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Facile and Eco-Friendly Synthesis of Silver Nanoparticles using *Semecarpus anacardium* and their Antibacterial and Anticancer Activity

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Plant extracts are utilized to synthesize nanoparticles containing bioactive compounds that can effectively decrease metal toxicity. In present study, the aqueous extracts of *Semecarpus anacardium* leaves and stems were used to reduce silver nitrate and synthesize silver nanoparticles (AgNPs), which have potent antibacterial and anticancer effects. The physico-chemical methods such as XRD, UV-Vis, Fourier transform IR and SEM were used to characterize the biogenci silver nanoparticles. These methods validated the synthesis of AgNPs with smaller size and homogeneously sphere-shaped AgNPs (10-50 nm) after incubation with *S. anacardium* leaf and stem extract. Stem extract derived AgNPs showed the significant cancer fighting abilities in the human breast (MDA-MB-231) and prostate adenocarcinoma (PC-3) cells compared to that of derived AgNPs. Stem extract derived AgNPs exhibits the significant antibacterial activity against *E. coli* and *B. subtilis* with respect to the gentamycin. Additionally, the study demonstrated that green synthetic AgNPs may be investigated for use as a treatment against bacterial infections.

Keywords: *S. anacardium*, Silver nanoparticles, Antibacterial, Anticancer activity, MDA-MB-231, PC-3 lines.

INTRODUCTION

Inorganic nanoparticles have raised great attention due to its high volume ratio and specificity followed by sustained release [1-4]. The development of nanoparticles through green route regarded as reasonably priced and environmental friendly process was confirmed to exist an improved method due to the property of slower dynamics, stability and safe handling [5-7]. Previous reports showed that the synthesized nanoparticles using plant extracts exhibits the antibacterial [8,9], antioxidant [10], anticancer [11] and anti-inflammatory [12] activities of green synthesized nanoparticles.

According to WHO, by 2020, cancer will be one of the top 15 causes of mortality worldwide. Breast cancer accounts for 28% of all cancer cases and it is the most common cause of deaths for women out of every cancer patient. Cancerous glandular tissue is known as adenocarcinoma and for men, prostate cancer is the second most common malady [13]. As the immunity lowered or suppressed condition these harmless organisms turned into opportunistic form. The bacterium *Bacillus subtilis* is responsible for food poisoning [14] and has also been shown to generate bacteremia [15] in cancer patients. An antagonistic bacterium *viz. Escherichia coli* can lead to the significant issues including kidneys and urinary tract infections [16].

Involvement of metal nanoparticles treatment for cancer be additional advantageous compared to conventional therapeutics [17]. Alternative medicines are extremely important since radiation and chemotherapy have serious adverse effects [18]. Silver is lethal for humans and other animals when compared to other metals [19,20]. Silver nanoparticles are biocompatible and have the ability to selectively kill cancer cells by interrupting DNA damage induced by the mitochondrial DNA pathway, leading to the synthesis of ATP and the formation of

reactive oxygen species [21,22]. AgNPs exhibits potential bactericidal action that is directed at both Gram-positive and Gram-negative, due to its high ratio of surface area to volume, which makes them widely used in fragrances, medical equipment and water purification systems [23].

Semecarpus anacardium belongs to the Anacardiaceae family. Each and every part of the plant has been exploited in Ayurvedic medicines for various types of ailments include cardiovascular [24], neurological [25], dermatological [26,27] and cancer diseases [28] owing to the existence of wide variety of bioactive compounds such as bhilwanol, jeediflavone, semecarpuflavone, gulluflavone, anacardoside, biflavanone and anacardic acid [29]. This work assessed the antimicrobial activity against *B. subtilis* and *E. coli*, as well as *in vitro* cytotoxicity against the mesenchymal lineages of cancer cells, and synthesized AgNPs utilizing *S. anacardium* leaf and stem extractions.

EXPERIMENTAL

Fresh plant leaves and stem materials of *S. anacardium* were collected and the taxonomic authentication was done by comparing to voucher specimen obtainable in the herbarium, at Department of Botany, Andhra University, Visakhapatnam, India.

