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RESEARCH ARTICLE

Evaluation of Antioxidant and Antimicrobial Potential of Green Synthesized Ag Nanoparticles from Ethanolic Leaf Extract of *Thespesia populnea*

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Abstract

Thespesia populnea is a variety of small tree found mostly in the tropical regions of the world belonging to the family of Malvaceae. According to ancient and traditional medicine such as Ayurveda this plant is used to treat various diseases such as bloody diarrhea, fracture healing, urinary tract problem, abdomen swelling, pleurisy, skin disorders, high fever and menstrual issues. This study focuses on the evaluation of antioxidant and antimicrobial activities of synthesized silver nanoparticles from aqueous leaf extract of *Thespesia populnea* plant. Reducing capability of synthesized nanoparticles was examined by carrying out various antioxidant assays such as reduction of DPPH substrate through radical scavenging. It showed the maximum reduction of DPPH as 92.77% at 120 µg/mL concentration. At 120 µg/mL concentration, phosphomolybdenum showed highest contraction percentage ranging 51.47 % and followed by Fe^{3+} reduction at 28.78 %. Studies on antimicrobial activity has revealed that the maximum zone of inhibition was exhibited by *Proteus vulgaris* with distinct zone formation up to 19 mm diameter.

Keywords: Antioxidant, Antimicrobial, DPPH· Radical, Phosphomolybdenum

1. Introduction

Thespesia populnea (Figure 1) is an evergreen, medium sized flowering plant commonly found in tropical coastal regions and commonly denoted as Portia tree. This plant has its origin in ancient world tropics and was exported to South America and some Southern parts of Northern America. *Thespesia populnea* tree's trunk measures about 20 to 30 cm in average. In seas it grows at an elevation to 275 m and in land it reaches about 6 to 10 m in height. It obtains rainfall about 500 to 1,600 mm. Its leaves are simple, heart-shaped with specific tips. Although this tree grows widely in costal environment, it prefers neutral soils of pH range 6–7.4[1]. The major component present in *Thespesia populnea* is gossypol which produces anti-fertility effects in human beings and rats. The Portia tree has a reddish-brown heart wood with a dense crown[1]. Extracts from roots are applied externally for scabies, psoriasis and related skin diseases[2]. In coastal regions like Mauritius, dried bark of this tree is used as detoxifying agent and to cure stomach disorders. The fruit obtained from this plant is used in Ayurveda to control diabetics. The compound oil containing capsules and bark is used in treating urethritis and gonorrhea[3]. The concoctions prepared from the tree bark is used as topical cleansing agent for skin disease and to treat hemorrhoids. The barks and leaves of the plant are used in producing oil which helps in treatment of fracture wound and boils as folk medicine[3]. Certain research finding also reported the antioxidant and anti-hepatotoxicity of bark extracts[3]. The main constituent of leaves are glycosides,

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flavonoids, rutins, phytosterols, alkaloids and lupeal[4]. Leaf extracts obtained from this plant is used as the topical application in swollen joints for arthritis as anti-inflammatory agent. The nano particle and herbal extract have been proved beneficial in tissue damage reduction as it can enter deep inside the cell[5].

1.1 Systematic Positions

Kingdom :	Plantae
Subkingdom :	Tracheobionta
Super division :	Spermatophyta
Division :	Magnoliophyta
Class :	Magnoliopsida
Subclass :	Dilleniidae
Order :	Malvales
Family :	Malvaceae
Genus :	Thespesia
Species :	populnea

2. Constituents and Procedure

2.1 Sample Preparation

The leaves of *Thespesia populnea* were gathered from Madhuvinkarai, Guindy, Tamil Nadu (preserved in a hygienic, sterilized pliable sealed container) and the authentication was done by Dr. Annamalai, Plant Biologist, Coimbatore, and the voucher specimen (BIT/BT/MAC0519/2019) was deposited in Department of Biotechnology, Bannari Amman Institute of Technology Sathyamangalam, India. This plant is selected because it contains higher antibacterial and antioxidant properties and also efficient against multi drug resistance bacteria[6]. The leaves were cleaned with distilled water to remove any dirt, before pulverizing into fine powder the washed leaves were dried in shade at room temperature. Out of which ten grams of leaf powder was suspended in ethanol and left for 3 days. Using rotary evaporator, the obtained ethanolic extract was concentrated at 50 °C which yields a greenish gluey concentrate.

