 1. (a) Leaf extract of *Dryopteris barbiger* (b) Change in colour after reaction of leaf extract with AgNO_3 solution.

3.2. UV- Visible studies

The synthesis of AgNPs in aqueous suspensions was examined by using UV-Vis spectroscopy [49]. AgNPs generally exhibit surface plasmon resonance (SPR) band at 400-480 nm [48,50]. In our study an absorbance peak at 418 nm was observed (Fig. 2). The broadened peak indicates polydispersed nature of nanoparticles [51].

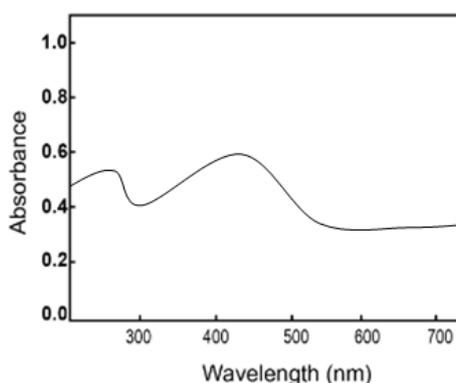


Fig. 2. Absorption spectra of AgNPs synthesized using *D. barbiger* leaf extract.

3.3. Optimization of different parameters

Various parameters including reaction time, concentration of leaf extract, concentration of AgNO_3 and temperature were optimized for synthesis of AgNPs.

3.4. Effect of leaf extract on synthesis of AgNPs

The different concentrations of *Dryopteris barbiger* leaf extract 1 ml, 2 ml, 3 ml, 4 ml and 5 ml were added separately to 9 ml of 1 mM AgNO_3 solution for synthesis of AgNPs. The color intensity changed with increase in leaf extract concentration from light to deep brown at 418 nm. The surface plasmon resonance band got shifted to higher absorbance and wavelength with the increase in the leaf extract concentration (Fig. 3) This signifies increase in size of AgNPs [52]. At 2 ml, 3 ml, 4 ml and 5 ml of leaf extract concentration agglomeration of AgNPs was noticed after 3 days of incubation. Stable AgNPs were obtained at 1 ml of leaf extract as no agglomeration was found at this concentration. One ml of leaf extract was therefore, used for further studies. Same concentration of leaf extract has been used earlier for synthesis of stable nanoparticles [53].

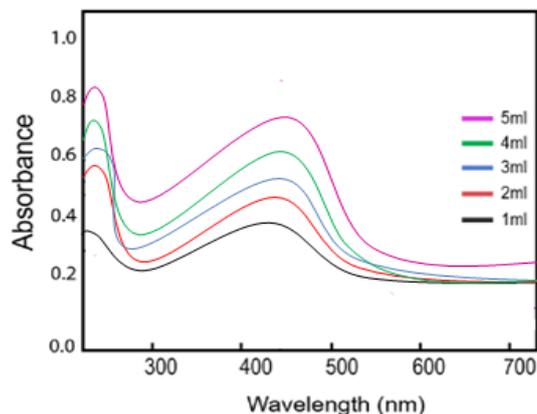


Fig. 3. UV-Vis spectrum of AgNPs showing effect of concentration of leaf extract.

3.5. Effect of AgNO_3 concentration on synthesis of AgNPs

The effect of silver nitrate concentration was investigated with varying concentration from 1 mM to 5 mM. The ratio used was 1:9 for leaf extract and AgNO_3 . Synthesis of AgNPs was indicated by change in color from golden to dark brown at 1 mM concentration at 418 nm. Increase in color intensity and shift in surface plasmon resonance band towards higher absorbance and wavelength at 2 mM, 3 mM, 4 mM and 5 mM was noticed as AgNO_3 concentration was increased (Fig. 4). The shift in peak may be due to increase in size of AgNPs[52]. There was agglomeration of AgNPs after three days of incubation with 2 mM, 3mM, 4mM and 5 mM concentration of AgNO_3 , whereas, no agglomeration was found with 1 mM concentration of AgNO_3 . One mM of AgNO_3 concentration was, therefore, used for the synthesis of AgNPs[51, 53].

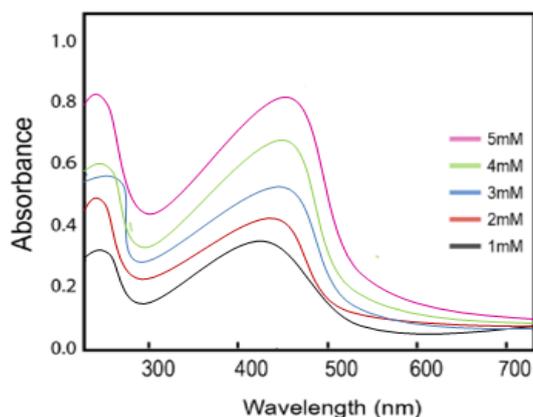


Fig. 4. UV-Vis spectrum of AgNPs showing effect of concentration of AgNO_3 .

3.6. Effect of temperature on synthesis of AgNPs

The reaction mixture was incubated with 1 mM AgNO_3 at 37° C, 50° C, 60° C, 80° C and 100° C to determine the effect of temperature on the synthesis of AgNPs. There was a change in color from golden brown to dark brown at 418 nm at 37° C (Fig. 5). The surface plasmon resonance band got shifted to higher absorbance and wavelength at temperature above 60° C, which may be due to increase in particle size of AgNPs[52]. High temperatures lead to biomolecules destruction resulting in formation of large sized nanoparticles.

