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Microwave assisted biosynthesis of silver nanoparticles using banana leaves extract: Phytochemical, spectral characterization, and anticancer activity studies

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ABSTRACT

Microwave-assisted biosynthesis of nanoparticles has been a cost-effective, environmentally benign, and alternative to the chemical method. In this context, we report eco-friendly and robust nanoparticles synthesized using the bio-waste (Banana leaves) extract material through a microwave method. The newly synthesized Banana Leaves extract-Silver Nanoparticles (BL-AgNPs) is confirmed by using the UV-Visible, FT-IR spectroscopy, and Scanning Electron Microscopy (SEM) techniques. UV-Vis spectrum shows the widening of the band around 476 nm, which confirms the polydispersed nature of BL-AgNPs. FT-IR spectroscopy explores that, hydroxyl and carbonyl groups in the Banana Leaves extract play a vital role in the reduction of silver ions and also attach with AgNPs. The phytochemical studies reveal that, the polyphenols and alkaloids present in the BL extract act as reducing and stabilizing agent, which is responsible for the reduction of Ag+ (silver ions) to Ag (BL-AgNPs) and the stabilization of BL-AgNPs. This confirms the formation of silver nanoparticles (AgNPs). SEM results revealed that, bead shapes of BL-AgNPs with a particle size of 80 to 100 nm. In conclusion, BL-AgNPs exhibits promising anticancer activity against lung cancer and breast cancer cell line by endorsing inhibition of cell migration and proliferation on low concentrations.

Keywords: Biosynthesis; UV-Visible spectroscopy; Nanoparticles; Anticancer activity; Phytochemical screening

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INTRODUCTION

Nanoparticles from a metal source such as zinc (Zn), titanium (Ti), copper (Cu), gold (Au), and silver (Ag) play an important role in Nanoscience technology. Among these, silver nanoparticles (AgNPs) attracted many scientists, working on various sections of biomedical, pharmacological, and food sciences towards green nanotechnology compared to other metal source nanoparticles. This is because, silver nanoparticles (AgNPs) possess unique physical, biological and chemical properties

such as conductivity, catalytic, mechanical, thermal, electronic, and optical properties due to their biocompatibility, high stability, low toxicity, heat transfer, spatial confinement, high areavolume ratio, and small size. Therefore, it is widely used in biomedical and nutraceuticals such as biosensing, bioimaging, catalysis, and drug delivery systems, etc. Hence, AgNPs gain close attention in the nanomedicine field [1-5]. At present, the production of AgNPs from the chemical and physical methods include hydrothermal, lithography, sonochemical, aerosol, sol-gel, UV

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irradiation, pulsed laser deposition, chemical vapor deposition, and thermal evaporation. These involve harsh reaction conditions, toxic and expensive chemicals, which is assigned as major drawback and threats to the environment, health, and its usage. Hence, there is an urgent need to develop green techniques/methods without using expensive and toxic chemicals. Synthesis of eco-friendly AgNPs is economical and fast increasing research. Therefore, green nanoparticles synthesized from the eco-friendly species gained popularity as it generates minimal waste, costeffective and eco-friendly nature. Many green species can be employed as a reducing and capping agent for the synthesis of environmentally benign nanoparticles without using hazardous chemicals and other purification techniques [6-8]. Therefore, scientists focused on closed reactor eco-friendly techniques and green solvents (for example, water) alternative to toxic chemicals such as ethylene glycol, sodium borohydride, dimethylformamide, and hydrazine hydrate. Thus, it is very much important to develop green nanoparticles to solve the environmental contamination issues. For the synthesis of green nanoparticles, the chemicals such as plant extract species, biopolymers, sugars, vitamins, and microorganisms are generally used as reducing and capping agents. In recent years, the nanoparticles prepared from the plant extract species are more suitable for large-scale and stable particle productions. Furthermore, the reduction of metallic ions is fast in the presence of plant extract species compared to the other methods. The plant extract possesses free-radical scavenging species such as alkaloids, terpenoids, tannins, fats, carbohydrates, and polyphenols in their moieties, which contains carbonyl and hydroxyl groups. This will act as reducing and stabilizing agents. The advantages of plant extract species are i) stable against temperature and pH, ii) no need for special storage conditions, iii) no environmental contamination, and iv) cheap and easily available. The greater stability is due to the formation of a bond in between the phytochemicals and nanoparticles. The nucleation centers are formed due to the reduction of metal ions which leads to the formation of nanoparticles [9-12]. In recent years, diagnostic and therapeutic approaches through the green nanoparticles show good anticancer activity compared to the chemical nanoparticles. Chougule et al., (2020) prepared silver nanoparticles by using Moringa oleifera plant extract for food

