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Research Article

In-Vitro Antimicrobial and Anticancer Properties of Green Synthesized Gold Nanoparticles Using Securinega leucopyrus Leaves Extract

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ABSTRACT

The aqueous leaves extract obtained was investigated for the preparation of gold nanoparticles. The obtained AuNPs were characterized by UV–Visible spectroscopy, FTIR, DLS and Zeta potential analysis. Results indicated that the green synthesized AuNPs showed good antibacterial effect against gram positive, gram negative and exhibited % viability of 45.717 and % of cytotoxicity 54.282 on breast cancer (MCF-7) at a maximum concentration of 100 mg/ml.

1. Introduction

Nanotechnology is a promising arena dealing with the design, manipulation and application of nanoparticles in generating new applications (1). The unique property of NP includes its extreme microscopic size, high surface to volume ratio, an optical, thermal and catalytic property that promotes interactions between microbes and biological agents (2). Since 2015, nanomedicine involving gold and silver nanoparticles has become a new sensation for researchers in developing alternative solutions for biological applications (4). Gold nanoparticles in particular has been used extensively in biomedical and imaging procedures (5). The biggest choice of using gold nanoparticles for biological application is that it can be synthesized easily and have less toxicity (6). Among the several methods available for the synthesis of AuNPs, the procedures using naturally occurring plant extracts are consider to be bio compatible, cost effective and requires less workers (7). Green synthesis involves the presence of flavonoids and polyphenols which act as reducing agents and stabilizing and capping agents (8). In the current study, we have selected *securinega leucopyrus* leaves extract. In a previous study, photosynthesis of AgNPs using leaves of *securinega leucopyrus* leaves extract was reported (9). Many reports claimed that the nanoparticles synthesized using green approaches have many advantages over the chemical method of synthesizing the nanoparticles. The conversion of nanoparticles was environmental friendly and has fewer side effects to the human. Alternatively the green synthesized nanoparticles mode of action was highly effective. However, there has been no previous report on antibacterial activity against Gram Negative, Gram positive and cytotoxic effect against breast cancer cell lines. Therefore, in the current study, we have synthesized and characterized the gold nanoparticles (AuNPs) applying *Securinega leucopyrus* leaves extract and assessed their antibacterial and cytotoxicity activity.

2. Materials and Methods

2.1. Chemicals and reagents

Chemicals of analytical grade were procured from Sigma Aldrich USA, laboratories *securinega leucopyrus* leaves extract were collected from the Kakatiya university area, Warangal district, The microbial strains *Escherichia coli*, *Pseudomonas putida, Staphylocaccus aureus* and *Micrococcus luteus* were revived at Department of Biochemistry, Kakatiya university, Telangana state, India.

2.2. Preparation of dried powder

Freshly collected leaves of *Securinega leucopyrus* were thoroughly washed with distilled water, shade dried (5

days), pulverized using ball mill and the dried powder was stored at 37°C Room temperature.

2.3. Synthesis of gold nanoparticles (AuNPs)

Dried powder (10 g) of *Securinega leucopyrus* leaves extract and 100 ml of distilled water was blended in a beaker, stirred on a magnetic stirrer (400 rpm) overnight (Room Temperature). After incubation, the extract was filtered through a whatman filter paper and lyophilized to obtain moisture free powders. The lyophilized extract powder (0.5 g) was dissolved in double distilled water. To the 5 ml of *Securinega leucopyrus* leaves extract, 20 ml of chloroauric acid (0.01 M) was added drop wise under continuous stirring (400 rpm) on a magnetic stirrer and the whole mixture was incubated overnight (9). The color change (yellow to ruby red) was seen after the incubation period and was subjected for spectroscopic and bioactivity study.

2.4. Characterization of green synthesized AuNPs

UV–Visible spectroscopy (UV-VIS spectrophotometer), Particle size analyzer (Malvern Instruments zeta sizer), DLS and Zeta potential were used for characterization of the green synthesized AuNPs. Further, functional groups present in the Gold nanoparticles (AuNPs) were identified using FTIR analysis.

