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Bioinformatics-Based Preparation and Characterization of Silver Nanoparticles Synthesized from Pterocarpus Marsupium

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Abstract

Silver nanoparticles were synthesized using Pterocarpus marsupium bark extract in combination with silver nitrate solution through a green synthesis method. Silver nanoparticles formation was confirmed by the formation of dark brown from solution of silver nitrate, where the reduction of silver ion occurs which leads to the formation of silver nanoparticles. The UV–visible spectra showed a peak at 429 nm, confirming the reduction of silver ions and the subsequent synthesis of silver nanoparticles. FTIR analysis of Pterocarpus marsupium Roxb. identified hydroxyl, carboxyl, and phenolic groups responsible for effectively capping and stabilizing the Ag nanoparticles through their reducing and capping properties. Additionally, drug entrapment efficacy testing demonstrated a high percentage of 95% for drug entrapment efficiency.SEM analysis was conducted to examine the structural and morphological characteristics of the synthesized Pterocarpus marsupium silver nanoparticles, revealing the presence of polydisperse spherical shapes. Particle size distribution was evaluated using a zeta sizer, indicating an average particle size of 36.44 nm with a polydispersity index of 0.825 and an intercept of 0.781. Zeta potential measurement was found to be -22.6 mV with the peak areas of 100% intensity and these values indicate formulated nanoparticles are stable. In vitro drug release data were examined at various time intervals, and the release kinetics were assessed using zero-order, Higuchi, and Korsmeyer-Peppas models. The analysis indicated that the Higuchi model provided the best fit for the release data, with an R2 value of 0.9877. The Korsmeyer-Peppas equation demonstrated strong linearity, characterized by a release exponent "n" of 0.167, which verified that the formulation followed Fickian diffusion kinetics.

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INTRODUCTION

Nanoscience involves manipulating materials at the nanometer scale, where their properties differ significantly from bulk materials. Nanotechnology, a rapidly growing field over the past decade, encompasses research, development, and industrial activities worldwide [1].

The combination of biotechnology and nanotechnology has led to the emergence of bio-nanotechnology, promoting environmentally friendly and biologically derived approaches to producing nanomaterials. Nanoparticles, assemblies of atoms ranging from 1 to 100 nm in

size, particularly metallic ones like gold, magnesium, titanium, copper, zinc, and silver, exhibit unique electrical, chemical, optical, and catalytic properties, with silver nanoparticles noted for their strong antimicrobial activity due to their high surface area to volume ratio (2, 3, 4, 5, 6, 7).

Nanotechnology finds applications across diverse fields such as military defense, medicine, astronomy, and computing, enabling precise control over molecules and atoms. Innovations include DNA silicon chips and various consumer products like eyeliner, sunscreen, sterile socks, and self-cleaning glass (8). Nanotechnology also promises advancements in drug delivery, enhancing precision in injecting drugs into the human body, and potential benefits in nanofood for improved digestion and appetite control [9].

Metformin, an antihyperglycemic drug, reduces blood glucose levels in type II diabetes by increasing insulin sensitivity without causing hypoglycemia. Glimepiride, used in managing type 2 diabetes mellitus, stimulates insulin production in the pancreas and improves insulin utilization in the body.

This study focuses on formulating and characterizing green-synthesized silver nanoparticles from the aqueous extract of Pterocarpus marsupium, aiming to explore their potential applications and properties.

MATERIALS

Table 1. Materials are used

S. No	Materials Used	Source
1	Silver Nitrate	Qualigens fine chemicals, Mumbai
2	Pterocarpus marsupium Roxb Bark	Natural Source., Thalassery
3	α- amylase enzyme	Himedia, Mumbai
4	Drugs	Microlabs

PREFORMULATION STUDY

Solubility Test

About 1 mg of Pterocarpus marsupium Roxb. bark extract powder was taken in a test tube and solubility in ethanol, water, chloroform and diethyl ether, dimethyl sulphoxide were checked.

Uv- visible spectral analysis of pterocarpus marsupium roxb bark extract

1ml of Pterocarpus marsupium Roxb. bark extract was taken in a 10 ml standard flask and diluted with distilled water. then Uv visible spectra were taken in the range of 200- 400 nm using phosphate buffer at pH 7.4 as blank

Preparation of Calibration Curve of Pterocarpus Marsupium Roxb Bark Extract

Twenty-five milligrams of crude extract were dissolved in phosphate buffer with a pH 7.4 and further diluted to 50mL of solvent, in volumetric flask to get a concentration of 500 µg/mL. This was treated as stock solution. Various aliquots of stock solution were diluted further to get different concentrations. The resultant solutions were scanned for λ max in the range of 200-400nm using UV-spectrometer.

Ftir spectroscopy of pterocarpus marsupium roxb bark

50 mg each of dried Pterocarpus marsupium Roxb bark and wood were mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm-1 range.

Ftir Spectroscopy of Silver Nitrate

100mg of silver nitrate was mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm- 1 range.

GREEN SYNTHESIS OF SILVER NANOPARTICLES

Preparation of Stock Solution

1 mg of aqueous extract was weighed and diluted to 10 ml with distilled water.

Preparation of 1mM silver nitrate aqueous solution 0.017g of silver nitrate was dissolved in 100 ml of distilled water to prepare 1mM solution of silver nitrate and stored in amber colored bottle until further use Synthesis of silver nanoparticles

An aliquot (1ml, 2ml,3ml, 4ml, 5ml) of aqueous plant extract sample was separately added to 10ml of 1mM aqueous $AgNO_3$. To drive nanoparticle formation the reaction mixtures were kept in magnetic stirrer with constant stirring at 120 rpm. Color change of the reaction mixtures were monitored to determine silver nanoparticle formation which is indicated by a colloidal brown color.
Separation of AgNPs:

Centrifuged technique was used to separate the nanoparticles from the solution (AgNO3 + plant extract). The reaction mixture (10ml AgNO3 + 5ml leaf extract sample) was split into two equal parts and transferred to pre-weighed sterile 15ml centrifuge tubes (United Scientific, South Africa). The preparations were then centrifuged at 4000rpm for 20 mins. (Eppendorf centrifuge 5810 R, Germany). The centrifuged supernatant liquid was collected and then centrifuged twice at 10,000 rpm for 30 min. The suspended pellet was purified using ethanol. The purified pellets were then dried and the powder was taken which is used for further characterization.

