

Green synthesis of AgNPs using Rauvolfia tetraphylla leaf extract: Invitro antidiabetic potential and characterization using FTIR, UV-Vis spectrophotometry and SEM analysis

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Research Article

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Abstract

Diabetes mellitus is a serious health concern and the synthetic drugs used are effective with adverse effects. In this glimpse, plants become indispensable due to their distinctive physical, chemical and biological characteristics to counteract their negative effects. *Rauvolfia tetraphylla* leaves holds the essential phytochemical that plays crucial role in the management of diabetes. The present research aims to investigate the antioxidant capacity, antidiabetic property (α -amylase inhibition) using aqueous leaf extract and green synthesized silver nanoparticles. Metallic nanoparticle are essential in achieving target delivery of plant metabolite also improve insulin sensitivity. The silver nanoparticles were characterized by FT-IR, UV-Vis spectroscopy and SEM analysis. The aqueous leaf extract and biosynthesized AgNPs both displayed significant enzyme inhibition, and the AgNPs proved to be potent and more efficacious emphasizing their existence in disease management. FT-IR spectra revealed functional groups accountable for nanoparticle reduction and stabilization, and UV-Vis spectroscopy indicated absorption peaks at 230–250 nm and 350 nm. SEM images revealed rod-shaped and needle-like nanostructures with a crystalline morphology, indicates viable phytochemical-mediated synthesis. The increased bioactivity of AgNPs, resulting from their larger surface area and stabilization by plant biomolecules, suggests their potential as novel agents for treating diabetes and a potent antioxidant.

1.Introduction

Diabetes mellitus (DM) emerged as widespread chronic medical condition and their complication continues to pose significant burden on population and affects life expectancies [1]. Meanwhile, many researches are concentrating on therapeutic regimen of other non-communicable disease. Inevitably DM is progressively increased over the time. International diabetes federation estimated the incidence and its projection by 2045 as shown in Fig. 1, where India accounts for considerable number of cases [2, 3].

In addition to synthetic drugs, plants based pharmaceuticals are indispensable source of new therapeutic compounds that paves way for state-of-art in pharmacology field. Plant - derived substances has gained attention especially in the management of diabetes mellitus due their affordability, widespread availability, fewer side effect and also provide distinctive physiological effects on humans [4]. Moreover, plant derived metabolites has ability to inhibit carbohydrate – hydrolysing enzyme such as α -amylase and α -glucosidase. This inhibition slows the carbohydrate digestion and glucose digestion, that aids in post prandial blood sugar management [5]. Based on this, the plant *Rauvolfia tetraphylla* contains significant alkaloid and their therapeutics plays substantial role in economy of developing countries like India. Predominantly these plants were used in the treatment of snake bite, later scientific community have started research on treatment of various other ailments [6].

Emergence of nanotechnology has become pivotal in pharmaceutical sector. In recent years green synthesis of nanoparticles emerged as a breakthrough strategy in the management of diabetes due to its eco-friendly nature and sustainability [7]. This is the first reported study on *Rauvolfia tetraphylla* aqueous leaf extract and green synthesized silver nanoparticles (AgNps) utilized to investigate

antioxidant, invitro antidiabetic activity (α -amylase inhibition potential) and characterization of AgNps using FTIR ,UV-Vis spectrophotometry and SEM analysis. Combination of plant based compounds and silver nanoparticle could bridge the major lacunae in disease management. Green synthesized silver nanoparticles offer a promising sustainable, affordable alternative to conventional therapeutics.

2. Materials and Methods

2.1 Collection of plant material

Fresh leaves of *Rauvolfia tetraphylla* were procured from the outskirts of Tirupattur district, Tamilnadu, India. The leaves were washed under running tap water and with sterile distilled water. The leaves were shade dried and ground into coarse powder. The samples were stored in airtight container for further use.

2.2 Preparation of aqueous plant extract

Aqueous plant extract was prepared using [8] with minor modification, 5g of coarsely powdered plant leaves were extracted by 100ml of distilled water at room temperature for 24hrs, then the suspension was filtered using Whatman filter paper. And the supernatant was used for further analysis.