Preparation of extract from leaves and stems: Leaves and stem material surface was cleaned thoroughly, dried and were thinly crushed using mortar and pestle. Powdered leaves and stems being individually disintegrated in pure water before being heated at 70-80 ºC for 30 min and then filtered thoroughly using Whatmann No. 1 filter paper. The collected filtrate was used for the preparation of silver nanoparticles.

Synthesis of AgNPs: Leaf and stem extracts (20 mL) were separately mixed with 80 mL (1 mM) $AgNO_3$ solution and heated for 5 h. By periodically monitoring the colour change of the blend of pale yellow and dark brown shades, the reaction was arrested and centrifuged the sample at 10,000 rpm for 8 min. Deionized water was used pellet washing and allowed to dry for 3 h at 60 ºC, which was used for further characterization. For *in vitro* cytotoxicity and antibacterial studies, Before the drying process, the AgNPs were transformed in DMSO.

Characterization: The optimization for synthesized AgNPs was monitored using UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan). The FTIR analysis (Prestige21, Shimadzu, Japan) was performed in the spectrum range of 4000-400 cm^{-1}. The X-ray diffraction (XRD) studies on thin films of biosynthesized AgNPs were carried out using a PANalytical model with Cu$K\alpha$ ($\lambda = 0.1546$ nm). Using SEM, the AgNPs' mean particle size and shape of the surface were ascertained with the help of JSM6610LV, Jeol Asia PTE Ltd. model.

Antibacterial activity: The antibacterial efficacy of biosynthesized AuNPs of varying particle sizes was assessed using the agar well diffusion method. Sterile petri dishes were utilized to prepare the agar media and allowed to solidify overnight. Bacterial inoculums were prepared by combining 0.1 mL of bacterial culture with 10 mL of AgNPs and incubated aerobically at 37 ºC for 24 h to promote bacterial growth. A 0.1 mL of bacterial culture suspension with a concentration of 0.5 McFarland units was spread twice on Mueller-Hinton Agar (MHA) plates. The specified bacteria were then inoculated into the agar plates and left at room temperature for 10 min. Identical wells, each measuring 4 mm in diameter, were developed in the MHA agar using a sterile stainless steel cork borer. Au NPs were added to each well in varying quantities ranging from 100 ppm to 0.097 ppm in 25 µL volume. The experiments were replicated in three petri plates (n=3). The plates were kept at room temperature for 10 min and then incubated aerobically overnight at 37 ºC. The minimum inhibitory concentration (MIC) was determined by measuring and recording the diameter of inhibition zones after incubation. The positive and negative controls were generated by filling one well with gentamicin and another well with deionized water.

Anticancer activity

Cell culture with maintenance: The cell lines of breast epithelial adenocarcinoma (MDA-MB 231) and penile cancer were obtained from the National Centre for Cell Science (NCCS) in Pune, India. In a humid environment with 5% CO_2 at 37 ºC, 80% of the cells were grown to confluence in DMEM supplemented with 10% FBS and streptomycin-penicillin (100 µg/mL).

MTT assay: The cells were nourished in a 96-well plate (1×10^4 cells/well) and allowed to developed at 37 ºC in dark with 5% CO_2 5%. Following the incubation period, cells were exposed to AgNPs at concentrations ranging from 20 to 100 mg/mL for periods of 24 and 48 h. After treatment, the media was removed and 20 µL of MTT liquid (0.5 mg/mL) was added, incubated for 3 h and then solubilized with 100 µL of DMSO. The absorbance be recorded at 590 nm with micro plate ELISA reader (BioRad). The cytotoxicity percentage was calculated as follows:

$$\text{Cytotoxicity (\%)} = \frac{\text{O.D of treated wells} - \text{O.D of vacant wells}}{\text{O.D of control wells} - \text{O.D of blank wells}} \times 100$$

Statistical analysis: Using SPSS software (version 16.0; Chicago, IL, USA), the disc diffusion methods had been explained as means ± the standard deviation (S.D.). The student used verification to reconcile the results of the disc diffusion test, considering P-values below 0.01 ($p < 0.01$) as significant.