RESULTS & DISCUSSION

Sample Collection

The bark of Pterocarpus marsupium collected from Chittor district from AP and it is cleaned with distilled water to remove any substance on the surface of the bark.

Drying and pulverizing

The collected bark was shade dried & it was grounded into fine powder with the help of electronic blender. Then the powder obtained was stored in well closed container and kept in dry place.

Preparation Of Aqueous Extract of Pterocarpus Marsupium Roxb

50g of Pterocarpus marsupium bark powder was stirred with 500 mL of deionized water and kept at 65 °C for 30mins. Then the extracts were filtered by using Whatman No. 1 filter paper after cooling to room temperature. The extract was kept in air tight container & stored at 4 °C for future use

PREFORMULATION STUDIES

Physical Characteristics

Pterocarpus marsupium was checked for its color, odor and texture. It is light yellow colored powder in appearance and has a pleasant odor. The results are shown in Table. 2

Table 2. Phytochemical analysis of Pterocarpus marsupium Roxb aqueous bark extract

Chemical Constituent	Tests	PM Ag NPs
Tannins and Phenolics	Ferric chloride Test	+
	Lead acetate Test	+
Flavonoids	Alkaline reagent Test	+
	Shinoda Test	+
	Lead acetate test	+
Alkaloids	Mayer's Test	+

	Dragendroff's Test	+
Proteins	Warming Test	-
	Biuret Test	-
	Ninhydrin Test	-
Starch	Iodine Test	+
Saponins	Foam Test	+
Steroids	Salkowski's Test	+

(+) Presence of Phytoconstituents and (-) Absence of Phytoconstituents

Preliminary phytochemical screening of plant provided information regarding chemical nature of plant such as presence and absence of various phytoconstituents. The phytoconstituents screening was done for extracts of Pterocarpus marsupium.

Phytochemical screening identified the presence of various constituents such as alkaloids, flavonoids, tannins, phenols, starch, steroids, saponins, pterostilbene, and (-)-epicatechin in Pterocarpus marsupium bark. These compounds are responsible for the antidiabetic effects observed in Pterocarpus marsupium bark

Solubility studies

Solubility test for Pterocarpus marsupium bark was carried out in different solvents such as ethanol, water, chloroform, Diethyl ether, Dimethyl sulphoxide and results are given in Table.3

Table 3. solubility studies of Pterocarpus marsupium bark in different solvents

S. No	Solvent	soluble	Sparingly soluble	Insoluble
1	Ethanol	□	-	-
2	Water	□	-	-
3	Chloroform	-	□	-
4	Diethyl ether	-	□	-
5	Dimethylsulphoxide	-	□	-

The solubility studies revealed that Pterocarpus marsupium bark extract dissolves readily in water and ethanol, while it shows limited solubility in chloroform, diethyl ether, and dimethyl sulphoxide. Therefore, Pterocarpus marsupium bark extract exhibits higher solubility in polar solvents and lower solubility in non-polar solvents.

Uv Visible Spectral Analysis of Pterocarpus Marsupium Roxb Bark Extract

The Pterocarpus marsupium stock solution of concentration 500µg/mL were prepared and the UV-visible absorption spectra for the sample were taken in the range of 200 - 800nm for λmax. using double beam UV Spectrophotometer.

Figure.1 Uv- Visible absorption spectra of pterocarpus marsupium roxb bark extract

The maximum absorption of Pterocarpus marsupium Roxb bark extract was found to be at 342 nm and hence it is selected as the λmax for further studies.

Calibration Curve of Pterocarpus Marsupium Roxb Bark Extract

To construct a calibration curve, 50 – 250 µg/ml of Pterocarpus marsupium bark extract was taken & checked the linearity at 342 nm. The calibration data is shown in the Table.4

Table 4. Calibration data of Pterocarpus marsupium bark extract was measured at 342 nm using Uv – visible absorption spectroscopy

S.no	Concentration(µg/ml)	Absorbance at 342 nm
1	50	0.1066

2	100	0.3063
3	150	0.5157
4	200	0.7129

Construction of calibration curve of Pterocarpus marsupium bark

In the calibration curve, linearity was obtained between 50-250 µg/ml concentration of Pterocarpus marsupium bark and the regression value was found to be $R^2 = 0.9992$.