packaging, photocatalytic degradation, antimicrobial applications. Authors synthesized silver nanoparticles from 10 ml of Moringa oleifera leaf extract and 90 ml of 1 mM silver nitrate. Plant extract is mixed with silver nitrate and heated at 80 ⁰ C for 20 minutes. Change in the color is observed and it is centrifuged for ten minutes. The residue is washed with distilled water and dried [13]. Laura Carson et al., (2020) reported the antimicrobial activity of microwave-assisted Phyla dulcis plant extract-silver extract. Silver nanoparticles were prepared by the microwave method at different time intervals followed by centrifugation. They prepared pellet silver nanoparticles for antimicrobial activity [14]. Pooja Moteriya et al., (2020) synthesized silver nanoparticles from Caesalpinia pulcherrima leaf extract and screened their antimicrobial, cytotoxic, and genotoxic potential (3-in-1 System). Silver nanoparticles were prepared by adding 3 ml of a leaf extract into 40 ml of 1 mM of silver nitrate solution. The nanoparticles were obtained after centrifugation by using optimized parameters [15]. According to the American Cancer Society, lung cancer and breast cancer are considered the most dangerous diseases due to their high death rate. Hence, researchers focused on developing new anticancer agents on cancer cells. There is no specific report on the Banana leaves extract-silver nanoparticles as anticancer agents; therefore, this motivated us to determine the anticancer activity of Banana leaves extract-silver nanoparticles on A549 (lung cancer cell line) and MCF7 (breast cancer cell line) cancer cells. The green chemicals from the plant extract species can be successfully isolated by various extraction techniques. Among these, the Soxhlet apparatus used for green chemical extraction was compared to other normal extraction methods. This is because; Soxhlet apparatus is very simple, a high percentage of yield (in terms of extraction) with a low amount of solvent can be achieved; which is very imperative in terms of financial inputs, period, and energy. Hence, in the current investigation, we selected Soxhlet extraction apparatus for the isolation of green species in the Banana leaves. The schematic representation of the Soxhlet extraction apparatus is shown in Fig. 1.

Phytochemical studies carried out on Banana leaves extract. A microwave technique was employed for the synthesis of silver nanoparticles by using Banana leaves extract. The newly synthesized Banana leaves extract Silver Nanoparticles (BL-

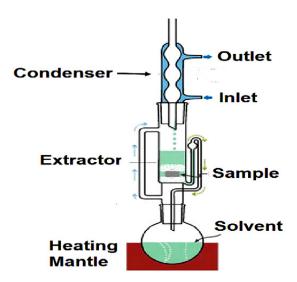


Fig. 1. Schematic representation of Soxhlet extraction apparatus

AgNPs) were characterized by using Ultraviolet-Visible (UV-Vis), Fourier Transform-Infrared (FT-IR) spectroscopy, and Scanning Electron Microscopy (SEM) techniques. Furthermore, biosynthesized BL-AgNps were evaluated for anticancer activity against A549 (lung cancer cell line) and MCF7 (breast cancer cell line). The work plan of the present investigation is shown in Fig. 2.

EXPERIMENTAL SECTION

Plants and chemicals

Banana leaves [Fig. 3] are the leaves of the banana plant which belong to Musaceae and the plant is classified as Kingdom: Plantae, Order: Zingiberales, and Genus: Musa. The plant is grown worldwide, including in Asian countries such as India, Bhutan, Sri Lanka, Burma, Indonesia, China, and Pakistan. Fresh Banana leaves are collected from the rural area in Hubballi, Karnataka (India). These leaves were washed with tap water followed by double distilled water to remove the dust particles. Cleaned leaves were kept to sun dry for about fifteen days. After that, they were powdered well by grinding. Silver nitrate (AgNO₃)- 99 %, HCl, NaOH, and FeCl, were purchased from Sigma-Aldrich. Double distilled water and glassware such as glass rods, beaker, petri dish, condenser, and round bottom flask were used throughout the experiment where necessary.

Preparation of plant extraction

The banana leaves powder of 250 grams placed in the Soxhlet extraction chamber and solvent

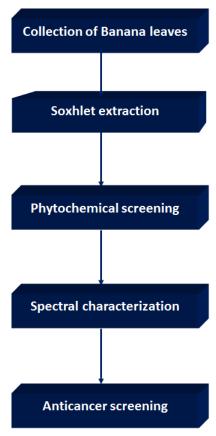


Fig. 2. Work plan of present investigation

(water) of 250 ml is placed in the round bottom flask along with boiling chips. The vaporized double distilled water undergoes condensation in the water-cooled condenser. The condensed water



Fig. 3. Banana leaves used in the present investigation

dropped on the Banana leaves to powder and water extracts the green chemicals by its contact with the Banana leaves powder. When the water reaches the overflow level, then the whole contents are discharged to the distillation flask (round-bottom flask). The extraction could be successfully achieved by repeating the operation several times. After that, the whole extracted solution was filtered to remove the impurities and kept in the refrigerator to prevent biochemical side reactions [16].

Phytochemical screening

Identification of medicinally active species in the plant extract is very important because some green species such as phenolic, tannin, carotenoids, alkaloids, and flavonoids play a very important role as reducing agents in the preparation of nanoparticles. Hence, the identification of these groups in the plant extract is very important. Therefore, in this study, the preliminary phytochemical test was used to find out major components present in the Banana leaves extract by standard procedures.

- a) **Test for Saponins:** 5 ml of Banana leaves extract was added to 5 ml of double-distilled water by vigorous shaking, which leads to the formation of foam. This confirms the presence of saponins in Banana leaves extract [17].
- b) **Test for Alkaloids:** 2 ml of hydrochloric acid added to the 5 ml of Banana leaves extract.

