



IN VITRO PHARMACOLOGICAL EVALUATION OF BRYOPHYLLUM PINNATUM SILVER NANOPARTICLES IN BREAST CANCER THERAPY

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ABSTRACT

Breast cancer remains one of the most prevalent malignancies among women worldwide, highlighting the need for innovative, effective, and less toxic therapeutic strategies. Conventional treatments are often associated with systemic toxicity, drug resistance, and high costs, prompting exploration of alternative approaches. This research focuses on the green synthesis of silver nanoparticles (AgNPs) using the medicinal plant *Bryophyllum pinnata*, known for its rich phytochemical profile and anticancer properties. Plant bioactive compounds act as natural reducing and stabilizing agents to produce biocompatible AgNPs. The synthesized nanoparticles were comprehensively characterized for their physicochemical properties and evaluated in vitro against human breast cancer cell lines. Mechanistic studies revealed apoptosis induction through ROS generation and DNA damage. This eco-friendly approach aligns with green chemistry principles and demonstrates promising potential for plant-mediated AgNPs in

breast cancer nanomedicine.

KEYWORDS: Silver Nanoparticles, *Bryophyllum pinnata*, anticancer activity, Nano medicine.

INTRODUCTION

Cancer is one of the most serious health challenges worldwide and continues to be a major cause of death across different populations. It is not a single disease but a group of disorders that occur when normal cellular regulatory mechanisms fail, leading to uncontrolled growth and division of abnormal cells. These cells can invade surrounding tissues and spread to other parts of the body through a process known as metastasis. Over the years, the incidence of cancer has increased significantly due to factors such as aging, environmental pollution, unhealthy dietary habits, tobacco and alcohol use, and sedentary lifestyles. As a result, there is a growing need to develop more effective, safer, and affordable treatment strategies.^[1]

Among all types of cancer, breast cancer is the most commonly diagnosed cancer in women and is one of the leading causes of cancer-related deaths globally. In India, the burden of breast cancer has been rising steadily, with many cases being detected at advanced stages due to lack of awareness and limited access to early screening facilities. Breast cancer mainly develops from the epithelial cells of the mammary ducts or lobules and is considered a heterogeneous disease, meaning that it consists of different subtypes with varying biological characteristics and responses to treatment. These subtypes are commonly classified based on the presence or absence of hormone receptors such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Understanding these molecular differences is essential for selecting appropriate treatment approaches. The development of breast cancer is influenced by multiple factors, including genetic, hormonal, environmental, and lifestyle-related components. Genetic mutations, particularly in genes such as *BRCA1* and *BRCA2*, significantly increase the risk of developing breast cancer. Hormonal factors such as early onset of menstruation, late menopause, and prolonged exposure to estrogen also play an important role. In addition, lifestyle factors like obesity, physical inactivity, alcohol consumption, and reduced breastfeeding have been linked to increased risk. These factors together highlight the complex nature of breast cancer and emphasize the importance of both prevention and early detection.^[2,3]

Currently, breast cancer treatment involves a combination of approaches including surgery,

chemotherapy, radiation therapy, hormone therapy, and targeted therapy. While these treatments have improved survival rates, they are often associated with several limitations such as systemic toxicity, drug resistance, high cost, and adverse side effects. Chemotherapy, for instance, not only affects cancer cells but also damages healthy cells, leading to complications like hair loss, fatigue, and immune suppression. Similarly, targeted therapies, although more specific, are expensive and may not be accessible to all patients. These challenges have encouraged researchers to explore alternative and complementary treatment strategies that are more selective, cost-effective, and less harmful.^[4]

In recent years, nanotechnology has emerged as a promising field in cancer research, offering new possibilities for diagnosis and treatment. Nanoparticles, due to their extremely small size and large surface area, can interact with biological systems at the molecular level. This allows them to be used as carriers for drug delivery, enabling targeted treatment of cancer cells while minimizing damage to normal tissues. Among various types of nanoparticles, silver nanoparticles (AgNPs) have gained significant attention due to their unique physicochemical properties and strong biological activities, including antimicrobial and anticancer effects. Studies have shown that AgNPs can induce cancer cell death through mechanisms such as generation of reactive oxygen species (ROS), DNA damage, and activation of apoptotic pathways.^[5]

Despite their potential, conventional methods for synthesizing silver nanoparticles often involve the use of toxic chemicals and require high energy input, making them less environmentally friendly. To overcome these limitations, green synthesis methods have been developed using natural resources such as plant extracts. This approach is simple, cost-effective, and eco-friendly, as it utilizes phytochemicals present in plants as reducing and stabilizing agents. These bioactive compounds not only facilitate the formation of nanoparticles but also enhance their biological activity, making them more suitable for biomedical applications.

