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Research Article

Green Synthesis of Silver Nanoparticles and Characterization using *Peltophorum pterocarpum* Leaf Extract and Cancer Cell Line Studies

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ABSTRACT

Peltophorum pterocarpum [Pp], family Fabaceae, native of tropical South Eastern Asia is used to treat many diseases like Stomatitis, Insomnia, Skin troubles, Constipation etc**.** Silver nano particles [AgNps] were synthesised using aqueous leaf extract of *Peltophorum pterocarpum* and its anti-toxic activity was studied on human skin cancer lines-A431. Further Fourier Transform Infra-red studies [FTIR], Ultra Violet studies [UV] and Field emission Scanning electron microscopic studies were carried out for the AgNps present in the leaf extract. Reduction of Ag^{+} to Ag^{0} was visually observed from yellow to dark brown colour and confirmed the presence of silver nanoparticles at 450nm in UV. The phytochemicals present were also investigated and cytotoxic activity was studied on A431 human skin cancer cell lines which showed that synthesized AgNps revealed 96% activity.

Keywords: *Peltophorum pterocarpum*, silver nanoparticle, cell lines.

INTRODUCTION

Nanotechnology is rapidly growing field with its application in science and technology for the purpose of manufacturing novel materials at the nano scale level [1]. Noble metal nanoparticles have been gaining a lot of significance in the past few years due to their applicability in the field of physics, chemistry, medicine, biology and material science[2]. Metal nanoparticles, particularly noble metals, have been studied mainly because of their strong optical absorption in the visible region caused by the group excitation of the free electron gas [3]. Due to reduction in size and large surface areas, the nanoparticles have wide range of applications in catalysis, photonics and medical field [4].

Silver nanoparticles occupies a significant place in medical domain other than its application in non-linear optics, spectrally selective coating for solar energy absorption, bio-labelling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions and as anti-bacterial capacities [5-7]. Even though there are many physico-chemical routes available due to the involvement of toxic chemicals, high temperature, pressure and production of hazardous by-products there is aneed of alternative green approach for the synthesis of nanoparticles. Plant extracts being nano factory for synthesizing silver and other nanoparticles as it is potentially advantageous over microorganisms due to the ease of scale up, less biohazard, eco-friendly and elaborate process of maintaining cell cultures [8]. Bioactive compounds are rich in plant extracts which have recently been used for the synthesis of nanoparticles. Many different plant leaves and herbs for the synthesis of nanoparticles have been reported [9].

A lot of biological studies have been carried out using green synthesis of silver nanoparticles. The green rapid syntheses of spherical shaped silver nanoparticles with dimensions of 50–100 nm were observed using *Alternanthera dentate* aqueous extract which revealed anti-bacterial and anti-cancer activities [10]. The leaves of *Moringaoleifera*[11], *Acalyphaindica*[12] and tea extract[13] have been successfully used for the synthesis of silver nanoparticles of size ranging from 0.5-90nm with spherical shape and having anti-bacterial activity.

Peltophorum pterocarpum [Pp](Copperpod, Golden Flamboyant, Yellow Flamboyant, Yellow Flame Tree, Yellow Poinciana and Radhachura in Bangla; Synonyms: *Peltophoruminermis* and *Peltophorum ferrugineum*) is a family of Fabaceae, native to tropical southeastern Asia and a popularly ornamental tree grown around the world[14]. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm and its flower extract is known to be a good sleep inducer and used in insomnia treatment [15-17].

The synthesis of silver nanoparticles using *Peltophorum pterocarpum* flower extracts as both reducing and capping agents were performed to study anti-bacterial activity against fish pathogenic microorganism by agar-well diffusion method [18].The anti-bacterial activity of the silver nanoparticles was demonstrated against *Escherichia coli* and anti-coagulant activity was also studiedfrom the aqueous extract of pods of *Peltophorum pterocarpum* [19].

Current study focusses on the synthesis of silver nanoparticles from aqueous extract of the leaves of *Peltophorum pterocarpum* and characterized by UV, FTIR and FESEM. Further the phytochemical investigation followed by cytotoxic studies was carried out using human skin cancer cell lines.

