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ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING LEAVES AND STEM BARK OF *PTEROCARPUS INDICUS* WILLD

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ABSTRACT

In the present study, synthesis of silver nanoparticles (AgNPs) using leaves and stem bark of *Pterocarpus indicus* were compared by studied for alpha amylase, alpha glucosidase inhibition assay and also examined for its antioxidant activities by using free radical 1,1 diphenyl -2 picrycl hydrozyl DPPH scavenging method under *in vitro* model separately. These synthesized silver nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) and zeta potential for the further confirmation. The synthesized silver nanoparticles were checked with the colour variation and it was confirmed by UV-vis spectral analysis. The morphology of the synthesized nanoparticles was analyzed using SEM. The XRD was done to find out the crystalline structure of the compound. FTIR measurements are carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver NPs synthesized using *Pterocarpus indicus*. The result revealed that the leaves-AgNPs

showed significant inhibitory effect on alpha amylase (77.1%) and for the stem bark-AgNPs (74.2%). Alpha glucosidase showed inhibitory effect on leaves-AgNPs (77.5%) and for stem bark-AgNPs (75.7%) were compared with standard acarbose drug. The antioxidant activities by DPPH method showed activity for leaves-AgNPs (70.15%), and for the stem bark-AgNPs (75.92%) were compared with standard ascorbic acid by measuring the percentage inhibitory effect at the concentration of 100µg/ ml respectively.

Maximum inhibition was observed for reducing power assay at (79.27 %) for stem bark and for leaves (78.37 %) and compared to the standard drug ascorbic acid (84.68 %) at a concentration of 100 µg/ml respectively. Therefore, it is suggested that the synthesized silver nanoparticles (AgNPs) using leaves and stem bark of *Pterocarpus indicus*is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetes mellitus.

Keywords: leaves-AgNPs and stem bark-AgNPs of *Pterocarpus indicus*, alpha amylase inhibitory activity, alpha glucosidase inhibitory activity, antioxidant (DPPH) activities

INTRODUCTION

Nanotechnology is a rapidly growing in the field of science for synthesizing and utilizing nano-sized particles **[1]**. For the synthesis of silver nanoparticles, such as thermal decom-position, electrochemical, micro-wave assisted process and green chemistry methods are available **[2]**. Many of the nanoparticle synthesis or production methods of nanoparticles involve the use of hazardous chemicals, low material conversions and high energy requirements **[3]**. So, there is need to develop an eco-friendly techniques and methods for nanoparticle synthesis without using any toxic chemicals. Thus, synthesising silver nanoparticles from plant extracts are gaining importance and also are considered as a simple, economic and viable alternative to chemical synthetic procedure **[4]**. Silver nanoparticles reduces toxicity and is involved in lowering of blood glucose level, higher serum insulin, higher glucokinase activity and boost up immune system, reduces kidney crystal formation and higher the expression of insulin level **[5]**, Silver nanoparticles used in wide range of application such as antimicrobial**,** anti-inflammatory**,** antiviral and anti- diabetic and also involved in the prevention of diabetic wound healing (ointments) **[6]**.

Diabetes mellitus is a universal endocrine metabolic disorder that affects people in both developed and developing countries. Due to lack of insulin secretion and action, leads to the metabolic changes of carbohydrates, proteins, fats and lipids **[7]**. It is designated by hyperglycemic condition (high blood sugar levels), because the pancreas do not produce enough insulin/ cells do not respond for the production of insulin **[8]**. Genetic factors and lifestyle may be the causes of diabetes **[9]**.

Type-1 diabetes mellitus occurs due to the deficiency of insulin secretion which is produced by β-cells of pancreas. Type-2 diabetes mellitus is caused by insulin resistance/β-cell dysfunction **[10]**. The treatment for this disorder is insulin injection and antidiabetic drug therapy. α -amylase and α- glucosidase are the two important digestive enzymes involved in the breakdown of carbohydrates and helps in intestinal absorption. α- amylase is responsible for the breakdown of long chain carbohydrates and α- glucosidase results in breakdown of starch and disaccharides into glucose **[11]**. α -amylase and α- glucosidase inhibitors are useful for lowering the process of glucose absorption and decreases glucose level in blood **[12]**. During hyperglycemic condition, reactive oxygen species (ROS) gets generated and leads to lipid-peroxidation, membrane damages and production of secondary complications such as kidney, eye, blood vessel and nerve damage **[13]**. Antioxidant plays a vital role in the inhibition of lipid peroxidation radical chain reaction, scavenging of free radicals, active oxygen species by rising a reaction cycle and to chelate heavy metal ions **[14]**.

Pterocarpus indicus belongs to the family Fabaceae popularly known as vengai maram and it's a medicinally valuable species widely distributed in the region of tropical and subtropical south Asia as Malaysia, Philippines, Brunei, Thailand, and Indonesia **[15]**. In the ayurvedic Pharmacopoeia of India, *Pterocarpus indicus*used in the treatment of krmiroga (worm infection), kustha (leprosy), prameha (diabetes), pandu (anemia), and medodosa (obesity), strong antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, and anticancer activities and is used for the treatment of diabetes, jaundice, ulcer, gastritis **[16]**. Their phytochemical studiescontain iso-flavonoids, terpenoids and related phenolic compounds, ß-sitosterol, lupenol, epicatechin and glycosides **[17,18]**.

Therefore, this study was designed to synthesize AgNPs using leaves and stem bark of *Pterocarpus indicus* and to evaluate for *in vitro* antioxidant and antidiabetic activity separately.

MATERIALS AND METHODS:

Collection of Plant Material:

The *Pterocarpus indicus* bark and leaves were collected in the month of December from the Senthankudi Village, Pudukkottai, Tamil Nadu, India. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu for identifying the plants.

Chemicals and Reagents:

Alpha (α)-Glucosidase, porcine pancreas alpha(α)-amylase, p-nitrophenyl-α-D-glucopyranose (p-NPG), 3,5-dinitrosalicylic acid (DNS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate (NaH2PO4), Di-sodium hydrogen phosphate (Na2HPO4) sodium chloride, sodium hydroxide, potassium ferricyanide, ferric chloride (FeCl3) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Instruments:

Lambda 35, Perkin Elmer Spectrophotometer, Malvern zetasizer version 2.2., XPERT-PRO Machine and TEM, JEOL-JEM 2100

Ethanolic extract of plant preparation:

The *Pterocarpus indicus* leaves and stem barks were collected and made into small pieces. The collected plant parts were thoroughly washed with tap water and air dried in a shadow that is free from sunlight till it becomes dried nicely. Then it is crushed in an electrical grinder and the powder was separated, which were stored individually in air-tight containers and kept in a cool, dark and dry place for further study. The ethanol extract was prepared by taking 20g of the powdered sample each and it is soaked in 40ml ofethanol for 24hours. Then it was extracted using hot percolation method. The extract was filtered using whatman No.41 filter paper in a clean beaker and used for the further study **[19]**.