2.3 Qualitative phytochemical screening

Phytochemical analysis was performed to identify the presence of bioactive compounds in aqueous leaf extract of *Rauvolfia tetraphylla*. Standard chemical test were done as per the procedure described by [9, 10] with slight modifications.

2.4. Invitro assessment of antioxidant potential

Invitro antioxidant activity [11] of *Rauvolfia tetraphylla* leave extract was performed using Phosphomolybdenum complex formation technique. 5ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate) were transferred into fresh test tubes. In each test tubes, 1 ml of plant extract was added in varying concentration and incubated for 90 minutes at 95°C in water bath to promote formation of complex. The samples are cooled to room temperature. The absorbance of resulting solution is measured at 695 nm in spectrophotometer and reagent blank as reference.

2.5. Green synthesis of silver nanoparticles

90 mL of 1 mm aqueous silver nitrate solution was mixed with 10 mL of leaf extract [12] with slight modifications. The solution mixture was placed in shaker for 24 or 48 hours to observe the colour shift from yellow to dark brown and cooled to room temperature. The aqueous silver nitrate solution was filled in petri plates, centrifuge tubes and optimal temperature was maintained overnight in a hot air oven at 20°C.

2.6. Characterization of silver nanoparticle

The green synthesized silver nanoparticles (AgNPs) from *Rauvolfia tetraphylla* leaves encompasses an eco-friendly reduction of the silver ions, wherein phytoconstituents act as reducing as well as stabilizing agents. Characterization of AgNPs includes FT-IR spectroscopy for the identification of functional groups (e.g., flavonoids, phenols) responsible for reduction and capping, UV-Vis spectroscopy for determining nanoparticle formation by observing surface plasmon resonance peaks between 400–450 nm, and SEM analysis for characterizing morphology and particle size distribution [6].

2.7. α -Amylase Inhibition Assay

2.7.1. Inhibition with aqueous plant extract

500 μ L of plant extract and 500 μ L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α -amylase solution (0.5 mg/mL) were incubated for 10 minutes at 25°C. After pre-incubation, 500 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5-second intervals [11]. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 1.0 Mol of a di-nitro salicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted by adding 10 mL of distilled water and the absorbance was measured at 540 nm using a UV/VIS spectrophotometer.

2.7.2. Inhibition with green synthesis of silver nanoparticles

500 μ L of silver nanoparticles and 500 μ L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α -amylase solution (0.5 mg/mL) were incubated for 10 min at 25°C [13]. After pre-incubation, 500 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5-second intervals. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 1.0 Mol of di-nitro salicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature [12]. The reaction mixture was diluted by adding 10 mL of distilled water and the absorbance was measured at 540 nm using a UV/VIS spectrophotometer.

3. Results and Discussion

3.1. Identification of *Rauvolfia tetraphylla*

Rauvolfia tetraphylla leaves were collected from their natural habitat, authenticated using taxonomic keys, and confirmed by morphological features. The plant material was washed, shade-dried, and powdered for further use.

3.2. Qualitative phytochemical screening

The phytochemical analysis of *Rauvolfia tetraphylla* confirmed the presence of bioactive compounds as given in Table.1 such as alkaloids, flavonoids, terpenoids, saponins, tannins, phenolics, glycosides,

steroids and carbohydrates that holds many therapeutic applications. Since ancient times the leaves were used in various treatments like hypertension, anxiety, microbial infection and many more.

3.3. Antioxidant Activity

The Phosphomolybdenum complex formation assay measured the antioxidant activity of *Rauvolfia tetraphylla* at various concentrations. Results revealed concentration-dependent activity, where the lowest concentration showed the highest inhibition (87.5%) as shown in Table .2. The antioxidant activity was compared to ascorbic acid, confirming its high free radical scavenging potential. This is also represented in Fig. 2 plotted between absorbance at 695 nm and varying concentration of plant extract. The presence of significant amount of antioxidant is paramount because oxidative stress play a significant role in pathogenesis of metabolic syndrome, capable of preventing cell damage and also capable of inhibiting lipid oxidation an important factor in lifestyle diseases [14]. These results prove the competence of *Rauvolfia tetraphylla* as a natural antioxidant, showing its prospective use in therapeutics.