MATERIALS AND METHODS

Collection of *Peltophorum pterocarpum* leaves

Fresh leaves of *Peltophorum pterocarpum* [Pp] (Fig 1) were collected near lake side of SRMIST, Kattankulathur. It was confirmed by Dr. M. Ganeshan, Asst. Prof [S.G], IIISM, SRMIST. The leaves were rinsed with distilled water thrice followed by Milli-Qwater to remove the dust and other contaminants then shade dried at room temperature to remove the moisture for 2 hours. Later it was coarsely grinded (Fig 2).

Figure 1: Photograph of *Peltophorum pterocarpum*

Figure 2: Coarsely grinded leaves

Preparation leaf extract

10gms of coarsely grinded leaves were taken in 300ml Erlenmeyer flask along with 100ml of sterilized distilled water and then boiled the mixture for 10min. The extract was filtered using Whatman filter paper no.1 and the filtrate was stored at 4-5^{0}C overnight for further studies.

Synthesis of Silver nanoparticles using aqueous extract

Silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich chemicals, Chennai, India. 10ml and 20ml of 1mM solution of $AgNO_3$ were added to 20ml of leaf extract separately. After 30min the solution changed from pale yellow colour to dark brown colour (Fig 3). The colour change indicates the reduction of Ag^+ to Ag^0 [20, 21]. After centrifuging the solution at 10,000 rpm for 15 min, the Ag NPs were separated washed and stored at 4^{0}C for further analysis.

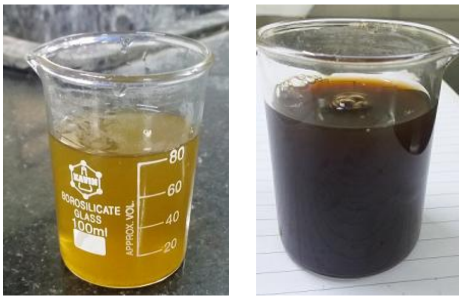


Figure 3: Synthesis of silver nanoparticles

Phytochemical analysis-Qualitative studies

Again 10gms of coarsely grinded leaves of *Peltophorum pterocarpum* was extracted using distilled water by orbital shaker method and evaporated the solvent in rotary evaporator. Then the residue dried in oven and initial stock of the aqueous extract was made at the concentration of 10 mg/ml using 1:1 (DMSO: H_2O). This was subjected to various biochemical tests to screen for the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, tannins, carbohydrates and glycosides [22].

Characterization of silver nanoparticles in the leaf extract

The UV-Visible spectral measurements were carried out on double beam UV-Visible spectrophotometer-LMSP-UV 1900S ranging from 190-1100nm. FTIR analysis was performed using Alpha-T FT-IR spectrophotometer operated at the resolution of 2cm^{-1}in the diffuse reflectance mode.Further the size and shape of Ag NPs were studied by Field emission scanning electron microscope (FE I Quanta FEG 200).

Cytotoxic studies- MTT Assay

Stock solution of aqueous leaf extract of *Peltophorum pterocarpum* was prepared in ethanol (5mg/ml). 20000 cells of A431 cell lines were seeded in 96 well plates and incubated overnight. Cells were treated with 10ml and 20mlof Ag NPs separately followed by 24 hrs incubation. 20µl of MTT along with 5% FBS were added. Incubated for 3hrs, finally 100µl DMSO added and quantified by Thermofisher at 570nm [23].

RESULTS AND DISCUSSION

Green synthesized silver nanoparticles were reddish brown in colour. The colour of the extract was changed from light yellowish to reddish brown after addition of $AgNO_3$ (Fig 3).This was due to coherent excitation of all the free electrons within the conduction band, leading to a phase oscillation[4] and the colour was stable after 3 hours which clearly indicates the completion of reduction reaction.

UV analysis

The UV spectral studies revealed that there is absorption at 450nm (Fig 4a and 4b) which in turn infers that silver nanoparticles have free electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of silver nanoparticles in resonance with light wave.