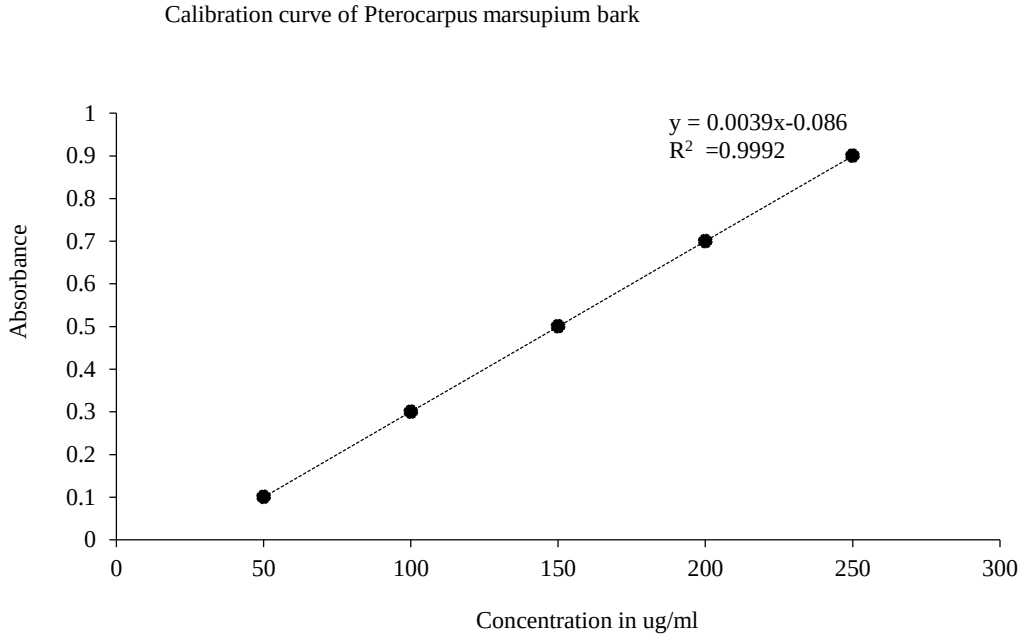

Figure 3. Calibration curve of pterocarpus marsupium bark extract

Hence, the sample of Pterocarpus marsupium bark obeys Beer Lambert's Law at the concentration between 50-250 µg/ml.

Green Synthesis of Silver Nanoparticles

A 5 mL aliquot of aqueous plant extract sample was combined with 10 mL of 1 mM aqueous AgNO3 solution drop by drop. The mixture was placed on a magnetic stirrer with continuous stirring at 120 rpm. The formation of silver nanoparticles was monitored by observing the color change of the reaction mixture, which turned colloidal brown, indicating the presence of nanoparticles.

Separation of silver nanoparticles

The colloidal solution was subjected to centrifugation at 4000 revolutions per minute (rpm) for a duration of 20 minutes. Following centrifugation, the pellets containing silver nanoparticles were carefully collected and washed extensively with deionized water to separate them from other components. Subsequently, the washed nanoparticles were dried in a desiccator to prevent moisture and dust contamination from the air. Once dried, the formulated Pterocarpus marsupium silver nanoparticles were stored in an airtight container and reserved for further evaluation studies

Characterization of Synthesized Pterocarpus Marsupium Roxb Silver Nanoparticles

Visual examination

In the experiment, the addition of Pterocarpus marsupium plant extract to beakers containing aqueous silver nitrate solution resulted in a color change from yellowish to reddish-brown.

This change in color signified the reduction of silver ions within the reaction medium, resulting in the creation of elemental silver nanoparticles sized in the nanometric range. Five formulations (F1, F2, F3, F4, and F5) of Pterocarpus marsupium silver nanoparticles were developed through the environmentally friendly synthesis method.By adding different volumes (1 to 5 mL) of bark extract to a constant 10 mL solution of 1 mM silver nitrate, the color of the solution transitioned from light yellow to colloidal brown, confirming the synthesis of silver nanoparticles. Therefore, formulation F5 was selected for further evaluation studies due to its successful formation of colloidal brown nanoparticles. nanoparticles by green synthesis method

UV Visible Spectral Analysis of Pterocarpus Marsupium Silver Nanoparticles

UV-Visible spectral analysis was used to monitor the formation and completion of silver nanoparticles using a UV-Visible spectrophotometer. The reduction of silver ions in the solution was tracked by periodically sampling aliquots at intervals of 30 minutes, 210 minutes, and 24 hours. Distilled water was used as a blank during measurements taken across the wavelength range of 200 to 800 nm.

Figure. 2 uv- visible absorption spectra of pterocarpus marsupium roxb. silver nanoparticles at different time interval

The reduction of silver ions to silver nanoparticles was evidenced by a change in color from yellow to brown, which was consistent with the spectral data obtained using a UV-Visible spectrophotometer. This color change is attributed to the surface plasmon resonance (SPR) phenomenon of silver nanoparticles, characterized by an absorption peak around 429 nm, specifically observed for Pterocarpus marsupium bark silver nanoparticles

Ftir Spectroscopy of Pterocarpus Marsupium Silver Nanoparticles

FTIR spectroscopy aids in identifying the various functional groups within compounds responsible for the reduction of silver ions to silver nanoparticles and their subsequent capping or stabilization. FTIR spectra of Pterocarpus marsupium -AgNPs were obtained to analyze and identify these functional groups

Table 5. FTIR Interpretation of Pterocarpus marsupium silver nanoparticles

Materials	Test wave number cm^{-1}	Functional group assessment
Pterocarpus marsupium silver nanoparticles	3689.95	O-H stretching
	3592.54	O-H stretching
	3544.32	O-H stretching
	3450.77	O-H stretching
	3037.99	C-H bending
	1729.24	C=O stretching
	1738.89	C=O stretching
	1124.54	C-O stretching
	1159.26	C-O stretching
	1218.09	C-O stretching
	1230.63	C-O stretching
	1316.46	N-O stretching
	1323.21	N-O stretching
	1354.07	N-O stretching
	1361.79	N-O stretching
	1367.58	N-O stretching

	1373.36	N-O stretching

FTIR interpretation results are given in the above Table.5 and the spectrum of Pterocarpus marsupium silver nanoparticle was shown in the Figure. 3

