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Original Article

BIOSYNTHESIS, CHARACTERIZATION AND EVALUATION OF SILVER NANOPARTICLES FROM THE LEAF EXTRACT OF *PREMNA INTIGRIFOLIA* L. AS A POTENTIAL ANTICANCER AGENT

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ABSTRACT

Objective: In this study, plant-based silver nanoparticles were synthesized and characterized from *Premna integrifolia* leaf extract to test the viability towards anticancer properties.

Methods: Preliminary identification of silver nanoparticles was validated by Visual observation and confirmed for the characterization by Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy **(**EDX) and Fourier-transform Infrared Spectroscopy (FTIR) analysis. Further synthesized nanoparticles were evaluated against non-small lung cancer cells (A549) by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

Results: Aqueous leaf extract of *Premna intigrifolia* was synthesized for silver nanoparticles and showed an average size from 35 nm to 100 nm through SEM studies. EDX showed a strong signal confirming the formation of silver nanoparticles in the metallic silver region at 5Kev, and the FTIR spectrum showed changes in some peaks of the aqueous extract with functional groups. The newly synthesized silver nanoparticles showed significant anticancer properties targeting lung cancer A549 cell line against the standard drug Epotoside with a 50% Inhibitory Concentration (IC50) value of 78.431 µg.

Conclusion: The results affirm that biosynthesized silver nanoparticles can be used as an alternative to chemical medicines to cure cancer.

Keywords: Green synthesis, Nanoparticles, Anticancer, Plant, Therapy

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INTRODUCTION

Nanoparticles are a broad spectrum of materials containing special compounds which will be characterized with at least one dimension smaller than 100 nm [1, 2]. The significance of these materials became evident when scientists discovered that the physical and chemical properties of the materials are impacted by their size [3]. Nanoparticles can be synthesized chemically as well as biologically. Chemically generated procedures have a lot of negative impacts since some hazardous substances are absorbed on the surface [4]. The green chemistry approach of nanoparticle manufacturing methods using different types of microorganisms, enzymes, and plant extracts an environmentally acceptable alternative approach to conventional chemical and physical processes [5, 6]. Various kinds of life exist on earth, which can be utilized in developing towards green chemistry. The creation of these ecologically benign techniques for nanoparticle synthesis, particularly for Silver Nanoparticles, is developing as a crucial area of nanotechnology with numerous applications [7].

Silver Nanoparticles are very much significant and captivating nanomaterials that have find extensive usage in the field of biomedical applications, among the several metallic nanoparticles used in this field [8, 9]. Silver Nanoparticles are crucial to the fields of nanoscience and nanotechnology, particularly nanomedicine [10]. Silver Nanoparticles have gained significant attention in cancer diagnostics and its therapy, despite the use of other noble metals for various applications. Silver Nanoparticles are regarded as a potent and sophisticated chemical instrument for the identification and management of serious diseases like cancer, Human Immunodeficiency Virus (HIV) and other infectious disorders [11]. The most important application of nanotechnology has significant importance in cancer treatment, which relies on the early detection of tumours and the analysis of cancer cells by nanodevices [12].

One of the most widespread medical conditions, cancer, causes more fatalities than usual globally. The development of alternative and more effective cancer theranostics treatment plans are significantly aided by nanomedicine. Enabling cell visualization, drug administration, use of photo-thermal therapy, and application of bio-imaging techniques in nanoparticles are interesting to note and play a crucial role in the detection and diagnosis of tumours in the early stages [13]. Because of its targeting, which is crucial for targeted chemotherapy, the nanocarrier approach is used. Due to increased binding activity between tumour cells and particles, nanoparticles with positive charges can quickly enter tumour cells [14]. Lung cancer came in second place among the many types of cancer in both men and women. Epithelial cells are where bronchiogenic carcinoma, often known as lung cancer, develops. Lung cancer is the most aggressive disease when compared to other cancers, and it is challenging to treat [15]. Products made from plants are becoming more and more important in the fight against lung cancer. In this regard, several plant extracts have been employed to successfully produce Silver Nanoparticles by biogenic means [16].