Figure 1. Taxonomic classification of *Thespesia populnea*.

2.2 Silver Nanoparticle Preparation

Initially, 0.1 M of silver nitrate was prepared for 100 ml. The prepared silver nitrate was placed in the magnetic stirrer. To that, the prepared extract was added dropwise until the color fluctuates. The above mixture was centrifuged at 10,000 rpm for 900 seconds[7]. Then, formed pellet was collected and stored in hot air vacuum. Silver nanoparticles was scraped and stored.

2.3 *In vitro* Assays for Antioxidant Activity

2.3.1 Radical Scavenging Activity - DPPH Assay

DPPH free radical reduction method was carried out to measure the antioxidant activity of silver nanoparticles extracted using ethanolic extracts of *T. populnea* leaves[8]. To measure the activity, the leaf extract silver nanoparticles was taken at various concentrations from 20 up to 120 µg/mL at a constant volume of 1 ml and mixed with 1 ml of methanol based 0.1 M DPPH solution and left undisturbed at dark for 30 min. The mixture of methanol and DPPH at 0.1 M concentration was used as control. UV-Vis Spectrophotometer ranging at 517 nm was used to measure the decreasing absorbance with ascorbic acid as standard reference. The antioxidant activity was measured as the percentage of radical inhibition using the below formula.

$$\% \text{ of DPPH radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

2.3.2 Antioxidant Activity - Phosphomolybdenum Assay

Mo^{6+} reduction method was used to estimate the reducing capacity of silver nanoparticles formed using *T. populnea* leaf extract[9]. The nanoparticle extract at

varying concentrations ranging from 20 µg/mL to 120 µg/mL was blended with concoctions containing 4 mM ammonium molybdate, 28 mM sodium phosphate and 600 mM sulphuric acid at the volume of 1 ml. The resultant mixture was allowed undisturbed at 95 °C for 1 ½ hours in water bath for the reaction to take place. UV-Vis spectrophotometric analysis was carried out to measure the OD value of the reaction mixture at the wavelength of 695 nm with ascorbic acid as the standard reference. The reduction capability was determined as:

$$\% \text{ of phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

2.3.3 Assay for Ferric (Fe^{3+}) Reduction Activity

Fe^{3+} reduction method with slight modification was used to study the reducing property of silver nanoparticles produced using ethanolic extracts of *T. populnea* leaves[10]. 1 ml of silver nanoparticle extract at diverse concentration ranging from 20 µg/mL up to 120 µg/mL was utilized for this assay. 0.2 M phosphate buffer at pH 6.6 and 1 % (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] solution at constant volume of 1 ml each was mixed with the green synthesized silver nanoparticle and incubated for 30 min at 50 °C in water bath. After incubation 10 % trichloroacetic acid (w/v) at the volume of 1 ml was added to each mixture which is followed by addition of 1 ml of freshly prepared $FeCl_3$ solution at the concentration of 0.1% (w/v). UV-Vis spectrophotometric analysis was carried out at 700 nm to measure the absorbance with the standard reference being ascorbic acid. The degree of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

2.4. Antibacterial Activity

2.4.1 Micro Organisms

A non-pathogenic strain of microorganisms such as *Proteus vulgaris, Micrococcus luteus, Escherichia coli*, and *Staphylococcus aureus* were used for the determination of resistance towards microbial growth by *Thespesia populnea* leaf-based silver nanoparticles. To compare and validate the activity the nanoparticle mixture along with the solvent was mixed with solidifying agar and used as a control. An antibiotic solution namely tetracycline was used as positive control to check and validate the zone formation.

2.4.2 Media Preparation

As per the standard methodologies nutrient agar medium was prepared containing 1 g of peptone, 0.6 g of yeast and 1 g of NaCl was suspended in 200 ml of water followed by addition of 4 g of agar in 500 ml conical flask, melted using a microwave oven. Once the agar was melted, the mixture was steam sterilized at 121˚C for 15 min under 15 lbs pressure. The sterile plates were filled with hot medium and allowed to stand for 15 min in the aseptic laminar chamber for solidification.