There was agglomeration of AgNPs after 3 days of incubation at temperature 50° C and 60° C with no agglomeration at temperature 37° C, therefore, selected for further studies for the synthesis of NPs [53, 54].

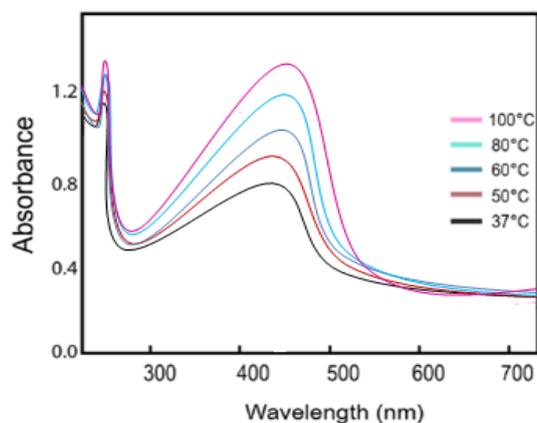


Fig. 5. UV-Vis spectrum of synthesized AgNPs at different temperatures.

3.7. FT-IR spectroscopy

The biomolecules involved in the reduction and stabilization of AgNPs were identified using FTIR spectroscopy. Four absorption peaks at wavenumber 3443 cm^{-1} , 1615 cm^{-1} , 1384 cm^{-1} and 1077 cm^{-1} were observed (Fig. 6). The peak at 3443 cm^{-1} is attributed to O-H stretching of hydrogen bonded phenolic group [55]. The peak at 1615 cm^{-1} can be assigned to C=C stretch in aromatic ring confirming the presence of aromatic group [56]. Peak at 1384 cm^{-1} is assigned to C-H bending vibration while the band at 1077 cm^{-1} can be attributed to C-N stretching of amines group [57].

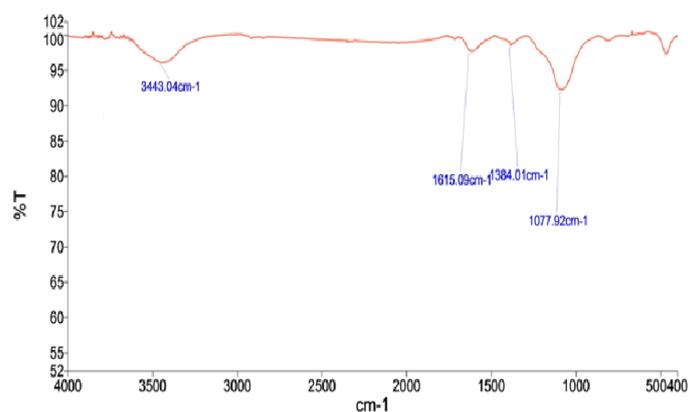


Fig.6. FT-IR spectrum of biosynthesized AgNPs.

FT-IR study thus revealed that phenols and amines could have interacted with the surface of AgNPs and might be responsible for reduction of Ag^+ to Ag^0 and stabilization of AgNPs.

3.8. XRD studies

The structural parameters (phase, crystallite size and lattice parameter) of biosynthesized AgNPs using *D. barbiger* aqueous leaf extract were investigated with XRD (Fig.7). The presence of intense peaks in the XRD spectrum indicates the crystalline nature of synthesized nanoparticles. Four obvious diffraction peaks appearing at 38.3°, 44.4°, 64.6° and 76.8° values of 2θ confirm the formation of face-centered cubic AgNPs (JCPDS card number: 04-0783). These peaks can be

assigned to (111), (200), (220) and (311) crystallographic planes of AgNPs, which is further supported by SAED pattern obtained in the TEM study. Presence of some additional peaks (marked with stars) at 27.9°, 32.3°, 46.3° and 57.6° was also shown by XRD. Similar crystalline peaks at 32.2°, 46.2°, 54.8°, and 67.4° were reported due to presence of phytochemicals [56,58, 59].

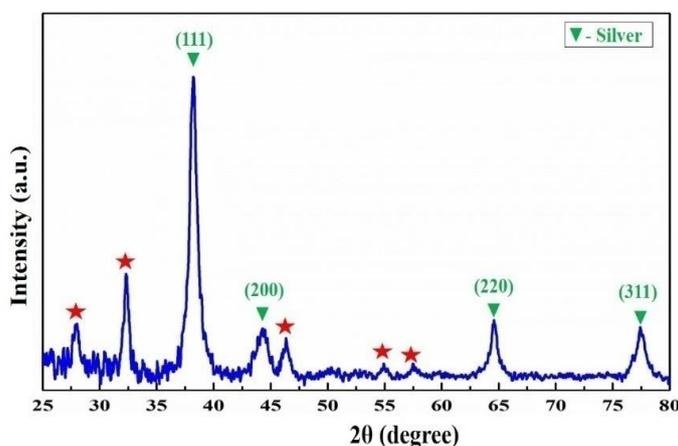


Fig.7. X-ray diffraction pattern of synthesized AgNPs.

