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Research Article

Hepatoprotective, antioxidant and amylase-inhibitory activities of silver nanoparticles synthesized from leaf extract of *Pedilanthus (Euphorbia) tithymaloides*

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Abstract
During the past one or two decades, the biosynthesis of metal nanoparticles especially the silver nanoparticles, using plant extracts, and their diverse biomedical applications, including antioxidant and hepatoprotective, has gained the attention of researchers. The present work includes the green synthesis and characterization of silver nanoparticles (AgNPs) from the aqueous leaf extract of *Pedilanthus (Euphorbia) tithymaloides*. The biosynthesis employed a bottom-up approach, in which the secondary metabolites of the leaf extract served as reducing, stabilizing and capping agents. The characterization was performed using various techniques, including X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM) coupled with energy-dispersive spectroscopy (EDS), Dynamic light scattering (DLS), and Zeta potential analysis. The biosynthesized silver nanoparticles were further evaluated for their *in-vitro* antioxidant and amylase -inhibitory properties, as well as their *in-vivo* hepatoprotective efficacy in albino Wistar rats. The findings demonstrated that the biosynthesized silver nanoparticles exhibit strong antioxidant and amylase-inhibitory properties, and significantly reduce the oxidative stress-induced hepatotoxicity, reflected by improved liver function parameters, such as serum levels of bilirubin (from 2.86±0.38 mg/dL in hepatotoxic to 1.34±0.18 mg/dL in treated rats), total protein (from 7.44±0.96 to 12.63±2.14 g/dL), albumin (from 2.22±0.57 g/dL to 3.14±0.46 g/dL), key enzymes like alanine aminotransferase [ALT] (from 172.38±14.97 IU/L to 85.19±11.63 IU/L), aspartate aminotransferase [AST] (from 144.29±8.93 IU/L to 61.57±11.26 IU/L), serum alkaline phosphatase [ALP] (from 154.78±16.92 IU/L to 114±13.57 IU/L), and key antioxidant enzymes in hepatic tissues, such as reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and oxidative stress parameter like malondialdehyde (MDA).

Keywords: Amylase-inhibitory, Antioxidant, Hepatoprotective, *Pedilanthus* (*Euphorbia*) *tithymaloides*, Silver nanoparticle

INTRODUCTION

The liver carries out a variety of essential and vital functions, such as the metabolism of food molecules, the detoxification of toxicants, drugs, or metabolic waste products like ammonia, the recycling of RBC breakdown products and bile synthesis, the synthesis of plasma proteins, including clotting factors, the maintenance of blood glucose levels through glycogenesis and glycogenolysis, and numerous other biochemical and physiological processes (Kumar *et al*., 2019). Therefore, any agent that can adversely affect liver function will lead to serious health consequences, which may sometimes be fatal. The modern-day lifestyle, dietary

habits, alcoholic beverages, and irrational use of drugs and medications are among the most common but potent factors of liver damage, resulting in various health issues and diseases, making it one of the most prevalent and widely distributed health issue worldwide. For assessment of liver damage, many quantitative tests and imaging techniques are used in practice; however, some parameters, such as serum bilirubin, serum or liver tissue levels of enzymes like alanine aminotransferase [ALT], aspartate aminotransferase [AST], serum alkaline phosphatase [ALP], and serum albumin, are used to evaluate liver functions primarily (Das and Sattegiri, 2018; Sharma, 2022). One of the most significant causes of liver dysfunction is oxidative stress, which has been implicated in the molecular and cellular mechanisms leading to several pathophysiological states of liver damage, such as alcoholic liver injury, exposure to hepatotoxins, intrahepatic cholestasis, viral hepatitis, liver ischaemia, biliary disease, and liver necrosis (Stehbens, 2003; Adeyemi, 2014). In most cases of liver dysfunction, where oxidative stress is involved, the pathophysiological states are characterized by a progressive change from steatosis to chronic hepatitis, fibrosis, cirrhosis and sometimes hepatocellular carcinoma (Kodavanti *et al*., 1989).

One of the common pathophysiological conditions involving high blood glucose levels, mostly in the case of diabetes mellitus, is intricately related to the abnormal metabolic or physiological functions of the liver. Diabetes mellitus is a chronic metabolic disorder, extensively distributed across the world that is mostly associated with postprandial hyperglycemia (Ponnusamy *et al*., 2011). Postprandial hyperglycemia is a state of abnormally elevated blood glucose level after one to two hours of eating (postprandial state), which may contribute to the development of type 2 Diabetes mellitus (Khalafi *et al*., 2022). It has been reported that the activity of pancreatic α-amylase (a calcium metalloenzyme) within the human intestine correlates with an increase in postprandial glucose levels. Therefore, controlling α-amylase activity may be a crucial aspect of treating hyperglycemic conditions in diabetes (Khadayat *et al*., 2020).

Nanotechnology is gaining substantial interest as an emerging field of science focused on the advancement of nanomaterials and nanoparticles for their application in various areas, including catalysis, electrochemistry, biomedicine, pharmaceuticals, sensors, food technology, cosmetics, water treatment, and more (Velez, 2017; Bera and Belhaj, 2016). Among all noble metal nanoparticles, silver nanoparticles (AgNPs) are one of the Klaus physicochemical properties, and most importantly, their biomedical applications like antioxidant, antibacterial, antiviral, antifungal, and anti-inflammatory potentialities, among others, have generated endless interest (Ahmad *et al.*, 2003; Klaus-Joerger *et al*., 2001). The versatility of silver nanoparticles in medical applications has gained significant value when combined with medical practices producing a fascinating field referred to as Nanomedicines (Ghavanloo *et al.*, 2023).