2.4.3 Agar Well Diffusion Method

Agar well diffusion method carried out to identify the antimicrobial activity of the green synthesized silver nanoparticles[10]. Spread plate method carried out to smear the inoculum on the solid surface of NA plates evenly. To carry out well diffusion assay, the surface of solid medium was punctured using a sterile well borer to make 5 wells each of 8 mm diameter. Three wells were inoculated with silver nanoparticles with varying concentrations such as 100, 150 and 200 µg/mL. The range of concentration is increased to check the antimicrobial activity effectiveness of the sample. The remaining two wells were inoculated with positive and negative control. The positive control was the antibiotic solution namely tetracyclin was taken at the concentration of 30 µg. Once inoculated, the plates were incubated for one whole day at 37˚C for formation of inhibition zone based on which the antibacterial activity was assessed.

3. Results and Analysis

3.1 Radical Scavenging Activity - DPPH Assay

The antioxidant activity of most of the plant-based compounds can be easily assessed using this assay. This assay measures the antioxidant activity through

Table 1. Antioxidant activity of silver nanoparticles obtained from leave extracts of *Thespesia populnea*

S. No	Concentration µg/mL	% of inhibition
1	20	20.91±0.72
2	40	46.07±0.15
3	60	47.14±0.62
4	80	53.23±0.28
5	100	58.17±0.59
6	120	92.77±0.72

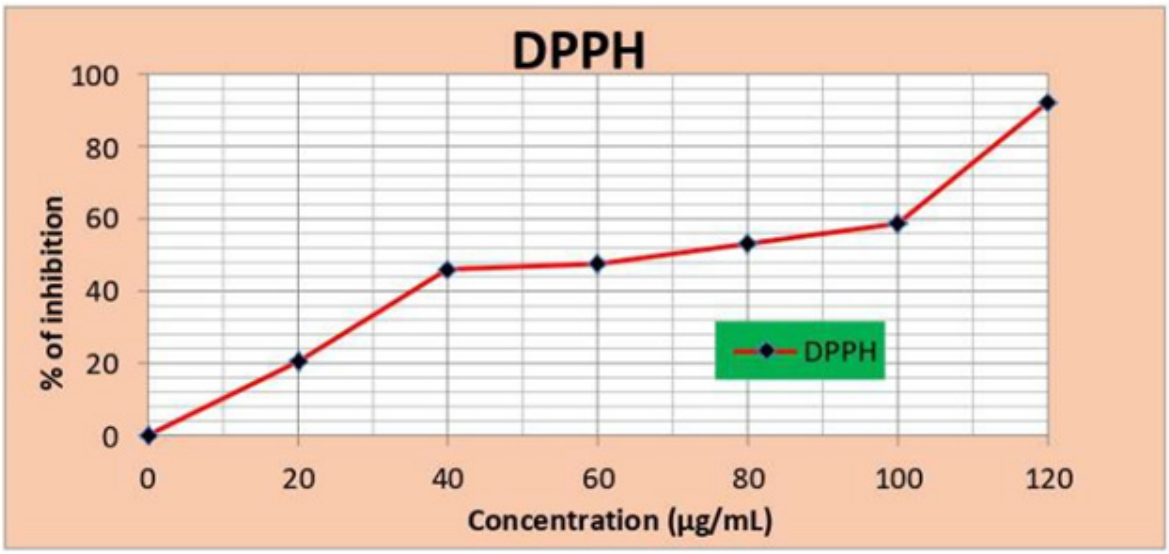

Figure 2. Graph showing antioxidant activity of silver nanoparticles obtained from the leaf extracts of *T. populnea*.

monitoring the DPPH scavenging capacity of test sample. On reduction by antioxidants, purple color of the DPPH (1,1-Diphenyl-2-picrylhydrazyl) substrate changes to yellow color 1,1-diphenyl-2-picrylhydrazine. At 517 nm, the concentration at which higher activity is achieved can be measured using UV-Vis Spectrophotometer[11]. The maximum reduction of silver nanoparticles extract obtained from leaves of *Thespesia populnea* to scavenge free radicals was found to be 92.77±0.72 % at 120 µg/mL concentration (Table 1 and Figure 2). On comparing the standard reference as ascorbic acid used for this assay which has half maximal inhibitory concentration equal to 6.31 µg/mL, the half maximal inhibitory concentration was 83.88 µg/mL.