Premna integrifolia is a large shrub or small tree, up to 10 m tall, trunk diameter up to 30 cm, with numerous branches, sometimes spiny, scaly bark and earthy grey colour. Leaves broadly elliptic, oblong or ovate, obovate to sub-orbicular, obtuse, with very short, pointed tips, glabrous, entire or the upper part dentate, base rounded or sub-acute. In addition, early phytochemical studies of *Premna integrifolia* extracts indicated the presence of carbohydrates, alkaloids, amino acids, flavonoids, glycosides, steroids, tannins, and phenolic compounds [17]. *Premna integrifolia* has shown pharmacological effects, which have exhibited various properties, including hepatoprotective, anti-diabetic, antioxidant, anti-obesity, anti-tumor and various other biological activities [18]. *Premna intigrifolia* will be used in the study to synthesise and characterise plant-mediated silver nanoparticles. The *in vitro* anticancer potential of synthesised nanoparticles was evaluated at various concentrations.

MATERIALS AND METHODS

Plant material collection and formulation of leaf extract

Premna intigrifolia L. was collected from the Anshi Forest range with 14 °59'55"N 74 °21'33"E, elevation of 635 m located in the Western Ghats region of Uttar Kannada District of Karnataka, India. Plant

material was identified and authenticated using standard manuals. Voucher specimen (18/P/JSSCACS/2022) was deposited in the herbarium of the Post Graduate Department of Botany, JSS College of Arts, Commerce and Science, Mysuru, Karnataka. Fresh leaves were selected and, washed with running tap water and dried in the shade area. The dried leaves were then ground into a coarse powder using a mixer grinder. The fine powder was stored at room temperature in sealed containers until it could be used for a basic solvent extraction. 250 ml of distilled water was combined with about 25 g of leaf powder, and then the mixture was left to boil for 20 min. The extracts were filtered three times using Whatman No. 1 filter paper to obtain clear solution, which were then chilled (4 °C) to eliminate the residue. The aqueous extract acts as a stabilizer and reducing agent [19].

Synthesis of silver nanoparticles

1 ml of aqueous leaf extract was added to 10 ml of 1 mmol Silver Nitrate solution. To prevent unwanted photochemical reactions, the mixture was kept in dark condition so that the reaction can undergo in room temperature. The colorless reaction mixture turned dark brown after the incubation and reaction time, indicating the oxidation/reduction reaction had taken place [20]. After the desired reaction time, the aqueous mixture containing the Silver Nanoparticles was centrifuged repeatedly at 10,000 rpm for 10 min. The mixture was combined with double distilled water to in order to eliminate any traces of the aqueous extract in the freshly synthesized Silver Nanoparticles. The colloidal mixture was then sealed and stored in a container for later use [19].

Characterization of the newly synthesized silver nanoparticles

Several techniques were used to characterise the synthesized Silver Nanoparticles such as UV-visible spectroscopy, FTIR analysis, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) [21].

UV–visible spectroscopy analysis

UV-visible spectroscopy was used to validate and analyze the reduction of silver ions. From the colloidal solution of about 1 ml aliquot sample was examined in a quartz cuvette with a wavelength scan ranging from 200 to 700 nm, and distilled water was used as a reference. While 1 mmol of Silver Nitrate was used as a blank.

FTIR analysis

The functional groups that are responsible for biological reduction, which is attached to the silver surface and involved in the synthesis of Silver Nanoparticles, were found using FTIR spectroscopy [22]. Silver Nanoparticles were extracted after a 72 h of incubation period by centrifuging of the reaction mixtures for about three to four times at 10,000 rpm for 15 min. The supernatant was replaced with Deionized water, and the particle was then pulverized and stored. Dried Silver Nanoparticles were analysed using FTIR spectroscopy, using the potassium bromide pelletization method with a ratio of 1:100 [23].

Scanning electron microscopy analysis

The surface morphology of nanoparticles was analysed using Scanning Electron Microscopy (SEM). After 4-6 h of reaction, the colloidal solution was centrifuged at 10,000 rpm for 5 min to prepare the sample [24]. The pellet was redispersed in deionized water, then centrifuged several times and dried to produce a dry pellet. To prepare the suspension, the purified Silver Nanoparticles were sonicated for 5–10 min in one cycle. A drop of the suspension was then carefully placed on the carbon-coated grid. The sample material was subjected to drying process by keeping under a lamp until it attained complete dryness. The processed sample was examined by SEM analysis [21, 25].

Energy dispersive X-ray spectroscopy analysis

The elemental composition of the dried reduced Silver Nanoparticles on a carbon-coated copper grid was determined using Energy Dispersive X-ray method [26].