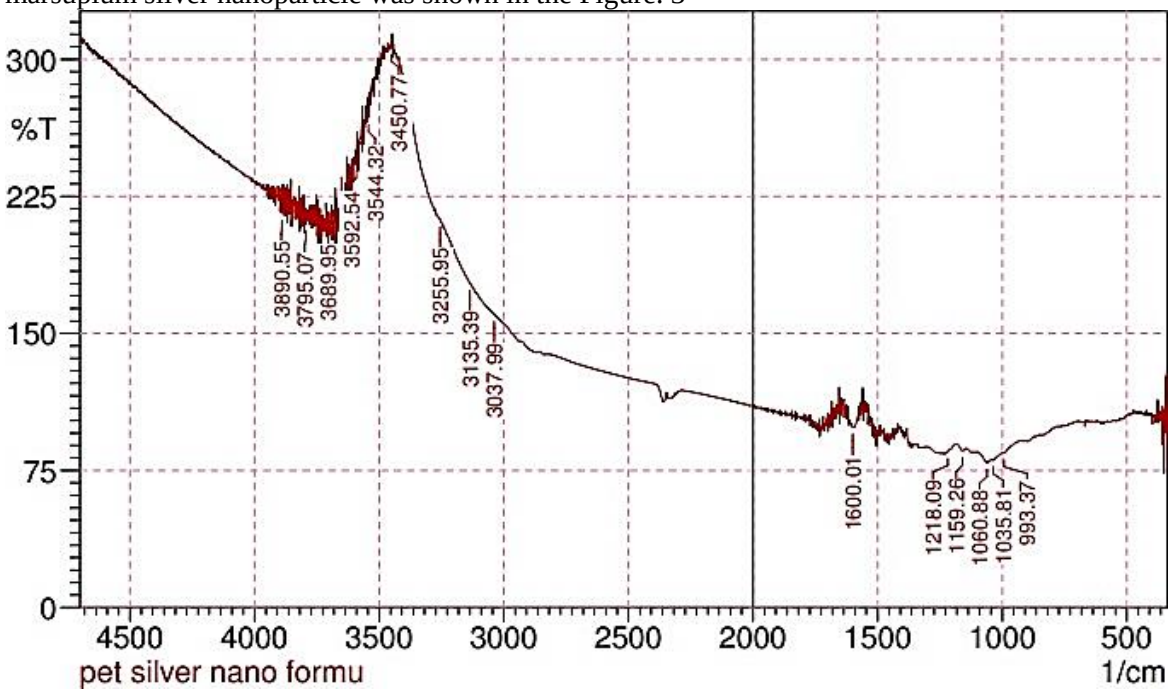

Figure 3. FTIR spectrum of Pterocarpus marsupium silver nanoparticles

Based on the spectral analysis above, it was determined that there is no incompatibility between the drug and excipients. This conclusion was drawn because the peaks observed in the drug spectrum were also present in the spectra of the drug combined with the excipients. Therefore, these excipients can be selected for the preparation of silver nanoparticles for further analysis. The presence of hydroxyl, carboxyl, and phenolic groups was confirmed in the spectra.

Drug entrapment efficiency

Drug entrapment efficacy measures the drug loading capacity of the system. It can be determined by analyzing the supernatant liquid of Pterocarpus marsupium bark silver nanoparticles after centrifugation using UV spectrophotometry at 429 nm.

Table 6. drug entrapment efficacy of five formulation

S. No	Formulation code	% Drug entrapment
1	F1	72%
2	F2	78%
3	F3	81%
4	F4	86%

The drug entrapment efficiency of Pterocarpus marsupium Roxb. silver nanoparticles was found to be 72% for F1, 78% for F2, 81% for F3, 86% for F4, and 95% for F5. The highest drug entrapment was observed in formulation F5, with an efficiency of 95%, and it displayed a colloidal brown color, indicating the formation of Pterocarpus marsupium silver nanoparticles. Based on these results, formulation F5, which had the highest drug entrapment capacity, was selected for further evaluation studies.

Scanning Electron Microscopy (Sem)

SEM was used to analyze the surface morphology and size details of the silver nanoparticles. Images were captured at various magnifications, including 1500X, 3000X, 5000X, and 15000X.

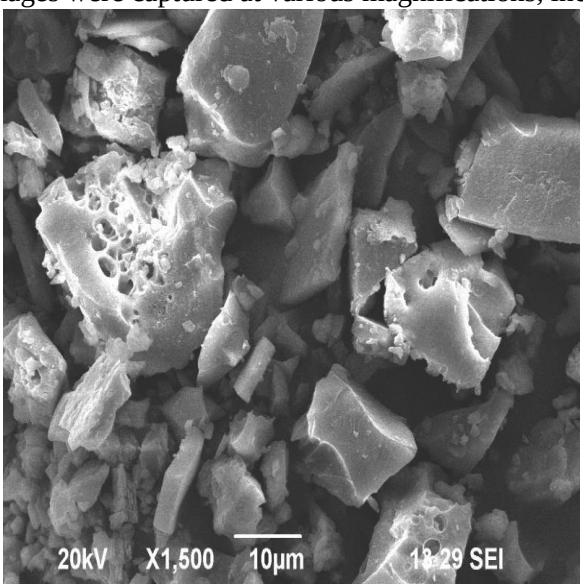

Figure 4. SEM analysis of Pterocarpus marsupium Roxb. Silver nanoparticle of 1500X magnification

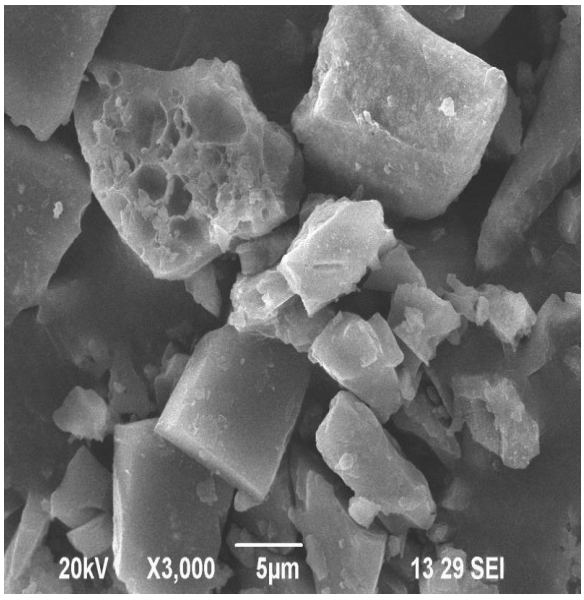

Figure 5. SEM analysis of Pterocarpus marsupium Roxb. Silver nanoparticle of 3000X magnification