Optimization and Synthesis of Silver Nanoparticles:

1mM silver nitrate was prepared in a 50 ml standard flask. 2.5 ml of the ethanolic extract of each leaves and stem bark of *Pterocarpus indicus*sample (25µl, 50µl, 75µl

and 100µl) was mixed with 1mM of silver nitrate solution without any contamination with continuous and constant stirring which react at an ambient condition and Ag get reduced in to Ag+ ion.

The colour change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. The presence of reduction of Ag+ ion was confirmed overtime by the UV-Spectral analysis **[20]**.

Characterization Techniques:

Characterization of synthesized nanoparticles were carried out to learn the characteristic wavelength and functional group bound to silver nanoparticles by UV-Vis spectra and FTIR and its size, crystalline nature and elemental composition using SEM, XRD and zeta potential **[21, 22]**.

UV-Visible Analysis:

The optical properties of silver nanoparticles were characterized using UV-Vis spectrophotometer. Silver nitrate was added to the ethanolic extract of leaves and stem bark of *Pterocarpus indicus* separately, the colour change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. UV was taken after 24 hours of addition. The absorbance was recorded between 350-500nm

FTIR analysis:

Fourier Transform Infrared Spectroscopy is otherwise called as FTIR Analysis or FTIR Spectroscopy. The synthesized nanoparticle can be scanned by infrared light and chemical properties like organic, polymeric and inorganic materials were observed by this method. Fourier Transform Spectrometer absorbs infrared spectra within the range of 400-4500cm^{-1}. At a particular frequency, multiple functional groups may be absorbed and it gives rise to different characteristic absorptions.

XRD:

X-ray diffraction (XRD) analysis is used to study the nanomaterials (with structural features in the range of 1-100 nm). The structure of nanomaterials has been probed by XRD method.The position of values of product (crystallinity or amorphous nature) can be identified by this technique. With, respect to d-spacingvalues; the fingerprint regions of relative intensity are found in XRD analysis.

SEM Analysis:

SEM analysis of synthesized silver nanoparticles were performed to evaluate the surface morphology of nanoparticles. Silver nanoparticles were prepared and dried well to remove the moisture content and images

were taken by using FEI Quanta 250 FEG SEM operating at 10 kV.

Zeta Potentiometer:

The zeta potential was measured by using Zeta Sizer (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles.

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus* were examined on the basis of the scavenging effect on the stable DPPH free radical activity **[23]**. 300 µl of ethanolic solution of DPPH (0.05 mM) was added to 40 µl of each leaves-AgNPs and stem bark-AgNPs with different concentrations of 20 - 100 µg/ml separately and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectro-photometrically at 540 nm. Ethanolwas used to set the absorbance zero. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation **[24]**.

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100

Where B and A are the absorbance values of the test and of the blank sample, respectively.

MEASUREMENT OF REDUCING POWER

The ethanolic extracts of leaves and bark of *Pterocarpus indicus* were taken in different concentrations in phosphate buffer (0.2 mol /L, pH 6.6) and incubated with potassium ferricyanide (1 g /100 mL water) at 50^{o}C for 20 min. the reaction was terminated by adding TCA solution (10 g /100 mL water), centrifuged at 3000 rpm for 10 min and the supernatant was mixed with ferric chloride (0.1 g/100 Ml water), the absorbance measuredat 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

Percent (%) inhibition of DPPH activity = [(A – B) / A] x 100Where B and A are the absorbance values of the test and of the blank sample, respectively **[24, 25]**.

Alpha-Amylase Inhibitory Assay

A total of 250μL of synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus* (20-100 µg/ml) was placed in a tube separately and 250 μL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was preincubated at

25ºC for 10 min, after which 250 μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at for 25ºC for 10min. The reaction was terminated by adding 500 μL of dinitro salicylic acid reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water **[25]**.

The α-amylase inhibitory activity was calculated as percentage inhibition:

% Inhibition = [(Abs control – Abs extract) / Abs control] x 100

Alpha-Glucosidase Inhibitory Assay

The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, and pH 6.9. 100 μL of α- glucosidase (1.0U/mL) was preincubated with 50 μL of the different concentrations 20-100 µg/ml of the synthesized silver nanoparticles of leaves and stem bark of *Pterocarpus indicus*for 10min separately. Then 50 μL of 3.0mM (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37ºC for 20min and stopped by adding 2mL of 0.1M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-colouredpara nitrophenol released from pNPG at 540 nm. The results were expressed as percentage of the blank control **[26]**.

The α-glucosidase inhibitory activity was calculated as percentage inhibition:

%Inhibition = [(Abs control – Abs extract) / Abs control] x 100

Statistical Analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Visual colour change and UV-Vis spectroscopy:

The presence of silver nanoparticle was analysed by using UV-Vis spectral technique at room temperature. The gradual change in the colour of samples from colourless to dark brown colour was observed and the bioreduction of Ag+ in the solvent extract. The UV visible absorption spectrum was noted at the range of 435 nm for the leaves-AgNPs and at the range of 439 nm for the stem bark-AgNPs **(Figure 1)**. The previous studies suggested that the silver nanoparticles are known to exhibit UV-Visible absorption in the range of 400-500 nm. The sharp absorption

bands of *Pterocarpus marsupium* silver nanoparticles were observed around 431 nm **[27]**.

Functional group determination using FT-IR spectroscopy:

The FT-IR spectrum of synthesized gives details about the functional group involved in the silver ions reduction **(Figure 2)**. The FT-IR spectra ofsynthesized silver nanoparticles by using *Pterocarpus indicus* leaf and barkextract are shown in (**Tables 1 and 2)**. Some of the peaks appeared in the FT-IR spectrum of stem bark-AgNPs **(Figure 2b)**, which were disappeared in FT-IR spectra of leaves-AgNPs **(Figure 2a)**. This disappearance of peaks is due to phytochemicals present in the bark extract, involves silver nanoparticles reduction. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.From the FTIR spectral analysis it is concluded thathydroxyl and carboxyl groups present may act asreducing and stabilizing agent and phenolic grouppresent may act as capping agent **[28, 29]**. The previous studies reported that the main components such as steroids, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides present in the leaf extract of *P. santalinus* are prime responsible for the observed reduction and capping during the synthesis of Ag NPs **[30]**.