Determination of cell viability by MTT assay

The standard colorimetric MTT assay used the application of 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyl tetrazolium bromide dye [27], to measure the impact of the aqueous leaf extract of *Premna intigrifolia* and the synthesised Silver Nanoparticles on the viability of non-small lung cancer cells (A549). The growth inhibition percentage was calculated using the following formula, while the values for test compound's 50% Inhibitory Concentration (IC50) were obtained from the dose-response curves for each cell line [28]. The fundamental concept behind this test is that mitochondrial dehydrogenase in cells changes MTT into a purple formazan product [29, 30].

$$\text{Percentage of Inhibition} = \text{OD of test sample}/\text{OD of control x } 100$$

Statistical investigation

The data has been presented in the form of the mean±standard deviation and standard error. Analytical measurements were made in triplicates (n=3).

RESULTS

Premna integrifolia L. has a wide geographical distribution, including tropical and subtropical regions. The current study successfully synthesized silver nanoparticles from plants, which were characterized using several spectroscopic and microscopic analyses to elucidate their properties.

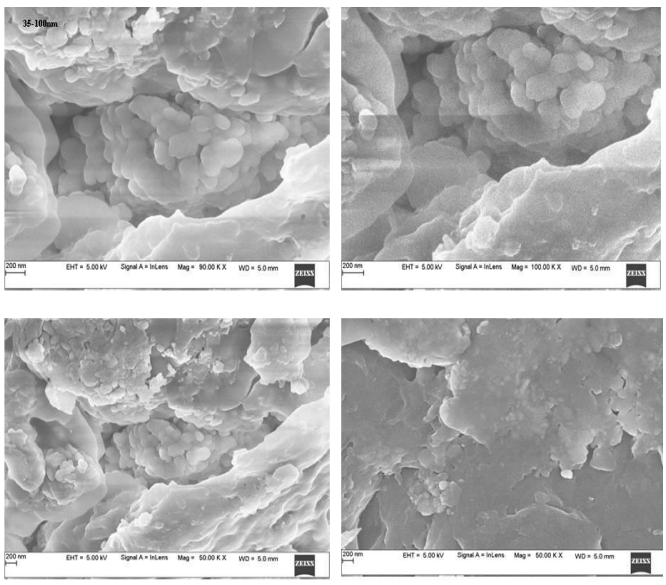


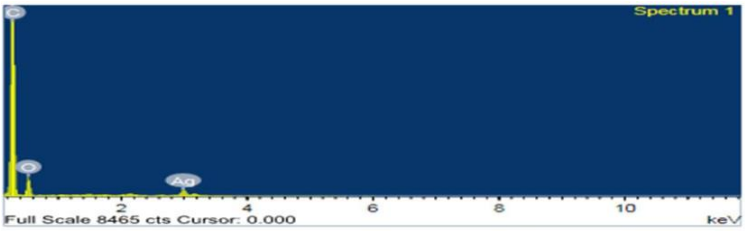
Fig. 1: SEM images of synthesized silver nanoparticles from *Premna intigrifolia*

SEM and EDX studies

SEM images revealed the existence of silver nanoparticles with structures and dimensions ranging from 35 to 100 nm (fig. 1). The occurrence of metallic silver region at 5Kev in the aqueous leaf extract of *Premna integrifolia* confirms the formation of silver nanoparticles. According to investigations on elemental composition, several elements, such as carbon and oxygen, were present in percentages of 30.71% and 61.41%, respectively, and silver in 7.88% (fig. 2).

FTIR analysis

FTIR spectra of aqueous plant extract and the synthesized Silver Nanoparticles displayed a range of functional groups including alkyl halides, alkynes, aromatics amines, aliphatic amines, esters, ethers, alcohols, carboxylic acids and nitro compounds. These functional groups were identified on the presence of correlating peaks at 554.85 cm^{-1}, 1039.87 cm^{-1}, 1265.41 cm^{-1}, 1458.00 cm^{-1}, 1541.04 cm^{-1}, 1635.00 cm^{-1}, 1745.22 cm^{-1}, 2860.83 cm^{-1} and 3434.14 cm^{-1} which acts as capping agents and formation of Silver Nanoparticles (fig. 3 and 4).