The resulting mixture boiled for about 30 minutes, cooled and filtrated solution treated with Hager's reagent. This results formation of yellow color, which indicates the presence

of alkaloid groups in the Banana leaves

extract [18].

c) **Test for Phenol:** 5 ml of Banana leaves extract was treated with 10 drops of ferric chloride

(FeCl₃) solution. The bluish color evidence the presence of Phenolic compounds in Banana leaves extract [19].

d) **Test for Flavonoids:** 5 ml of Banana leaves extract was treated with 10 % sodium hydroxide, solution changes to intense yellow color, which confirms the presence of Flavonoids in the extract [20].

Preparation of silver nanoparticles by using Banana leaves extract

Biosynthesis of silver nanoparticles was carried out by adding 10 ml of Banana leaves extract in 40 ml of known strength (0.0001 M) of an aqueous solution of silver nitrate (AgNO₂) with constant stirring. During this time, no appreciable color change was noticed. After that, the resulting solution was heated in the domestic microwave for about five minutes at 160 °C, and a noticeable color change was observed. The color of the solution is due to the formation of silver nanoparticles. The intense brown color on heating indicates the formation of Banana Leaves extract-Silver Nanoparticles (BL-AgNps) [Fig.4]. The obtained solution is placed in the petri dish and dried at 60 °C in a vacuum oven for one day. After that, the powder form of the sample is collected and stored in the desiccator and used for spectral characterizations and anticancer activity studies. The mechanism involved in the synthesis of silver nanoparticles from the Banana leaves extract under reducing and capping action is shown in Fig. 5.





Fig. 4. Banana Leaves extract -silver nanoparticles (BL-AgNps) obtained by microwave assisted biosynthesis

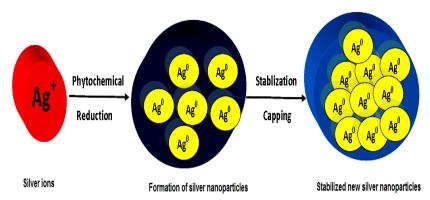


Fig. 5. The schematic representation of formation of silver nanoparticles from the plant extract

Spectral characterization of biosynthesized silver nanoparticles

The BL-AgNPs prepared by microwave-assisted biosynthesis were characterized by using the UV-Visible spectrophotometer (cytxon, biosolution, pvt. Ltd, Hubli) and Fourier-Transform Infrared (FT-IR) spectroscopy (KUD, Dharwad) techniques. The Uv-Visible spectrum was recorded in the range of 650 nm to 300 nm for three systems, i.e. double distilled water, BL extract, and BL-AgNps respectively. The green chemical species in the Banana leaves extract responsible for the capping agents and reduction of silver from Ag⁺ to Ag⁰ of silver nanoparticles was screened by using the FT-IR spectroscopy in the range of 400 to 4000 cm⁻¹.

Scanning electron microscopy (SEM) technique

SEM is an electron microscope technique that captures the image of the silver nanoparticles by scanning with a high-energy beam of electrons. These electrons undergo an interaction with atoms of silver nanoparticles and producing signals that provide information about composition, particle size, roughness, smoothness, surface topography,

and electrical conductivity. The SEM technique is widely considered as a gold standard for silver nanoparticle characterization. Hence, in the current investigation, BL-AgNPs were placed in the sample chamber of scanning electron microscopy and scanning was carried out at different magnifications.

Anticancer activity studies

The MTT reduction assay was carried out to screen the anticancer activity of BL-AgNPs. The cells were seeded on the 96 well microtitre plate with 200 μ L of DMEM HG medium. At 310 K, the plate was incubated with five percentages of carbon dioxide for twenty-four hours. After twenty-four-hour incubation with BL-AgNPs, the spent media was aspirated. The 200 μ l of five different concentrations namely 20, 40, 60, 80, and 100 μ g/ml of test drugs were placed into the respective cells and incubated at 310 K in a humidified chamber with five percentages of carbon dioxide. Then, the spent medium is replaced with a 10 % MTT reagent to each well to get 0.5 mg/ml of final concentration and incubated at 310 K with five percentages of

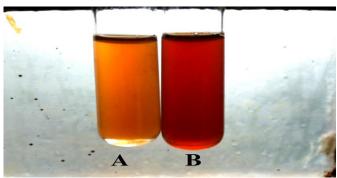


Fig. 6. Reaction mixture color change from light yellow (A) into intense brown color (after five minutes), which confirms biosynthesis of BL-AgNPs (B)

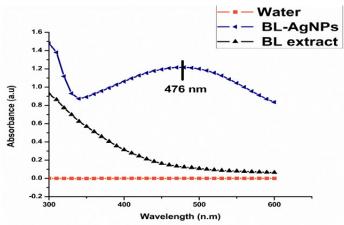


Fig. 7. UV-Visible spectroscopy of water, BL extract and BL-AgNPs

carbon dioxide for three hours. After the specified time, MTT was removed without troubling the crystals generated and 100 μL of dimethylsulfoxide (DMSO) was added to the plates. The absorbance was read at 570 nm and also at 630 nm with a microplate spectrophotometer. The % grown inhibition was calculated [21-25].

RESULTS AND DISCUSSION

In the present investigation, we reported the microwave-assisted biosynthesis of BL-AgNPs using Banana leaves extract. The Banana leaves extract act as a capping and reducing agent which reduces the silver ions (Ag+) into nanosilver (AgNPs). The reaction mixture contains 40 ml of 0.0001 M AgNO₃ and 10 ml of Banana leaves extract, which turns into intense brown color from light yellow color after 5 minutes of microwave irradiation [Fig.6]. This color change is a clear indication of the formation of green BL-AgNPs. Further, the biosynthesis of BL-AgNPs was

confirmed by UV-Visible spectroscopy analysis of colloidal reaction mixture.