3.2 Antioxidant Activity - Phosphomolybdenum Assay

The silver nanoparticles obtained from ethanolic leaf extracts of *Thespesia populnea* forms green colored phosphate in complex with Mo (V) at acidic pH by reducing Mo (VI) to Mo (V) at the maximum test sample concentration of 120 µg/m. Similarly, the highest rate of reduction of phosphomolybdenum was

Table 2. Phosphomolybdenum reduction of silver nanoparticles synthesized from leaves of *Thespesia populnea*

S. No	Concentration µg/mL	% of reduction
1	20	33.10±0.28
2	40	36.53±0.44
3	60	37.73±0.85
4	80	49.48±0.72
5	100	50.74±0.79
6	120	51.47±0.39

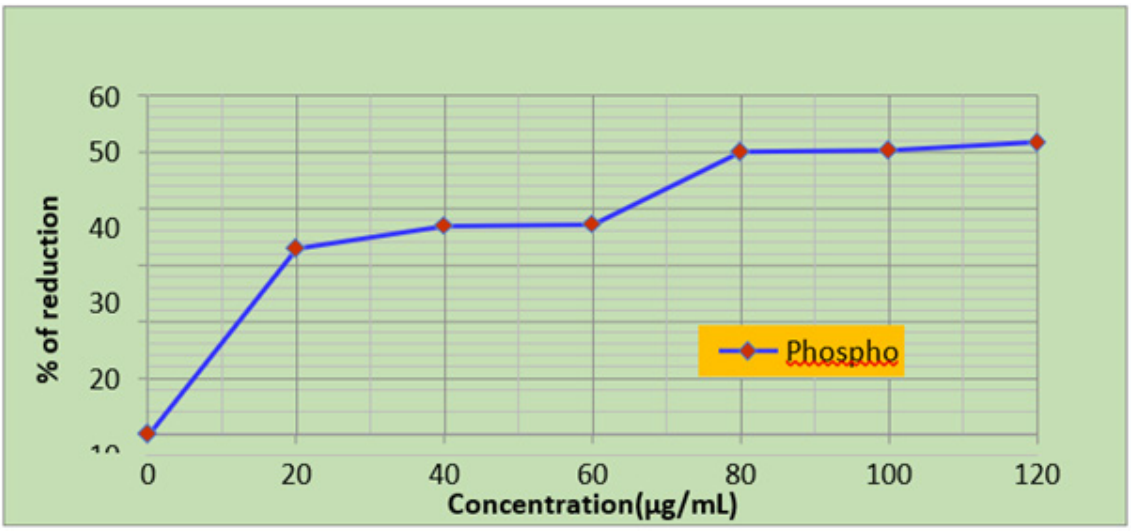

Figure 3. Phosphomolybdenum reduction of silver nanoparticles synthesized from leaves of *Thespesia populnea*

51.47±0.39 % (Table 2, Figure 3) with the half maximal inhibitory concentration equal to 56.35 µg/mL with standard reference as ascorbic acid which has the half maximal inhibitory concentration equal to 6.34 µg/mL.

3.3 Assay for Ferric (Fe^{3+}) Reduction Activity

The reducing power of phenolic antioxidant present in our silver nanoparticle obtained from leaf extract of *Thespesia populnea* can be assessed by its capability of reducing Fe^{3+} to Fe^{2+} with subsequent formation of a complex containing ferro-ferric since this reduction can proceed only in the presence of reductions such as antioxidants. The silver nanoparticles obtained from leaf extracts of *Thespesia populnea* showed the reduction ability which is deemed to increases with increase in concentration. The pinnacle point of Fe^{3+} reduction was 28.78±0.60 % at 120 µg/mL concentration with the half maximal inhibitory concentration equal to 125.31 µg/mL (Table 3 and Figure 4). The Ascorbic acid was used as the standard reference which has the half maximal inhibitory concentration equal to 7.72 µg/mL.