The AgNPs can be synthesized using chemical, physical and biological methods, employing natural products, microbes or plant extracts. The biogenic production of AgNPs has proven to be among the most significant applications of green chemistry methods, as these methods are environment friendly, cost-effective and less labor intensive. In plant-mediated methods different parts of plants, such as leaves, fruits, seeds or stem extracts, are used for the synthesis of AgNPs (Rafique *et al*., 2017). Plants contain plethora of bioactive metabolites, such as polyphenols, flavonoids, terpenoids, tannins, proteins, polysaccharides, alkaloids, amines, ketones and aldehydes, among others, which act as capping, stabilizing and reducing agents and has a role in conversion of metallic ions to metallic nanoparticles, leading to synthesis of desired nanoparticles with defined features (Vanlalveni *et al*., 2021; Castillo-Henriquez, 2020).

The plant *Pedilanthus tithymaloides*, also known as *Euphorbia tithymaloides,* is a succulent shrub belonging to the family Euphorbiaceae. It is widely distributed across tropical and subtropical regions worldwide. In India, it is traditionally used as a source of medicinal agents and a folk medicine for the treatment of several diseases. Different parts of this plant, like root, stem, leaf and latex, have already been reported to possess various pharmacological activities, including antioxidant, anti-inflammatory, antiviral, anti-diabetic, antibacterial, haemostatic, anticancer and anti-tuberculosis, among others (Abreu *et al.,* 2006; Srivastava and Soni, 2019). The leaves of this plant have been reported to possess a plethora of pharmacologically active and potent secondary metabolites, including flavonoids, terpenoids, alkaloids, phenolic compounds and fatty acids, among others (Rani and Sisodia, 2023; Prakash and Tripathy, 2023). Furthermore, the majority of phytochemicals present in the leaf extracts of *P. tithymaloides* have been reported to exhibit strong antioxidant activity (Falode *et al*., 2019).

Silver nanoparticles synthesized from various plant extracts have been reported to exhibit strong hepatoprotective efficacy against hepatotoxicity caused by drugs or other hepatotoxic agents, primarily due to their high antioxidant properties (Zhang *et al*., 2019). CCl_4 is commonly used in animal experiments as a hepatotoxic agent as it can induce hepatopathy by increasing oxidative stress in liver tissues (Lu *et al*., 2017). The present study aimed to synthesize silver nanoparticles using the aqueous extract of *P. tithymaloides* leaves (Pt-AgNPs), to *in-vitro* estimate the antioxidant and amylase-inhibitory activities, and to evaluate the hepatopro-

tective activity of Pt-AgNPs against CCl_4-induced hepatotoxicity in albino Wistar rats (*Rattus norvegicus*), as the leaf extract of *P. tithymaloides* contains bioactive antioxidant compounds that act as capping and stabilising agents for Pt-AgNPs.

MATERIALS AND METHODS

Collection of samples and preparation of the extract

Fresh leaves of the plant were handpicked and brought to the Department of Botany, Cooperative College, Jamshedpur, for identification based on available herbaria and literature (Steinmann, 2003). The leaves were washed properly and dried under shade. After complete drying for five to six days, the leaves were powdered and sieved. 10 g of dried leaf powder were mixed with 500 mL of distilled water and subjected to solvent extraction using a Soxhlet apparatus. Following extraction, the extract is centrifuged at 3000 rpm for 5 minutes, filtered, and the supernatant is collected and stored at 4ºC for further use.

Synthesis of silver nanoparticles

A 1 mM solution of silver nitrate ($AgNO_3$) was prepared by dissolving 0.169 g of $AgNO_3$ crystals in 1000 mL of distilled water while stirring continuously. The solution was carefully protected from exposure to the sunlight. Then, 10 ml of the extract was added to 100 ml of a 1 mM $AgNO_3$ solution and mixed thoroughly and gently using a stirrer. The mixture was then incubated at 80°C for 8 hrs in an incubator. The change in the colour of the solution preliminarily indicates the formation of Pt-AgNPs.

The Pt-AgNPs solution is then centrifuged at 10,000 rpm for 10 minutes, and the pellet was collected. The pellet was dried, and the powder was collected as a virgin Pt-AgNPs powder, which was stored for further analysis. From the powdered Pt-AgNPs, solutions of varied concentrations of Pt-AgNPs can be formed for further experiments.