UV-Visible spectroscopy

Uv-Visible spectroscopy of water, BL extract, and BL-AgNPs are shown in Fig. 7. The visual inspection of BL-AgNPs spectra showed the absorption band at 476 nm. The broad absorption band around 476 nm related to the interaction between silver ions and conjugate aromatic systems, polyphenols, and flavonoids in the BL extract. After the addition of BL extract to silver ion solution, the green silver nanoparticles are formed. This can be confirmed by the presence of broadband around 476 nm and phenomena of color change into an intense brown color, which is an indication of silver ion reduction. The adsorption broadband at 476 nm is a Surface Plasmon Resonance (SPR) band and it is the characteristic band of BL-AgNPs. SPR band mainly depends on the distribution of green nanoparticles, size, and morphological shape. The characteristic

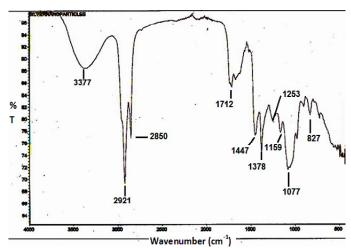


Fig. 8. FT-IR spectrum of BL-AgNPs exposed the presence of functional groups

SPR band is an indication of interaction in between the hydroxyl and aromatic rings with silver ions. SPR reflects the unique interaction of light in which metallic nanoparticles free electrons undergo oscillations concerning the metallic lattice in the presence of electromagnetic fields of light. No broadband at 476 nm was observed in BL-extract due to the absence of silver nanoparticles (AgNPs). The broad applications of the silver nanoparticles are due to their optical properties related to dielectric constant, adsorbed species on the surface, chemical surrounding, surface bioconjugation with molecular probes, and localized SPR phenomenon [26-28]. BL extract exhibits no band, whereas BL-AgNPs show broadband at 476 nm, which is attributed due to the formation of large-sized silver nanoparticles. In the reaction mixture, the BL extract act as a strong reducing agent that abruptly accelerates the nucleation and effect of growth silver nanoparticles. Based on these, 10 ml of BL extract can be considered as an optimum concentration for the production of large-size silver nanoparticles [24]. Further, no drastic color change is observed in the reaction mixture at ambient temperature (303 K) and Uv-Visible spectra displayed no broad or sharp band in the range of 400-500 nm. The silver nanoparticles synthesized microwave-assisted biosynthesis produce broadband at 476 nm. Compared to conventional heating, microwave-assisted biosynthesis facilitates fast heating and nucleation of silver nanoparticles, which accelerates the formation of green silver nanoparticles. Hence, microwave-assisted biosynthesis is considered for favorable kinetics and rapid green silver nanoparticles synthesis [29]. The

stability of green BL-AgNPs was studied by using UV-Visible spectroscopy after 35 days. No change in the UV-Vis spectrum was observed, which confirms the good stability of silver nanoparticles (BL-AGNPs) at ambient temperature (303 K) as no significant variation in the position and shape of the absorption band. According to the Mie theory, green silver nanoparticles with spherical shapes have a single SPR band, whereas, the green silver nanoparticles with different and asymmetric shapes have more shoulders in SPR bands [30].

FT-IR spectroscopy

The FT-IR spectroscopy provides information about how to plant extract species involved in the transformation of silver ions into silver nanoparticles. The plant extract species have properties such as reducing agents, capping, and stabilizing during the synthesis of silver nanoparticles. The FT-IR spectroscopy is an appropriate technique to screen chemical adsorption and the functional groups that are responsible for the reduction of Ag+ ions in the newly synthesized silver nanoparticles. It exhibits the characteristics of functional moieties and surface structure present in the reduction of silver ions and possible interaction between BL extract and silver ions. The FT-IR analysis of BL-AgNPs exhibited prominent bands at 3377, 2921, 2850, 1712, 1447, 1378, 1253, 1159, 1077, and 827 cm⁻¹ [Fig.8], which suggests binding the active AgNPs to the surface.

The band at 3377 cm⁻¹ shows the presence of OH stretching, which is attributed to the hydroxyl group (-OH) of the flavonoids or phenolic

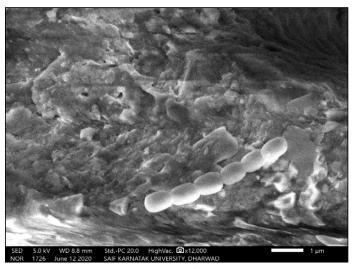


Fig.9. SEM image of BL-AgNPs

compounds, and the band at 2921 cm⁻¹ is assigned to the C-H stretching vibrations of the -CH, group. The presence of carbonyl group (C=O) is confirmed due to the presence of a band at 1712 cm⁻¹. The band at 1447 cm⁻¹ indicates the presence of stretching vibrations of carbonyl groups in the aromatic compounds. The band at 1378 cm⁻¹ is related to the presence of aromatic rings in flavonoids and polyphenol compounds in the reaction mixture. The band at 1077 cm⁻¹ hints at the presence of a carboxylic group in the sample. FT-IR spectrum confirms that polyphenolic, flavonoids and other active species are actively involved in the reduction and stabilization (capping agent) of silver ions (Ag+) into green silver nanoparticles (BL-Ag⁰NPs) by chelating of silver ions with BL extract hydroxyl and carboxyl groups [31-34].