Medicinal plants have been used for centuries in traditional systems of medicine and continue to be an important source of therapeutic agents. Many plant-derived compounds have shown significant anticancer properties, including the ability to inhibit cell proliferation, induce apoptosis, and reduce oxidative stress. In this context, the use of plant-mediated nanoparticles represents a combination of traditional knowledge and modern technology, offering a novel approach for cancer treatment.^[6]

In the present study, the medicinal plant *Bryophyllum pinnata* has been selected for the green synthesis of silver nanoparticles. This plant, commonly known as “Miracle Leaf” or “Pattharchatta,” is widely used in traditional medicine and is known for its rich phytochemical composition, including flavonoids, phenolic compounds, and glycosides. These compounds play a crucial role in reducing silver ions to nanoparticles and stabilizing them. Moreover, *Bryophyllum pinnata* has been reported to exhibit various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects.

The silver nanoparticles synthesized using *Bryophyllum pinnata* are expected to combine the therapeutic properties of both silver and plant bioactive compounds. These nanoparticles may exhibit enhanced cytotoxic effects against breast cancer cells by inducing oxidative stress, damaging cellular components, and triggering apoptosis. Additionally, due to their nanoscale size, they may achieve better cellular uptake and targeted action, thereby reducing systemic toxicity.^[7]

MATERIALS AND METHOD

Plant Profile^[8]

Bryophyllum pinnatum



Fig: *Bryophyllum pinnatum* plant.

Common Name

Table: Synonyms of *Bryophyllum pinnatum*.^[8]

Language	Common Name
English	Life Plant, Miracle Leaf, Air Plant, Cathedral Bells
Hindi	Pattharchatta, Ajuba
Sanskrit	Parnabeeja, Hemalata
Marathi	Panphuti, Ghamari

Gas Chromatography and Mass Spectroscopy

PROCEDURE

Gas Chromatography-Mass Spectroscopy (GC-MS) in the green synthesis of silver nanoparticles (AgNPs) using *Bryophyllum pinnatum* extract, particularly for targeted breast cancer therapy, involves several key steps. First, fresh and healthy leaves of *Bryophyllum pinnatum* are collected, thoroughly washed with distilled water, and shade-dried. The dried leaves are then ground into a coarse powder and boiled in distilled water (typically 10 g in 100 mL) at 60–70°C for about 30 minutes to extract the phytochemicals. The mixture is filtered using Whatman No.1 filter paper, and the resulting aqueous extract is stored at 4°C for further use. For GC-MS analysis, the plant extract undergoes solvent extraction using ethyl acetate or methanol to isolate the bioactive compounds. The organic layer is collected, evaporated to dryness using a rotary evaporator or under nitrogen gas, and the residue is reconstituted in analytical-grade methanol. This solution is filtered through a 0.22 µm syringe filter and transferred into GC-MS vials. The GC-MS analysis is carried out using a system such as the Agilent 7890 GC coupled with a 5977 MS detector, equipped with a DB-5MS capillary column. Helium is used as the carrier gas at a constant flow rate of 1.0 mL/min. The injection is made in split mode (split ratio 10:1), using an injection volume of 1 µL at an injector temperature of 250°C. The oven temperature is programmed to begin at 60°C (held for 2 minutes), then increased at 10°C/min to 200°C (held for 5 minutes), followed by a slower ramp of 5°C/min to 280°C (held for 10 minutes). The mass spectrometer operates in electron impact ionization mode at 70 eV, scanning a mass range of m/z 50–600, with the ion source and interface temperatures set at 230°C and 280°C, respectively.

The resulting chromatograms and mass spectra are analyzed using GC-MS software, and the compounds are identified by comparing their fragmentation patterns to those in the NIST or Wiley spectral libraries. The retention times, molecular weights, and peak areas of the detected compounds are recorded. These identified phytochemicals such as flavonoids, phenolics, and terpenoids are evaluated for their roles in the reduction and stabilization of silver ions to form nanoparticles. Their known or predicted anticancer properties are also considered, helping to explain the therapeutic potential of the synthesized AgNPs in targeting breast cancer cells. This analysis forms a crucial part of understanding the biochemical mechanism of nanoparticle synthesis and their application in green nanomedicine for cancer therapy.^[9]

Synthesis of Silver Nanoparticles

Experimental procedures Preparation of Leaf Extract

1. The leaves/Powders were collected from respected area.
2. Then the leaves were washed using deionized water.
3. 4g of leaves were taken and crushed.
4. Crushed leaves were then mixed in 100ml of deionized water.
5. The mixture was then kept for boiling for 30 mins and then allowed to cool.
6. The extract was then filtered using Whatman filter paper.
7. Equal amount of solvent was then added to the filtrate.
8. Centrifuge at 7000 rpm for 10 min.
9. Supernatant was collected and stored at 40c.