RESULTS AND DISCUSSION

UV-visible studies: When *S. anacardium* leaf and stem extracts were added to an aqueous solution of silver nitrate, the colour changed from green to brown due to the reduction of silver ions ($Ag^+ \rightarrow Ag^o$). This change in color is a result of the excitation of the surface plasmon atmosphere in silver nanoparticles (AgNPs), causing the aqueous solution of AgNPs to exhibit a yellowish-brown colour [30]. The presence of silver nanoparticles was confirmed by detecting peaks in the UV-Vis spectrum within the wavelength range of 200-800 nm (Fig. 1). In view of the fact that the spectral investigation, the absorbance peak was obtaining at 446 nm for *S. anacardium* leaf derived AgNPs and 432 nm to facilitate of stem derived AgNPs, which was specific for silver nanoparticles [31]. An increase in absorption intensity at the absorption peak was observed in

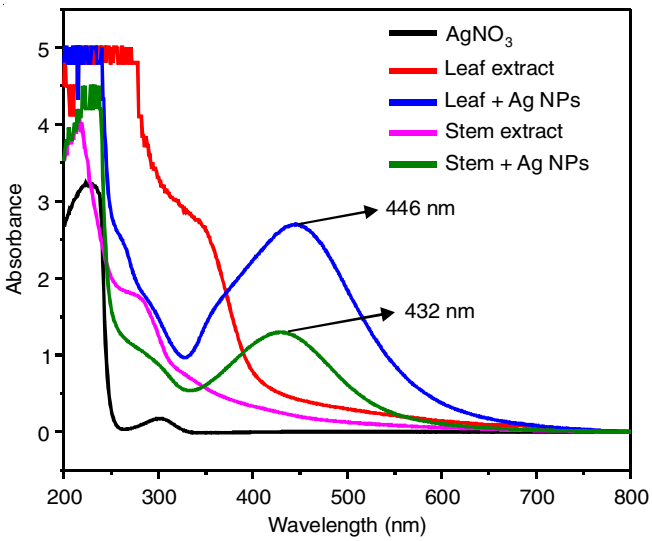

Fig. 1. AgNPs' UV-Vis spec produced from *S. anacardium* leaf and stem extracts

the obtained UV-Vis spectra, which is consistent with earlier findings [31]. The biomacromolecules found in leaf and stem extracts led to a decrease in the concentration of silver ions because of their biological functions.

XRD studies: In order to recognize the crystalline size, orientation, phase formation of nanoparticles by XRD revealed the distinct diffraction peaks associated with the silver crystalline position (Fig. 2). The Bragg's peaks (2θ) at 37.31º, 47.52º, 63.91º and 78.16º and corresponding miller indices (111), (200), (220) and (311) indicates that the AgNPs produced by plant extracts from the leaves and stems of *S. anacardium*, respectively. The results confirmed the purity of silver as face-centered cubic (FCC) crystalline and matched with the JCPDS file no. #04-0783). The bioorganic phases that are affixed to the surface of the produced nanoparticles crystallized, resulting in the observation of several undesignated peaks [32-34]. The lattice volume is 66.7788 $Å^3$ and the computed lattice parameter is a = 4.0569 Å. The Debye-Scherrer's formulae D = (kλ/βcos θ) was used to determine the nano-formulations. An average

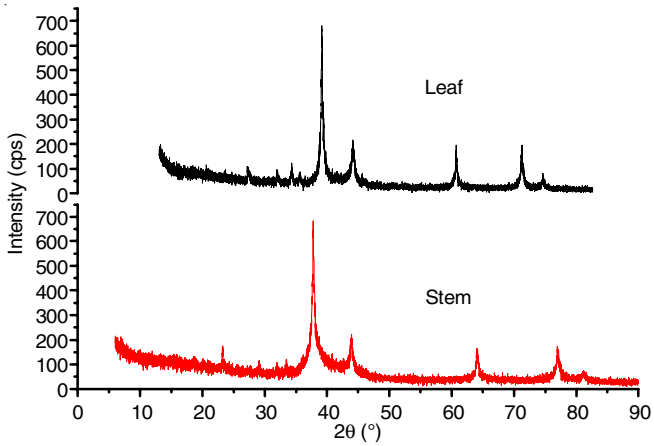

Fig. 2. XRD spectra of leaf and stem Ag-NPs of *S. anacardium*

crystalline size of 19 nm was found for the silver nanoparticles produced from *S. anacardium* leaf and plant extracts.