These results show that a strong bond is generated between the BL extract and silver nanoparticles. The C=O, aliphatic C=C, -OH, and -COOH functional groups in the flavonoids, polyphenols, and other aromatic compounds will be the key factor in the reduction of silver ions and stabilization of green BL-AgNPs. These active functional groups greatly facilitate the formation of stable green silver nanoparticles because of their greater affinity towards the metal ions. The present investigation suggests that BL extract contains many functional active centers required for the biosynthesis of stable silver nanoparticles. The stabilization of synthesized silver nanoparticles (BL-AgNPs) is due to the coordination of silver ions with C=C, C=O-C, and C=O groups of

Table 1. Measurement of the silver nanoparticles (BL-AgNPs) shape and size under SEM Technique

Characteristics	SEM
Size (nm)	80- 100
Shape	Bead

aromatic species in BL extract. Previous reports show that the FT-IR prominent band in the range of 1600-1700 cm⁻¹ shows the formation of silver nanoparticles and capped with various bioactive species [35-38]. The literature study also shows that the FT-IR spectrum of biologically synthesized silver nanoparticles from the plant extract has shown the presence of active biospecies on their surface [39].

Scanning electron microscopy (SEM) technique

The size and shape of the BL-AgNPs were measured by using the SEM technique. The SEM results reveal that most of the silver nanoparticles were bead shape and spongy with moderate variation in the particle sizes. The primarily synthesized silver nanoparticles are in the nanosize range. The particle size in the range of 80-100 nm and it is crystalline [Fig. 9 and Table 1]. The size variation in silver nanoparticles is due to the presence of molecules or proteins from the green extract species which bound on the surface of the silver nanoparticles. The agglomeration of silver nanoparticles exhibited in the SEM topography is related to the preparation technique in this measurement. Smaller-sized silver nanoparticles have many advantages such as chemical stability,

Concentration (µg/ml) of BL-AgNPs	A549 cells		MCF7 cells	
	OD	% Viability	OD	% Viability
0	1.720	100	0.523	100
20	1.358	78.84	0.468	89.52
40	1.050	60.86	0.375	71.56
60	0.937	54.30	0.334	63.78
80	0.852	49.31	0.270	51.48
100	0.792	45.81	0.182	34.48
Cisplatin- 15µg/ml	0.660	38.13	0.066	12.19

Table 2. Comparison of anticancer activity of Banana leaves extract-silver nanoparticles against A549 and MCF7 cells

catalytic, good conductivity, and anticancer activities. These make them appropriate for various applications. Further, the SEM topography exhibits green silver nanoparticle aggregates. The new silver nanoparticles are not in direct contact within the aggregates, showing the stabilization of silver nanoparticles by a capping agent. The remarkable potential property of BL-AgNPs is due to the presence of flavonoids, phenol, alkaloids, and saponins in the plant extracts which can act as stabilizing and reducing agents. The results obtained from the SEM obey the results of UV-Vis spectra concerning the shape and size of the silver nanoparticles.

Anti-cancer studies

The MTT assay was carried out by using BLAgNPs on two different types of cancerous cells which are A549 (lung cancer cell line) and MCF7 (breast cancer cell line). For each cell line, 200 μl of different test concentrations namely, 20, 40, 60, 80, 100 $\mu g/ml$ of silver nanoparticles (BL-AgNPs) were added to the 96 wells. The % of cell viability can be obtained by using optical density (OD) values as per the following relation:

% of cell viability =
$$\frac{\text{OD value of experiment samples}}{\text{OD value of experimental controls}} \times 100$$
 (1)

The results are given in Table 2 and Fig. 10,11 and 12, interestingly, we found that exposure of A549 cells and MCF7 cells to BL-AgNPs at five different concentrations for twenty-four hours greatly reduces the cell viability in concentration-dependent mode. The cell viability at $100 \,\mu\text{g/ml}$ was not significant. The increase in the concentration of BL-AgNPs from 20 to $100 \,\mu\text{g/ml}$ declines the % cell viability towards the lower value. For BL-AgNPs concentration from 20 to $100 \,\mu\text{g/ml}$, the cell viability percentage decreases from 89.529 to $34.486 \,(\text{MCF7} \,\text{cell} \,\text{line})$ and $78.844 \,\text{to} \,45.812 \,(\text{A549} \,\text{cell} \,\text{cell})$

cell line). The standard cis-platin drug decreases % viability to 12.1 (MCF 7 cell) and 38.1 (A549 cell line) at 15 $\mu g/ml$. The reason behind this trend is due to the stimulation of Reactive Oxygen Species (ROS) by BL-AgNPs and their potential activity on the cellular components which leads to cell death [40]. The cancer cell death enhanced with a rise in the concentration of the BL-AgNPs on A549 and MCF7 cells. The cytotoxicity activity of BL-AgNPs against A549 and MCF7 cell line, the IC50 values are 79.48 $\mu g/ml$ and 78.76 $\mu g/ml$ respectively.