2.5. Study of antibacterial activity of Gold nanoparticles (AuNPs)

This study was performed by following (10). The bacterial cultures were grown on nutrient agar broth and were swabbed on petri plates containing sterile nutrient agar medium. Three wells were bored on the agar surface using a sterile well cutter (6.0 mm diameter of whatman filter paper stips) on each agar plate. Thereafter, the AuNPs suspension (20 µg/20 µl, 40 µg /40µl, 60 µg/60 µl, 80 µg/80 µl and 100 µg/100 µl) was poured into two wells of the agar plates and 50 µg/50 µl of Ampicilline standard was added. The plates were incubated at 37°C and the zone of inhibition (in mm) was evaluated after incubation one day.

2.6. *In-vitro* % viability assays of the AuNPs on MCF 7 cells

Breast cancer cell line and Peripheral Blood Mononuclear Cells (PBMC) were procured from NCCS, India. The culturing was done in Amphotericin B (1 mg/mL), Gentamycin (100 mg/ml), Penicillin(250 mg/ml) /Streptomycin (250 mg/ml) and 10% FBS. Next, cells (1: 105 /well) were plated in 24-well plates and incubated to reach confluence Further, cell control (Con),and different concentrations (5 µg,10 µg, 25 µg.50 µg 100 µg , Doxorubicin Values(2µM) standard drug used and green synthesized AuNPs were loaded and incubated for 24 hours. After incubation, the samples were removed and rinsed with PBS buffer. The cells were incubated for a period of 4 hours. The absorbance (570 nm) were measured and the cell viability was calculated in percentage:

% Cell Viability = A570 of sample cells / A570 of control cells X 100

Graphs concentration weses cell viability was plotted on the X and Y-axis respectively. To compare the assessments the control and gold nanoparticles samples are included in the assay (11).

3. Results and Discussion

3.1. Green synthesis of Gold nanoparticles and characterization

The color conversion from yellow to ruby red **(Figure. 1)** indicates the development of AuNPs. This reaction may be due to surface Plasmon resonance (SPR) displayed by the green synthesized particles. The change of gold chloride and the change in color is due to the existence of chemical constituents in *securinega leucopyrus* leaves extract.

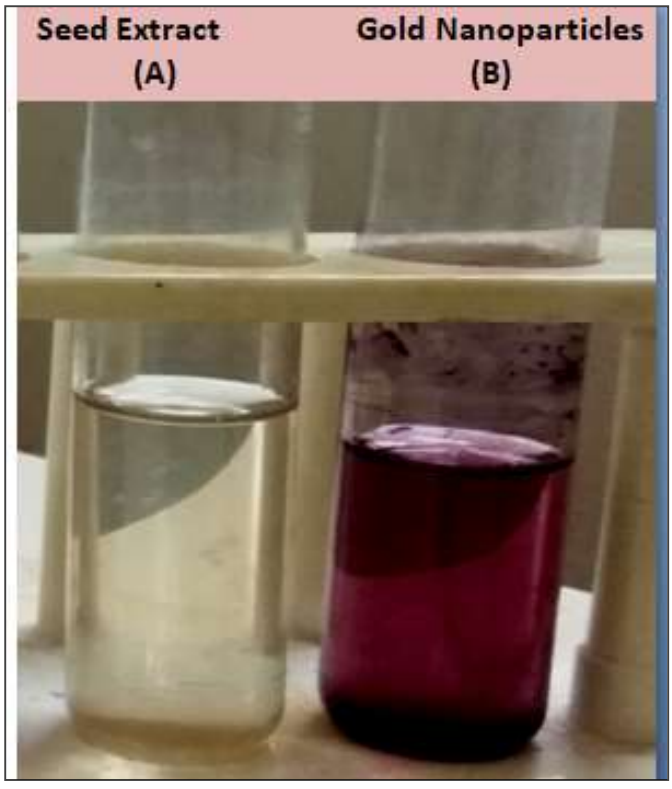

Figure 1. Green synthesis of gold nanoparticles from *securinega leucopyrus* leaves extract (A). The colour change from gold to ruby red indicates the formation of gold nanoparticles (B).