Biosynthesis of Silver Nano Particles

1. 6ml of silver nitrate (1M) was added in 40ml of extract and 154ml of deionized water.
2. The reaction mixture was kept at room temperature and was observed for colour change.
3. Reaction mixture when showed reddish brown colour it was centrifuged at 12000 rpm for 10 min.
4. The pellet obtained was then washed with deionized water (3x) and then with acetone.
5. Pellet is then dried and stored.

Characterization of Nanoparticles

Zeta Potential

Zeta potential is a measure of the electrical potential at the slipping plane of nanoparticles in a colloidal suspension. It reflects the surface charge of the particles and is an important indicator of colloidal stability. In a suspension, particles with high zeta potential (either positive or negative, typically $> \pm 30$ mV) tend to repel each other, reducing aggregation and resulting in a stable dispersion. In the context of AgNPs synthesized using *Bryophyllum pinnatum*, the presence of bioactive phytochemicals like flavonoids, phenolics, or terpenoids can influence the surface charge by acting as natural capping agents. Measuring zeta potential confirms the role of these compounds in stabilizing the nanoparticles. For example, a zeta potential of -25 mV to -35 mV suggests moderately high stability and good dispersion, which is crucial for biological applications where nanoparticle aggregation could reduce therapeutic efficacy or cause cytotoxicity.^[10]

Transmission Electron Microscopy (TEM)

TEM provides high-resolution images of nanoparticles, allowing for direct observation of their size, shape, and morphology at the nanoscale. For silver nanoparticles synthesized via *Bryophyllum pinnatum* extract, TEM analysis often reveals spherical or quasi-spherical shapes, although triangular or irregular shapes may also appear depending on synthesis conditions.

Typical TEM images show well-dispersed particles with a uniform distribution and average diameters ranging from 10 to 50 nm, which is an optimal size range for cellular uptake and tumor penetration in cancer therapy. TEM also enables the measurement of particle size distribution and the visualization of any aggregation or clustering. In some cases, a selected area electron diffraction (SAED) pattern may be included in the TEM analysis to confirm the crystalline nature of the nanoparticles, which supports findings from XRD analysis.^[11]

Antibacterial activity by well diffusion method Experimental procedure

1. The inoculum of the microorganism was prepared from the bacterial cultures. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.
2. 100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly.
3. Wells of 6mm in diameter were bored using a sterile corn borer. Solutions of the compounds (100µl/ml) were prepared in DMSO and 100µl of prepared test solutions (1mg/ml) and standard were added to the wells. The petri plates incubated at 37°C for 24 h.
4. Streptomycin (1mg/ml) was prepared as a positive control and DMSO was taken as negative control.
5. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.^[12]

Antioxidant Assay by DPPH Method

Experimental procedure

1. Antioxidant activity of the test sample was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals.
2. 1ml of different concentrations (20, 40, 60, 80, 100µg/ml) were taken in a test tubes.

1.5ml of 0.1% metabolic DPPH was added over the samples and incubated for 30 minutes in dark condition.

3. The samples were then observed for discoloration; from purple to yellow and read the absorbance on colorimeter at 510 nm. Radical scavenging activity was calculated by the following equation.^[13]

DPPH radical scavenging activity (%) = [(Absorbance of control- Absorbance of test sample) / (Absorbance of control)] x 100.

ANTI-INFLAMMATORY ASSAY

Experimental procedure

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of different concentration sample. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C ± 2) in a incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was used as reference drug and treated similarly for determination of absorbance.^[14] The percentage inhibition of protein denaturation was calculated by using the following formula.

Were,

% Inhibition = C-T/ C

T = absorbance of test sample C = absorbance of control.

MTT ASSAY

1. Experimental procedure MCF7 Cells were incubated at a concentration of 1×10^4 cells/ml in culture medium for 24 h at 37°C and 5% CO₂.
2. Cells were seeded at a concentration (100 µl) 104 cells/well) in 100 µl culture medium and 200-1000 µg/ml of Samples into micro plates respectively (tissue culture grade, and 96 wells).
3. Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at

37°C and 5% CO₂ in CO₂ incubator.