Further, lattice parameter of the synthesized AgNPs was calculated using the following relation for the cubic crystal system

$$d = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

Here, ‘a’ is the lattice parameter and ‘d’ is the interplanar spacing for the set of parallel planes represented by miller indices (**hkl**). The calculated lattice parameter was 0.408 nm which is in close agreement with reported lattice parameter of the silver [60]. The broadening of diffraction peaks in the XRD spectrum indicates the formation of nanoparticles. From the broadening of peaks, average crystallite size of the nanoparticles was estimated using the Debye–Scherrer’s equation

$$D = (K\lambda)/(\beta\cos\theta)$$

Here, K is a dimensionless constant which is generally assigned a value of 0.94[61], λ is the wavelength of X-ray radiation used, and β is the full width at half maximum (FWHM) of a diffraction intensity peak. Average crystallite size estimated using Debye-Scherrer’s equation was found to be 11.9 ± 2.9 nm, which is slightly less than that obtained from SEM, but close to TEM measurements.

3.9. FESEM and TEM analysis

FESEM micrograph of AgNPs shows that synthesized nanoparticles are spherical in shape (Fig. 8a). The agglomeration of few particles has also been observed in the SEM. The synthesized nanoparticles are smaller than 50 nm and having different sizes ranging from ~18 nm to ~42 nm. Similar results have been reported by Lee *et al.* [6].

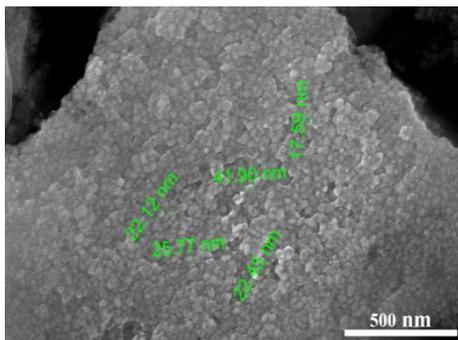


Fig. 8a. SEM image of synthesized AgNPs.

A representative TEM image collected for the AgNPs is shown in Fig. 8b. It confirms that most of the particles are spherical in shape as visualized with FESEM. The size of synthesized AgNPs varies from ~11–28 nm. The estimated average size was 17.7 nm, which is close to that calculated by XRD. Similar results have been reported using plant extract of *Adiantum lunulatum* with size range of 10 nm–60 nm [7].

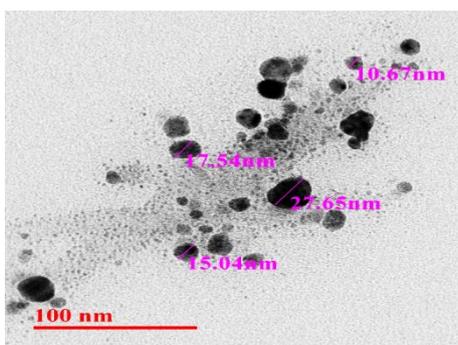


Fig. 8b. TEM image of synthesized AgNPs.

3.10. Selected Area Electron Diffraction (SAED) studies

SAED pattern is very useful to reveal the structure and distinguish between crystalline and amorphous nature of the material [62]. The SAED pattern (Fig. 9) obtained in the present study confirms the polycrystalline nature of synthesized AgNPs [63]. The brighter spots in the obtained SAED were grouped in concentric circles based on their distances from the centers. The d spacing calculated corresponding to the radii of these circles were found to be 0.238, 0.203, 0.143 and 0.121 nm which correspond to (111), (200), (220) and (311) crystallographic planes of face-centered cubic AgNPs.

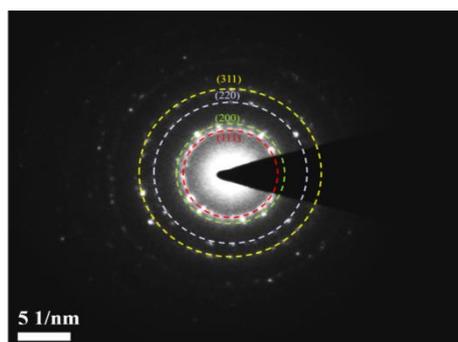


Fig. 9. SAED pattern of biosynthesized AgNPs.

3.11. EDX study

In the present study a strong signal peak at 3 keV was observed by EDAX spectroscopy suggesting the presence of silver as constituent element. Similar peak at 3 keV has also been reported by various researchers [49, 58]. The elemental studies of the AgNPs showed presence of Ag followed by C, Cu, Cl, Co, Fe, S, Si, Mg, P and O (Fig. 10). These peaks are due to biomolecules which are bound on the surface of AgNPs. The presence of copper (Cu) is due to copper grid which was used for TEM imaging and EDX investigation [64].

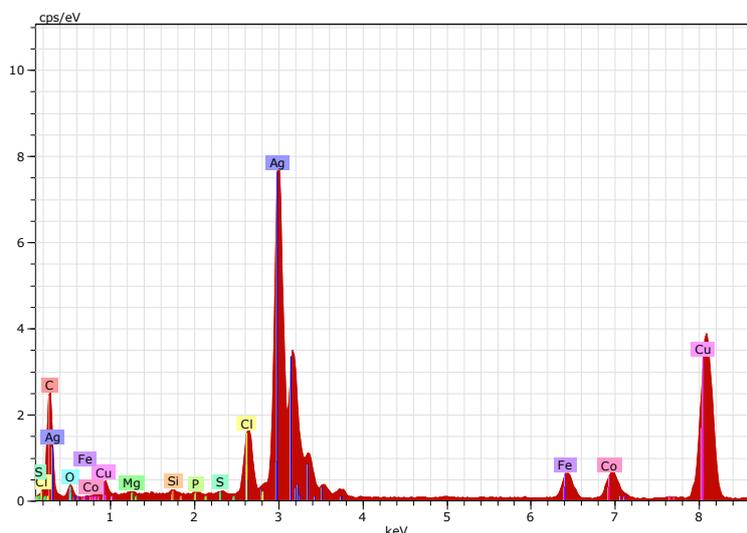


Fig.10. EDAX analysis of biosynthesized AgNPs.