Literature study shows that cytotoxicity activity of Syzygium aromaticum fruit extract-Silver Nanoparticles (SAF-AgNPs) against A549 cell line, the IC50 value is 70 μ g/ml [41] and, cytotoxicity activity of Alternanthera tenella leaf extract Silver Nanoparticles (ATL-AgNPs) against MCF7 cell line, the IC50 value is 42.5 μ g/mL [42].

The BL-AgNPs is found to be efficacious as seen by the low amount (100 µg/ml) at which 65.513 % and 54.187 % cancer cell death occurred in the case of MCF7 and A549 cells respectively. The new synthesized anticancer drug (BL-AgNPs) damages both malignant and normal cells alike. The presence of pharmacologicalogically active anticancer active species which includes polyphenols, flavonoids, and other aromatic compounds also reason for BL-AgNPs to exhibit efficacious anticancer activity. The previous report shows that the Banana leaves extract shows 48. 60 % cytotoxic effect on MCF 7 cell line, whereas in the present investigation, Banan leaves extract-AgNPs exhibit 65.514 % cytotoxic effect at 100 µg/ml. The strong interaction between the bio-green chemicals present in the BL extract and silver ions in the sample is the main reason for the superior cytotoxic effect (%) of BL-AgNPs on the MCF 7 cell line compared to Banana leaves extract [Table 3]. The mechanism of action of BLAgNPs as an anticancer agent on both A549 and MCF 7 cells is shown in Fig. 13. The results confirm

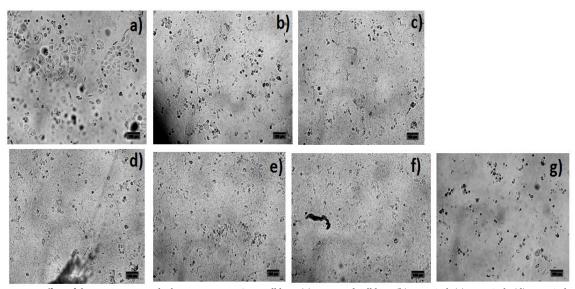


Fig. 10 Effect of the BL-AgNPs on the breast cancer MCF-7 cell line: (a) untreated cell line, (b) 20 μg/ml, (c) 40 μg/ml, (d) 60 μg/ml, (e) 80 μg/ml, (f) 100 μg/ml of BL-AgNPs (treated cell line) and (g) standard drug (Cis-platin)

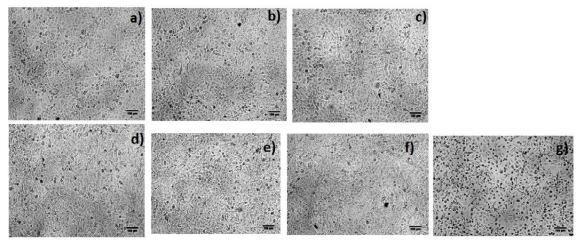


Fig. 11 Effect of the BL-AgNPs on the breast cancer A549 cell line: (a) untreated cell line, (b) 20 μ g/ml, (c) 40 μ g/ml, (d) 60 μ g/ml, (e) 80 μ g/ml, (f) 100 μ g/ml of BL-AgNPs (treated cell line) and (h) standard drug (Cis platin)

Table 3. Cytotoxic effects of Banana leaves extract- silver nanoparticles at five different concentrations and banana leaves extract at 100 $\mu g/ml$

	μg/1111		
Samples	Concentration (µg/ml)	MCF-7 (%)	Reference
Banana leaves extract-silver nanoparticles	20	10.47	Present paper
Banana leaves extract-silver nanoparicles	40	28.43	Present paper
Banana leaves extract-silver nanoparticles	60	36.21	Present paper
Banana leaves extract-silver nanoparticles	80	48.51	Present paper
Banana leaves extract-silver nanoparticles	100	65.51	Present paper
Banana leaves	100	48.60	[44]

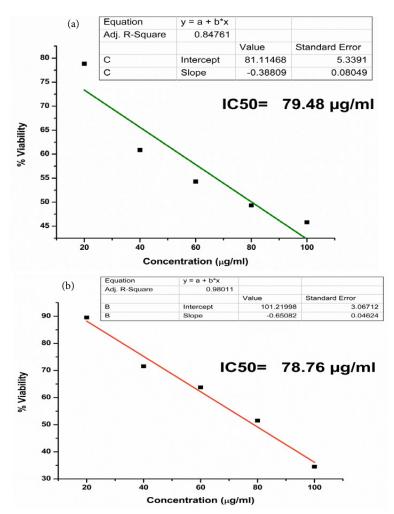


Fig. 12 Effect of concentration of BL-AgNPs on % viability, a) A549 and b) MCF7 cells

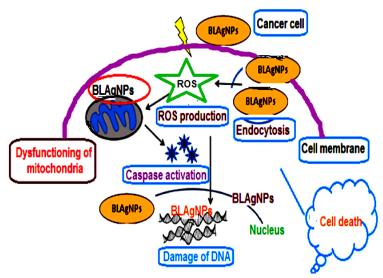


Fig. 13. Mechanism of anticancer activity of BLAgNPs on cancer cells

the enhanced potential of microwave-assisted biologically synthesized of BL-AgNPs against both lung and breast cancer cells without any side effects and this warrants further analysis.