4. After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/ml PBS). After addition of MTT, cells incubated for 4 hours at 37°C in CO₂ incubator.
5. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only.
6. After removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 37°C (wrapped with aluminum foil).
7. Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader at a wavelength of 550 nm.^[15]

CELL CYCLE ARREST ASSAY

Experimental procedure

1. Suspended approx. 10⁶ cells in 0.5 mL of PBS. Vortex gently (approx. 5s) or gently aspirate several times with a Pasteur pipet to obtain a mono-dispersed cell suspension, with minimal cell aggregation.
2. The cells were fixed by transferring this suspension, with a Pasteur pipet, into centrifuge tubes containing 4.5 mL of 70% ethanol, on ice. Kept the cells in ethanol for at least 2 h at 4°C. Cells may be stored in 70% ethanol at 4°C for weeks.
3. Centrifuged the ethanol-suspended cells for 5 min at 300g. Decant ethanol thoroughly. Suspended the cell pellet in 5 mL of PBS, wait approx. 30 s and centrifuge at 300g for 5 min.
4. Suspend the cell pellet in 1 mL of PI staining solution and 500µl/ml test sample. Keep in the dark at room temperature for 30 min, or at 37°C for 10 min.
5. Transferred the sample to the flow cytometer and measure cell fluorescence. Maximum excitation of PI bound to DNA is at 536 nm, and emission is at 617 nm. Blue (488 nm) or green light lines of lasers are optimal for excitation of PI fluorescence.^[16]

RESULT

A) Phytochemical analysis.

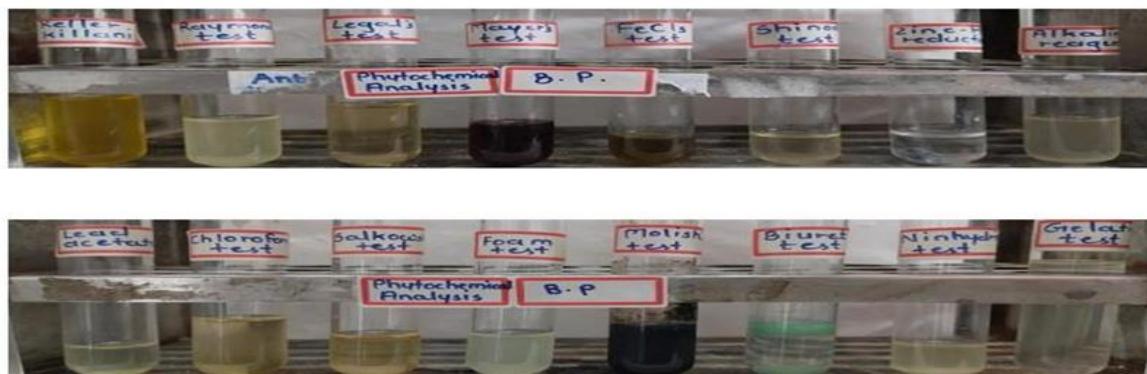
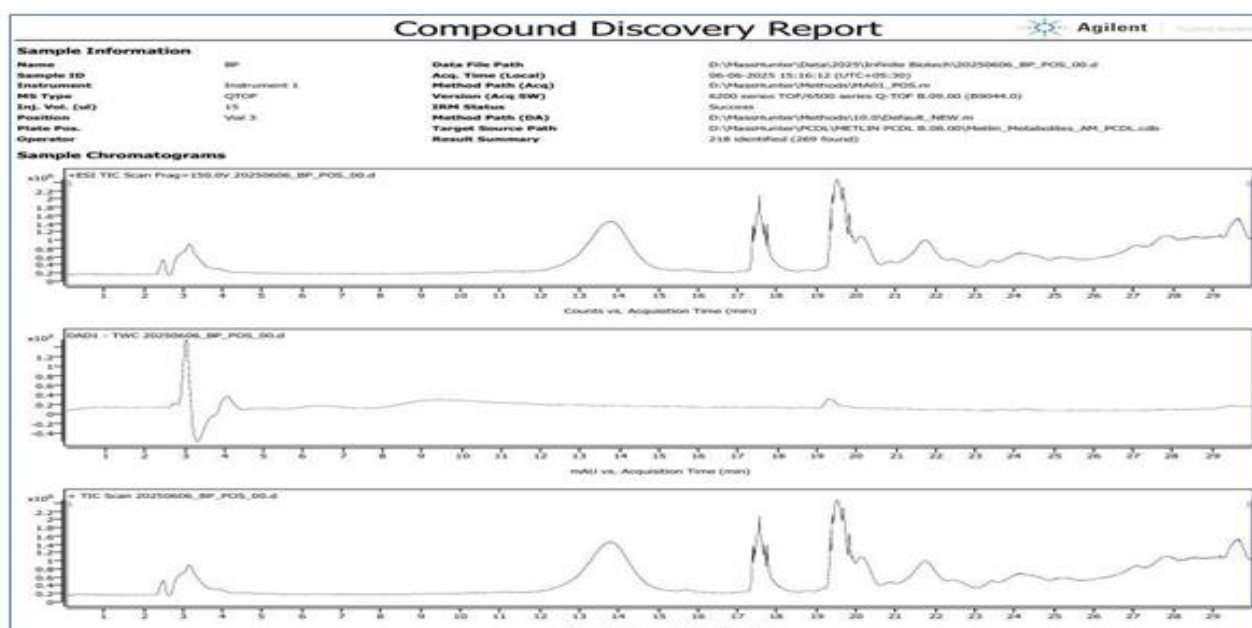


Fig: Phytochemical analysis.