Characterization of silver nanoparticles synthesized from *Pedilanthus tithymaloides* leaf extract (Pt-AgNPs)

The Pt-AgNPs were characterized using various techniques, including X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM) coupled with energy dispersive spectroscopy (EDS), dynamic light scattering (DLS) and zeta potential analysis. The reduction of silver ions by the secondary metabolites present in the leaf extract was preliminarily indicated by the colour change. The X-ray diffraction pattern was analyzed using an X-ray diffractometer (Rigaku, Japan; Smart Lab 9KW) for the structural analysis of biosynthesized Pt-AgNPs. The wavelength employed was 0.154 nm (the wavelength of the X-ray), covering a diffraction angle (2θ) range from 10º to 80º to obtain the XRD pattern. The FTIR spectrum was obtained using an FTIR spectrometer (Shimadzu Co., Japan; IR Prestige 21) in a wavelength range of 400 to 4000 cm^{-1} to confirm the presence of different functional groups in the secondary metabolites, which act as reducing and capping agents. The FESEM-EDX (Carl Zeiss Microscope Ltd., Germany; Sigma 300) was used to examine the shape, size, topographic, and morphological characteristics of Pt-AgNPs. DLS analysis was performed to determine the hydrodynamic diameter and surface charge of Pt-AgNPs (Malvern Instruments, UK; Zeta Sizer Nano ZS).

Antioxidant analysis

The antioxidant activity of the Pt-AgNPs solution was analyzed using the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay following the method of Luhata *et al*., (2022) with slight modifications. Different concentrations of sample solution were prepared, and 1 ml of DPPH (0.16 mM in MeOH) was added to each of them. The solutions were kept in the dark for about half an hour, and then the absorbance was read at a wavelength of 517 nm. The DPPH solution without the sample served as a control, and Vitamin C (Ascorbic acid) was taken as a reference standard. The calculation is as follows:

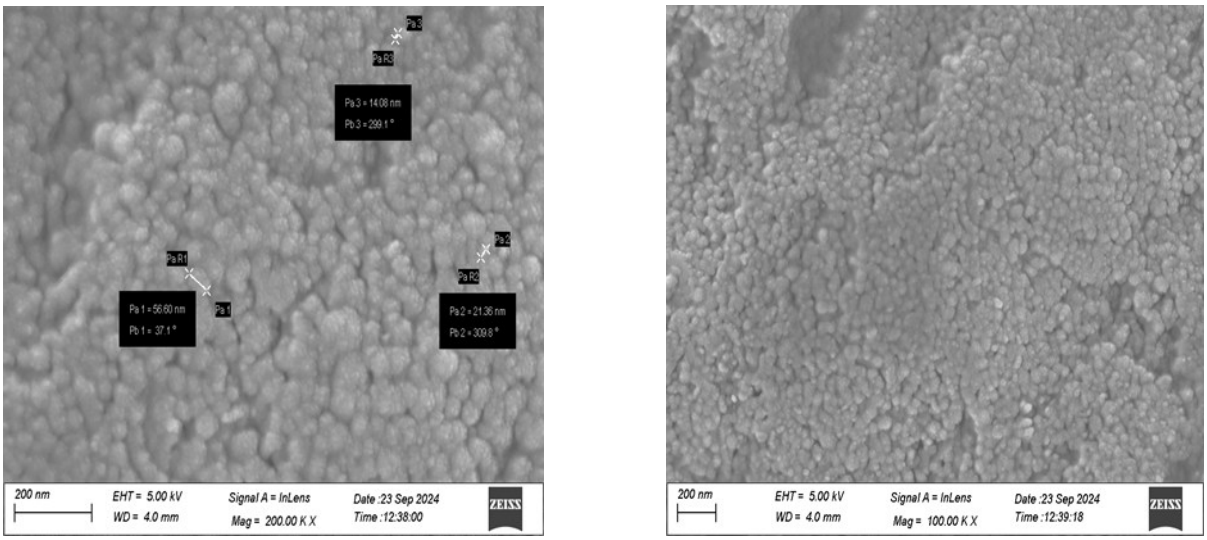
% Inhibition = (Absorbance of Control - Absorbance of Sample)/Absorbance of Control X 100

Amylase-inhibitory activity

The chromogenic DNSA (3,5-dinitrosalicylic acid) method was employed to measure the amylase-inhibition activity of Pt-AgNPs solution (Sudha *et al*., 2011; Kumar *et al*., 2018). The absorbance of test samples was recorded at a wavelength of 540 nm, using Acarbose as a reference standard.

Animals and acute toxicity studies

Male albino rats weighing approximately 200-250 gm have been used in the study. The rats were maintained at a temperature of 25±5 ºC, a 12-hour dark-light cycle, and a relative humidity of 50±15%. They were acclimatized for 10 days. The test animals were housed in polypropylene cages on a sawdust bed, and fed a commercial pellet diet using an oral feeding tube. The CPCSEA guidelines were followed throughout the entire experimental period (Committee for the Purpose of Control and Supervision of Experiments on Animals, India). The acute toxicity study has been carried out following the OECD guidelines (2004). Different groups of rats, each comprising 6 rats, were fed increasing concentrations of Pt-AgNPs solution. No mortality was observed at the maximum dose of 2 ml of 1000 µg/100



(A) (B)

Fig. 1. *FESEM images (A) and (B) of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs) showing the particle size and shape*

ml of Pt-AgNPs solution per kg body weight per day.

Ethical committee approval

The present work was approved by the Institutional Ethical Committee (IEC) of Kolhan University, Jharkhand, India, vide approval number KU/IEC/01/2021.

Animal groups and research design

The animals were divided into four groups, each containing six rats. The experiment was conducted for 14 days as follows:

Group 1: Control group, fed with a normal diet

Group 2: Hepatotoxic group: hepatotoxicity was induced by administering 1 ml/kg body weight of a mixture containing 30% CCl_4 and liquid paraffin (1:2 v/v) via intraperitoneal injection for the initial 3 days.