3.2. Characterization of AuNPs

Figure: 2(a) shows the maximum peak corresponding to the SPR at 545 nm. The result confirms the formation of AuNPs, since the peak at 540 nm is specific to AuNPs

(12). The UV analysis was repeated after 10 days to analyze the stability of the AuNPs.

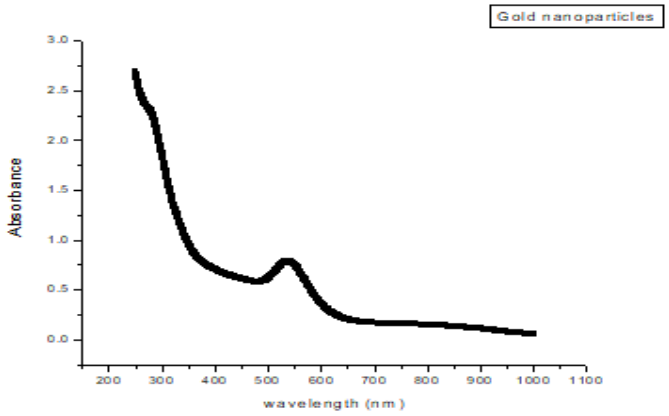

Figure. 2. **Ultraviolet–visible spectroscopy analysis of synthesized gold nanoparticles. The maximum peak was noted at 545 nm.**

The FT-IR spectra for the green synthesized AuNPs were recorded from 500 cm^{-1} to 4000 cm^{-1}. The observed strong peaks corresponding to -OH group in the spectra indicates that there may be phenolic groups present in the nanoparticles **(Figure. 3).** We also observed peaks for C=C in the spectra which may be due to the presence of flavonoids in the plant (13). Phenolic compounds possess antioxidant activity, act as protective agents against cancer and heart diseases, also neutralize the free radicals, and prevent them from causing cellular damage. In addition, the broad peak at 3374 cm^{-1} –3500 cm^{-1} in the plant**.** The sharp peak at 1639 cm-1 indicates the fingerprint region of C=C. Compared to the leaf extract, spectra of nanoparticles contains a reduced peak at 2468 cm^{-1}, the plant leaves extract of *Securinega leucopyrus* has contributed to the formation of AuNPs and indicate the presence and binding of phenolic groups to the synthesized nanoparticles (14). Therefore, the investigation the present study suggest advice that phytochemical compounds (phenols, flavonoids) present in *Securinega leucopyrus leaf* extract promotes the generation of AuNPs. Particle size analysis by DLS and Zeta potential were used for identified gold nanoparticles size 10-120 nm and stability of gold nanoparticles of the green synthesized AuNPs -27.2mV **(Figure.4).**

3.3. Antibacterial analysis

The zone of inhibition (mm) and gold nanoparticles show inhibitory effect at all different concentrations **(Table.1** and **Figure.5).** The reason for choosing these four strains is because they are as associated with food borne diseases, urinary tract infections and other wide range of ailments (15). In our study, the green synthesized AuNPs inhibited the bacterial growth and the proposed antimicrobial mechanism may be due to cell wall and membrane damage (16), damage in ribosomes and mitochondria (17), inhibition of thiol group in bacterial cells and cause cell decease (18). The increased activity of AuNPs could be due to the capping of the nanoparticles that have been confirmed by FTIR analysis. In addition, the antibacterial activities of the gold nanaoparticles (AuNPs) were higher in Gram-negative bacteria. This could be due to Gram negative bacteria having thinner cell wall in comparison with Gram-positive bacteria. AuNPs have durable electrostatic attractiveness to the negatively charged