X-ray diffraction (XRD):

The crystalline nature of silver nanoparticles was confirmed using X-ray crystallography. The X-ray diffractogram pattern of synthesized silver nanoparticles was represented in **Figure 3**. The diffraction peaks were obtained by leaves-AgNPs is observed at 38.4024, 46.3896, 65.1721 and 78.568 in the 2Ɵ range **(Figure 3a)**. The obtained XRD pattern for silver nanoparticles synthesized using *Pterocarpus indicus* bark extract showed the characteristic peaks 38.7965, 44.7206, 65.0686 and 78.2134 in the 2Ɵ range **(Figure 3b)**.

The intense diffraction peak is obtained at 2Ɵ values 27.7794, 32.1627, 38.0824, 44.7077, 65.0407, 78.187 and 78.4575. The peak corresponds to 38.0824, 44.7077 following diffraction facets are (111), (200) respectively. This pattern (111),(200),(220) and (311) reflection of the face cantered cubic structure for silver according to (JCPDS, File No. 04-0783). Unassigned peaks are also present in the graph this may be due to extract contains some phytochemicals which may be capping the nanoparticles surface. The XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag+ ions using *P. santalinus* leaf extract are crystalline in nature. Similar results

were reported for AgNPs in the literature **[31-33]**.

SEM image:

SEM images of the synthesized silver nanoparticles lie between 37.9-124.5 nm region in case of leaves-AgNPs **(Figure 4a)** and 98.70-126 nm in case of stem bark-AgNPs sample **(Figure 4b)**. SEM image reveals that most of the synthesized silver nanoparticles are nearly spherical in shape and cubic. The SEM image further ascertains that the silver nanoparticles are predominantly spherical in morphology with their sizes ranging from 20 to 300 nm was previously reported **[34]**. From the previous studies suggested that the SEM analysis it was found that *Pterocarpus marsupium* Roxb.silver nanoparticles have spherical shape and the assembling of silver nanoparticles on the surface **[35]**.

Particle Size Distribution and Zeta Potential Studies:

The average size of the nanoparticles, particle size distribution, and polydispersity index (PDI) of the synthesized silver nanoparticles was characterized using particle size analyzer.. The average particle size diameter of synthesized leaves silver nanoparticle is1842 nm with poly dispersity index of 0.247 **(Figure 5a)** and for the stem bark-AgNPs exhibited polydisperse mixture with the size ranging of 385 nm with poly dispersity index of 0.063 **(Figure 6a)**. From the average particle size and PDI value it is found that produced nanoparticles are monodispersed in nature.

Zeta potential is used for determining the stability of synthesized silver nanoparticles. For leaves-AgNPs zeta potential measured was found to be −13.1 mVwith peak area of 100% intensity (fig. 5b). The biosynthesized stem bark-AgNPs had a negative charge with a zeta potential value −16.3 mV **(Figure 6b)**. These values indicate the full stabilization of nanoparticles. The previous studies suggested that the particle size is analyzed by using zeta sizer and the average particle size was found to be 148.5 nm with polydispersity index 0.336 and intercept 0.963. Zeta potential measured was found to be -28 mV with peak area of 100 % intensity. These values indicate the stabilization of silver nanoparticles for the *Pterocarpus marsupium* silver nanoparticles **[36]**.

Antioxidant activity of silver nanoparticles synthesized using leaves and stem bark of *Pterocarpus indicus*by DPPH method

The result showed that thesilver nanoparticles synthesized using a *Pterocarpusindicus*bark and leaf sample of ethanolicextracthad better percentage antioxidant activities at high concentrations when compared with ascorbic acid **(Table 3)**. The synthesized silver nanoparticles showed

70.149% (leaves-AgNPs) and 75.929 (stem bark-AgNPs) activity at concentration 100 µg/ml while ascorbic acid gave 94.69 % at the same concentration. Methanol extract of *Pterocarpus marsupium* is found to possess highest DPPH radical scavenging activity followed by aqueous and ethyl acetate extracts **[37]**.

Antioxidant activity of stem barks and leaves of *Pterocarpus indicus* by reducing power method:

The reducing power of ethanolic extracts of leaves and bark of *Pterocarpus indicus* was performed and showed concentration dependent manner **(Figure 7)**. Ethanolic extract of stem bark of *Pterocarpus indicus* (78.37%) exhibits good reducing power activity among leaves extracts (79.27%) as we compared to the standard drug ascorbic acid (84.68%) at a concentration 100 µg/ml **(Table 4)**. It is believed that antioxidant activity and reducing power are related. Reductones inhibits LPO by donating a hydrogen atom and thereby terminating the free radical chain reaction.

***In vitro* alpha amylase inhibitory assay:**

In this study the *in vitro* alpha amylase inhibitory activities of the silver nanoparticles synthesized using a *Pterocarpus indicus*extract was investigated. The synthesized silver nanoparticles showed inhibitory activity from 65.954% to 80.369% for stem and 56.954%to 67.756% for leaves at concentration 100 µg/ml **(Table 5)**. Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882% at the same concentrations 100 µg/ml. A comparison of α–amylase inhibitory activity between the standard drug has been depicted in **(Figure 8)**.

Our results are in accordance with the previous study wherein, some mechanisms of antidiabetic drugs, such as suppress hepatic glucose production (biguanides), stimulate insulin secretion (sulfonylureas and glinides), and absorption of intestinal carbohydrates to maintain postprandial glucose level (α-glucosidase and α-amylase inhibitors), improve the sensitivity of insulin receptor and peripheral glucose uptake (thiazolidinediones and metformin) or insulin**[33]**. The previous study suggested an *in vitro* alpha-amylase inhibition model wasused to screen the *Pterocarpus marsupium* Roxb. silver nanoparticles to evaluatethe hypoglycemic effects **[34]**. Strong *in vitro* inhibitory effects of *Pterocarpus marsupium* Roxb. latex was observed on α-amylase and α-glucosidase

with IC50 of 2.97 and 0.54 µg/ml respectively **[38]**. Aqueous extract of the PM latex had shown a marked α-glucosidase inhibitory activity **[39]**.

