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SYNTHESIS OF PLANT-MEDIATED SILVER NANOPARTICLES USING *TYLOPHORA INDICA MERR.* (PITTAKARI) LEAF EXTRACT AND EVALUATION OF ITS ANTIMICROBIAL AND ANTICANCER ACTIVITY

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ABSTRACT

Nanotechnology and nanoscience has got tremendous applications in different areas like catalysis, medicine, chemicals and electronics. Plant mediated synthesis of nanoparticles offers a single step, easy and extracellular synthesis of nanoparticles. In this method, synthesis of silver nanoparticles was done using aqueous leaf extracts of *Tylophora indica* Merr. The phytoconstituents and reducing compounds present in the leaf extract reduces aqueous silver chloride rapidly to nanosilver. The bio fabricated nanoparticles were confirmed by Surface Plasmon Resonance (SPR) as characterized by UV-vis spectrophotometry and Fourier transform infrared spectroscopy (FTIR). The morphology and size of the silver nanoparticles were determined by Scanning Electron Microscopy. The silver nanoparticles synthesized were found to be effective against selected gram positive and negative bacteria (*Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli*). Anticancer activity of the nanoparticles was also done in vitro using MTT assay and its IC_{50} dose was found out. The cell line used in this assay was a breast cancer cell line, MCF-7. Thus, the biosynthesized nanoparticles possess potential applications in medicine and pharmaceutical fields.

KEYWORDS:Silver nanoparticles, *Tylophora indica* Merr, Phytoconstituents, Antimicrobial activity, Anticancer activity.

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INTRODUCTION

Metallic nanoparticles have received significant attention in recent years owing to their unique properties and practical applications[1,2]. Their applications in different areas like medicine, chemistry, catalysis and electronics are well known. Production of silver nanoparticles can be done by physical, chemical and biological methods. Amongst these methods, biological method of nanoparticles synthesis is eco-friendly as no toxic and harmful chemicals are utilized or produced. In this method plant based extracts, enzymes, proteins, antioxidants, triglycerides, saponins, glycoproteins, polysaccharides, phytochemical constituents like terpenes, flavonoids, and tannins are used for reduction and stabilization of nanoparticles[3]. Silver nanoparticles synthesis has been previously reported using extracts of *Trianthema decandra*, *Pisonia grandis,* lemon, sea weeds, jackfruit etc[4]. Nanoparticles have also emerged as an effective antimicrobial agent due to their high surface area to volume ratio and their unique physical and chemical properties, which increases their contact with microbes and their ability to permeate cells[5]. Silver is the only material whose plasmon resonance can be turned to any wavelength in the visible spectrum[6]. Studies carried out in the last few decades shows that silver nanoparticles exhibit a rare combination of valuable properties like catalytic activity, high electrical double layer capacitance etc. Nanosilver has also been used extensively as anti-bacterial agent in the health industry, food storage, textile coatings and a number of environmental applications[7]. *Tylophora indica* Merr. *(T. Asthmatica Wight & Arn,* Family: Asclepiadaceae) also called as Indian Ipecac is indigenous to India, growing in wild in the Western and Southern parts. The name of the plant has been derived from ancient greek words meaning, *Tylos*-'knot' and *phoros*-'bearing'. It has a longstanding reputation as a remedy for asthma. It is administered in respiratory infections, bronchitis and whooping cough, dysentery, osteoarthritis pain[8]. Dried leaves are emetic, diaphoretic and expectorant. It contains various phytochemicals like alkaloids, tannin, phenols, proteins, carbohydrates and tritepenes. The present paper deals with the biological synthesis of silver nanoparticles using leaf extracts of *Tylophora indica* Merr. Characterization of synthesized silver nanoparticles was done using spectrometry, SEM and FTIR analysis to confirm their presence. Anticancer activity of synthesized silver nanoparticles was done *in vitro* using breast cancer cell line, MCF-7. Antibacterial activity was also done to study the inhibitory effects of synthesized silver nanoparticles on different test organisms. These biological activities of synthesized silver nanoparticles can evaluate its potential application in the field of biomedical sciences and in drugs.