Group 3: Received 2 ml of 500 µg/100 ml of Pt-AgNPs solution per kg body weight per day after intoxication with CCl_4, i.e. from 4^{th} day

Group 4: Received the standard drug Silymarin 20mg/kg BW/day after CCl_4 intoxication

Estimation of biochemical parameters:

The experiment was continued for a total of 14 days. At the end of the 14th day, all rats were starved overnight, and then blood is collected in triplicate by puncturing the retro-orbital sinus under light anaesthesia. The blood samples were centrifuged at 3000 rpm for 10 minutes, and clear serum was collected for further biochemical analysis. The serum was analyzed for liver function parameters, including Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), serum bilirubin, total protein, and albumin levels, using standard kits (Kumar *et al*., 2021). After being dissected, the liver was cut into thin slices. The tissue was homogenized in a homogenizer with phosphate-buffered saline (PBS), and then the homogenate was centrifuged at 10,000 rpm for 5 minutes at 4º C in a refrigerated centrifuge (Remi CPR-24 Plus). After discarding the pellet, the supernatant was collected for the estimation of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) for the analysis of lipid peroxidation (Chandrashekhar *et al*., 2010; Maity *et al*., 2012; Johra *et al*., 2023).

RESULTS

Characterization of Pt-AgNPs

The reduction of silver ions by the secondary metabolites present in the *P. tithymaloides* leaf extract was the basic chemical reaction that resulted in the formation of Pt-AgNPs, which was indicated by the colour change of the solution.

Field emission scanning electron microscopy (FESEM) and Energy dispersive spectroscopy (EDS) analysis

The FESEM images reveal that the Pt-AgNPs are ovoid or quasi-spherical in shape, with a size range of 14.08 nm to 56.60 nm, as shown in Fig. 1. Furthermore, some large-sized particles can also be observed due to the natural tendency of aggregation of Pt-AgNPs, which may be a result of solvent evaporation (Hanachi *et al*., 2022). The presence of silver nanocrystals is implied by the EDS spectrum of the Pt-AgNPs, which displays a peak at around 3 keV (Salar and Kumar, 2016). The additional peaks in the EDS spectrum indicate the presence of other elements, such as oxygen, platinum, chlorine and potassium, which may be due to the other biomolecules that have been adsorbed onto the surface of Pt-AgNPs. The main peak for silver in an EDS spec-

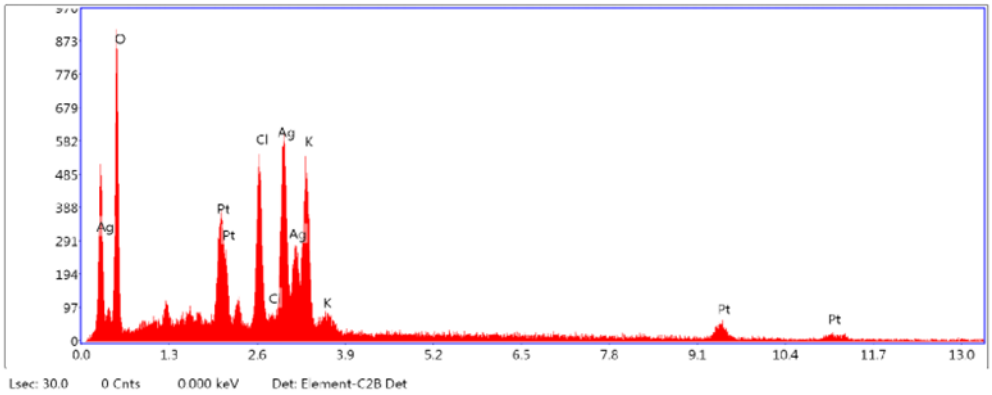


Fig. 2. *EDS analysis of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs) showing characteristic peaks of silver and other elements*

trum is a strong characteristic peak at approximately 3 KeV, which is a key indicator for the presence of silver in a sample (Fazil Khuda *et al*., 2023). In the present EDS spectrum, a prominent peak was seen at 3 keV, confirming that Ag was present in the sample under analysis (Fig. 2).

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectrum (Fig. 3) reveals the various functional groups of the secondary metabolites present in the Pt-AgNPs, serving as both reducing and capping agents. The wave numbers and the corresponding functional groups are listed in Table 1 (Coates, 2000; Nandiyanto *et al*., 2019).

X-ray Diffraction analysis (XRD) analysis

The XRD pattern reveals the crystalline nature of biosynthesised Pt-AgNPs. The diffraction peaks observed at 2θ values of 27.68º, 32.1º, 46.1º, 54.68º, 57.34º, and 76.6º corresponded to the Miller indices (110), (111), (200), (220), (222), and (311) of Bragg's reflection, respectively. These peaks corresponded to the JCPDS/ ICDD standard powder diffraction card (silver file), indicating that the biosynthesized silver nanoparticles are crystalline in nature (Fig. 4) (Elumalai *et al*., 2017; Anith Jose *et al*., 2022).