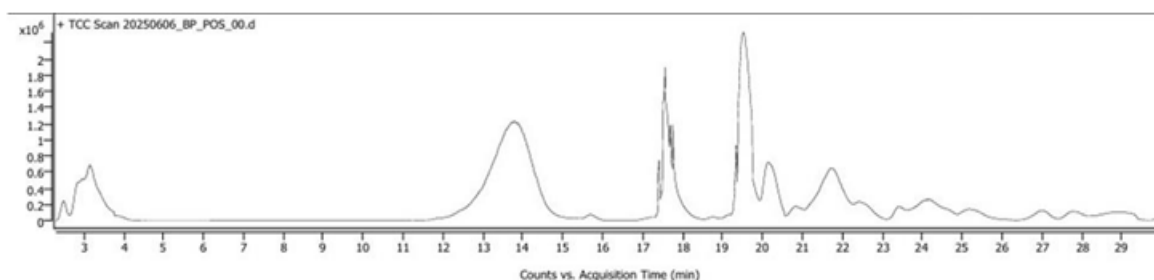
Qualitative phytochemical analysis.

Sr. No	Chemical Constituents	Tests	B.P.	Observation
1	Steroids	Chloroform test	++	Present
2	Triterpenoids	Salkowski test	++	Present
5	Carbohydrates	Benedict's test	++	Present
6	Alkaloids	Wagner's test	++	Present
7	Flavonoids	Lead acetate solution test	++	Present
8	Phenol	FeCl ₃ test	++	Present
9	Proteins	Xanthoproteic test	++	Present
10	Tannins	NaOH test	++	Present