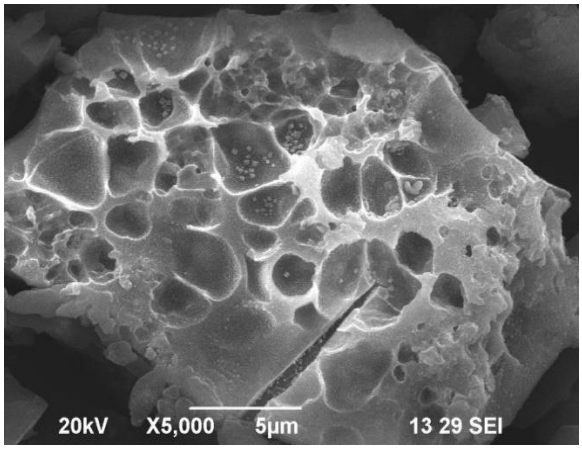

Figure 6. SEM analysis of pterocarpus marsupium roxb. silver nanoparticle of 5000x magnification

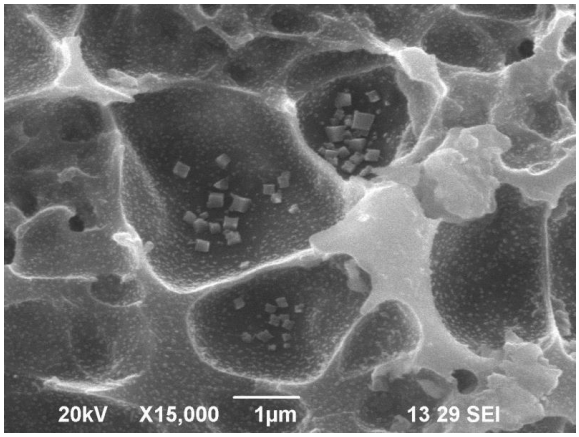

Figure 7. SEM analysis of Pterocarpus marsupium Roxb. Silver nanoparticle of 15000X magnification

The SEM micrographs of Pterocarpus marsupium silver nanoparticles revealed that the synthesized nanoparticles were polydisperse, spherical, and highly distributed with some aggregation. The SEM images confirmed the formation of silver nanostructures using the aqueous extract of Pterocarpus marsupium bark.

PARTICLE SIZE MEASUREMENT

The average particle size (z-average) and polydispersity index (PDI) of Pterocarpus marsupium silver nanoparticles were measured using dynamic light scattering with a zeta size analyzer from Malvern Instruments.The analysis revealed an average particle size (z-average) of 36.44 nm. The particle size analysis indicated the presence of nanoparticles with a PDI value of 0.825 and an intercept of 0.781.

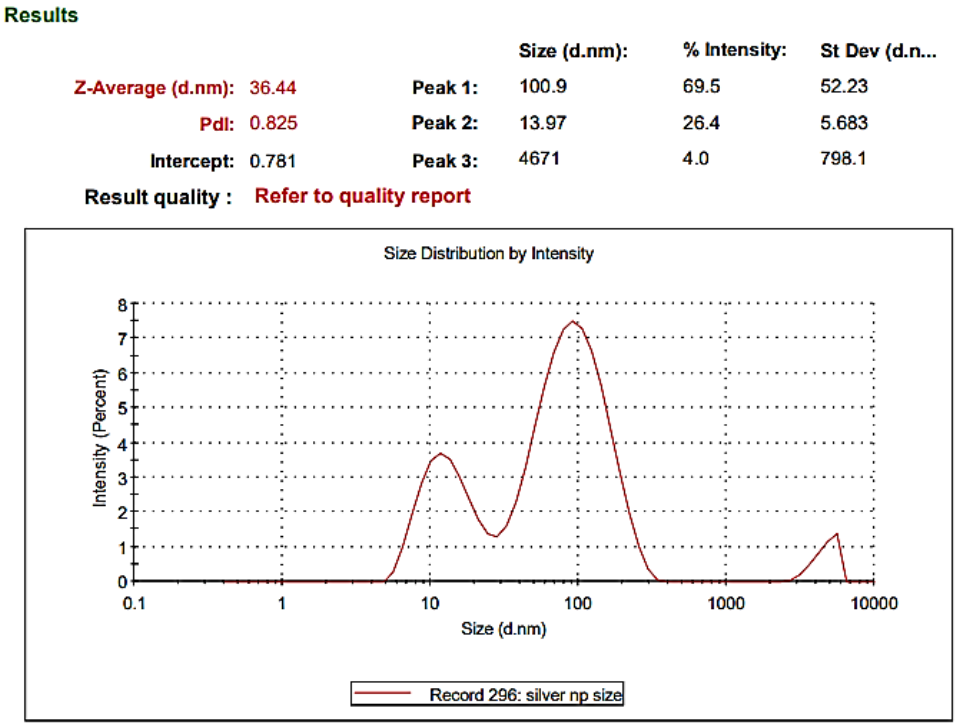

Figure 8. Particle size measurement of Pterocarpus marsupium silver nanoparticles

Zeta Potential

The zeta potential was determined using a Malvern zeta-sizer instrument to characterize the surface charge of the particles and the stability of the solution. For Pterocarpus marsupium silver nanoparticles, the zeta potential was found to be -22.6 mV with a peak area intensity of 100. These values indicate that the formulated Pterocarpus marsupium silver nanoparticles are stable. The zeta potential distribution of the silver nanoparticles is depicted in the analysis. Figure.9