Elem...	Weight%	Atomic%	Compd%	Formula
O K	0.80	61.41	1.10	O2C
Ag L	0.69	7.88	0.00	
C	0.30	30.71		
Totals	1.79			

Fig. 2: EDX of synthesized silver nanoparticles from *Premna intigrifolia*

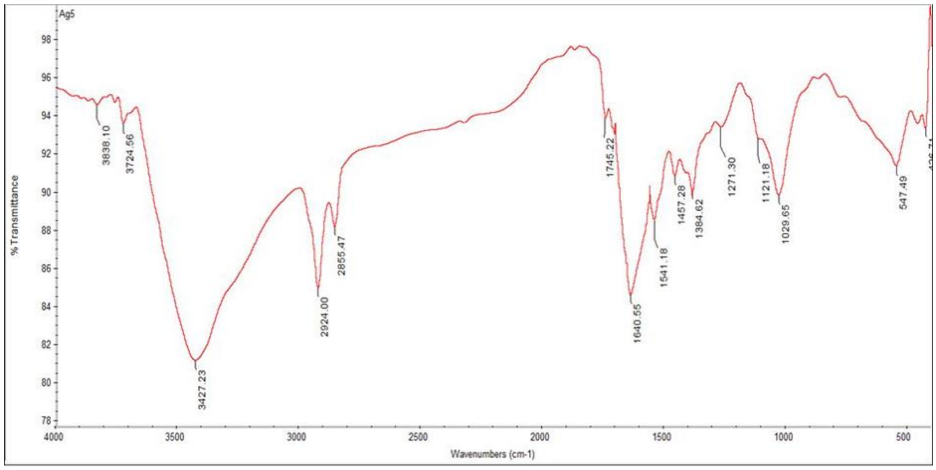


Fig. 3: FTIR analyses of silver nanoparticles synthesized from *Premna intigrifolia*

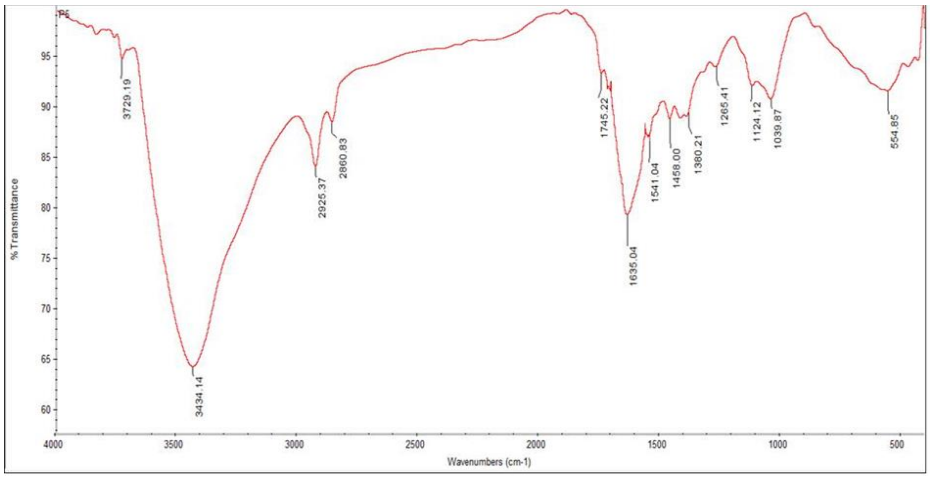


Fig. 4: FTIR analyses of aqueous leaf extract of *Premna intigrifolia*

Determination of cell viability by MTT assay

Cytotoxic potential of silver nanoparticles from *Premna intigrifolia* against A549 cells was examined in the current study using the MTT assay. The aqueous leaf extract of *Premna intigrifolia* and the A549 cell line treated with synthesized silver nanoparticles were taken as the treatment group, while the untreated A549 cell line was considered as the control group, whereas the positive control group consisted of the A549 cell line treated with the standard drug Epotoside. While normal cells seemed regular and in their typical shape, the treated group showed chromatin condensation, cell elongation, a drop in cell count and density, and other signs of apoptosis. The aqueous extract of *Premna intigrifolia* and the corresponding synthetic silver nanoparticles generated morphological alterations and cell shrinkage, which resulted in cell apoptosis, according to microscopic analysis (fig. 5 and 6).