Analytical Results: GC-MS of Bryophyllum Pinnatum



Compound Discovery Report 1



Compound Discovery Report 2

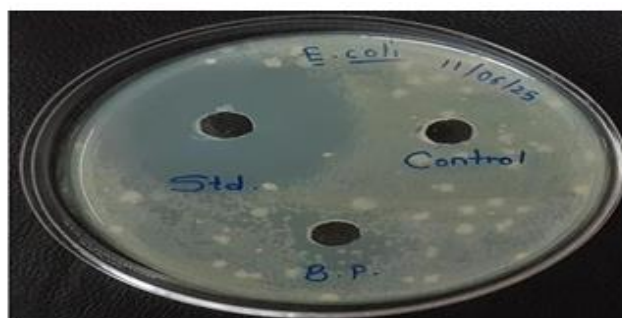
Compound Summary

Cpd	Name	Formula	RT	Mass	CAS	ID Source	Score	Score (Lib)	Score (DB)	Score (MFG)	Algorithm
8	2-Methylbutanal	C5 H10 O	2.857	86.0734	96-17-3	DBSearch	97.74		97.74		MFE
9	Dulcitol	C6 H14 O6	2.876	182.0795	608-66-2	DBSearch	97.45		97.45		MFE
15	Norvaline	C5 H11 N O2	3.107	117.0791	760-78-1	DBSearch	98.03		98.03		MFE
17		C5 H13 N O4 S	3.136	183.0566		MFG	99.53			99.53	MFE
20		C7 H15 N O4 S	3.158	209.0723		MFG	97.10			97.10	MFE
23	3beta,6beta-Dihydroxynortropine	C7 H13 N O2	3.232	143.0949		DBSearch	95.22		95.22		MFE
34		C26 H57 N4 O13	12.507	633.3922		MFG	92.61			92.61	MFE
37		C30 H63 N O15	12.739	677.4194		MFG	95.76			95.76	MFE
40		C32 H67 N O16	12.939	721.4457		MFG	98.77			98.77	MFE
41		C36 H77 N4 O18	12.942	853.5246		MFG	96.69			96.69	MFE
48		C47 H73 N O13 S	13.414	891.4814		MFG	91.24			91.24	MFE
49		C36 H77 N4 O18	13.462	853.5238		MFG	99.13			99.13	MFE
50		C41 H74 N6 O10 S	13.469	842.5187		MFG	94.02			94.02	MFE
52		C37 H75 N11 O14	13.603	897.5494		MFG	98.76			98.76	MFE
53		C36 H80 N5 O19	13.617	886.5443		MFG	96.17			96.17	MFE
55		C20 H47 N4 O11	13.652	519.3244		MFG	99.41			99.41	MFE
56		C40 H85 N4 O20	13.744	941.5755		MFG	99.40			99.40	MFE
57		C37 H78 N12 O15	13.755	930.5704		MFG	97.68			97.68	MFE
59		C39 H81 N14 O15	13.878	985.6013		MFG	98.91			98.91	MFE
91		C33 H74 N15 O13	15.086	888.5589		MFG	97.02			97.02	MFE
92		C35 H78 N15 O14	15.214	932.5854		MFG	96.55			96.55	MFE
93		C37 H82 N15 O15	15.330	976.6106		MFG	95.89			95.89	MFE
100	Ganglioside GM3 (d18:0/14:0)	C55 H102 N2 O21	15.644	1126.6984		DBSearch	91.90		91.90		MFE
114	3beta-Fluoro-5beta-pregn-20-one	C21 H33 F O	17.587	320.2508		DBSearch	91.38		91.38		MFE
115		C11 H19 N4 O13	17.928	415.0947		MFG	96.06			96.06	MFE
128		C16 H20 N5 O7 S2	19.559	458.0806		MFG	98.27			98.27	MFE
142	Moracin B	C16 H14 O5	20.787	286.0843	67259-16-9	DBSearch	92.95		92.95		MFE
159		C35 H75 N17 O8	21.565	861.5984		MFG	92.92			92.92	MFE
162		C39 H81 N14 O10	21.683	905.6260		MFG	98.85			98.85	MFE
163		C42 H91 N7 O16	21.689	949.6522		MFG	99.60			99.60	MFE
164		C37 H80 N15 O9	21.701	878.6260		MFG	97.32			97.32	MFE
174	Ganglioside GM3 (d18:1/26:0)	C67 H124 N2 O21	21.831	1292.8701	1354819-54-7	DBSearch	95.66		95.66		MFE
200	Chimyl alcohol	C19 H40 O3	23.807	316.2969	6145-69-3	DBSearch	92.42		92.42		MFE
212		C56 H94 N6 O9	24.182	994.7086		MFG	94.81			94.81	MFE
214		C40 H82 N22 O5	24.192	950.6835		MFG	95.72			95.72	MFE
255	4-Formylsalicylic acid	C8 H6 O4	27.770	166.0266	51572-88-4	DBSearch	98.64		98.64		MFE

Antibacterial activity by well diffusion method

Table: Antibacterial activity of test compound against.

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	32
3	B.P.	18

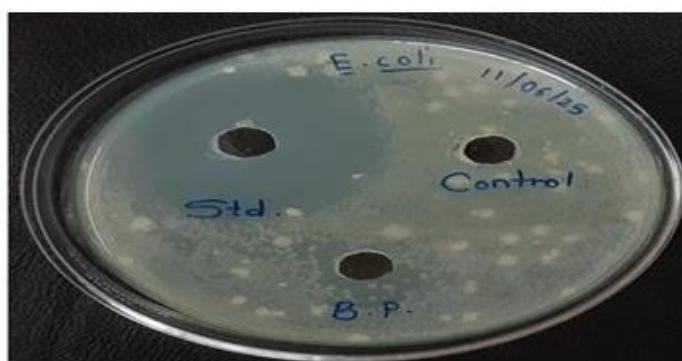


E. coli Antibacterial activity of test compound against E. coli.

Antibacterial activity of test compound against B. subtilis

Table: Antibacterial activity of test compound against B. subtilis.

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	27
3	B.P.	09



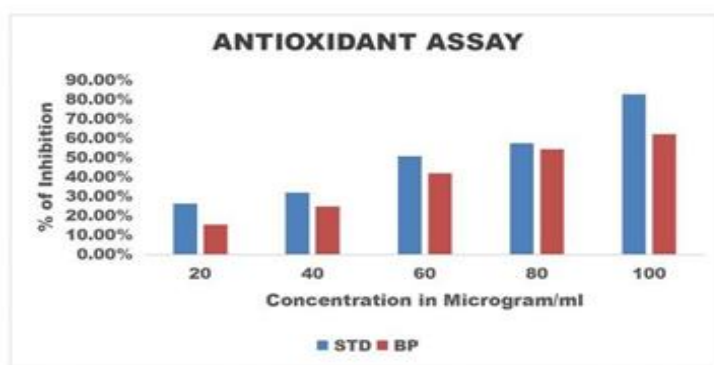
Antibacterial activity of test compound against B. subtilis.