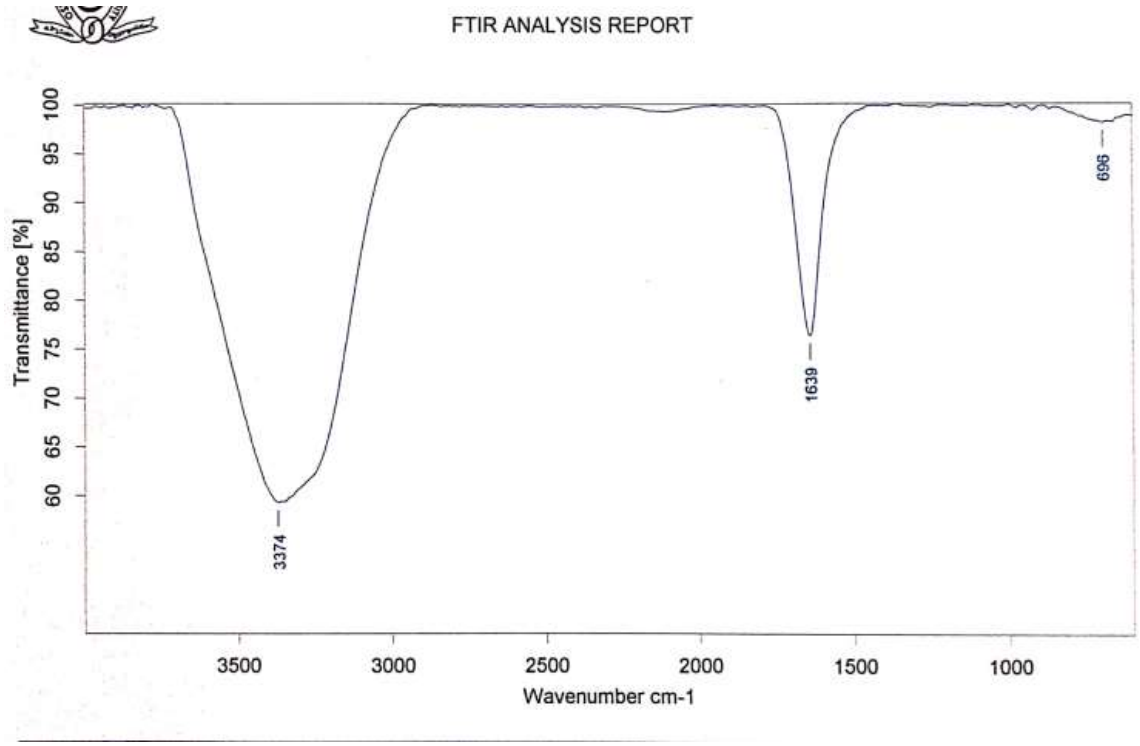

Figure. 3 FT-IR spectra of plant mediated gold AuNPs conformation.