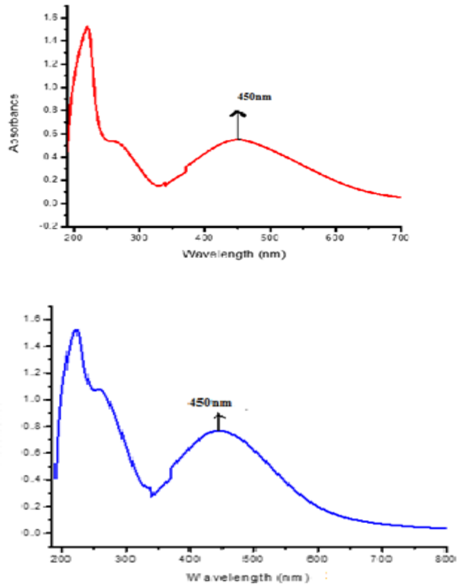


Figure 4a and 4b: UV-visible analysis of synthesized nanoparticles after 1 hr and 3 hrs

FTIR Analysis

FTIR studies (Fig 5) showed the absorption at 3331.18 cm $^{-1}$ due to the presence of stretching vibration of bonded or non-bonded -OH group and also might be broad secondary $–NH_2$ group. At 2123 cm $^{-1}$ and 1637 cm^{-1} corresponds to mono or di substituted alkynes and bending vibration of amide group respectively.

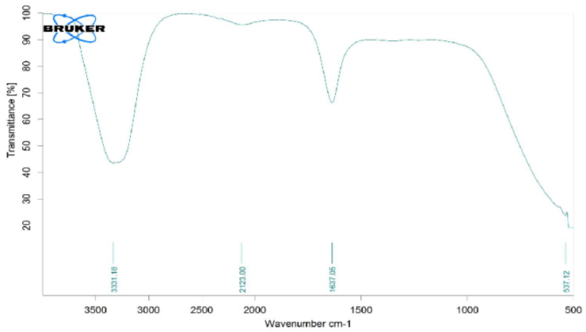


Figure 5: FTIR spectra of the Pp Ag NPs

FE SEM studies

FE SEM for the aqueous leaf extract of *Peltophorum pterocarpum* and silver nanoparticles are depicted in the figure 6 a, b, c, d, e and f at different magnifications. The image of the plant extract (Fig 6a and b) at high magnification showed a bulk matrix embedded with cotton like spheres and rods. It was free of any other characteristic features.

The images of Pp Ag Nps (Fig 6 c and d) for volume 10ml of $AgNO_3$solution revealed the presence of branched tree like structure of silver consist 100nm stem thickness, 40nm sub-stem thickness and 90-130nm particle sitting on the end of the branches.The presence of phenolic derivatives in the plant extract favours tree like growth of nanoparticles by combining with them and also infers that it might also be bonded with some carbohydrates to increase the viscosity of the medium that facilitates this type of structure.

The images of Ag Nps (Fig 6 e and f) for volume 20ml of AgNO3 solution revealed 300-400nm thickness. This image showed a significant increase in distribution of silver nanoparticles. In contrast to Ag NPs for 10ml, the dispersion is significant in 20ml of $AgNO_3$ solution.

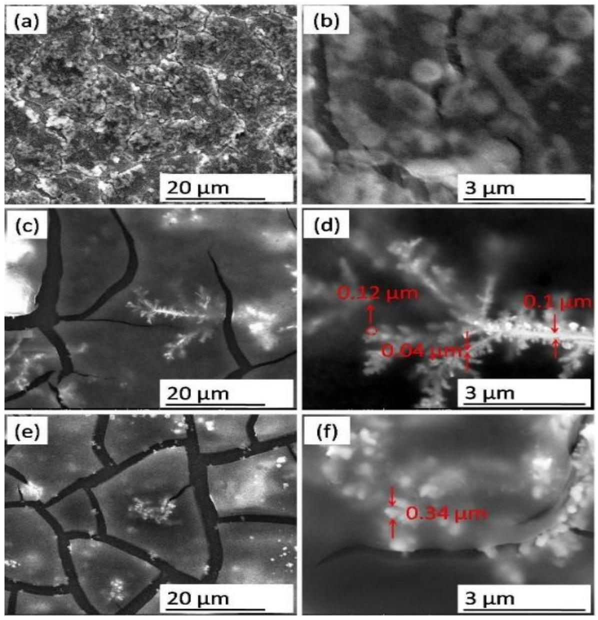


Figure 6: FESEM images (a) and (b) - Leaf extract images; (c) and (d) - PpAg NPs images for 10ml of $AgNO_3$ solution; (e) and (f) - PpAg NPs images for 20ml of $AgNO_3$ solution.