3.4 Antimicrobial Activity of Silver Nanoparticles

Antimicrobial activity of green synthesized silver nanoparticles was assessed through well diffusion assay. Four nonpathogenic microbial strains namely *Proteus*

Table 3. Fe^{3+}reduction of silver nanoparticles extracts of *Thespesia populnea*

S. No	Concentration µg/mL	% of reduction
1	20	5.42±0.54
2	40	7.86±0.13
3	60	14.98±0.22
4	80	17.34±0.41
5	100	23.11±0.70
6	120	28.78±0.60

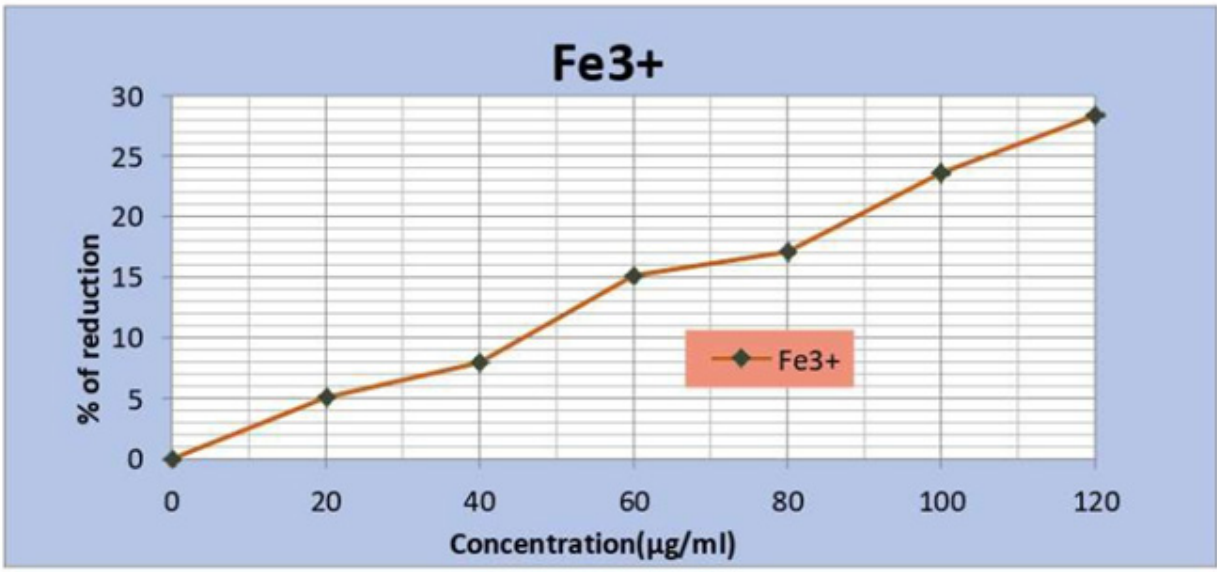

Figure 4. Fe^{3+} reduction of *T. populnea* leaves extract of silver nanoparticles.

Table 4. Zone of Inhibition of Organisms

S. No	Organisms	Zone of inhibition mm			Standard
		100 µg	150 µg	200 µg	
1	*Micrococcus luteus*	11	13	15	19
	Staphylococcus aureus	13	15	16	17
3	*Escherichia coli*	13	15	16	27
4	*Proteus vulgaris*	14	16	19	28