Dynamic light scattering (DLS) analysis

The DLS analysis is used to examine the dynamic variations in light intensity caused by the Brownian motion of the biosynthesised nanoparticles. Together with a PdI (polydispersity index) value of 0.531, the results demonstrated that the Pt-AgNPs had a Z-average of 530.9 d.nm (Fig. 5), indicating the possibility of formation of the aggregates in the solvent medium. The results actually depict the hydrodynamic diameter of the biosynthesized nanoparticles, which might be more than the size implied by the XRD and FESEM results, as hydrodynamic diameter could be affected by the interaction between the electric dipole of the solvent and the phytochemical constituent compounds present as capping agents (Ahluwalia *et al*., 2018). Such interactions might result in the formation of a hydration shell in the solvent medium over the surface of the nanoparticles (Mohammad and Hasan, 2024). According to

Table 1. Wave numbers in Fourier Transform Infrared Spectroscopy (FTIR) spectrum and their corresponding functional groups

S. no.	Wave no. (cm^{-1})	Corresponding functional group
1.	3876.56	-OH, -NH_2 (Hydroxy, Amino groups)
2.	3218.72	-C=C stretching, Aromatic compounds, -OH stretch
3.	2813.08	Linear hydrocarbons
2.	2314.3	Nitriles, thiocyanate, diazo compounds
3.	2118.9	Alkyne
4.	1558.01	-C-N, -C-C (aromatic ring, presence of proteins), secondary amines, NH bends
5.	1379.72	-C-O stretching/ O-H deformation, phenol or tertiary alcohol
6.	1275.59	Organic nitrates, Primary or secondary alcohols
7.	1013.56	-C-O stretching/ O-H deformation
8.	914.47	Aromatic –C-H
9.	532.56	-C-S stretch, aliphatic Iodo compounds -C-I stretch

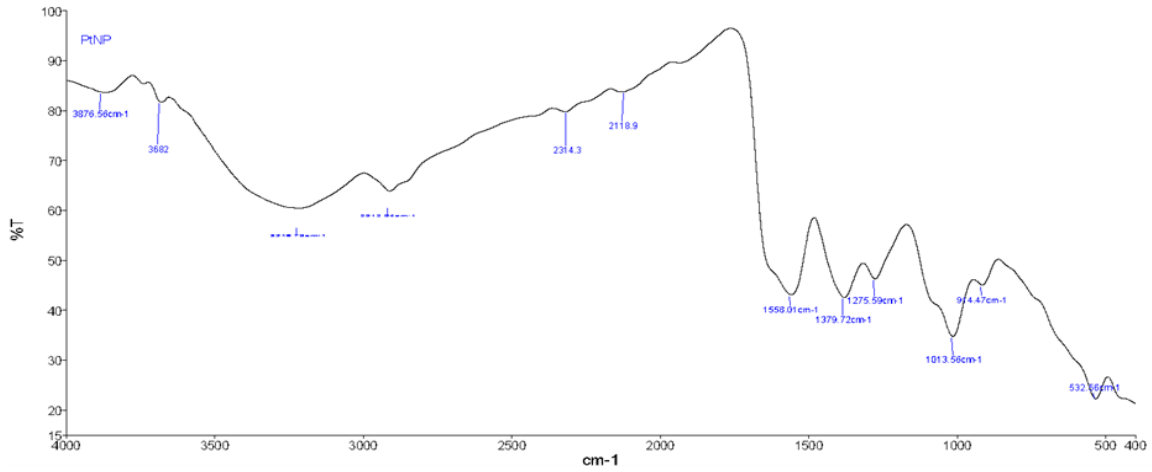


Fig. 3. *Fourier transform infrared spectroscopy* **(***FTIR) spectrum of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs) showing corresponding peaks of various functional groups*

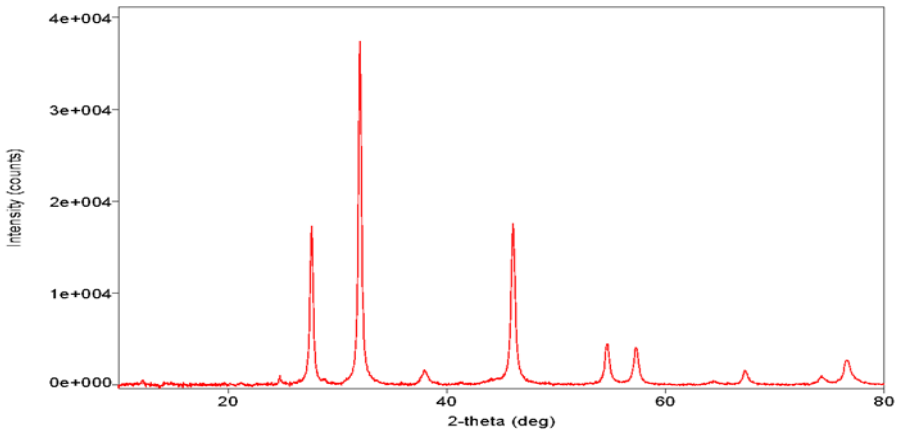


Fig.4: X-ray Diffraction analysis (*XRD) pattern of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs)*

previous scientific works (Khan *et al*., 2019) the particles having PdI values around or less than 0.50, are considered of good quality.

The Zeta potential of the biosynthesised Pt-AgNPs was found to be -19.6 mV (Fig. 6), which depicts that the biosynthesised nanoparticles have good stability since the negative charge results in the repulsion of the particles, which subsequently leads to the formation of colloids with good stability and marked dispersion (Hanachi *et al*., 2022).