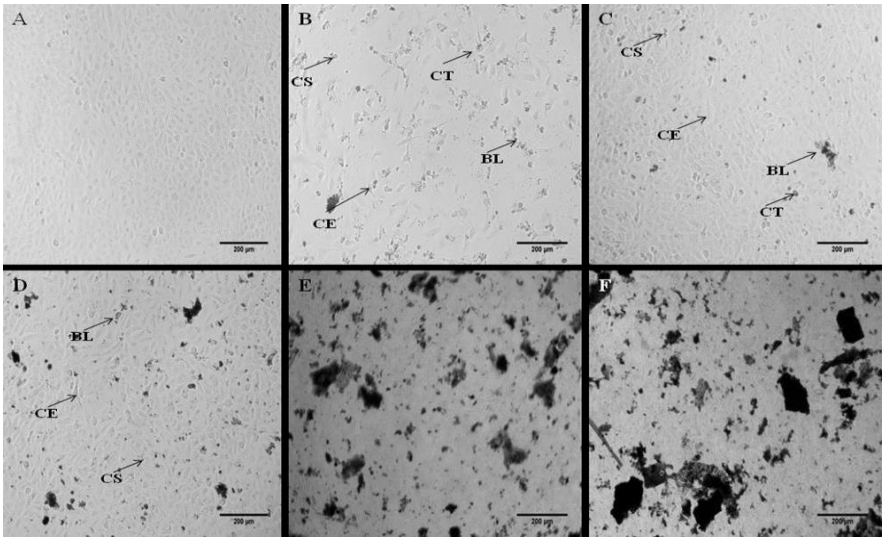


Fig. 5: Effect of aqueous leaf extract of *Premna intigrifolia* on the viability of A549 non-small lung cancer cells, Untreated (Control), B) STD (Epotoside 50µM), C) 50µg, D) 100µg, E) 200µg, F) 250µg, CE: Cell Expansion, CS: Cell Shrinkage, CT: Cell Turgidity, BL: Membrane Blabbing

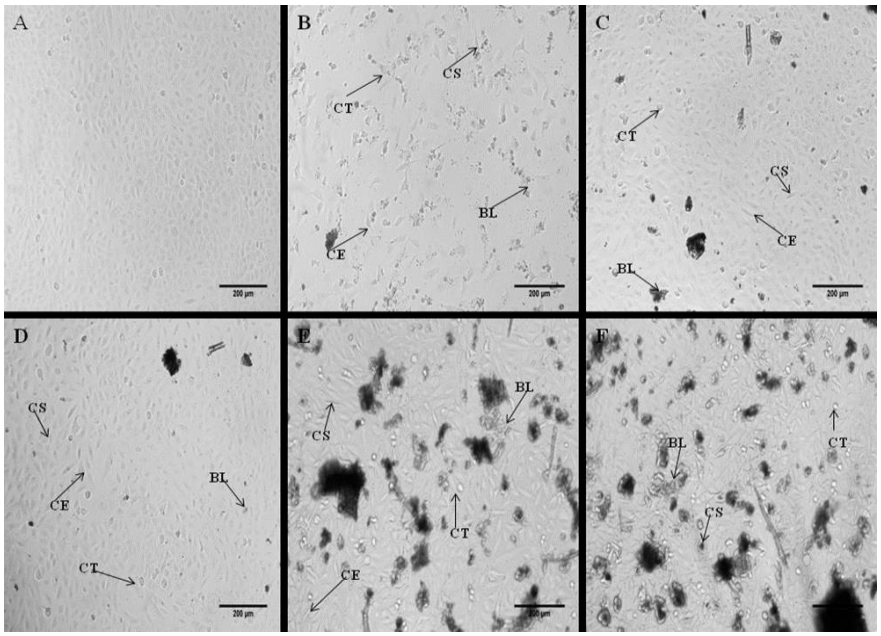


Fig. 6: Effect of silver nanoparticles derived from the aqueous leaf extract of *Premna intigrifolia* on the viability of A549 non-small lung cancer cells, A) Untreated (Control), B) STD (Epotoside 50µM), C) 5µg, D) 10µg, E) 40µg, F) 80µg, CE: Cell expansion, CS: Cell shrinkage, CT: Cell turgidity, BL: Membrane blabbing

When Compared with the cell viability of Epotoside treated cells, a common chemotherapy medication, with the prepared leaves aqueous extract of *Premna intigrifolia,* cell viability decreased with increasing concentration for all treated samples. The standard and extract's IC50 values were computed. For the common medication epotoside, a value of 89.28 µM for A549 was discovered. Overall, *Premna intigrifolia* leaves aqueous extract exhibited significant anticancer properties towards non-small lung cancer cells, specifically targeting A549 cells, which is evident by its IC50 value of 304.87 µg (table 1). The silver nanoparticles demonstrated a potent anti-cancer activity against A549 with an IC50 value of 78.431 µg (table 2).