MATERIALS AND METHODS

1. Plant Material

Tylophora indica Merr. was collected from Western ghats of Maharashtra, India. Healthy, young leaves of the plant were thoroughly washed with autoclaved distilled water and cut into small pieces and crushed to make 10 % (w/v) aqueous extract. This extract was filtered using whatmann filter paper no. 41 and used immediately for synthesis of silver nanoparticles.

2. Synthesis of silver nanoparticles

10 mM aqueous solution of silver nitrate ($AgNO_3$) (Fischer Scientific, USA) was prepared and used for the synthesis of silver nanoparticles. 100 ml of leaf extract was added to 100 ml of aqueous solution of 10 mM silver nitrate for reduction of silver nitrate into Ag^+ ions and kept at 37°C in a shaker incubator, overnight or till the colour change was observed. Synthesis of nanoparticles is confirmed by the change of colour of reaction mixture from pale yellow to dark brown.

3. Characterization of silver nanoparticles

1. UV-vis spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction mixture by diluting a small aliquot of the sample into distilled water over a range of 400-700 nm. UV-vis spectral analysis was done by using double beam

spectrophotometer (Shimadzu, UV 2450, Japan).

2. SEM (Scanning Electron Microscope)

The morphology of the obtained nanoparticles was characterized by using analytical scanning electron microscope (Jeol, JSM 6360). It has provided further insight into the morphology and size details of synthesized nanoparticles.

3. FTIR (Fourier Transform Infrared Spectroscopy)

FTIR analysis was performed to obtain wide spectrum of nanoparticles over a narrow range using Jasco FT/IR-16100. This method gives information about plant peptides that have coated the particles during synthesis procedure. For removing contaminants from the reaction mixture, the reaction mixture was centrifuged at 6000 rpm for 10 min and the suspension was redispersed in 50 ml distilled water. After repeating this several times a pure suspension obtained was dried to make its powder and this powder was analyzed by FTIR.

4. Antibacterial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was demonstrated using well diffusion technique against *E. coli, B. subtilis* and *S. aureus* as described by Thombre et. al[9]. 0.1 ml of test bacterial culture (Optical density at 600 nm = 0.5) was seeded on Muller Hinton agar plates (Hi Media). Five mm wells were made on agar surface with sterile cork borer to which 20 µl of each plant extract was added. Plates were incubated at 37°C for 24 h and zone of inhibition was measured.

5. Anticancer activity of silver nanoparticles

The anticancer activity of silver nanoparticles on MCF-7 cell line was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazoliumbromide) assay. The MCF 7 cell line was purchased from NCCS, Pune and was cultured on DMEM media. The cells were seeded in 96 well plate at a density of 1.0 X 10^4 cells. It was then incubated overnight at 37°C in a 5% CO_2 incubator. Nanoparticles with a concentration of 100 µg/ml were added to the wells. After incubation for desired period of time at 37°C, in an incubator, cell viability was assessed by MTT assay. The cells were incubated with MTT for 4 h, then MTT was removed and 100 µl DMSO containing 25 µl glycine buffer was added to each well. Absorbance was measured at 595 nm on micro plate reader (i mark ^{TM}). The experiment was carried out in triplicates and percentage of growth inhibition was calculated as follows: Growth Inhibition Rate: (% = Absorbance of Control-Absorbance of Test/Absorbance of Test)* 100. This formula gives viability using which % inhibition can be calculated[10].

RESULTS AND DISCUSSION

1. Synthesis of Silver nanoparticles

The silver nanoparticles were synthesized using the leaf extract of *Tylophora indica* Merr. plant by the method of reduction. This reduction of silver chloride to nano silver resulted in colour change (Figure 1).