Antioxidant activity

The result of the determination of antioxidant activity of Pt-AgNPs using the DPPH assay is shown in Table 2, which clearly depicts the strong free radical scavenging activity of Pt-AgNPs in comparison to the standard antioxidant agent Ascorbic acid, used in the experiment

Amylase-inhibitory activity

The results of the assessment of % amylase-inhibitory activity are shown in Table 3, which clearly indicate the marked amylase-inhibitory efficacy of Pt-AgNPs, compared to the standard i.e. Acarbose used in the test.

Hepatoprotective activity

The results of assessment of Hepatoprotective activity of biosynthesized Pt-AgNPs in CCl_4-induced hepatotoxicity in albino rats is shown in Table 4.

Impact of Pt-AgNPs on hepatic oxidative stress parameters

The results of assessing the of impact of Pt-AgNPs on oxidative stress parameters and enzymes in the CCl_4-induced hepatotoxic livers tissue are shown in Table 5.

DISCUSSION

Several previous reports have established that the synthesis of metal nanoparticles using plant extracts is an energy-efficient, cost-effective and eco-friendly approach (Kumar *et al*., 2024). In the present work, an aqueous leaf extract was used for the synthesis of silver nanoparticles, with the secondary metabolites present in the extract serving as both reducing and capping agents for the synthesized silver nanoparticles. The present study employed various techniques, in-

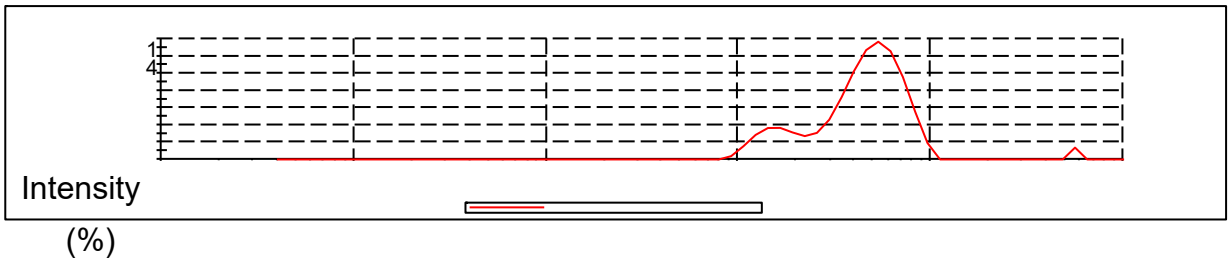


Fig. 5. *Showing Z-average peak of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs)*

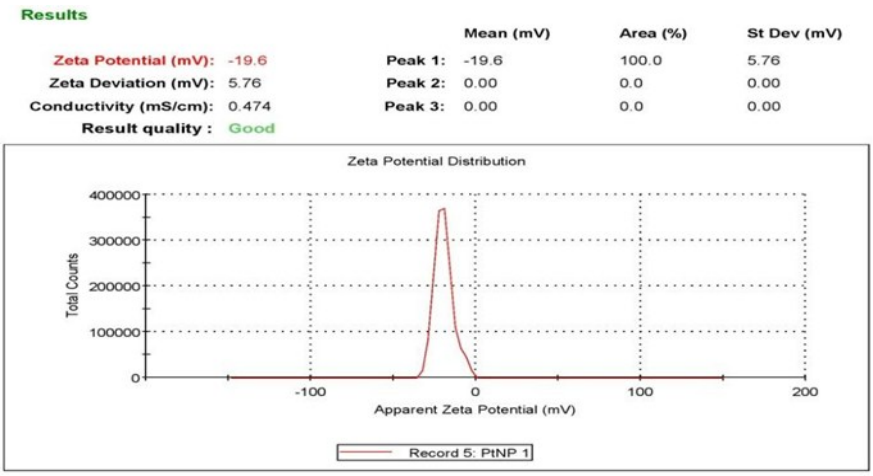


Fig. 6. *Showing Zeta potential distribution of silver nanoparticles synthesized from Pedilanthus tithymaloides (Pt-AgNPs)*

Table 2: Showing % free radical scavenging activity of silver nanoparticles synthesized from *Pedilanthus tithymaloides* (Pt-AgNPs) and the standard reference ascorbic acid

Concentration (µg/ml)	% Free radical scavenging activity of Pt-AgNPs	% Free radical scavenging activity of ascorbic acid
25	14.26	23.67
50	22.39	40.18
100	39.68	52.24

Table 3. Showing % amylase inhibitory activity of the silver nanoparticles synthesized from *Pedilanthus tithymaloides* (Pt -AgNPs) and the standard reference acarbose

Concentration(µg/ml)	% Amylase inhibitory activity of Pt-AgNPs	% Amylase inhibitory activity of Acarbose
100	22.87	28.32
200	41.58	50.96
500	57.64	62.52

Table 4. Showing improvement in liver function parameters in control, intoxicated and treated rats with reference to the standard drug Silymarin-treated rats (n=6, Mean±SE)

Animal groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (g/dL)	Albumin (g/dL)	Total bilirubin (mg/dL)
Group 1	56.34±3.76	63.19±8.47	102.47±12.73	14.82±1.36	4.16±0.68	0.94±0.13
Group 2	144.29±8.93^{a}	172.38±14.97^{a}	154.78±16.92^{a}	7.44±0.96^{a}	2.22±0.57^{a}	2.86±0.38^{a}
Group 3	61.57±11.26^{b}	85.19±11.63^{ab}	114±13.57^{ab}	12.63±2.14^{b}	3.14±0.46^{ab}	1.34±0.18^{a}
Group 4	76.24±9.73^{ab}	79.53±9.37^{ab}	120.39±17.18^{ab}	13.06±1.92^{b}	3.37±0.53^{ab}	1.18±0.22^{ab}

*a (significantly different from Group 1); b (significantly different from Group 2) at $p<0.05$

Table 5: Showing antioxidant enzymes activity in control, hepatotoxic and treated rats with reference to the standard drug silymarin treated rats (n=6, mean±S.E.)