Phytochemical analysis

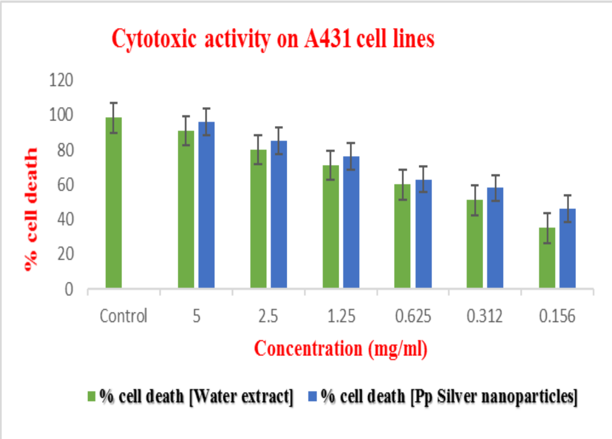
Qualitative analysis revealed (Table 1) the presence of proteins, carbohydrates, steroids, terpenoids, saponins, glycosides, phenols and tannins and absence of flavonoids.

Table1: Phytochemical analysis

Phytochemicals	Water extract
Proteins	√
Carbohydrate	√
Steroids	√
Terpenoids	√
Saponins	√
Flavonoids	X
Glycosides	√
Phenols	√
tannins	√

Cancer cell line studies

Cytotoxic studies on A431 human skin cancer cell lines revealed that Ag Nps has 96% of cell death when compared to aqueous extract which was only 91%. IC_{50} for PpAg Nps revealed 50% of cell death at concentration of 0.312mg/ml (Graph 1 and Fig 7).



Graph 1: MTT assay on A431 cell lines

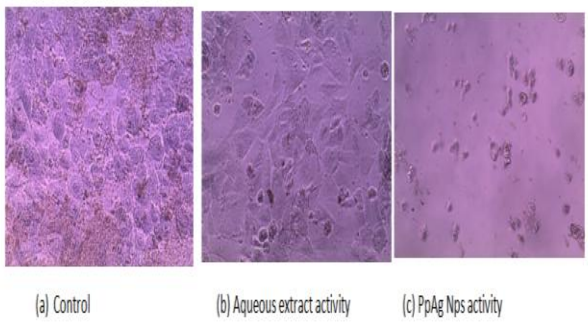


Figure 7: Cytotoxic activity of control, aqueous and synthesized Silver nanoparticles using Pp leaf extract

Current work reveals that silver nanoparticles of aqueous leaf extract of *Peltophorum pterocarpum* has appreciable cytotoxic activity. From previous studies it was known that the mechanism of anti-cancer activity of silver nanoparticles on cancer cell lines was through geno-toxicity, loss of the cell membrane integrity, oxidative stress and apoptosis [24]. Due to nanoparticles accumulation after cellular uptake inside lysosomes and endosomes, there is release of Ag^{+}ions from nanoparticles through oxidation and also simultaneous formation of reactive oxygen species (ROS). This might disrupts mitochondria of the cells and leads to cell damage. Silver nanoparticles and Ag^{+}ions can also directly affect the nucleus and trigger DNA abnormalities. This suggests that Ag Nps can trigger the mechanisms such as apoptosis and necrosis [25].

Further the presence of phytochemicals such as phenolic groups, terpenoids, proteins, steroids etc might induce apoptosis in a variety of human cancer cell lines and hence is important nutritional adjuvant therapeutics in the prevention of various human cancer diseases [26].

UV studies of current work reveals that the peak observed was little broader after one hour and after three hours the peak seems to be sharper at 450nm. This increase in the intensity of the peak i.e. absorbance with respect to time attributes to the completion of reduction reaction and also involvement of all biomolecules present in the leaf extract. Also FTIR analysis has revealed that proteins have good binding capacity with silver nanoparticles and forms a cap around it and thus stabilize the medium. TEM and SEM are the universally employed techniques for measuring the size of nanoparticles. This is due to their ability of providing all information directly related to the morphology of metallic nanoparticles[27].

CONCLUSION

An efficient green synthesis of silver nanoparticles was performed using aqueous leaf extract of *Peltophorum pterocarpum* which proved to have potent cytotoxic activity. Further studies will be conducted for anti-cancer activity by separating bioactive compound from this plant species.

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