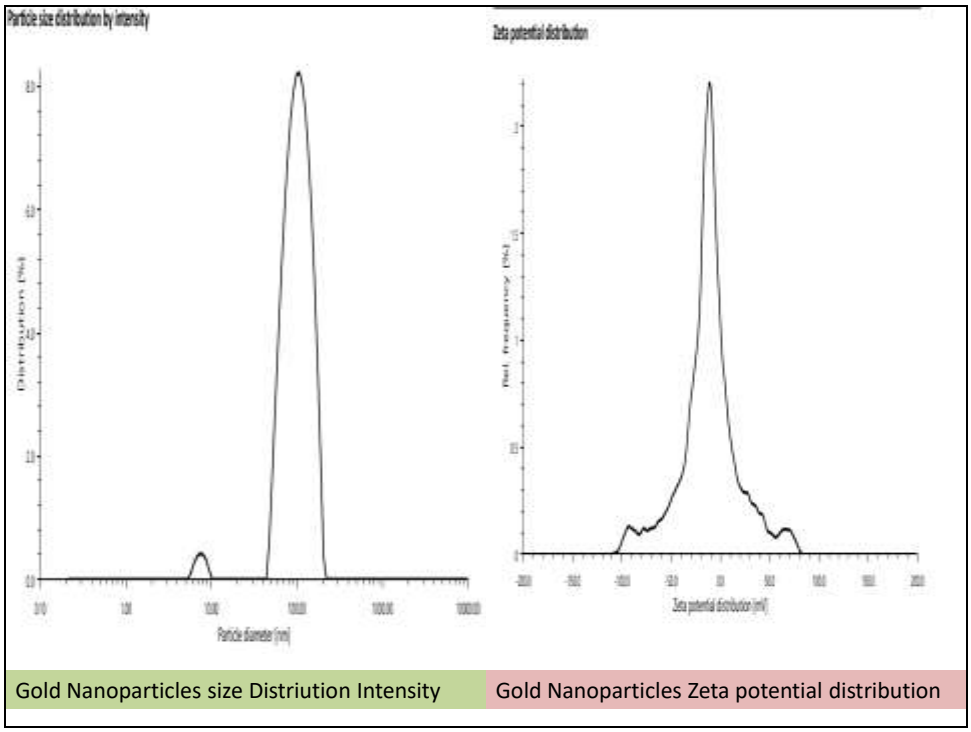

Figure.4 Gold nanoparticles distribution of size and zeta potential distribution.

Table 1: Effect of green synthesized AuNPs against on gram positive and gram negative zone of inhibition in (mm).

Antimicrobial activity of synthesis Gold nanoparticles						
Bacterial strains	20µg/20µl	40µg/40µl	60µg/60µl	80µg/80µl	100µg/100µl	Ampicillin (50µg/50µl)
E.coli	1.3	3.2	6.2	10.1	12	13.9
Pseudomonas putida	2.6	4.2	7.3	11.2	12.2	15.6
Staphylococcus aureus	3	4.6	6.8	10.5	12.4	13.1
Micrococcus luteus	3.4	4.8	7.6	9.5	11.1	12.4

bilayer, there by facilitating the diffusion of plant mediate gold nanoparticles (AuNPs) and cell lysis (19).

3.4. Cytotoxicity analysis of the AuNPs

To determinate % of viability the AuNPs, MTT assays were carried out. We observed cell % viability of 45.717 and % of cytotoxicity 54.282 at a concentration of 100 µg on MCF-7 cells in **(figure.6).** The IC50 values were calculated and were found to be 53.4 µg/ml for MCF-7 cells standard drug of doxorubicin values (2µl) % of viability 88.353 MCF-7 cancer cell line. The most important factor for developing an anticancer drug is its selective toxicity on cancer cells. Therefore, our results indicate that green synthesized gold nanoparticles treatment have shown selective cytotoxicity towards cancer cells than normal cells. Here in, we used MCF-7 cell line for cancer cell line for the studies on breast cancer for decades. Since, they maintain a strong similarity to the mammalian epithelium (20). Earlier reports of green synthesis of AuNPs from Sasa borealis (21), Illicum versus (22, 23, 24) etc.

4. Conclusion

This study reports synthesis of AuNPs by applying the leaf extracts of *Securinega leucopyrus* leaves extract. The synthesized AuNPs were of 10– 120 nm and spherical in shape. The green AuNPs elicited an increased activity against the pathogens, provided permissible levels of cytotoxicity towards normal cells and demonstrated high cytotoxicity towards MCF-7 cancer cells. The results indicate that the AuNPs synthesized by *Securinega leucopyrus* leaves extract can be safely applied for antibacterial applications.