3.12. Anticancer activity of AgNPs

Efficacy of synthesized AgNPs against cancer cell lines, MCF-7 and A549 was examined (Table 1 and Fig. 11, 12 and 13) and morphological changes were found in the cell leading to cell death at all the concentrations tested (25, 50, 100, 250 and 500 $\mu\text{g/ml}$). The IC₅₀ value for AgNPs was not achieved even up to 500 $\mu\text{g/ml}$ concentration suggesting that synthesized NPs might not be cytotoxic to A549 and MCF-7 cancer cell lines. The results were as per Patra *et al.*[32], and Zahoor *et al.*[65], who reported biocompatibility of AgNPs with B16F10, MCF-7, HNGC2, A549 and Hep G2 cancer cell lines.

However, there are reports on cytotoxicity of AgNPs towards cancer cell lines. *In-vitro* cytotoxic effect of *Cibotium barometz* leaf extract mediated AgNPson RAW264.7 murine macrophage and MCF-7 breast cancer cell lines was studied by Wang *et al.*[20]. The IC₅₀ value was found to be $\geq 10\mu\text{g/ml}$ for RAW264.7 cells and it was more than 10 $\mu\text{g/ml}$ for MCF-7 cell line. Jhonson *et al.*[66] treated *Cyathea nilgirensis* mediated AgNPs with hatched shrimps (*Artemia salina*) and inhibited the viability. The cytotoxic potential of the aqueous extract and AgNPs of *C. nilgirensis* showed varied percentage mortality with the LC₅₀ value 1533.28 $\mu\text{l}/10$ ml and 869.4 $\mu\text{l}/10$ ml, respectively. The AgNPs altered the cellular and molecular level functions of *Artemia salina* leading to mortality.

Table 1. Cell viability of A549 and MCF-7 cell lines when treated with different concentrations of AgNPs.

Tested concentration($\mu\text{g/ml}$)	OD at 570nm (A549)	%Cell Viability (A549)	OD at 570 nm (MCF-7)	%Cell Viability (MCF-7)
25	0.265	99.2	0.217	86.6
50	0.252	94.6	0.20	82.6
100	0.210	78.7	0.203	80.7
250	0.185	69.3	0.183	73.0
500	0.178	66.8	0.18	71.7
Control	0.266	100	0.250	100

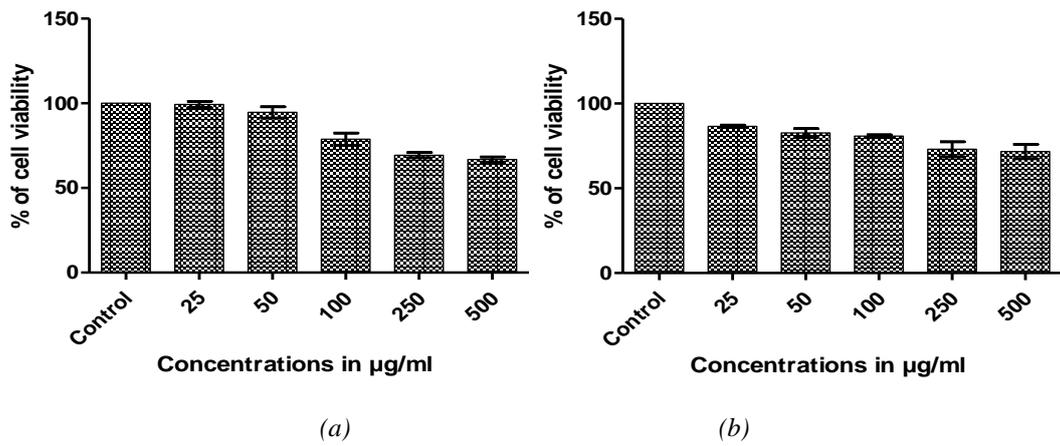


Fig. 11. Cell viability of (A) A549, (B) MCF-7 after treatment with AgNPs.