Animal groups	GSH (nM/mg of tissue protein)	CAT (U/mg of tissue protein)	SOD (U/mg of tissue protein)	LPO (nM of Malonaldehyde/ mg of tissue protein
Group 1	2.96±0.08	42.68±0.34	106.39±12.58	3.18±0.36
Group 2	0.77±0.09[a]	16.95±2.13[a]	54.27±8.69[a]	18.43±1.67[a]
Group 3	2.22±0.14[ab]	38.44±3.57[ab]	97.48±16.43[ab]	4.64±0.63[ab]
Group 4	2.08±0.16[b]	41.16±3.48[b]	96.79±13.53[ab]	3.96±0.47[ab]

*a (significantly different from Group 1); b (significantly different from Group 2) at $p<0.05$

cluding FESEM, EDS, XRD, DLS, zeta potential, and FTIR, to characterize the biosynthesized silver nanoparticles. These techniques confirmed that the various biochemical constituents of the leaf extract served as stabilizing and capping agents (confirmed with FTIR spectral peaks) and that the silver nanoparticles had a size range of 14 to 56 nm (as shown in FESEM images, further confirmed by Scherrer's equation). The EDS peaks indicate the presence of silver, along with other elements, and the DLS and zeta potential measurements reveal the size of hydration shells formed by the biosynthesized silver nanoparticles in the solution. The present results are comparable and are in accordance with several previous findings of the synthesis of metal nanoparticles using plant extracts or other natural products (Akhter *et al*., 2024; Kirubakaran *et al*., 2025). The biosynthesized silver nanoparticles have been reported to have marked hepatoprotective efficacy due to their strong antioxidant properties, which might be attributed to the bioactive phytochemicals present as their capping and stabilizing agents (Bhubaneshwari *et al*., 2014; Zhang *et al*., 2019; Kumar *et al*., 2021). These phytochemicals are actually the secondary metabolites present in the plant extract, which mainly include several groups of biochemical compounds with marked antioxidant properties, such as alkaloids, flavonoids, phenolics, and terpenoids, among others.

In the present study, the biosynthesized Pt-AgNPs exhibited a free radical scavenging activity of 39.68% at a concentration of 100 µg/ml. This result is comparable to that of the reference standard, ascorbic acid, which demonstrated a free radical scavenging activity of 52.24% at the same concentration. The determination of DPPH radical scavenging activity is a widely used method for the assessing the antioxidant potential of a test sample, and it involves a combined assay of hydrogen transfer (HAT) and electron transfer (ET) (Apak *et al*., 2016). Keshari *et al.* (2020) have reported that the AgNPs synthesized from leaf extract of *Cestrum nocturnum* showed 29.55% free radical scavenging activity, in comparison to 24.28% free radical scavenging activity of ascorbic acid. Several previous works have reported that biosynthesized silver nanoparticles exhibit marked antioxidant potency, which might be attributed to their natural product components acting as both reducing and capping agents (Flieger *et al*., 2021).

The pathophysiological conditions of the liver are intricately related to abnormalities in the body's vital physiological mechanisms, including postprandial hyperglycemia, or abnormally increased blood glucose levels (Ling *et al*., 2007; Gonzalez *et al*., 2023). Previous findings have elucidated that biosynthesized silver nanoparticles exhibit marked amylase-inhibitory properties, suggesting they may play a significant role in counteracting the conditions of post-prandial hyperglycemia and potentially mitigating hyperglycemia-induced oxidative stress in the body (Perumalsamy *et al*., 2022). According to Dey and Lakshmanan (2013), factors or agents with antioxidant properties can play a significant role in ameliorating hyperglycemia-mediated oxidative damage in hepatic tissues by reducing oxidative protein and nucleic acid damage, inflammation, and improving the insulin signaling, as well as restoring mitochondrial structure and functions. El-Baz *et al*. (2024) have reported that the silver nanoparticles synthesized using aqueous extract of Cinnamon exhibited a protective role in hyperglycemia and oxidative stress in the liver in diabetic rats. The results of the present work showed that the biosynthesized Pt-AgNPs have a strong amylase-inhibitory capacity compared to the standard Acarbose, which suggests that Pt-AgNPs may play a significant role in reducing post-prandial hyperglycemia and thereby counteracting the abnormally increased blood glucose levels in the body and the subsequent oxidative stress in the hepatic tissues.