Conclusion of the study: The antibacterial profile of B.P. was evaluated by measuring the zone of inhibition against E. (ATCC 9637), B. subtilis coli (ATCC 6051) bacterial strains via well diffusion method. The compound B.P. exhibited good antibacterial activity as compared to the standard streptomycin.

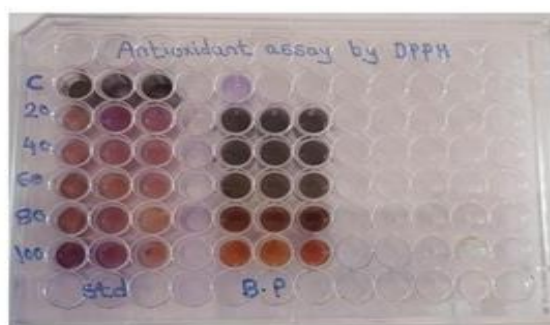
A) ANTIOXIDANT ACTIVITY

Observation Table: Antioxidant Activity

SR.NO	Sample Code	Concentration (µg/ml)	Absorbance at 510nm				% Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	
2	Standard	20	1.45	1.42	1.40	1.42	26.42%	57.67
	(Ascorbic Acid)	40	1.31	1.29	1.33	1.31	32.12%	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.82	57.51%	
		100	0.35	0.32	0.32	0.33	82.90%	
3	B.P.	20	1.62	1.65	1.64	1.63	15.54%	75.60
		40	1.46	1.44	1.45	1.45	24.87%	
		60	1.11	1.11	1.15	1.12	41.96%	
		80	0.89	0.86	0.90	0.88	54.40%	
		100	0.74	0.75	0.71	0.73	62.17%	



Graphical data



Antioxidant Assay

Conclusion of the study: The antioxidant profile of compounds B.P. was evaluated by measuring the percent of inhibition against DPPH reagent via test tube method. The compound B.P. exhibited good antioxidant activity against DPPH scavenging reagent and however the concentration increases also the antioxidant activity of the compound increases as compared to the standard ascorbic acid.

B) ANTI-INFLAMMATORY ASSAY.

SR. NO	Sample code	Concentration (µg/ml)	Protein denaturation assay					IC50 (µg/ml)
			Absorbance at 660nm				% Inhibition	
			Test 1	Test 2	Test 3	Mean		
1	Control		1.54	1.54	1.54	1.54	-	70.91
2	Standard(Diclofenac Sodium)	20	1.32	1.30	1.34	1.32	16.98%	
		40	1.20	1.18	1.22	1.20	24.52%	
		60	0.91	0.93	0.88	0.91	42.76%	
		80	0.65	0.68	0.62	0.65	59.11%	
		100	0.21	0.23	0.19	0.21	86.79%	
3	B.P.	20	1.43	1.44	1.43	1.43	7.14%	70.91
		40	1.29	1.26	1.30	1.28	16.88%	
		60	0.79	0.81	0.77	0.79	48.70%	
		80	0.65	0.62	0.62	0.63	59.09%	
		100	0.55	0.54	0.57	0.55	64.28%	

Table: Anti-Inflammatory by Protein Denaturation Assay.**C) ANTI-INFLAMMATORY BY PROTEIN DENATURATION ASSAY: Images.**

Conclusion: At the different concentrations the samples B.P. shows the good anti-inflammatory activity as compared to standard Diclofenac Sodium.