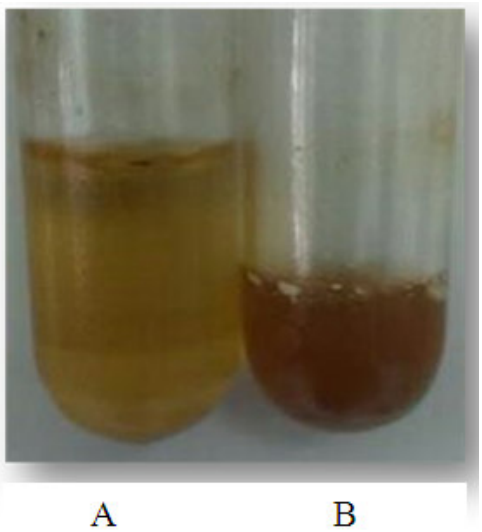

Figure 1
Change in colour of the plant extract from pale yellow to dark brown

A. Plant extract B. Colour change due to addition of $AgNO_3$ to the plant extract. In the case of *Tylophora indica*, it took about 24 h for synthesis of silver nanoparticles in contrast to other plant sources like eucalyptus, *Phytolacca decandra* that took only few hours for synthesis[4].

2. Characterization of silver nanoparticles
1. UV-visible Spectroscopy
Reduction of silver ions into silver nanoparticles using leaf extract of *Tylophora indica* Merr. was evidenced by the visual change of colour from pale yellow to dark brown due to excitation of surface plasmon vibrations in the nanoparticles. The UV-visible spectra showed a maximum absorbance at 422 nm. Similar results were obtained from silver nanoparticles synthesized from other plants as described by Thombre et. al[10].

2. SEM Analysis
SEM was used to study the topography and morphology nanoparticles. It gives the composition and information about electrical conductivity of nanoparticles. A typical SEM image of silver nanoparticles synthesized from *Tylophora indica* is depicted in Figure 2.

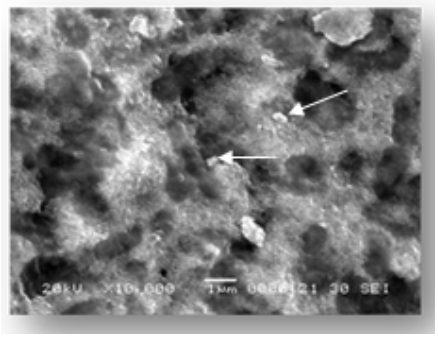

Figure 2
SEM image of synthesized silver nanoparticles using leaves of Tylophora indica Merr.

The arrows in the above figure show presence of circular nanoparticles in a range of 200-300 nm. It has been found that circular nanoparticles can be effectively used as a medicine for treatment of various ailments. Thus the circular shaped silver nanoparticles synthesized from *Tylophora indica* can also have use in this field.

3. FTIR analysis
Figure 3 reveals a typical FTIR graph of silver nanoparticles synthesized from *Tylophora indica*. Peaks at 1650 cm^{-1} indicates –C=O-

and –C=C- stretching. Peak at 1100 cm^{-1}, 1200 cm^{-1}, 1530 cm^{1} indicates presence of –C-N- stretching and methyl-amino stretching that suggests presence of plant proteins which may have played a role in stabilizing silver nanoparticles. The main mechanism underlying this reduction of silver nanoparticles is the interaction via carbonyl groups and oxidation of aldehyde groups present in carboxylic acids[11,12].