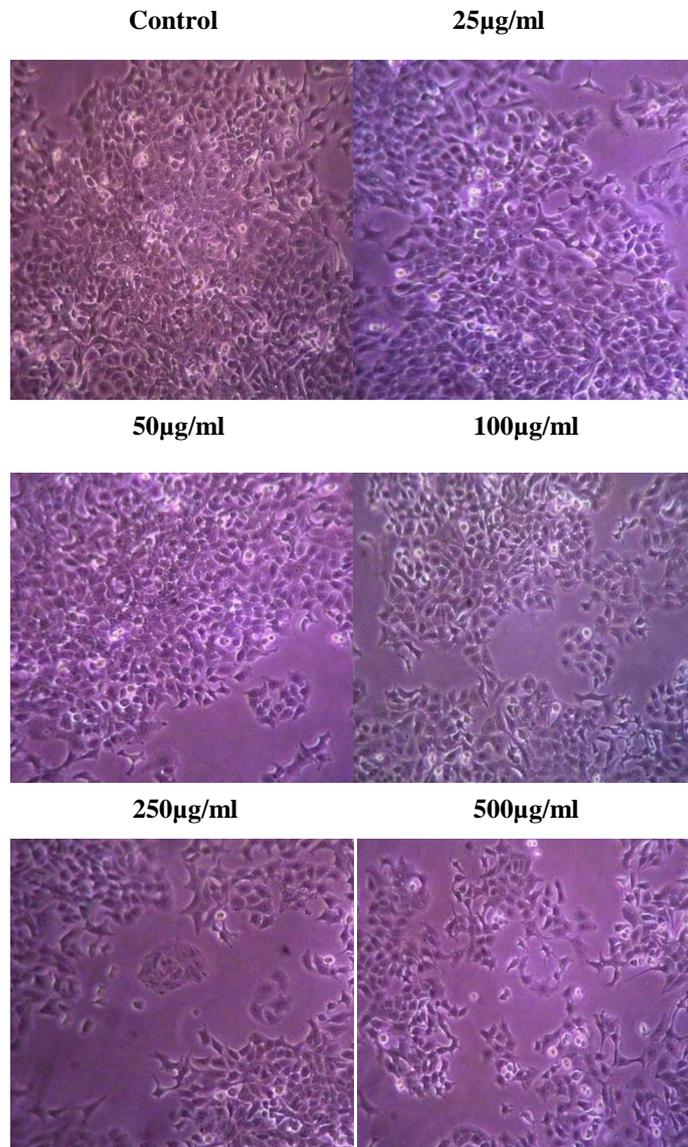


Fig.12. Cell viability of A431 after treatment with AgNPs.

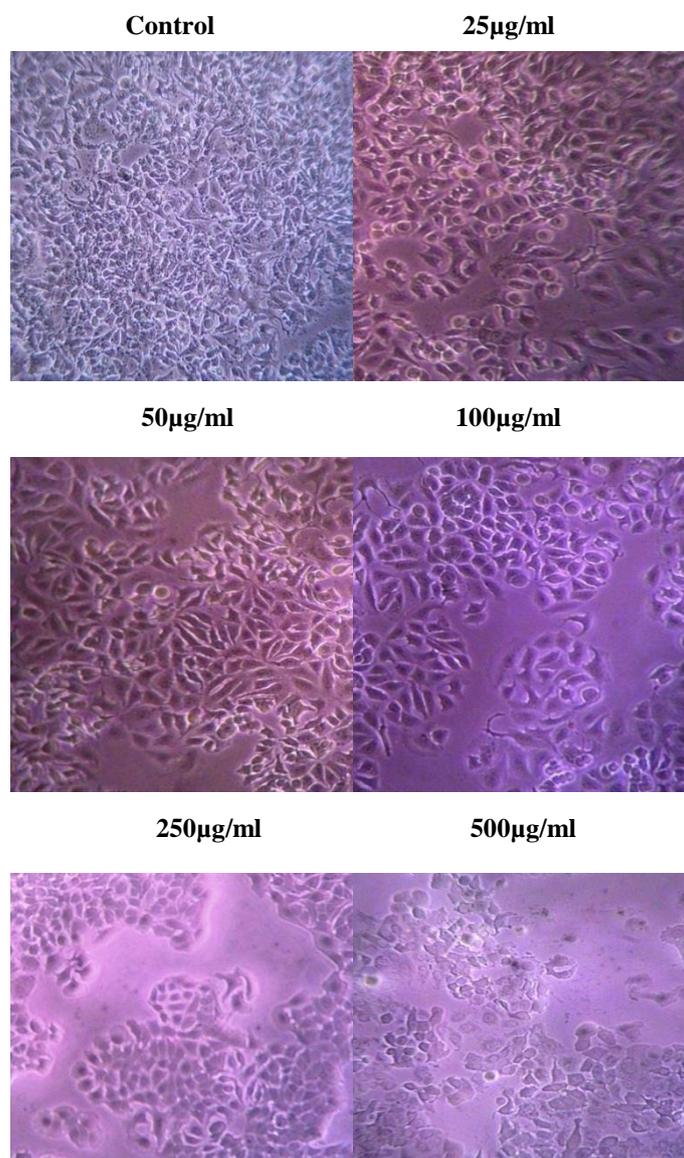


Fig. 13. Cell viability of MCF-7 after treatment with AgNPs

3.13. Antibacterial studies

In our study the AgNPs (DB-4) were analysed for antibacterial activity against one Gram positive bacterial strain *Staphylococcus aureus* and one Gram negative bacterial strain *Escherichia coli* by agar diffusion method. The synthesized AgNPs showed activity against both test organisms. Antibacterial activity of NPs for *S. aureus* and *E. coli* was observed at 50 µg/ml and 100 µg/ml concentration, respectively. Increased zone of inhibition was observed with increase in AgNPs concentrations. Ciprofloxacin was taken as a positive control. Also, antibacterial activity of plant extract (DB-1), AgNPs in aqueous solution just after the color change (DB-2) and agglomerated AgNPs which were incubated for three days (DB-3) were analyzed against the same bacterial strains (Table 2).

Table 2. Antibacterial activities of plant extract (DB1), AgNPs in aqueous solution (DB2), agglomerated AgNPs (DB3), synthesized AgNPs (DB4) and Ciprofloxacin as positive control.