Furthermore, the aberrant liver function is reflected in abnormal blood levels of specific liver function parameters, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), total protein, serum albumin and bilirubin (Das and Sattegiri, 2018). In several toxicological studies, the CCl_4 is used as a hepatotoxic agent to induce hepatotoxicity (Zhao *et al*., 2018). The CCl_4 can induce oxidative stress in the hepatic tissues through the excessive gen-

eration of free radicals, leading to an abnormal rise in lipid peroxidation, a major contributor to hepatotoxicity (Kumar *et al*., 2019). It can transform into highly reactive free radicals like the trichloromethylperoxy radical (CCl_3O_2) and the trichloromethyl radical (CCl_4), which are involved in the activation of the cytochrome 450 enzyme, increased lipid peroxidation, and rise in the expression of pro-inflammatory mediators such as TNF -α, TGF-β, IL-1β, and IL-6, which may lead to oxidative damage and subsequent necrosis in hepatic tissues (Huo *et al*., 2011; Johra *et al*., 2023). Due to the breakdown of the hepatic cell membrane and obstructions in the biliary system of the liver, the blood levels of liver function markers such as AST, ALT, ALP, and bilirubin rise abnormally (Huo *et al*., 2011). However, damage to intracellular components, such as mitochondria and the endoplasmic reticulum, as well as DNA, leads to a decrease in the quantity of albumin and protein in the blood (Uru *et al*., 2013). Accordingly, results of the present work (Table 4) clearly reveal that the CCl_4 intoxication resulted in a significant ($p<0.05$) increase in the blood levels of ALT, AST, ALP and serum bilirubin, associated with a significant ($p<0.05$) decrease in the blood levels of total protein and serum albumin, which may be attributed to CCl_4-mediated abnormal rise in oxidative stress in hepatic tissues. The administration of Pt-AgNPs resulted into significant ($p<0.05$) improvement in the serum levels of liver function biomarkers towards their normal values, indicating the marked potentiality of Pt-AgNPs as hepatoprotective agents.

Antioxidants, both exogenous and endogenous, can absorb free radicals and prevent hepatocellular damage. Oxidative stress in hepatic tissues results in abnormal levels of activity of specific antioxidant enzymes like CAT, SOD, and GSH, among others (Gheita and Kenawi, 2014; Chukwuebuka *et al*., 2021). The MDA levels are used to assess the extent of oxidative damage in the hepatic tissues caused by lipid peroxidation (El-Baz *et al*., 2024). In the present work, Table 5 shows that CCl_4 intoxication resulted in significantly ($p < 0.05$) decreased activities of antioxidant enzymes in hepatic tissue homogenate, like SOD, CAT, and GSH, associated with an abnormal rise in levels of MDA. The administration of Pt-AgNPs resulted in a significant ($p<0.05$) increase in the activities of antioxidant enzymes towards their normal values along with a significant ($p<0.05$) lowering of the MDA levels in the hepatic tissues, indicating that the Pt-AgNPs are involved in reducing the oxidative stress-mediated damage in the hepatic tissues. A number of earlier studies have reported the significant efficacy of plant extract-based silver nanoparticles, in conjunction with their plant-derived stabilizing and capping agents, in improving liver functions and repairing oxidative stress-induced hepatic injury brought on by hepatotoxic agents such as CCl_4 (Rakesh *et al*., 2020; Kumar *et al*., 2021; Uhuo *et al*., 2025).

In the present work, the administration of Pt-AgNPs resulted in a significant ($p<0.05$) improvement in the liver function parameters and the oxidative stress biomarker enzymes back towards their normal values, showing marked hepatoprotective efficacy in comparison to the administration of the standard drug silymarin, by reducing the oxidative stress and repair of hepatic cells (Hussain *et al*., 2017; Kumar *et al*., 2019). Several previous studies have reported that the plant extract-based silver nanoparticles can play a significant role in amelioration of oxidative stress-mediated hepatocellular damages by improving the antioxidant enzyme levels within the hepatic cells or tissues, thereby proving to be a valuable hepatoprotective agent (Laib *et al*., 2024). Das *et al*. (2024) have reported that the silver nanoparticles synthesized from aqueous leaf extract of *Premna esculenta* have a promising role as a hepatoprotective agent in CCl_4-induced hepatotoxicity in Swiss albino mice. Zhang *et al*. (2020) have reported the hepatoprotective potency of silver nanoparticles synthesized from the aqueous leaf extract of the *Rhizophora apiculata* through antioxidant defence mechanisms. The silver nanoparticles synthesized from aqueous leaf extract of *Punica granatum* (Kumar *et al*., 2021) and from *Cuscuta reflexa* (Rakesh *et al*., 2020) have also been reported to exhibit the hepatoprotective role in CCl4-induced hepatotoxicity in albino rats.

Conclusion

The silver nanoparticles synthesized from the leaf extract of the plant *P. tithymaloides* are stable and have the pharmacologically potent biochemical constituent compounds of the leaf extract of the plant as their capping and stabilizing agents. These biosynthesized Pt-AgNPs have marked amylase-inhibitory and antioxidant potentialities, and therefore these Pt-AgNPs were found to significantly improve the liver function parameters, and the antioxidant enzymes activities in hepatic tissues in CCl_4-mediated hepatotoxicity in albino wistar rats. Further studies at cellular and molecular levels will pave the way for development of new drugs or the more efficient mechanisms of drug delivery or the development of specifically designed nanomedicines, which can have more prompt biopotentiality in the treatment or improvement of impaired liver functions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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