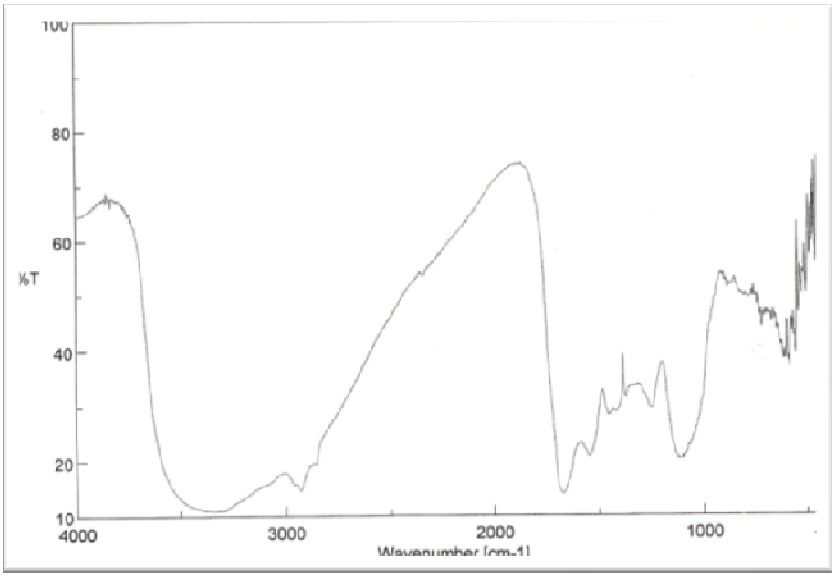

Figure 3
FTIR spectra of biostabilized silver nanoparticles

Figure 3 demonstrated peaks at different wavelengths and which are characteristic features of presence of certain plant peptides and groups.

4. Antibacterial activity

The antibacterial activity of silver nanoparticles was studied against *E.coli, S. aureus* and *B. subtilis.* The silver nanoparticles demonstrated a zone of inhibition against all test organisms with maximum inhibition against gram positive organism *S. aureus* (11 mm), followed by gram negative *E. coli* (6 mm) and gram positive *B. subtilis* (5 mm). One of the proposed mechanism for silver nanoparticle's bactericidal activity is they can inhibit cellular respiration and decrease membrane permeability. Atiyeh B. S. *et. al.*[13] suggested another mechanism of attachment of silver nanoparticles to the cell wall of bacteria which causes dispersion of proton motive force, destabilization of outer membrane and rupturing of cell leading to depletion of intracellular ATP levels. The antibacterial activity of other silver nanoparticles synthesized from *Eucalyptus champaniana* also showed good antibacterial activity against gram positive bacteria which is similar to the result presented here.

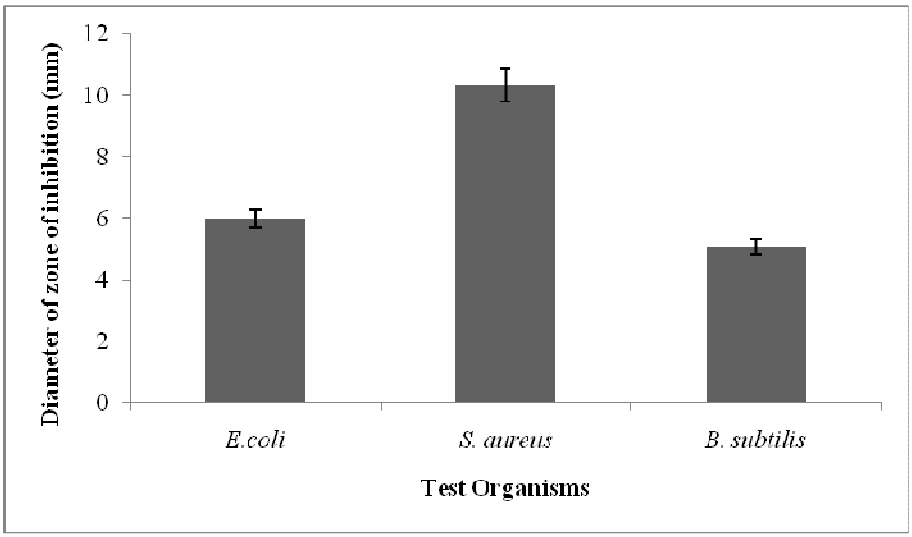

Figure 4
Antibacterial activity of silver nanoparticles against test organisms

The graph depicts antibacterial activity of synthesized silver nanoparticles against three different bacterial strains with maximum zone of inhibition against *S. aureus*. There was not much difference in zone of inhibition against *E. coli* and *B. subtilis.*