S.aureus

Samples	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
D.B1	0	0	0	5	10	12	250
D.B2	0	0	5	9	12	14	100
D.B3	0	0	0	0	0	6	1000
D.B4	0	5	9	12	13	16	50
Ciprofloxacin	13	18	21	25	27	*	25

E.coli

Samples	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
D.B1	0	0	0	0	0	5	1000
D.B2	0	0	5	10	13	15	100
D.B3	0	0	0	0	0	0	NF
D.B4	0	0	7	9	10	13	100
Ciprofloxacin	18	20	23	26	28	*	25

Note: In above tables, NF is MIC not found in the concentrations screened

*zones could not be measured due to merging

Zones ≥ 3 mm considered for MIC

These studies were supported by the results of Santoshkumar and Nagarajan [67] and Wang et al.[20]. Because of variations in size and shape of AgNPs, bacterial load, exposure time and nutrient media, there may be variation in antibacterial activities from species to species [55]. Antibacterial activity reports on AgNPs against Gram positive and Gram negative bacteria are contrary. Some reports showed AgNPs to be more sensitive to Gram negative bacteria when compared to Gram positive bacteria [68, 69] whereas reverse results were revealed by other reports [20, 55, 67].

4. Conclusions

A rapid, environmentally benign and inexpensive method of fabricating AgNPs using leaf extract of *Dryopteris barbigera* was developed in this study. UV-Vis spectroscopy, FTIR, XRD, SAED, EDX, TEM and FESEM characterization were used for determining absorbance peak, reduction mechanism, crystallinity, elemental composition and morphology of prepared AgNPs. The NPs were potent against both tested bacterial strains *Staphylococcus aureus* and *Escherichia coli*. In the present study synthesized AgNPs were found to be biocompatible towards MCF-7 and A549 cell lines, thereby forwarding our understanding towards use of AgNPs as drug delivery vectors.

References

- [1] I. Capek, Advances in Colloid and Interface Science 110(1-2), 49 (2004). <https://doi.org/10.1016/j.cis.2004.02.003>
- [2] A. Frattini, N. Pellegrini, D. Nicastro, O. De Sanctis, Materials Chemistry and Physics 94, 148 (2005) <https://doi.org/10.1016/j.matchemphys.2005.04.023>

- [3] S. Mohanbaba, S. Gurunathan, "Applications of Nanobiomaterials" Elsevier, Amsterdam 193, 2016. <https://doi.org/10.1016/B978-0-323-42864-4.00006-3>
- [4] M. P. Patil, G. D. Kim, Applied Microbiology and Biotechnology 101(1), 79 (2017) <https://doi.org/10.1007/s00253-016-8012-8>.
- [5] H. Korbekandi, S. Iravani, "The Delivery of Nanoparticles" InTech, 2012 <https://doi.org/10.5772/34157>
- [6] J. H. Lee, J. M. Lim, P. Velmurugan, Y. J. Park, Y. J. Park, K. S. Bang, B. T. Oh, J. Journal of Photochemistry and Photobiology B: Biology 162, 93(2016) <https://doi.org/10.1016/j.jphotobiol.2016.06.029>
- [7] A. Chatterjee, S. Khatua, K. Acharya, J. Sarkar, Digest Journal of Nanomaterials and Biostructures 14(2), 479 (2019).
- [8] N. A. Sutan, I. Fierascu, R. C. Fierascu, D. S. Manolescu, L. C. Soare, Industrial Crops and Products 83, 379(2016). <https://doi.org/10.1016/j.indcrop.2016.01.011>
- [9] X. Baskaran, A.V. Geo Vigila, T. Parimelazhagan, D. Muralidhara-Rao, S. Zhang, International Journal of Nanomedicine 11, 5789 (2016). <https://doi.org/10.2147/IJN.S108208>
- [10] S. Galdiero, A. Falanga, M. Vitiello, M. Cantisani, V. Marra, M. Galdiero, Molecules 16 (10), 8894 (2011). <https://doi.org/10.3390/molecules16108894>
- [11] H. H. Lara, E. N. Garza-Trevino, L. Ixtepan-Turrent, D. K. Singh, J. Nanobiotechnology 9, 30(2011). <https://doi.org/10.1186/1477-3155-9-30>
- [12] A. A. Zahir, A. A. Rahuman, Veterinary Parasitology 187(3-4), 511(2012) <https://doi.org/10.1016/j.vetpar.2012.02.001>
- [13] T. Santhoshkumar, A. A. Rahuman, A. Bagavan, S. Marimuthu, C. Jayaseelan, A. V. Kirthi, et al. Experimental Parasitology 132 (2), 156(2012). <https://doi.org/10.1016/j.exppara.2012.06.009>
- [14] R. Rajaganesh, K. Murugan, C. Panneerselvam, S. Jayashanthini, A. T. Aziz, M. Roni, et al. Research in Veterinary Science 109, 40(2016). <https://doi.org/10.1016/j.rvsc.2016.09.012>
- [15] G. Chitra, G. Balasubramani, R. Ramkumar, R. Sowmiya, P. Perumal, Parasitology Research 114(4), 1407(2015). <https://doi.org/10.1007/s00436-015-4320-7>
- [16] B. Paul, B. Bhuyan, D. D. Purkayastha, S. S. Dhar, Journal of Molecular Liquids 212, 813(2015). <https://doi.org/10.1016/j.molliq.2015.10.032>
- [17] A. Rostami-Vartooni, M. Nasrollahzadeh, M. J. Alizadeh, Journal of Alloys and Compounds 680, 309(2016). <https://doi.org/10.1016/j.jallcom.2016.04.008>
- [18] K. Satyavani, S. Gurudeeban, T. Ramanathan, T. J. Balasubramanian, Nanobiotechnology 9, 43(2011).
- [19] J. J. Antony, M. A. A. Sithika, T. A. Joseph, U. Suriyakalaa, A. Sankarganesh, D. Siva, et al. Colloids and Surfaces B: Biointerfaces 108, 185(2013). <https://doi.org/10.1016/j.colsurfb.2013.02.041>
- [20] D. Wang, J. Markus, C. Wang, Y.-J. Kim, R. Mathiyalagan, V. C. Aceituno, et al. Artificial Cells, Nanomedicine and Biotechnology 45(8), 1548(2016) <https://doi.org/10.1080/21691401.2016.1260580>
- [21] M. Kowshik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, S. K. Kulkarni, K. M. Paknikar, Nanotechnology. 14(1), 95(2003). <https://doi.org/10.1088/0957-4484/14/1/321>
- [22] M.-C. Daniel, D. Astruc, Chemical Reviews 104 (1), 293(2004). <https://doi.org/10.1021/cr030698+>
- [23] S. Iravani, Current Green Chemistry 13 (10), 2638(2011). <https://doi.org/10.1039/c1gc15386b>
- [24] P. Patel, P. Agarwal, S. Kanawaria, S. Kachhwaha, S. L. Kothari, "Nanotechnology and Plant Sciences: Nanoparticles and their Impact on Plants". Springer International, Switzerland 271, 2015 https://doi.org/10.1007/978-3-319-14502-0_13
- [25] K.B. Narayanan, N. Sakthivel, Adv. Journal of Colloid and Interface Science 156(1-2), 1(2010). <https://doi.org/10.1016/j.cis.2010.02.001>
- [26] T. Santhoshkumar, A. A. Rahuman, G. Rajakumar, S. Marimuthu, A. Bagavan, C. Jayaseelan, et al. Parasitology Research 108 (3), 693(2011). <https://doi.org/10.1007/s00436-010-2115-4>
- [27] E. C. Njagi, H. Huang, L. Stafford, H. Genuino, H. M. Galindo, J. B. Collins, et al. Langmuir 27(1), 264(2011). <https://doi.org/10.1021/la103190n>
- [28] A. Gangula, R. Podila, M. Ramakrishna, L. Karanam, C. Janardhana, A. M. Rao, Langmuir 27 (24), 15268(2011). <https://doi.org/10.1021/la2034559>