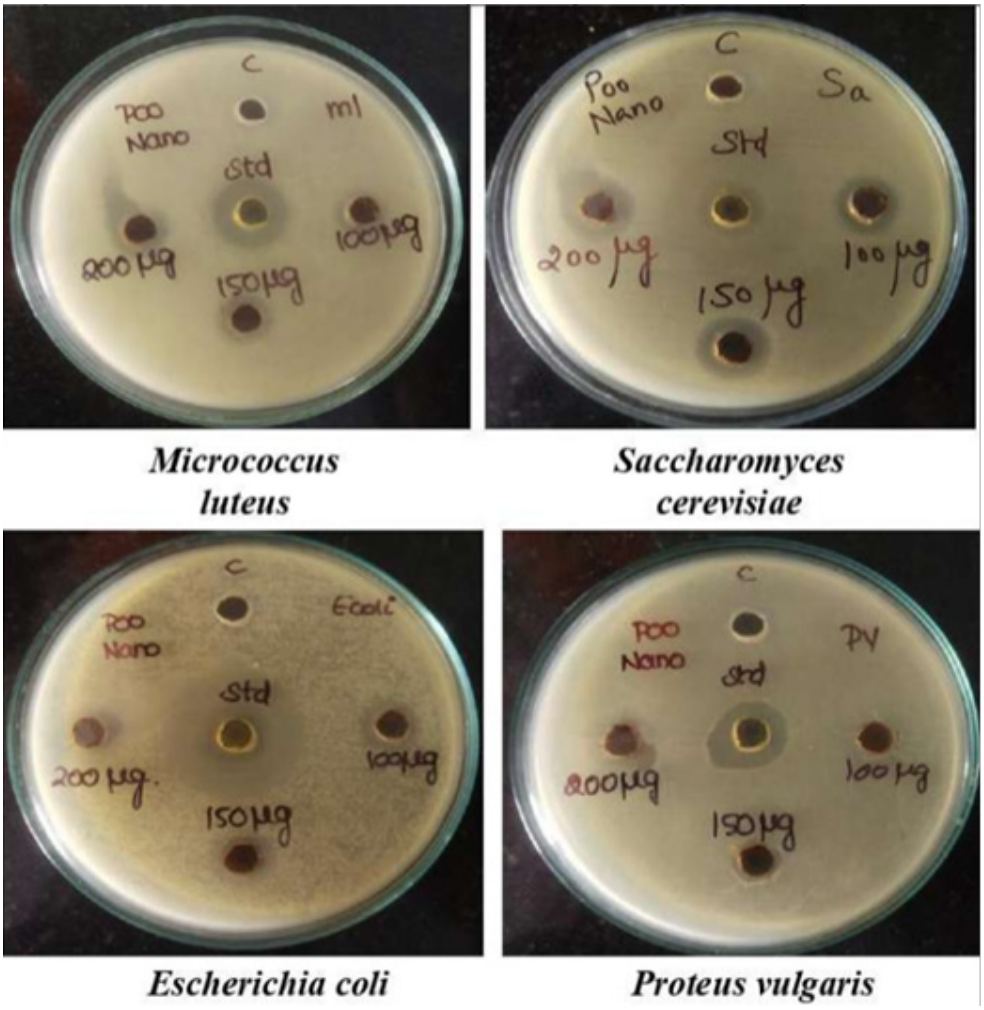

Figure 5. Antimicrobial activity of silver nanoparticles produced from leaves of *Thespesia populnea*.

vulgaris, Micrococcus luteus, Escherichia coli, and *Staphylococcus aureus* were used. All the strains have shown a distinct inhibition towards the synthesized silver nanoparticles in which *Proteus vulgaris* alone have shown highest percentage of hindrance towards our test sample at the concentration of 200 µg/mL and the diameter of the zone of inhibition is found to be 19 mm (Table 4, Figure 5).

4. Conclusion

Based on different characterization techniques, we can conclude that the biomolecules such as alkaloids, terpenoids, phenols and flavonoids present in the extracts of *Thespesia populnea* are mainly responsible for formation of silver nanoparticles by reduction of silver ions. When examining the strong absorption peak with FTIR the green synthesized silver nanoparticles exhibited 1040 cm^{-1} and confirmed presence of Ag-O stretching in the resultant nanoparticles. The nanoparticles showed significant antioxidant activity. The synthesized AgNPs showed better antibacterial activity against all four organisms with maximum the zone of inhibition of 19 mm against gram negative bacteria *Proteus vulgaris* at the concentration of 200 µg/mL, which suggests possible biomedical applications.

5. References

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