D) CELL CYCLE ARREST ASSAY

Cell line: MCF7

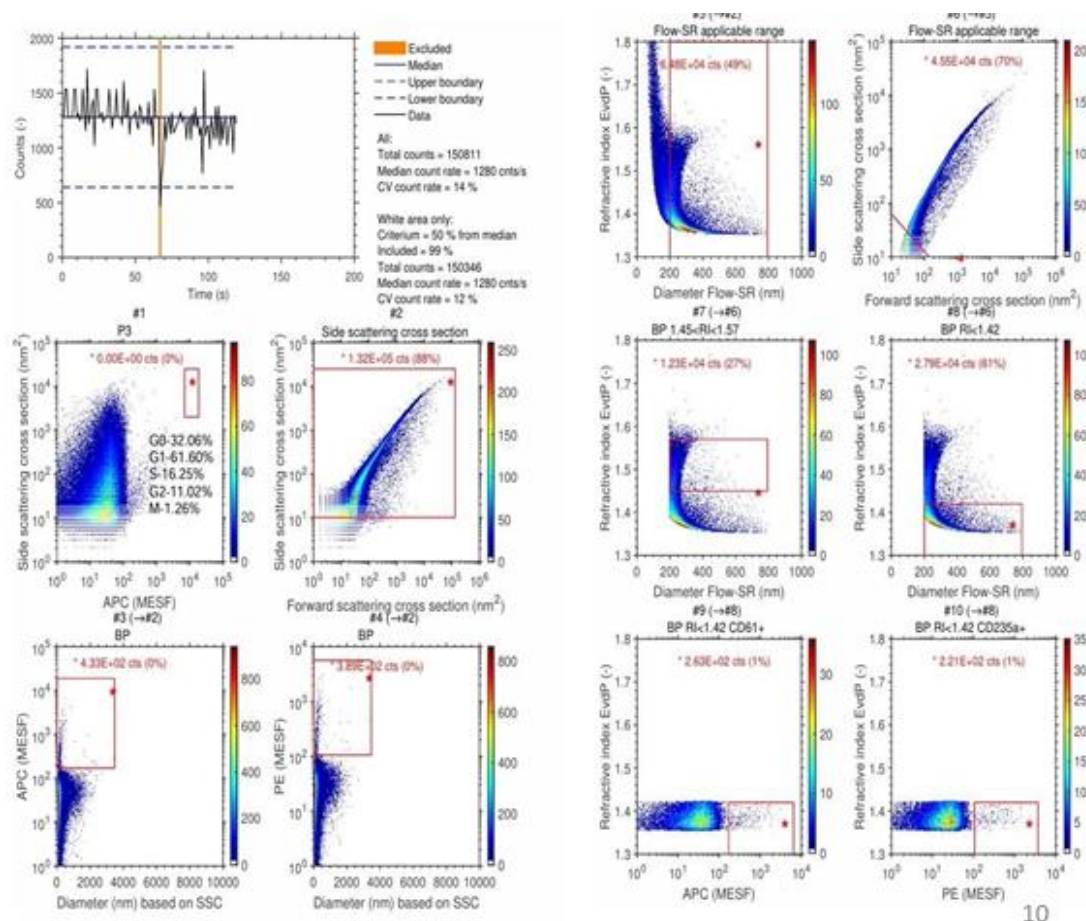
Media: DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No- 10270106 Antibiotic-Antimycotic 100X solution (Thermo fisher Scientific)-CatNo- 1524062.

Fig: Cell cycle arrest Assay-II

Synthesis of Silver Nano Particles:

Activity: Synthesis of Silver Nano Particles

Yield: 0.4gm from 2gm of extract.



DISCUSSION

The present study systematically evaluated the phytochemical composition and biological potential of *Bryophyllum pinnatum*-mediated silver nanoparticles (AgNPs) with emphasis on anticancer activity against breast cancer. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenols, alkaloids, steroids, and glycosides, which are known for diverse pharmacological properties. These phytochemicals played a crucial role in the green synthesis of AgNPs by acting as reducing and stabilizing agents. GC-MS analysis further validated the presence of functional bioactive compounds contributing to therapeutic efficacy. The successful synthesis of AgNPs was indicated by a visible colour change and confirmed through characterization studies. The green synthesis approach offers an eco-friendly, cost-effective alternative to conventional nanoparticle production methods. The synthesized AgNPs exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Antioxidant analysis demonstrated strong free radical scavenging potential in a concentration-dependent manner. The formulation also showed

notable anti-inflammatory activity by inhibiting protein denaturation. These properties collectively suggest its protective role against oxidative stress and inflammation-related disorders. Cytotoxicity studies on the MCF-7 breast cancer cell line revealed significant inhibition of cancer cell proliferation. The anticancer effect is likely mediated through ROS generation, mitochondrial dysfunction, and DNA damage. Additionally, the nanoparticles induced cell cycle arrest, highlighting their ability to disrupt cancer cell growth. The synergistic interaction between phytochemicals and silver nanoparticles enhances overall therapeutic performance. Overall, the findings indicate that *B. pinnatum* mediated AgNPs possess promising potential as a multifunctional nanotherapeutic agent for breast cancer.

CONCLUSION

In conclusion, this study not only underscores the efficacy of *Bryophyllum pinnatum*-mediated silver nanoparticles in breast cancer therapy but also highlights their multifunctional biological properties, including antioxidant, anti-inflammatory, and antibacterial activities. These findings support the use of green-synthesized AgNPs as a potent, biocompatible, and low toxicity alternative to conventional chemotherapeutic agents. Future work should involve in vivo studies to evaluate the pharmacokinetics, bio distribution, and long-term safety of these nanoparticles. Furthermore, surface modification with targeting ligands could enhance tumors specificity, providing an advanced Nano platform for targeted drug delivery in breast cancer treatment. Overall, this research contributes to the growing field of plant-based Nano medicine and offers a new direction in the development of safer and more effective cancer therapeutics.

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