SEM studies: The SEM investigation of structured samples at 80 ºC for the translucent AgNPs arrangements authenticated in continued for small and consistently sphere-shaped nanoparticles. The combination of nanoparticles was found to facilitate the production of well-built particles of AgNPs, which may have been caused by the solvent withdrawal [35]. The particle size ranges from 10.1 to 45 nm with sphere-shaped AgNPs (Fig. 3).

FT-IR studies: As evident from Fig. 4, the peaks at 3402 cm^{-1} in *S. anacardium* leaf and 3416 cm^{-1} in *S. anacardium* stem show that there is O-H and N-H groups of water and plant extracts. The C-H group stretching vibration of C-H group belongs to the peaks at 2924 cm^{-1} [36]. The carbonyl-group absorption corresponds at 1616-1608 cm^{-1} and the frequencies at 1124 cm^{-1}, 1134 cm^{-1}, which is associated to –C-O-C and C-O-H stretchings, respectively [37]. The N-H groups deformed mode relevant peaks was found at 1388 cm^{-1} [38]. The FT-IR spectra verified the binding of flavonoids, glycol and other phytochemicals [39]. The carbohydrates in the plant extract used externally on silver nanoparticles may be driving the reduction in silver ions response.

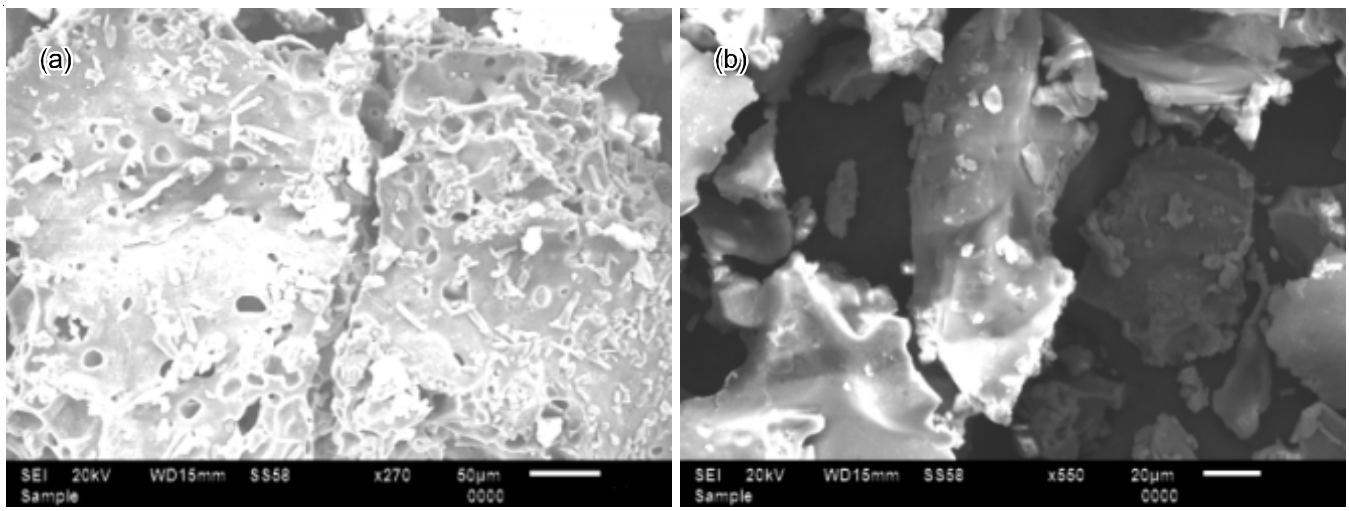

Fig. 3. SEM images (a) leaf extract derived AgNPs and (b) stem extract derived AgNPs prepared with *S. anacardium*