- [29] N. Mude, A. Ingle, A. Gade, M. J. Rai, *Journal of Plant Biochemistry and Biotechnology* 18, 83(2009). <https://doi.org/10.1007/BF03263300>
- [30] N. Ahmad, S. Sharma, V. N. Singh, S. F. Shamsi, A. Fatma, B. R. Mehta, *Biotechnology Research International* 454090, 1(2011). <https://doi.org/10.4061/2011/454090>
- [31] L. Rastogi, J. Arunachalam, *Materials Chemistry and Physics* 129(1-2), 558(2011) <https://doi.org/10.1016/j.matchemphys.2011.04.068>
- [32] S. Patra, S. Mukherjee, A. K. Barui, A. Ganguly, B. Sreedhar, C.R. Patra, *Materials Science and Engineering C* 53, 298(2015). <https://doi.org/10.1016/j.msec.2015.04.048>
- [33] M. J. Firdhouse, P. Lalitha, *Journal of Molecular Liquids* 222, 1041(2016) <https://doi.org/10.1016/j.molliq.2016.07.141>
- [34] E. B. Sessa, L. B. Zhang, H. Vare, A. Juslen, *Systematic Botany* 40(2), 387(2015) <https://doi.org/10.1600/036364415X688844>
- [35] X. Han, Z. Li, C.-Y. Li, W.-N. Jia, H.-T. Wang, C.-H. Wang, *Chemistry and Biodiversity* 12(8), 1131(2015). <https://doi.org/10.1002/cbdv.201400157>
- [36] Singh, P., Yasir, M., Khare, R., Shrivastava, R. *Toxicological Research* 9(5), 706(2020) <https://doi.org/10.1093/toxres/tfaa070>
- [37] T.C. Mittal, P.N. Mehra, *Journal of Pharmacy and Pharmacology* 12(1), 317(1960) <https://doi.org/10.1111/j.2042-7158.1960.tb12670.x>
- [38] L. T. C. Mittal, S. S. Bir, *Indian Fern Journal* 23(1-2), 133(2006).
- [39] R. N. Chopra, S. L. Nayar. I. C. Chopra, "Glossary of Indian Medicinal Plants (Including the Supplement)" CSIR, New Delhi, 3, 203, 1986.
- [40] D. Bown, "Encyclopaedia of Herbs and their Uses". Dorling Kindersley, London, 1995.
- [41] S. Ahmed, Saifullah, M. Ahmad, B. L. Swami, S. Ikram, J. Radiat. *Research Journal of Applied Sciences* 9(1), 1(2016). <https://doi.org/10.1016/j.jrras.2015.06.006>
- [42] S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, S. Thangamani, *Asian Pacific Journal of Tropical Biomedicine* 2(7), 574(2012). [https://doi.org/10.1016/S2221-1691\(12\)60100-2](https://doi.org/10.1016/S2221-1691(12)60100-2)
- [43] A. Sudha, J. Jeyakanthan, P. Srinivasan, *Resource-Efficient Technologies* 3(4), 506(2017) <https://doi.org/10.1016/j.reffit.2017.07.002>
- [44] C. J. Lovitt, T. B. Shelper, V. M. Avery, *Biology* 3(2), 345(2014) <https://doi.org/10.3390/biology3020345>
- [45] E. J. Threlfall, I. S. T. Fisher, L. Ward, H. Tschape, P. Gernersmidt, *Microbial Drug Resistance* 5(3), 195(1999). <https://doi.org/10.1089/mdr.1999.5.195>
- [46] R. D. Walker, "Antimicrobial Therapy in Veterinary Medicine" Iowa State University Press. Ames, IA 12, 2000.
- [47] X. Song, L. Zang, S. Hu, *Vaccine*. 27(17), 2306(2009). <https://doi.org/10.1016/j.vaccine.2009.02.040>
- [48] S. Medda, A. Hajra, U. Dey, P. Bose, N. B. Mondal, *Applied Nanoscience* 5(7), 875(2015) <https://doi.org/10.1007/s13204-014-0387-1>
- [49] M. H. Kahsay, D. R. Devi, Y. P. Kumar, B. S. Mohan, A. Tadesse, G. Battu, K. Basavaiah, *Open Nano* 3, 28(2018). <https://doi.org/10.1016/j.onano.2018.04.001>
- [50] M. Potara, M. Bawaskar, T. Simon, S. Gaikwad, E. Licarete, A. Ingle, M. Banciu, et al. *Colloids and Surfaces B* 133, 296(2015) <https://doi.org/10.1016/j.colsurfb.2015.06.024>
- [51] C. Krishnaraj, R. Ramachandran, K. Mohan, P. T. Kalaichelvan, *Spectroscopy* 93, 95(2012) <https://doi.org/10.1016/j.saa.2012.03.002>
- [52] A. A. Moosa, A. M. Ridha, M. Al-Kaser, *International Journal of Multidisciplinary and Current Research* 3(2015).
- [53] P. Balashanmugam, M. D. Balakumaran, R. Murugan, K. Dhanapal, P.T. Kalaichelvan, *Microbiological Research* 192, 52(2016). <https://doi.org/10.1016/j.micres.2016.06.004>
- [54] P. M. Mishra, S. K. Sahoo, G. K. Naik, K. Parida, *Materials Letters* 160, 566(2015) <https://doi.org/10.1016/j.matlet.2015.08.048>
- [55] R.W. Raut, V. D. Mendhulkar, S. B. Kashid, *Journal of Photochemistry and Photobiology B* 132, 45(2014). <https://doi.org/10.1016/j.jphotobiol.2014.02.001>
- [56] K. J. Rao, S. Paria, *Materials Research Bulletin* 48(2), 628(2010).