CONCLUSION

The green chemicals present in the BL were isolated by using the Soxhlet extraction apparatus. Phytochemical screening, UV-Vis, and FT-IR spectroscopy confirm the presence of polyphenols, flavonoids, and other aromatic green chemicals in the BL extract. The BL-AgNPs size was found to be 80 to 100 nm with spherical shape through SEM technique. Microwave-assisted BL-AgNPs proved as an environmentally benign anticancer agent against A549 (lung cancer cell line) and MCF7 (breast cancer cell line) with good IC50 values at low concentrations of silver nanoparticles. The superior anticancer agent property of BL-AgNPs against MCF 7 (breast cancer cell line) compared to BL extract is due to the strong interaction between the green chemicals in the BL extract and silver ions in the reaction mixture.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ETHICAL APPROVAL

This research work does not involve any human participants and/or animal subjectivity.

REFERENCES

- Dara PK, Mahadevan R, Digita PA, Visnuvinayagam S, Kumar LRG, Mathew S, et al. Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): in vitro studies on antioxidant and antibacterial applications. SN Applied Sciences. 2020;2(4):665.
- Manukumar HM, Umesha S, Kumar HNN. Promising biocidal activity of thymol loaded chitosan silver nanoparticles (T-C@AgNPs) as anti-infective agents against perilous pathogens. Int J Biol Macromol. 2017;102:1257-65.
- Reddy NV, Satyanarayana BM, Sivasankar S, Pragathi D, Subbaiah KV, Vijaya T. Eco-friendly synthesis of silver nanoparticles using leaf extract of Flemingia wightiana: spectral characterization, antioxidant and anticancer activity studies. SN Applied Sciences. 2020;2(5):884.
- Ravindran A, Chandran P, Khan SS. Biofunctionalized silver nanoparticles: Advances and prospects. Colloids Surf, B. 2013;105:342-52.
- Mukherjee S, Chowdhury D, Kotcherlakota R, Patra S, B V, Bhadra MP, et al. Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system). Theranostics. 2014;4(3):316-35.
- 6. Chand K, Cao D, Eldin Fouad D, Hussain Shah A, Qadeer

- Dayo A, Zhu K, et al. Green synthesis, characterization and photocatalytic application of silver nanoparticles synthesized by various plant extracts. Arabian J Chem. 2020;13(11):8248-61.
- Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat Res Appl Sci. 2016;9(1):1-7.
- Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Rai MK. Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Lett Appl Microbiol. 2009;48(2):173-9.
- Parsons JG, Peralta-Videa JR, Gardea-Torresdey JL. Chapter 21 Use of plants in biotechnology: Synthesis of metal nanoparticles by inactivated plant tissues, plant extracts, and living plants. In: Sarkar D, Datta R, Hannigan R, editors. Developments in Environmental Science. 5: Elsevier; 2007. p. 463-85.
- Nasrollahzadeh M, Maham M, Mohammad Sajadi S. Green synthesis of CuO nanoparticles by aqueous extract of Gundelia tournefortii and evaluation of their catalytic activity for the synthesis of N-monosubstituted ureas and reduction of 4-nitrophenol. J Colloid Interface Sci. 2015;455:245-53.
- Nasrollahzadeh M, Sajadi SM, Maham M. Tamarix gallica leaf extract mediated novel route for green synthesis of CuO nanoparticles and their application for N-arylation of nitrogen-containing heterocycles under ligand-free conditions. RSC Adv. 2015;5(51):40628-35.
- Raghavendra N, Ishwara Bhat J. An Environmentally Friendly Approach Towards Mitigation of Al Corrosion in Hydrochloric Acid by Yellow Colour Ripe Arecanut Husk Extract: Introducing Potential and Sustainable Inhibitor for Material Protection. Journal of Bio- and Tribo-Corrosion. 2017;4(1):2.
- 13. Chougule SS, Gurme ST, Jadhav JP, Dongale TD, Tiwari AP. Low density polyethylene films incorporated with Biosynthesised silver nanoparticles using Moringa oleifera plant extract for antimicrobial, food packaging, and photocatalytic degradation applications. J Plant Biochem Biotechnol. 2020
- 14. Carson L, Bandara S, Joseph M, Green T, Grady T, Osuji G, et al. Green Synthesis of Silver Nanoparticles with Antimicrobial Properties Using Phyla dulcis Plant Extract. Foodborne Pathogens and Disease. 2020;17(8):504-11.
- 15. Moteriya P, Chanda S. Green Synthesis of Silver Nanoparticles from Caesalpinia pulcherrima Leaf Extract and Evaluation of Their Antimicrobial, Cytotoxic and Genotoxic Potential (3-in-1 System). J Inorg Organomet Polym Mater. 2020;30(10):3920-32.
- Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanopart Res. 2007;9(3):479-89.
- Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters. 2015;7(3):219-42.
- 18. 18.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J IMMUNOL METHODS. 1983;65(1):55-63.
- Alley M, Scudiero D, Monks A, Czerwinski M, Shoemaker R, Boyd M, editors. Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug



- sensitivity of human-tumor cell-lines. P AM ASSOC CANC RES; 1986: AMER ASSOC CANCER RESEARCH PUBLIC LEDGER BLDG, SUITE 816, 150 S
- Venkatesan B, Subramanian V, Tumala A, Vellaichamy E. Rapid synthesis of biocompatible silver nanoparticles using aqueous extract of Rosa damascena petals and evaluation of their anticancer activity. Asian Pacific Journal of Tropical Medicine. 2014;7:S294-S300.
- Suriya J, Raja SB, Sekar V, Rajasekaran R. Biosynthesis of silver nanoparticles and its antibacterial activity using seaweed Urospora sp. African Journal of Biotechnology. 2012;11(58):12192-8.
- Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, et al. Non-cytotoxic Silver Nanoparticle-Polysaccharide Nanocomposites with Antimicrobial Activity. Biomacromolecules. 2009;10(6):1429-35.
- Kelly KL, Coronado E, Zhao LL, Schatz GC. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. The Journal of Physical Chemistry B. 2003;107(3):668-77.
- 25. Kreibig U, Vollmer M. Optical properties of metal clusters: Springer Science & Business Media; 2013.
- Feiner L-F. Crossing boundaries and borders. Nat Nanotechnol. 2006;1(2):91-2.
- Kumari M, Mishra A, Pandey S, Singh SP, Chaudhry V, Mudiam MKR, et al. Physico-Chemical Condition Optimization during Biosynthesis lead to development of Improved and Catalytically Efficient Gold Nano Particles. Sci Rep. 2016;6(1):27575.
- Pal J, Deb MK, Deshmukh DK. Microwave-assisted synthesis of silver nanoparticles using benzo-18-crown-6 as reducing and stabilizing agent. Applied Nanoscience. 2014;4(4):507-10.
- Netala VR, Kotakadi VS, Domdi L, Gaddam SA, Bobbu P, Venkata SK, et al. Biogenic silver nanoparticles: efficient and effective antifungal agents. Applied Nanoscience. 2016;6(4):475-84.
- Netala VR, Kotakadi VS, Nagam V, Bobbu P, Ghosh SB, Tartte V. First report of biomimetic synthesis of silver nanoparticles using aqueous callus extract of Centella asiatica and their antimicrobial activity. Applied Nanoscience. 2015;5(7):801-7.
- Netala VR, Kotakadi VS, Ghosh SB, Bobbu P, Nagam V, Sharma K, et al. Biofabrication of silver nanoparticles using aqueous leaf extract of Melia dubia, characterization and antifungal activity. Int J Pharm Pharm Sci. 2014;6:298-300.
- 32. , Anindita Chowdhury SK, Chiranjib Bhattacharjee and Ranjana Chowdhury. Green synthesis and characterization of biocompatible silver Nanoparticles using Brassica Ol-

- eracea l. leaf extract. International Journal of Current Research. 2014;6(4):6166-74.
- Sathishkumar P, Preethi J, Vijayan R, Mohd Yusoff AR, Ameen F, Suresh S, et al. Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using Coriandrum sativum leaf extract. J Photochem Photobiol, B. 2016;163:69-76.
- Harshiny M, Matheswaran M, Arthanareeswaran G, Kumaran S, Rajasree S. Enhancement of antibacterial properties of silver nanoparticles–ceftriaxone conjugate through Mukia maderaspatana leaf extract mediated synthesis. ECOTOX ENVIRON SAFE. 2015;121:135-41.
- Sharma P, Pant S, Rai S, Yadav RB, Dave V. Green Synthesis of Silver Nanoparticle Capped with Allium cepa and Their Catalytic Reduction of Textile Dyes: An Ecofriendly Approach. J Polym Environ. 2018;26(5):1795-803.
- Kalpana D, Han JH, Park WS, Lee SM, Wahab R, Lee YS. Green biosynthesis of silver nanoparticles using Torreya nucifera and their antibacterial activity. Arabian J Chem. 2019;12(7):1722-32.
- Arulkumar S, Sabesan M. Rapid preparation process of antiparkinsonian drug Mucuna pruriens silver nanoparticle by bioreduction and their characterization. Pharmacognosy Res. 2010;2(4):233-6.
- Mubarak Ali D, Thajuddin N, Jeganathan K, Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf, B. 2011;85(2):360-5.
- Ibrahim EH, Kilany M, Ghramh HA, Khan KA, ul Islam S. Cellular proliferation/cytotoxicity and antimicrobial potentials of green synthesized silver nanoparticles (AgNPs) using Juniperus procera. Saudi Journal of Biological Sciences. 2019;26(7):1689-94.
- 40. Venugopal K, Rather HA, Rajagopal K, Shanthi MP, Sheriff K, Illiyas M, et al. Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum. J Photochem Photobiol, B. 2017;167:282-9.
- 41. Sathishkumar P, Vennila K, Jayakumar R, Yusoff ARM, Hadibarata T, Palvannan T. Phyto-synthesis of silver nanoparticles using Alternanthera tenella leaf extract: an effective inhibitor for the migration of human breast adenocarcinoma (MCF-7) cells. Bioprocess Biosyst Eng. 2016;39(4):651-9.
- 42. Salama ZA, Aboul-Enein AM, Gaafar AA, Asker MS, Aly HF, Ahmed HA. In-vitro Antioxidant, Antimicrobial and Anticancer Activities of Banana leaves (Musa acuminata) and Olive leaves (Olea europaea L.) as by-products. Research Journal of Pharmacy and Technology. 2020;13(2):687-96.