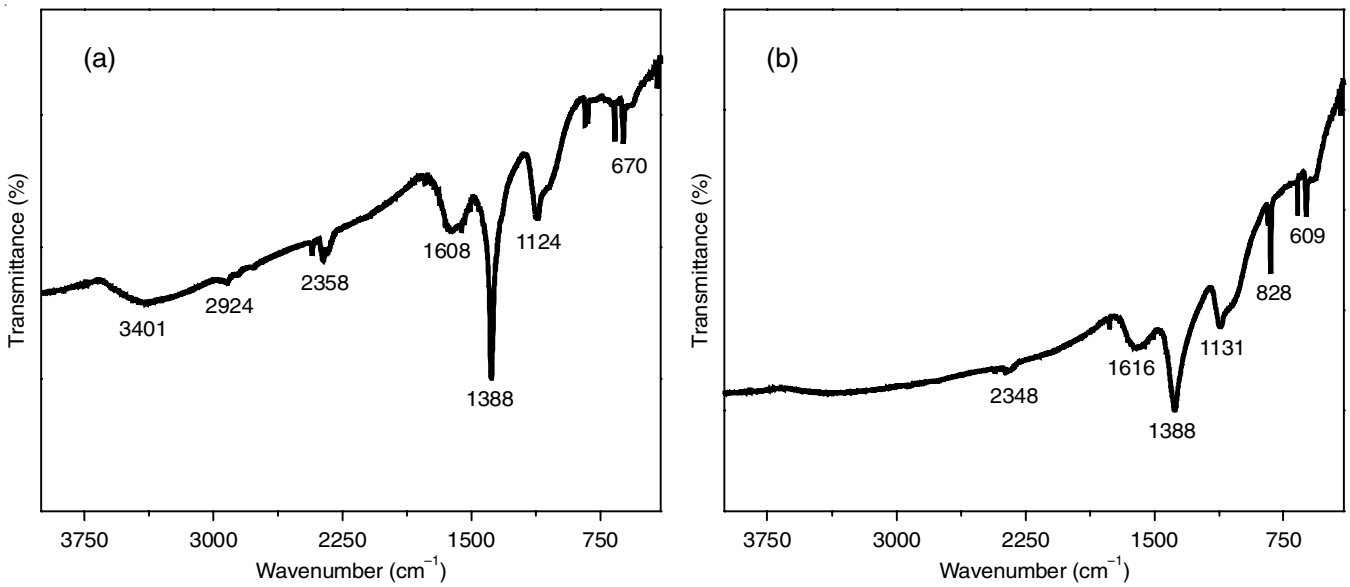

Fig. 4. FT-IR spectra of (a) leaf extract derived AgNPs and (b) stem extract derived AgNPs prepared with *S. anacardium*

Antibacterial activity: The antibacterial property of the biosynthesized silver nanoparticles was evaluated using the disc diffusion method, showing increased inhibition against the Gram-negative *E. coli* and the Gram-positive *B. subtilis* (Table-1). Leaf along with stem extracts of *S. anacardium* leaf against *E. coli* did not exhibits every region of inhibition, whereas folio and stalk extracts *S. anacardium* derived AgNPs demonstrated greater areas of inhibitions. AgNPs obtained from leaf and stem extracts of S. Anacardium (5, 10 µg) and positive control (gentamycin) resulted in significantly higher inhibition zones compared to untreated controls ($p < 0.001$). The inhibition zone levels were much larger for the AgNPs produced by the stem extract compared to the leaf extracts. When compared to the untreated control, the AgNPs of *S. anacardium* leaf and stem extracts (5, 10 µg) and the positive control (gentamycin) exhibited significantly higher resistance zones [40,41]. The results indicate that silver nanoparticles isolated from stems exhibited better bactericidal activity compared to those derived from leaves and the positive control.