***In Vitro* α-glucosidase inhibitory assay:**

The results of antidiabetic activity using α- glucosidase inhibitory assay of the silver nanoparticles synthesized using a *Pterocarpus indicus* extract. The synthesized silver nanoparticles showed inhibitory activity from 48.837% to 64.648% for stem and 58.747%to 72.855% for leaves at concentration 100 µg/ml **(Table 6)**. Acarbose is a standard drug for α-amylase inhibitor. Acarbose at a concentration of (20-100 µg/ml) showed α-amylase inhibitory activity from 70.270% to 82.882%at the same concentrations 100 µg/ml **(Figure 9)**.

The previous study suggested, one therapeutic approach for treating diabetes is decreasing post-prandial hyperglycemia by delaying glucose absorption through carbohydrate-hydrolyzing enzymes inhibition, α-glucosidase, in the digestive tract. Inhibitors of these enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. **[40, 41]**).

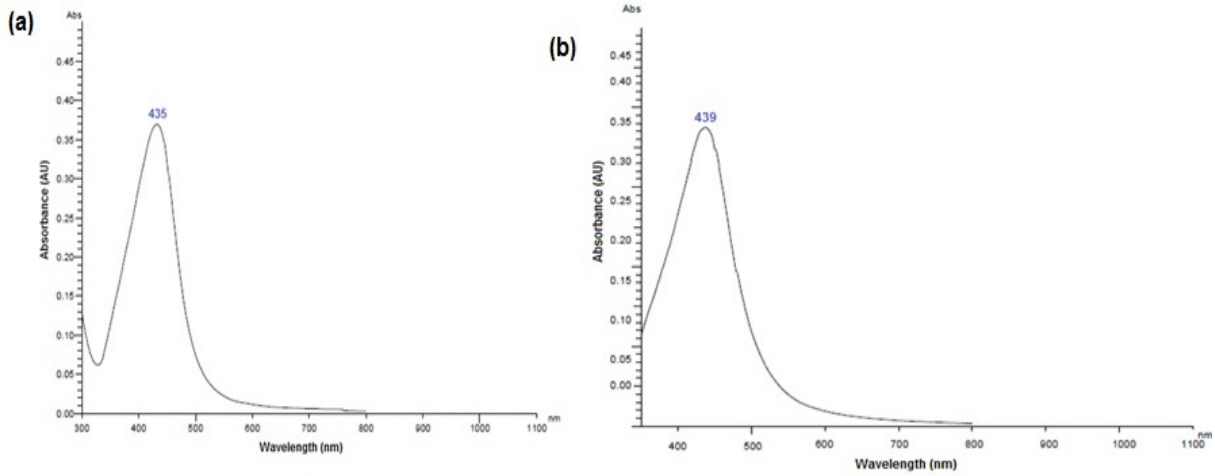


Figure 1: UV-VIS spectrum of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark-AgNPs

Table 1: FTIR band values of synthesized silver nanoparticles using of leaves of *Pterocarpus indicus*

Functional group	Band	Frequency. Cm^{-1}
Primary amine	Medium band	3438.11 cm^{-1} corresponds to N-H stretching vibrations
Isothiocyanate	Strong band	2085.17 cm^{-1} corresponds to N=C=S stretching vibrations
Alkene	Medium band	1637.86 cm^{-1} corresponds to C=C stretching vibrations
Amine	Medium band	1109.08 cm^{-1} corresponds to C-N stretching vibrations
Halo compound	Strong band	670.15 cm^{-1} corresponds to C-Br stretching vibrations

Table 2: FTIR band values of synthesized silver nanoparticles using of stem bark of *Pterocarpus indicus*

Functional group	Band	Frequency. Cm^{-1}
Primary amine	Strong band	3441.16 cm^{-1} corresponds to broad O-H stretching alcohol
Isothiocyanate	Strong band	2077.79 cm^{-1} corresponds to N=C=S stretching vibrations
Alkene	Medium band	1634.81 cm^{-1} corresponds to C=C stretching conjugated alkene
Amine	Medium band	1405.54 cm^{-1} corresponds to O-H bending alcohol
Alcohol	Strong band	1114.45 cm^{-1} corresponds to C=O secondary alcohol
Anhydride	Strong band	1030.7 cm^{-1} corresponds to CO-O-CO anhydride
Halo compound	Strong band	662.74 cm^{-1} corresponds to C-Br stretching vibrations

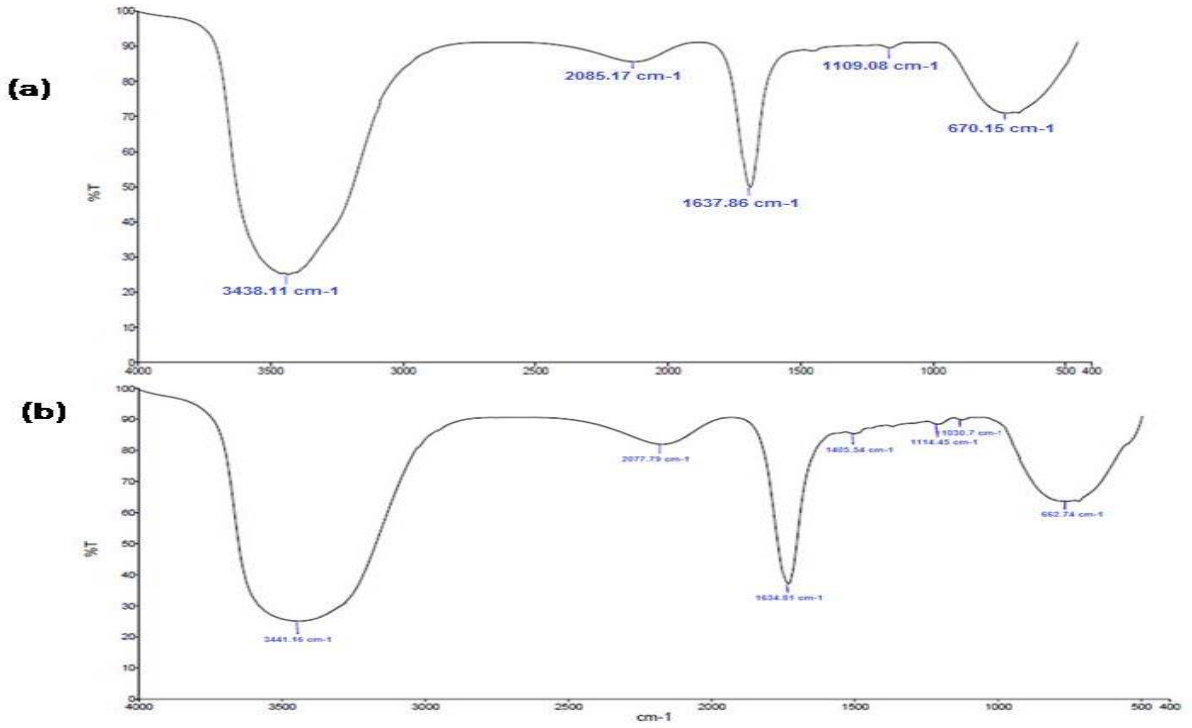


Figure 2: FTIR spectrum of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves-AgNPs (b) stem bark-AgNPs