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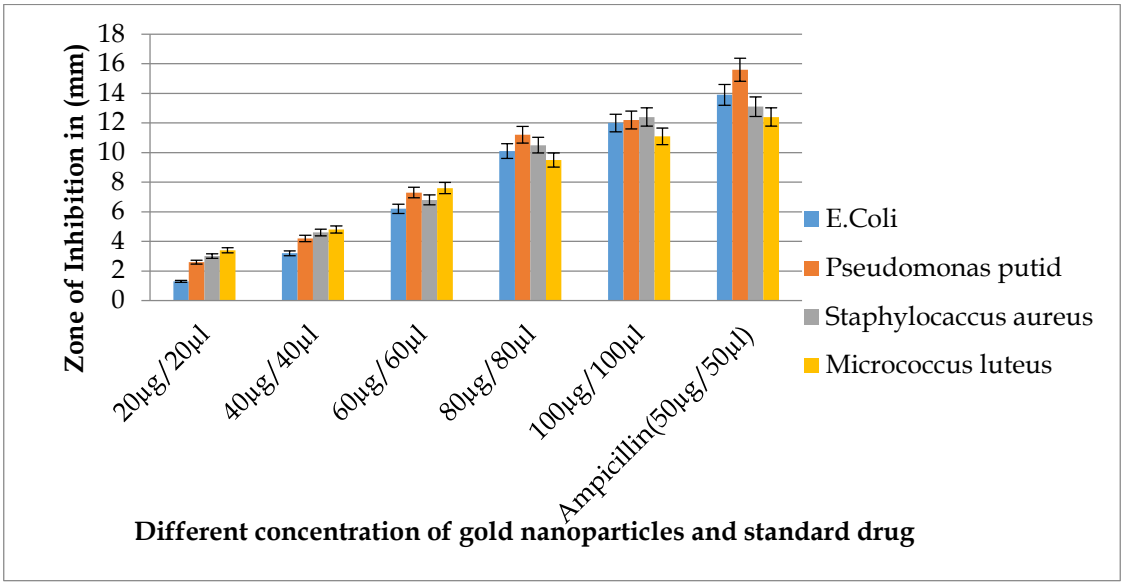

Figure.5. Zone of inhibition (mm) of green synthesized gold nanoparticles in gram positive and gram negative bacteria.

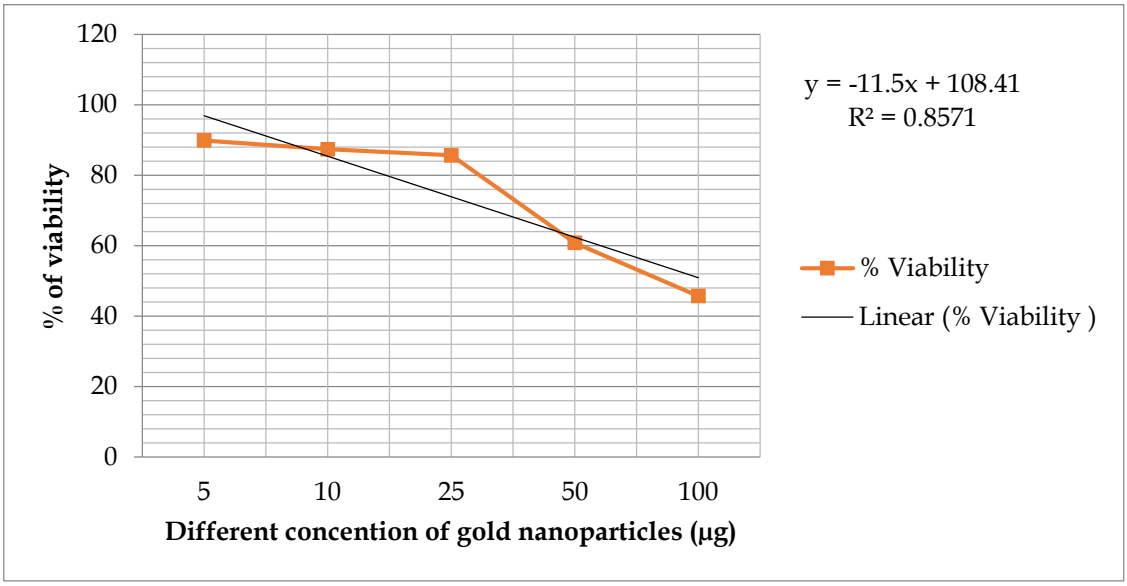

Figure.6 % of viability of green synthesized AuNPs against MCF-7 cancer cell line.

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Conflicting Interests

The authors have declared that no conflicting interests exist.

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