5. Anticancer activity

The cytotoxic effect of silver nanoparticles was studied *in vitro* on breast cancer cell line MCF-7 using MTT assay. A definite concentration range of nanoparticles was used for cytotoxicity testing ranging from 10 µg/ml to 100 µg/ml. Half maximal inhibitory concentration (IC_{50}) dose of silver nanoparticles was determined from this and it was found to be 50 µg/ml (Figure 5). This Similar biological action of silver nanoparticles synthesized from different plant sources was also studied on different cell lines like THP-1, HeLa, HL-60 and in each case the IC_{50} dose is found to be different depending upon the plant source and the cell line used.

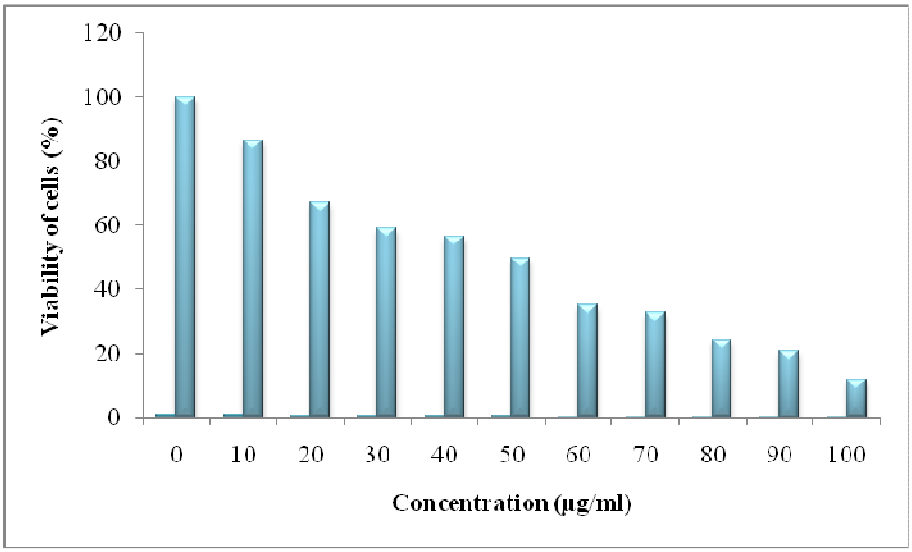

Figure 5
Cell viability of MCF-7 cell line with different concentrations of silver nanoparticles

The above graph shows the viability of cancer cells in terms of their percentage against a concentration range of silver nanoparticles. The dose at which half of the cell population

dies i.e. 50% cell death is seen is found out to be at 50 µg/ml (Figure 5). Many recent studies have proved that nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in medicine[14-19]. In this study, the synthesized nanoparticles demonstrated potent antibacterial activity against selected gram positive and gram negative bacteria. The results obtained also revealed the anticancer potentials of the silver nanoparticles produced by extracts of *Tylophora indica* Merr. as demonstrated by MTT assay. The inhibitory effect of synthesized silver nanoparticles on bacteria as well as cancer cells demonstrates its potential role in the field of medicines and as pharmaceutical products. *Tylophora indica* Merr. plant being mainly used in treatment of asthma, the silver nanoparticles synthesized from them can also be successfully used as a medicine against asthma.

CONCLUSION

The biostabilized silver nanoparticles were synthesized using leaf extract of *Tylophora indica* Merr. The nanoparticles were spherical and were characterized using UV-visible spectrometry, SEM and FTIR analysis. The biological method adopted in the present study precludes the use of hazardous chemicals, thus proving eco-friendly. The nanoparticles thus synthesized can be used in many medical applications effectively.

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