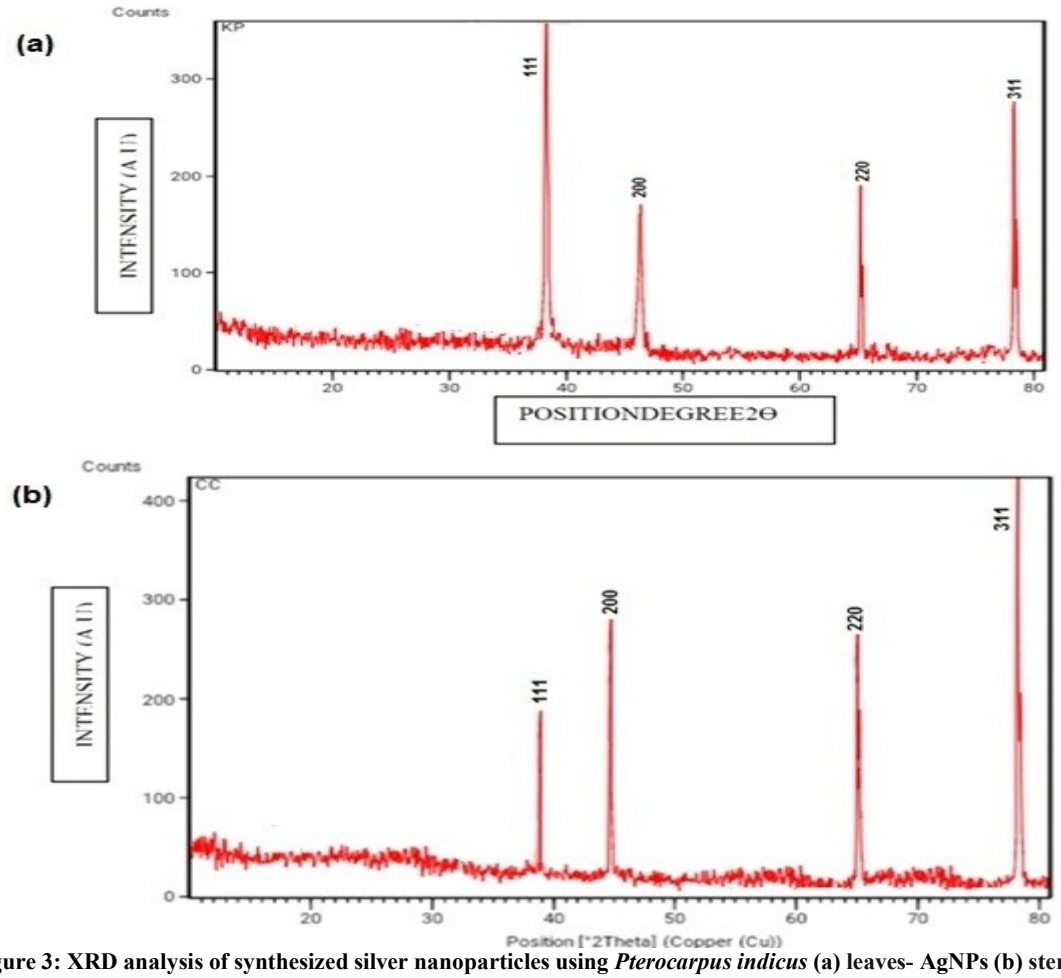


Figure 3: XRD analysis of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark-AgNPs

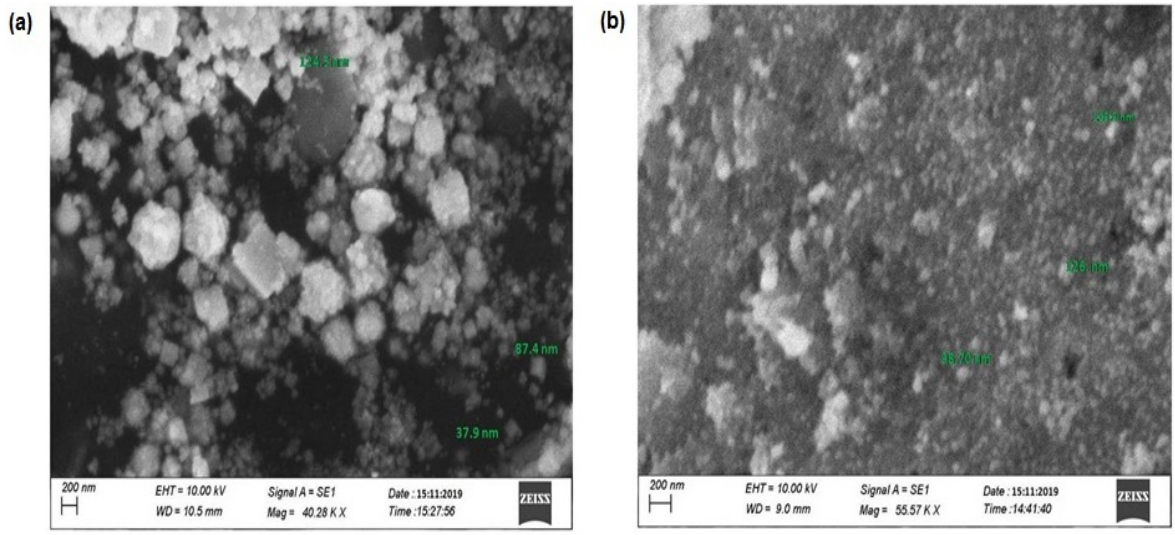
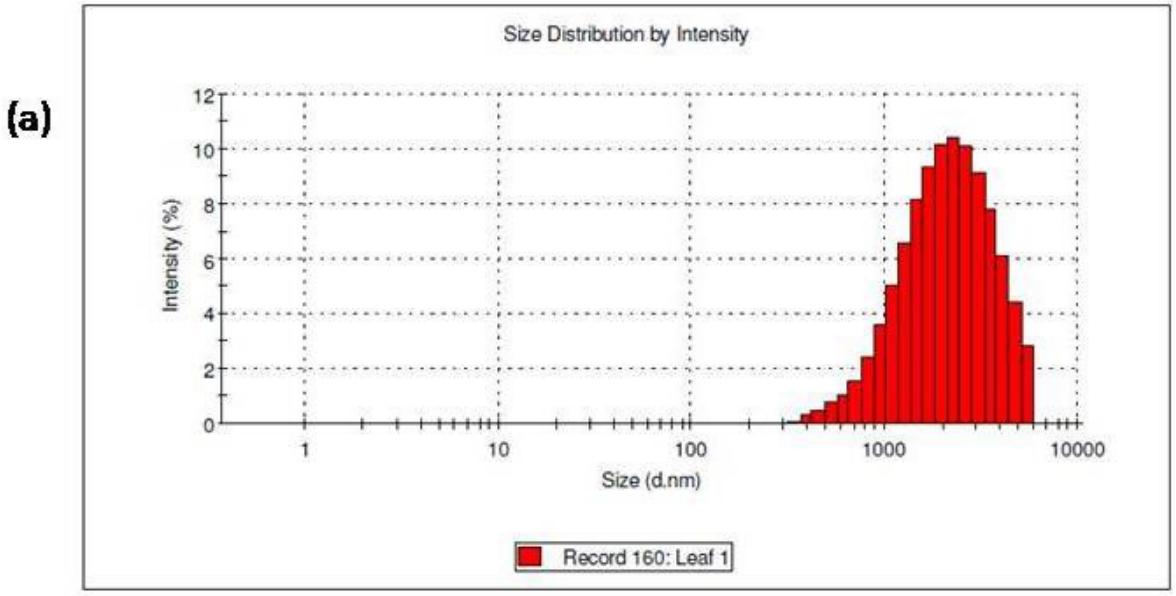