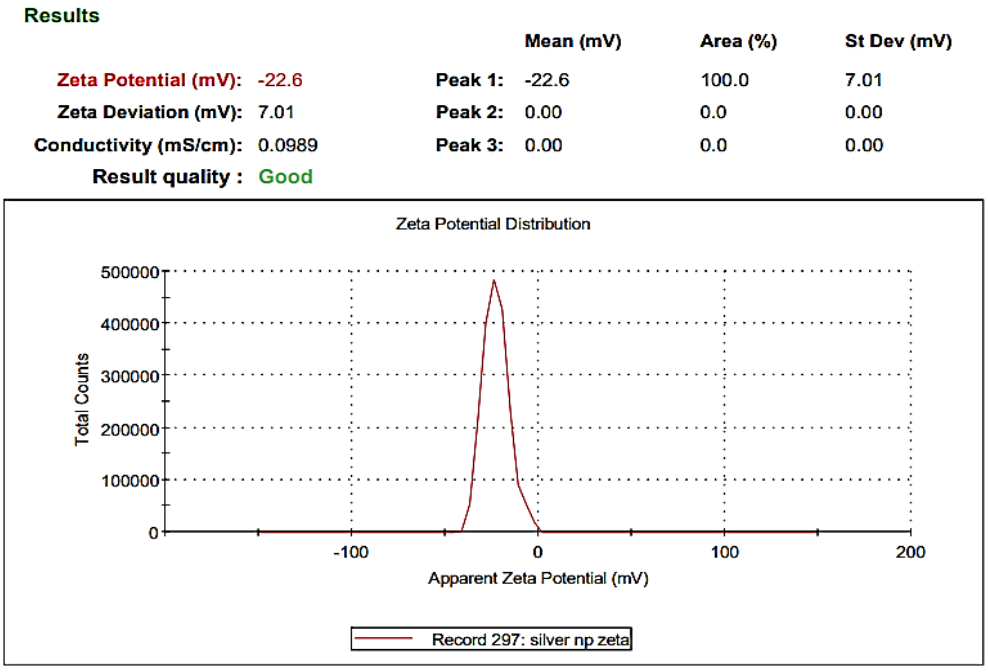

Figure 9. Determination of zeta potential of Pterocarpus marsupium silver nanoparticles

In vitro drug release study

The in vitro drug release from the nanoparticle was measured using the dialysis bag diffusion method in a phosphate buffer (pH 7.4). The amount of drug released was observed at various time intervals, specifically at 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, and 24 hours.

Table 7. In vitro drug release study of Pterocarpus marsupium Roxb silver Nanoparticles

Time (in hrs)	Log time	Square root of time	Cumulative amount of drug released	Log Cumulative amount of drug released	Cumulative% Of drug released	Log cumulative% of drug released
0	0.000	0.000	0	0.000	0	0.000
1	0.000	1.000	27.1	1.433	13.55	1.132
2	0.301	1.414	41.9	1.622	20.95	1.321
3	0.477	1.732	56.21	1.750	28.10	1.449
4	0.602	2.000	72.98	1.863	36.49	1.562
5	0.699	2.236	91.11	1.960	45.55	1.658
24	1.380	4.899	181.91	2.260	90.95	1.959

The in vitro drug release data were fitted into various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The different profiles were evaluated based on the correlation coefficient (R2). The model with the highest correlation coefficient indicates the most suitable mathematical model for describing the drug release kinetics

Drug Release Data Fitted to Various Kinetic Models

Zero order

Time vs Cumulative percentage release were plotted and shown in Figure.10 From the graph R^2 value were found to be 0.8966

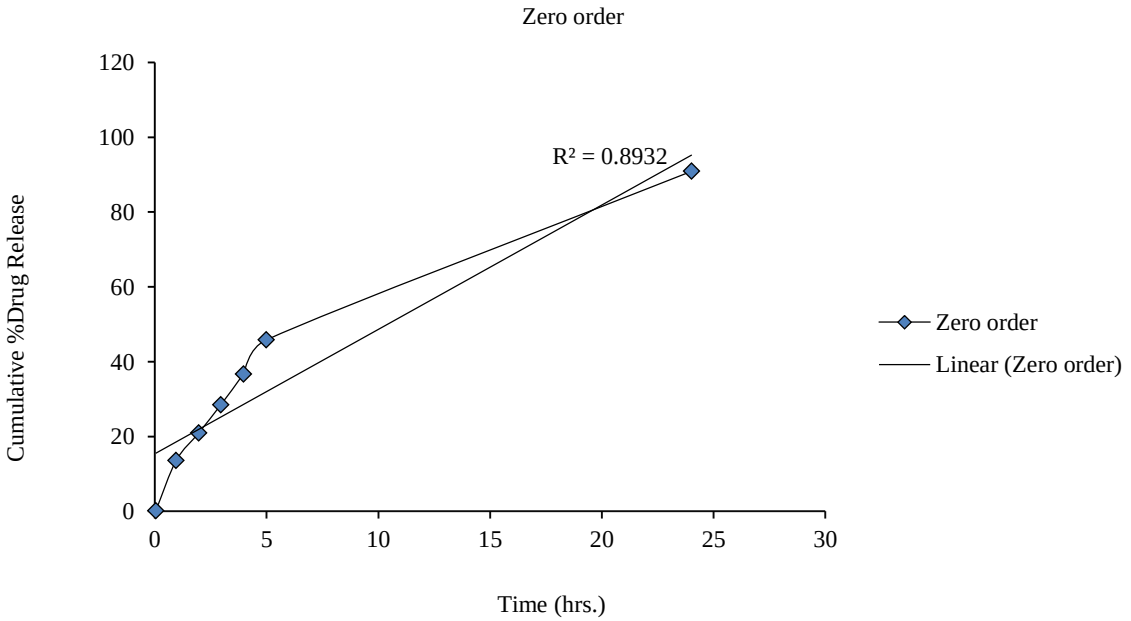

Figure 10. Zero order plot

First Order

Time vs log cumulative percentage were plotted and shown in Figure.11 From the graph R^2 value found to be 0.3223