Table 1: Comparison of *Premna intigrifolia* aqueous extract's impact on A549 cell viability and the standard drug Epotoside

Treatment	Cell line	Concentration in µg	Cell viability in %*	IC_{50}
Aqueous leaf extract of *P. intigrifolia*	A549	25	98.3082±0.01800	304.87 µg
		100	93.3455±0.00150	
		150	89.8897±0.01650	
		200	72.7058±0.02400	
		250	61.5882±0.01200	
Standard Epotoside	A549	50 µM	61.4411±0.00240	89.2857 µM

*Data represent as mean±SD n =3

Table 2: Comparison of *Premna intigrifolia* synthesised silver nanoparticles extract impact on A549 cell viability and the standard drug Epotoside

Treatment	Cell line	Concentration in µg	Cell viability in %*	IC_{50}
Synthesized Silver Nano particles from leaf extract of *P. intigrifolia*	A549	5	95.3676±0.00200	78.431 µg
		10	91.0661±0.09155	
		20	83.6764±0.01400	
		40	69.1958±0.01650	
		80	49.5830±0.00282	
Standard Epotoside	A549	50 µM	61.4411±0.00240	89.2857 µM

*Data represent as mean±SD n =3

DISCUSSION

For drug delivery and diagnostics, cardiovascular disease therapy, healing of wounds, and the creation of antimicrobial agents, nanomaterials are effectively used in medicine [31]. Nanomaterials use in tissue engineering, cancer therapy, cell labelling, biological tagging, and DNA and protein detection has recently increased [32]. The creation of high-potency Nanoparticles can be achieved through the green synthesis of Silver Nanoparticles utilising medicinal plants, which are easy to execute and environmentally benign [33]. *In vitro* tests on human cervical cancer cell lines using silver nanoparticles from *Premna integrifolia* demonstrated promising antioxidant and cytotoxic effects [34]. The formation of silver nanoparticles was determined by colour change, and it confirmed by UV-Vis spectra observed in the range of 500-600 nm [35]. The SEM analyses provided confirmation for the shape and surface features of synthesized Silver Nanoparticles [36]. *Premna odorata* (50–100 nm), *Premna herbacea* (10–30 nm), and *Premna serratifolia* (22.97 nm) are just a few of the species in the genus that can produce Silver Nanoparticles [37, 38]. Energy dispersive X-ray data was utilized for the identification of the presence of silver ions in the biosynthesized nanoparticles [39]. The purity and elemental makeup of silver nanoparticles were ascertained through the utilization of EDX studies [40]. FTIR is commonly used analytical technique to identify functional groups present in herbal medicines [41]. The MTT assay is regarded as one of the most trustworthy techniques for determining a compound's principal impact on cell lines [42]. The morphological alterations and cell proliferation were investigated in two distinct A549 non-small lung cancer cell lines using several quantities of the conventional medication [44], the aqueous extract of *Premna intigrifolia*, and the corresponding synthesised Silver Nanoparticles. Apoptosis is indicated by morphological changes in the nucleus. Because nanoparticles are taken up by cells by pinocytosis and endocytosis, Silver Nanoparticles have been shown to be harmful to cell lines [45]. According to one investigation, NPs are cytotoxic when cell viability is less than 50% [46]. Between the extract and the reference medication, the IC50 value varied [21]. The MTT assay was utilised in a cytotoxicity experiment to ascertain the anticancer activity of *Caulerpa taxifolia* produced Silver Nanoparticles against A549 lung cancer cells [47]. At concentrations greater than 250 g/ml of Silver Nanoparticles, *Prosopis farcta* has been demonstrated to kill 50% of cells [48]. The creation or synthesis of Silver Nanoparticles and their cytotoxic effects have been the subject of numerous investigations.

CONCLUSION

The current approach showed that using *Premna intigrifolia* aqueous leaf extract as a cost-effective substitute for bioreducing silver ions to silver nanoparticles was possible. Many different malignancies can be prevented and treated with the help of plants and plant products. One of the main causes of lung cancer is alveolar adenocarcinoma, which is linked to a high death rate not only in developing nations but also globally. Using SEM, EDX, and FTIR, *Premna intigrifolia* was successfully employed to create stable Silver Nanoparticles. Silver Nanoparticles can be created quickly and easily using the suggested process, which uses no toxic or dangerous compounds. Comparing the Silver Nanoparticles IC50 values for A-549 cells to those of the common medication Epotoside, the findings of our research supported the therapeutic efficacy in cells causing cancer in lungs.

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Nil

AUTHORS CONTRIBUTIONS

Conceptualization, methodology, validation, formal analysis, investigation, and writing were done by First (SK) and Second author (AKS). Review and editing, supervision of work, finalized and manuscript draft was conducted by Corresponding author (RS)

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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