Figure 4: SEM photograph of synthesized silver nanoparticles using *Pterocarpus indicus* (a) leaves- AgNPs (b) stem bark AgNPs



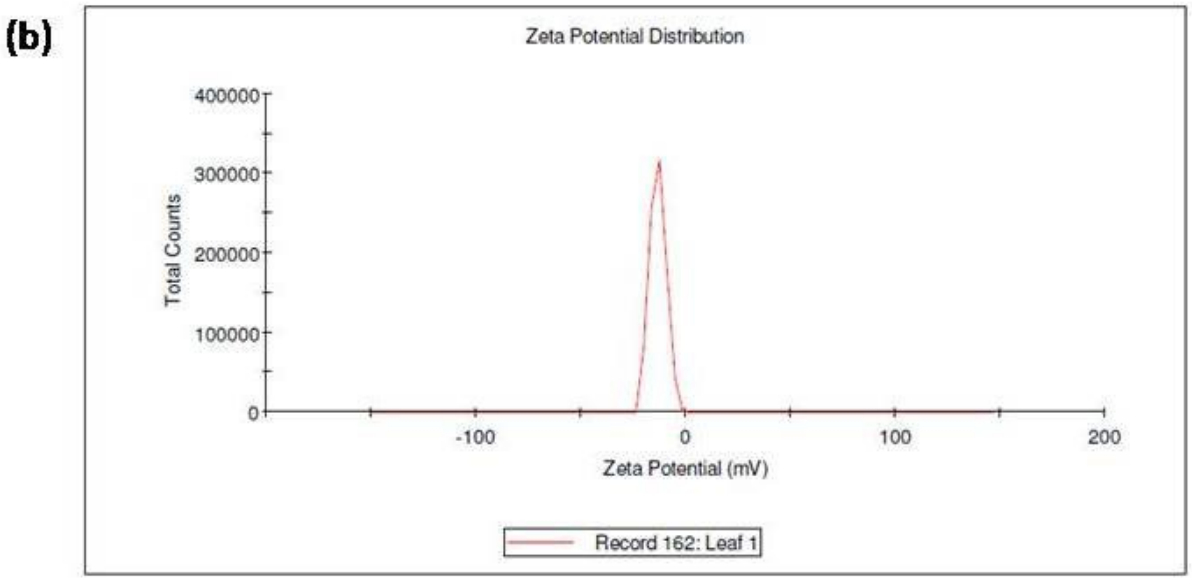


Figure 5: (a) particle size distribution, and (b) zeta potential measurement of the biosynthesized AgNPs using *Pterocarpus indicus* leaves extract