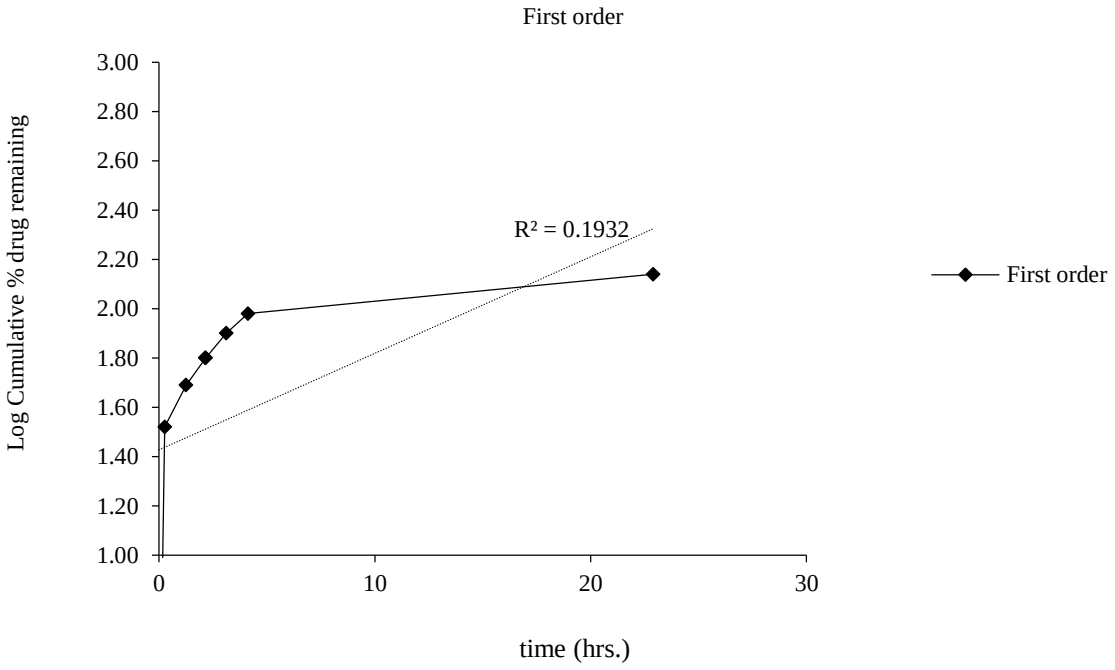

Figure 11. First order plot

Higuchi's plot

Square root of time vs cumulative percentage release and shown in the Figure.12 From the graph R^2 value was found to be 0.9877

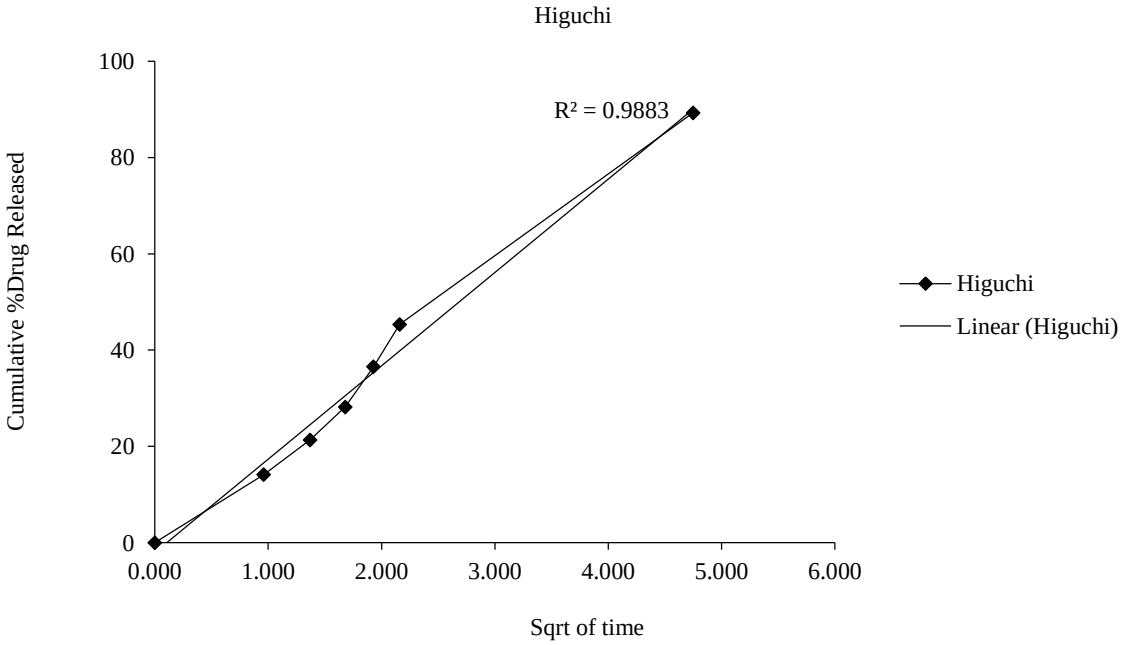

Figure 12. Higuchi plot

Korsmeyer Peppas

Log time vs log cumulative percentage and shown in the Figure. 13 From the graph was found to be 0.9692 and n value is 0.167