TABLE-1
INHIBITORY READINGS FOR AgNPs MADE BY *S. anacardium* LEAF AND STEM EXTRACTS

Sample (µg)		Zone of inhibition (mean ± SD) (mm)	
		B. subtilis	*E. coli*
Untreated		–	–
AgNPs prepared with leaf extract of *S. anacardium*	5	18 ± 3.00*	22 ± 3.00*
	10	21 ± 3.00*	24 ± 3.00*
AgNPs prepared with stem extract of *S. anacardium*	5	21 ± 3.61*	22 ± 3.00*
	10	24 ± 2.00*	25 ± 3.00*
Gentamycin	10	22 ± 2.10*	27 ± 2.15*

Dunnett's post test revealed a significant difference between the treated samples and the untreated control group *$p < 0.001$.

Due to their small size and high surface area to volume ratio, AgNPs produced from aqueous extracts of S. anacardium leaves and stems have a strong correlation with the microbial barrier. AgNPs have the property of releasing silver ions, which bind firmly to the thiol groups of biological enzymes and limit the development of bacterial cells [42]. The enhanced antibacterial effects of AgNPs on Gram-negative bacteria compared to Gram-positive bacteria may be due to the presence of a thinner and brittle peptidoglycan layer, permitting their easy penetration into the bacterial cell wall [43].

Anticancer activity: In MTT assay, NAD(P)H reliant cellular oxidoreductase antibodies diminish the MTT, a tetrazolium dye to deep purple colour formazan crystals [44]. The cytotoxic effect of leaf and stem derived AgNP's was evaluated at different concentrations *viz*. 20, 40, 60, 80, 100 µg/mL for 24 and 48 h of handling. The percent of cytotoxicity increases in a dose time dependent manner. The AgNPs obtained from leaf exhibited 41.85% and 71.60%, whereas stem made showed 55.30% and 72.44% (Table-2) uptake in breast epithelial cell line MDA-MB 231 after being treated with a 100 µg/mL solution for 24 and 48 h, respectively [45].

TABLE-2
AgNPs' CYTOTOXIC ACTIVITY AGAINST MDA-MB 231 IN LEAF AND STEM EXTRACTS FROM *S. anacardium*

Concentration (µg/mL)	% of Cytotoxicity against MDA-MB 231			
	Leaf		Stem	
	24 h	48 h	24 h	48 h
20	9.35	9.55	10.08	11.77
40	13.24	21.25	24.19	28.41
60	23.39	32.35	35.90	44.23
80	32.52	48.02	48.44	67.37
100	41.85	71.60	55.30	72.44

In PC-3 cells, leaf extract derived AgNPs showed 56.75%, 69.75% and stem extract derived AgNPs showed 56.91% and 71.59% of cytotoxicity at 100 µg/mL of concentration (Table-3) 24 and 48 h, respectively. On the PC-3 cell line, cytotoxicity has grown in a dose- and time-dependent way. Similar growth-limiting effects of AgNPs synthesized by different ways earlier

TABLE-3
CYTOTOXIC EFFECT OF AgNPs LEAF AND STEM EXTRACTS AGAINST PC-3

Concentration (µg/mL)	% of Cytotoxicity against PC-3			
	Leaf extract derived AgNPs		Stem extract derived AgNPs	
	24 h	48 h	24 h	48 h
20	8.44	16.57	12.81	14.82
40	17.25	27.23	20.65	26.16
60	28.94	38.03	35.06	44.39
80	39.15	48.09	44.07	57.90
100	56.75	69.75	56.91	71.59

against the currently evaluated cell lines were observed in the previous investigations [46]. The AgNPs cause cellular damage and kneejerk oxygen species (ROS) in cancer cells, which ultimately results in cell death [47].

Conclusion

Silver nanoparticles (AgNPs) synthesized from the aqueous extract of leaves and stems of *Semecarpus anacardium* are advantageous for their potent synthesis of AgNPs with small dimensions and a high surface area to volume ratio. When tested against *E. coli* and *B. subtilis*, the synthesized AgNPs showed strong antibacterial action. The use of synthetic, antibacterial AgNPs that are both environmentally benign and effective in medical treatments has many advantages over more traditional methods.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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