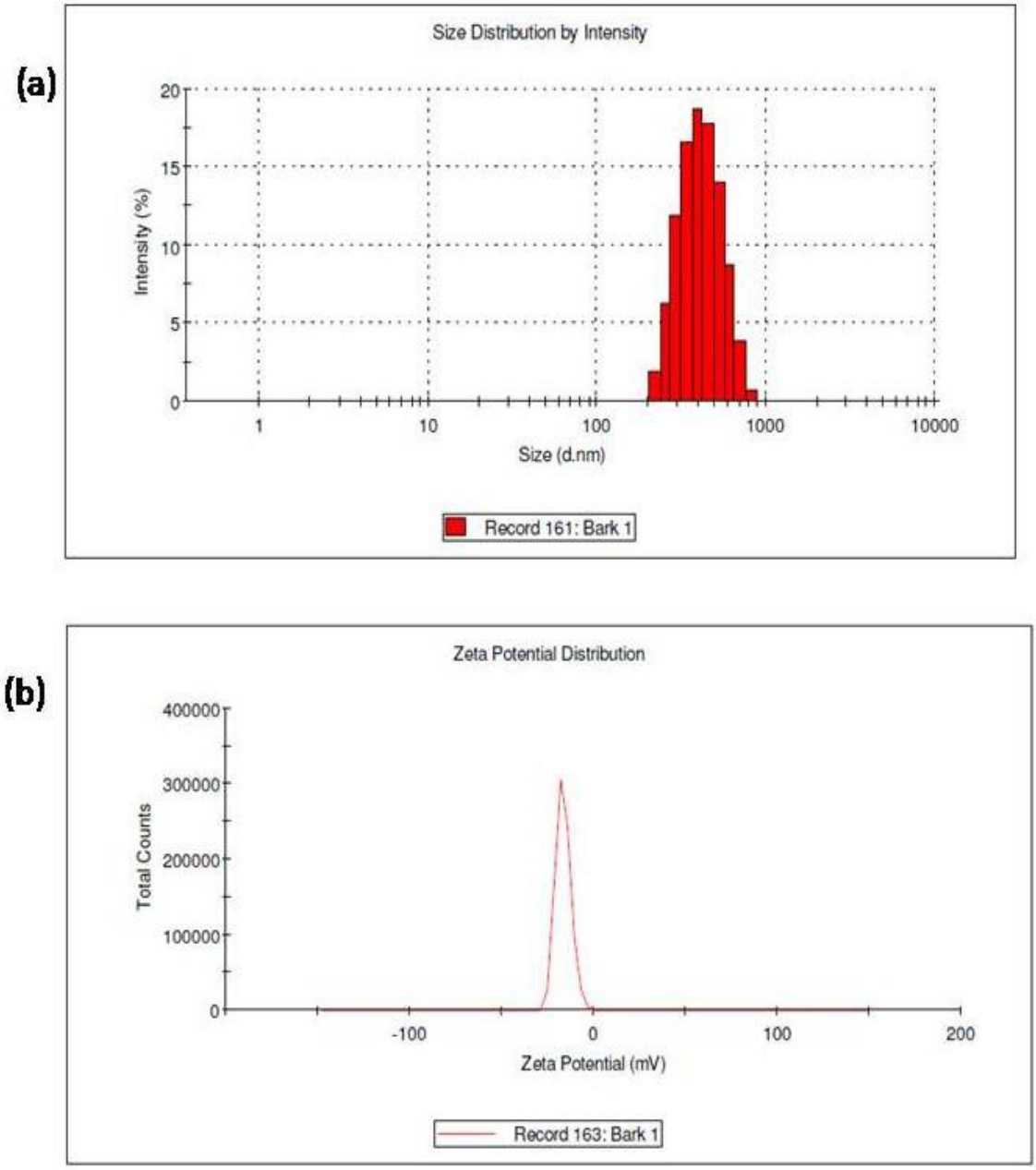


Figure 6: (a) particle size distribution, and (b) zeta potential measurement of the biosynthesized AgNPs using *Pterocarpus indicus* stem bark extract.

Table 3: Antioxidant activity of silver nanoparticles synthesized using a *pterocarpus indicus* by DPPH activity

S. No.	Concentration	DPPH ACTIVITY%		
		Leaves-AgNPs	Stem bark-AgNPs	Acarbose
1	20 (µg/ml)	55.22±0.812	57.76±0.812	61.68±0.824
2	40 (µg/ml)	57.46±0.824	62.98±0.808	72.85±0.824
3	60 (µg/ml)	59.70±0.824	69.47±0.808	79.74±0.808
4	80 (µg/ml)	64.17±0.824	71.46±0.824	82.34±0.824
5	100 (µg/ml)	70.14±0.008	75.92±0.824	94.69±0.808

At the 0.05 level, the population means are significantly different

Table 4: Antioxidant activity of stem barks and leaves of *Pterocarpus indicus*by reducing power activity

S. No.	Concentration	REDUCING POWER ACTIVITY %		
		Leaves-AgNPs	Stem bark-AgNPs	Acarbose
1	20 (µg/ml)	52.25±0.808	53.15±0.824	64.86±0.826
2	40 (µg/ml)	56.75±0.824	61.26±0.824	66.66±0.824
3	60 (µg/ml)	68.46±0.824	67.56±0.824	70.27±0.832
4	80 (µg/ml)	73.81±0.824	75.67±0.808	81.08±0.828
5	100 (µg/ml)	78.37±0.808	79.27±0.808	84.68±0.824

At the 0.05 level, the population means are significantly different

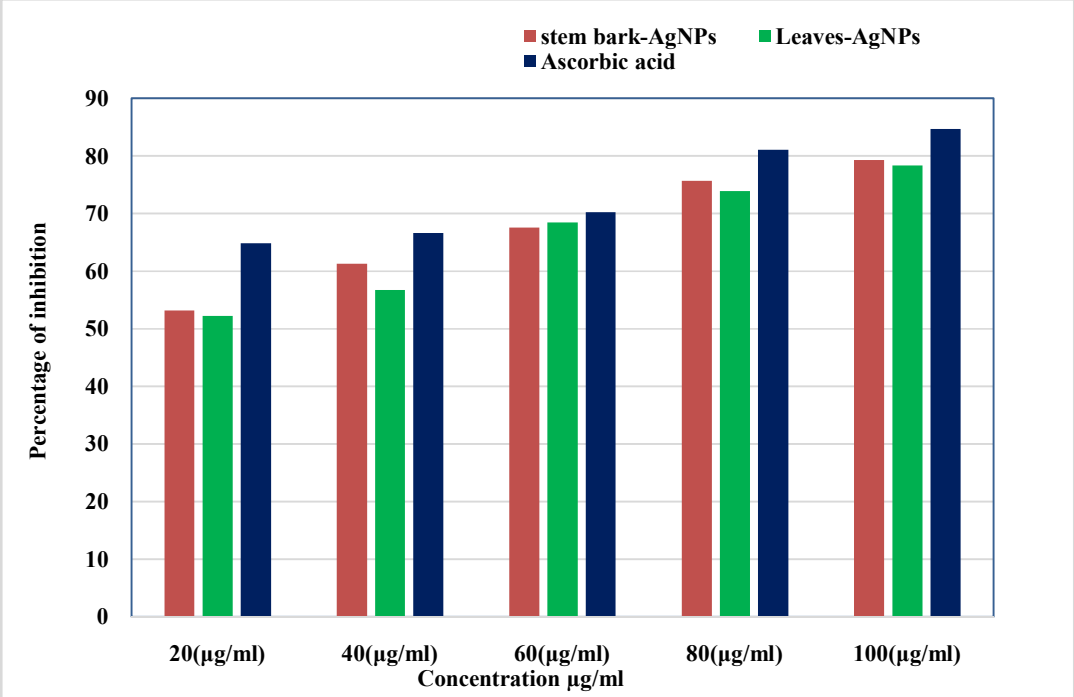


Figure 7: Antioxidant activity of stem bark and leaves of *Pterocarpus indicus* by reducing power activity

Table 5: *In vitro* antidiabetic activity of the silver nanoparticles synthesized using a *Pterocarpus indicus* extract using alpha amylase method and comparison with standard drug acarbose

S. No.	Concentration	Alpha amylase (%)		
		Leaves-AgNPs	Stem bark-AgNPs	Acarbose
1	20 (µg/ml)	56.04±0.832	65.95±0.832	70.27±0.824
2	40 (µg/ml)	59.64±0.824	67.75±0.824	74.77±0.824
3	60 (µg/ml)	60.54±0.832	73.16±0.832	80.18±0.824
4	80 (µg/ml)	65.95±0.824	78.56±0.824	81.98±0.824
5	100 (µg/ml)	67.75±0.824	80.36±0.824	82.88±0.824