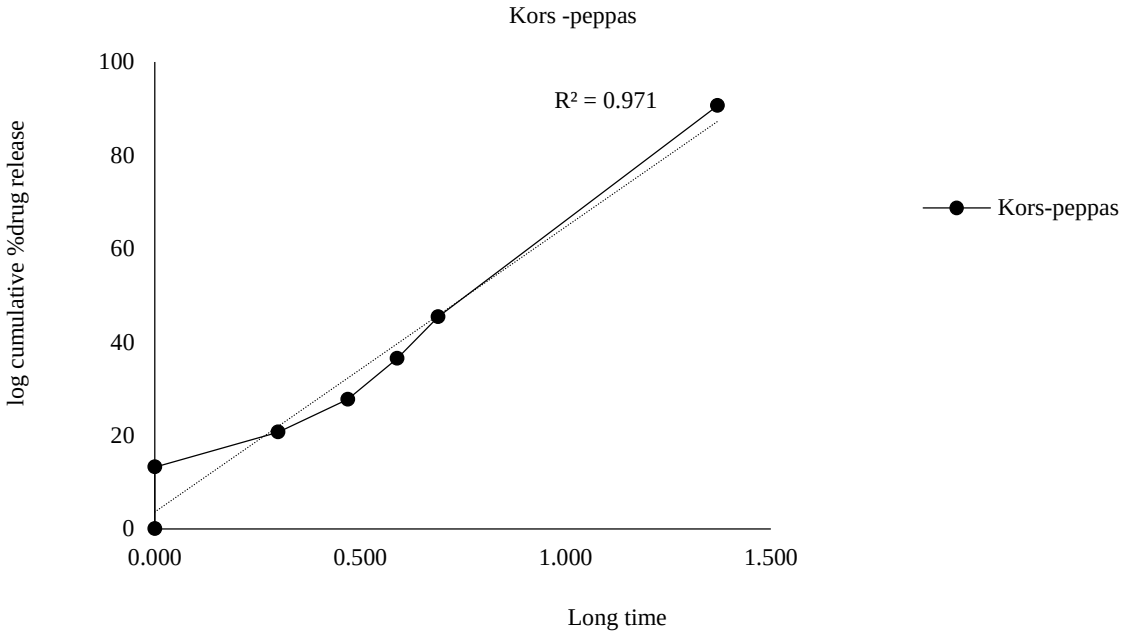

Figure 13. Korsmeyer peppas

Table 8. R^2 value obtained for various kinetic models

Formulation	**Correlation Coeffeicient (R^2)**				
Pterocarpus marsupium silver nanoparticles	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer peppas	
	0.8966	0.3223	0.9877	R^2	n
				0.9692	0.167

The release kinetic analysis indicated that the data best fit the Higuchi model, with an R2 value of 0.9877. To further understand the drug release mechanism, the data were also analyzed using the Korsmeyer-Peppas equation, which helps identify the type of diffusion involved. The Korsmeyer-Peppas plot exhibited good linearity, and the release exponent "n" was determined to be 0.167, indicating that the formulation followed Fickian diffusion kinetics.

Based on the release kinetics data, it can be concluded that the Pterocarpus marsupium Roxb. silver nanoparticle formulation showed the highest correlation (R2) with the Higuchi model, followed by the Korsmeyer-Peppas, zero-order, and first-order models.

SUMMARY & CONCLUSION

Nanotechnology is progressing swiftly owing to its distinct capabilities and broad range of applications. Within nanomedicine, researchers are investigating the use of nanotechnology to prevent, treat, diagnose, and manage various diseases. Diabetes mellitus, a cluster of metabolic conditions marked by high blood glucose levels resulting from insulin deficiency or resistance, is a significant focus area. With diabetes on the rise, it has emerged as a crucial area of scientific inquiry.

Silver nanoparticles with well-defined nanostructures were synthesized using a green chemistry approach with Pterocarpus marsupium Roxb. bark extract. This method is particularly important in nanomedicine due to its numerous advantages, offering a cost-effective and environmentally friendly alternative to traditional chemical and physical synthesis methods.

The study aims to investigate the antidiabetic effects of green-synthesized silver nanoparticles derived from the aqueous extract of Pterocarpus marsupium. Preformulation studies revealed that the bark is soluble in polar solvents and sparingly soluble in non-polar solvents. The UV-visible

absorption spectrum for the bark extract showed a λmax at 342 nm. The calibration curve of the bark extract was linear, with an R2 value of 0.999 for concentrations between 50-250 µg/ml.

Green synthesis of silver nanoparticles was conducted using the bark extract and a silver nitrate solution. The formation of silver nanoparticles was indicated by a color change to dark brown, confirming the reduction of silver ions. UV-visible spectroscopy revealed an absorption peak at 429 nm, confirming the presence of silver nanoparticles. FTIR analysis identified hydroxyl, carboxyl, and phenolic groups in the bark extract as responsible for capping and stabilizing the nanoparticles.

The drug entrapment efficacy test showed a 95% efficacy rate. SEM analysis revealed that the synthesized nanoparticles were polydisperse and spherical. Particle size distribution analysis using a zeta sizer showed an average particle size of 36.44 nm, with a polydispersity index of 0.825 and an intercept of 0.781. Zeta potential measurement was -22.6 mV, indicating stable nanoparticles.

In vitro drug release data were collected at various time intervals and analyzed using zero-order, Higuchi, and Korsmeyer-Peppas models. The Higuchi release model provided the best fit with an R2 value of 0.9877, and the Korsmeyer-Peppas model showed good linearity, with a release exponent (n) of 0.167, indicating Fickian diffusion kinetics.

This study showcases the environmentally friendly production of silver nanoparticles through the use of Pterocarpus marsupium bark extract, serving as both a reducing agent and stabilizer. The nanoparticles were extensively characterized using methods such as visual inspection, UV-visible spectroscopy, FTIR spectroscopy, spectroscopy, drug entrapment efficacy testing, particle size determination, zeta potential measurement, SEM analysis, in vitro antidiabetic study, and in vitro drug release kinetics study, were employed.

Studies in the field of diabetes mellitus suggest that patients who use both herbal medicines and conventional synthetic drugs may encounter interactions between the two. Therefore, this research aimed to explore potential pharmacokinetic interactions involving green-synthesized Pterocarpus marsupium silver nanoparticles and the antidiabetic medications metformin and glimepiride using equilibrium dialysis.

The study evaluated changes in the percentage of protein binding of metformin and glimepiride when administered individually and in combination with silver nanoparticles over time. These changes affected drug concentrations at various intervals. Scatchard plots were employed to analyze the number of binding sites and affinity constants involved in these interactions.The interaction studies revealed an increase in free drug concentration of both metformin and glimepiride in the presence of the silver nanoparticles.

Therefore, it is advised to refrain from simultaneously administering Pterocarpus marsupium silver nanoparticles with metformin and glimepiride, as it may affect the pharmacokinetic behavior of these drugs.

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