- [57] N. H., Mohamed, M. A., Ismail, W. M. Abdel-Mageed, A. A. M. Shoreit, Asian Pacific Journal of Tropical Biomedicine 4(11), 876(2014) <https://doi.org/10.12980/APJTB.4.201414B216>
- [58] K. Anandalakshmi, J. Venugobal, V. Ramasamy, Applied Nanoscience 6(3), 399(2016) <https://doi.org/10.1007/s13204-015-0449-z>
- [59] C. Dong, C. Cao, X. Zhang, Y. Zhan, X. Wang, X. Yang, et al. Optik 130, 162(2017) <https://doi.org/10.1016/j.ijleo.2016.11.010>
- [60] T. Shiozawa, T. Kobayashi, Physica Status Solidi (A) 116, 513(1989) <https://doi.org/10.1002/pssa.2211160208>
- [61] K., Jemal, B.V., Sandeep, S. Pola, Journal of Nanomaterials, 4213275, 11(2017) <https://doi.org/10.1155/2017/4213275>
- [62] B., Ajitha, Y. A. K., Reddy, P. S. Reddy, Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy 128, 257(2014). <https://doi.org/10.1016/j.saa.2014.02.105>
- [63] K. Jeeva, M. Thiyagarajan, V. Elangovan, N. Geetha, P. Venkatachalam, Industrial Crops and Products 52, 714(2014). <https://doi.org/10.1016/j.indcrop.2013.11.037>
- [64] P. Saini, S. K. Saha, P. Roy, P. Chowdhury, S.P.S. Babu, Experimental Parasitology 160, 39(2016). <https://doi.org/10.1016/j.exppara.2015.11.004>
- [65] I. Zahoor, F. Jan, U. Sharma, K. K. Sahu, A. Sharma, S. Pareek, D. Shrivastava, P. S. Bisen, Anti-Cancer Agents in Medicinal Chemistry 21(10), 1266(2021) <https://doi.org/10.2174/1871520620999201001201230>
- [66] M. Jhonson, A. Santhanam, S. Thangaiah, J. Narayanan, Particulate Science and Technology 36(5), 578(2017). <https://doi.org/10.1080/02726351.2016.1278292>
- [67] S. Santhoshkumar, N. Nagarajan, International Journal of Pharmaceutical Sciences Research 5(12), 5511(2014).
- [68] A. S. Dehnavi, A. Raisi, A. Aroujalian, Synthesis and Reactivity in Inorganic Metal-Organic and Nano-Metal Chemistry 43(5), 543(2013). <https://doi.org/10.1080/15533174.2012.741182>
- [69] M. Zhang, K. Zhang, B. D. Gusseme, W. Verstraete, R. Field, Biofouling 30(3), 347(2014). <https://doi.org/10.1080/08927014.2013.873419>