At the 0.05 level, the population means are significantly different

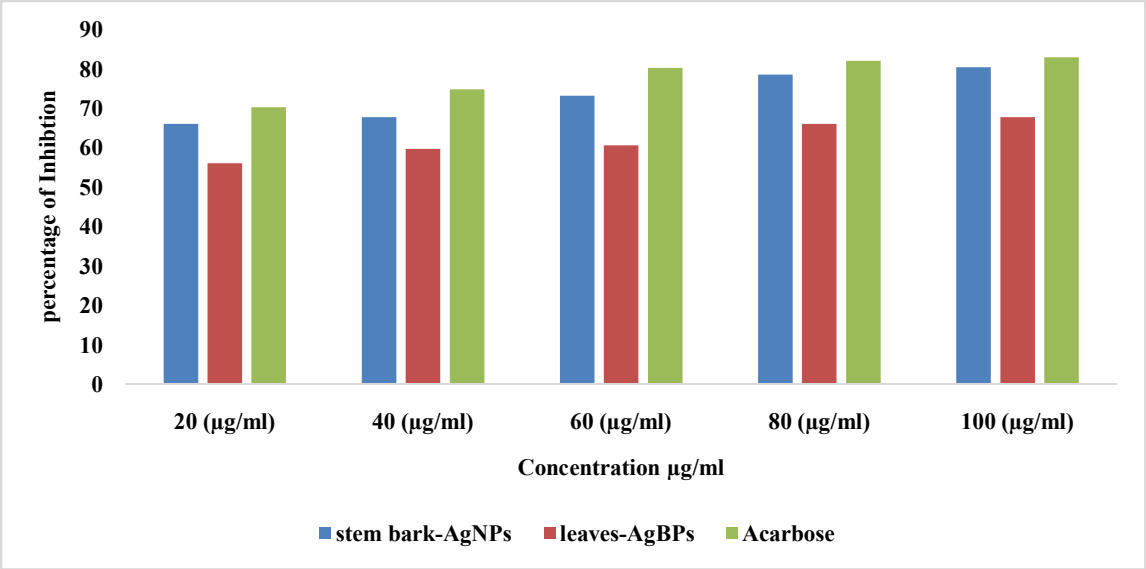


Figure 8: α-Amylase inhibitory activity of Acarbose vs silver nanoparticles synthesized using a *Pterocarpus indicus*

Table 6: *In vitro* antidiabetic activity of the synthesized silver nanoparticles using alpha glucosidase method and comparison with standard drug acarbose

S. No.	Concentration	Alpha Glucosidase (%)		
		Leaves-AgNPs	Stem bark-AgNPs	Acarbose
1	20 (µg/ml)	48.83±0.824	58.74±0.832	70.27±0.808
2	40 (µg/ml)	50.63±0.832	62.35±0.832	74.77±0.808
3	60 (µg/ml)	55.14±0.823	64.15±0.824	80.18±0.832
4	80 (µg/ml)	60.74±0.824	68.95±0.832	81.98±0.824
5	100 (µg/ml)	64.64±0.824	72.85±0.824	82.88±0.824

At the 0.05 level, the population means are significantly different

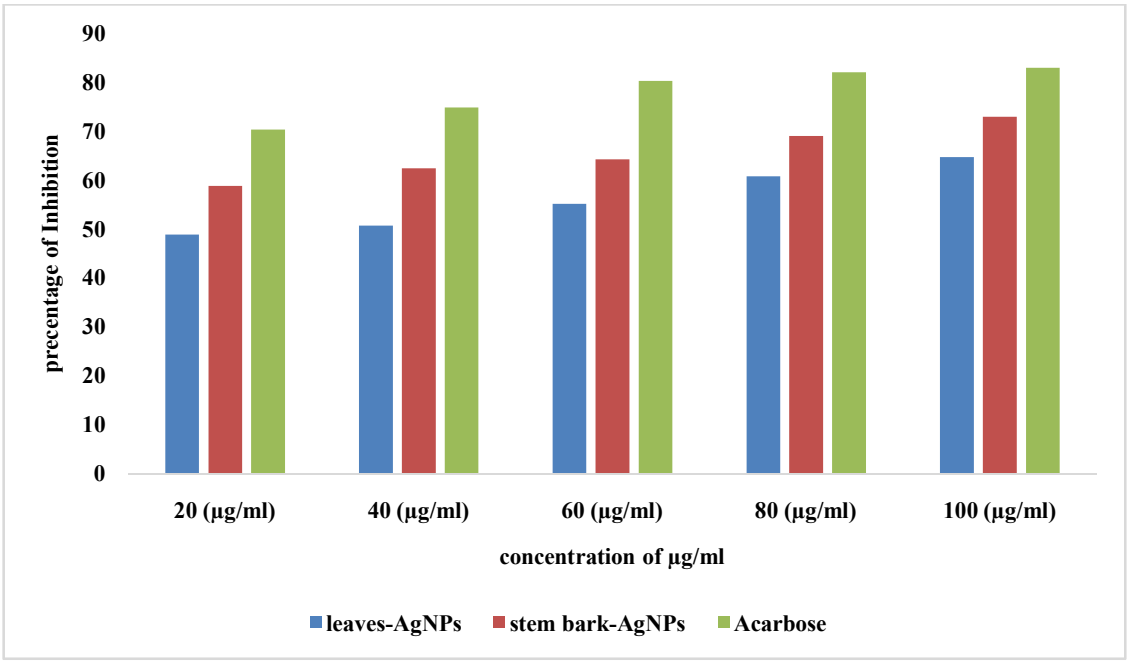


Figure 9: α- glucosidase inhibitory activity of Acarbose vssilver nanoparticles synthesized using a *Pterocarpus indicus*

CONCLUSION

From the present study, synthesis of silver nanoparticles using leaves and stem bark of *Pterocarpus indicus* showed maximum inhibitory activity of antioxidant and antidiabetic activity under *in vitro* condition. Synthesized AgNPs from the *Pterocarpus indicus* are characterized using UV-Visible spectroscopy, zeta potential for particle size analysis and SEM analysis are spherical with sizes in the ranges from 37.9-126 nm. Alpha amylase inhibitory action decreases the digestion of carbohydrates and alpha glucosidase reduces glucose level in blood. As a result, we found that the synthesized leaves-AgNPs and stem bark-AgNPs have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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