3.4. α -Amylase Inhibition assay

3.4.1. Plant Extract

Plant extract was introduced into α -amylase in sodium phosphate buffer in different concentrations to assess its in vitro carbohydrate break down. Tubes were incubated at 25°C for 10 minutes, and 500 μ L of 1% starch was added every 5 seconds. The process was terminated with dinitrosalicylic acid. Tubes were kept at room temperature for 5 minutes in a water bath, and absorbance was measured using a UV Spectrophotometer at 540 nm. As shown in Table.3 and Fig. 3, the lowest concentration 0.20 μ l produced highest inhibition of about 90%. Alpha amylase is one of the therapeutic target in the management of diabetes and prominent in pancreas responsible for the hydrolysis of carbohydrate [15], hence inhibiting this enzyme with plant extract will definitely aid in post prandial blood sugar by reducing the carbohydrate and glucose digestion.

3.4.2. Silver Nanoparticles

Silver nanoparticles were added to a sodium phosphate buffer solution containing α -amylase to evaluate their in vitro effect on α -amylase activity. The tubes were incubated at 25°C for 10 minutes, followed by the addition of 500 μ L of 1% starch and 1 mL of dinitrosalicylic acid. After cooling to room temperature, the absorbance was measured in a UV spectrophotometer at 540 nm shown in Table .4 and Fig .4 AgNp, a green manufacturing silver nanoparticle, showed a greater degree of inhibition than plant extract, indicating that the plant *Rauvolfia tetraphylla* exhibited the most significant level of anti-diabetic activity. As reducing and capping agent green synthesized AgNPs showed promising alpha-amylase inhibition compared to their non-nanoparticle counterparts [16], which may suppress carbohydrate breakdown and reduce postprandial glucose levels. These features render them a brilliant choice to create potent antidiabetic drug. The synergism of *R. tetraphylla* bioactive compounds and the improved bioactivity of

AgNPs [17] makes them excellent prospects for the development of novel and potentially safer therapeutic agent to manage diabetes.

3.5. Characterization of Silver Nanoparticles

3.5.1. FT-IR Analysis

The FT-IR spectrum of *Rauvolfia tetraphylla* revealed the presence of various functional groups that contributed to its phytochemical composition. As shown in Table.5 and Fig .5 Broad absorption peak at $\sim 3421\text{ cm}^{-1}$ are the representative of O–H stretching vibrations, indicating the presence of hydroxyl groups associated with phenols and flavonoids. C–H stretching at $\sim 2926\text{ cm}^{-1}$ indicates the presence of aliphatic chains. A pointed peak at $\sim 1735\text{ cm}^{-1}$ are due to C = O stretching vibrations, establishing the occurrence of carbonyl groups in esters or carboxylic acids. The band at $\sim 1614\text{ cm}^{-1}$ are due to aromatic C = C stretching, and peaks at $\sim 1400\text{--}1050\text{ cm}^{-1}$ are due to C–H bending, C–O, and C–N stretching, marking the occurrence of glycosides, ethers, and amines. Lower-frequency peaks at 875, 790, and 667 cm^{-1} are associated with aromatic out-of-plane C–H bending, thereby establishing the aromatic substitution patterns. Overall, FT-IR examination confirmed the presence of alcohols, phenols, carbonyls, aromatics and alkaloid-associated groups, thereby establishing the bioactive and medicinal value of *Rauvolfia tetraphylla*. Furthermore, peaks indicating capping and reducing agent confirms that *Rauvolfia tetraphylla* are not affected by the interaction of silver ions.

3.5.2. UV-Vis Spectroscopy

The UV-Visible absorption spectrum of *Rauvolfia tetraphylla* has two distinct high absorbance peaks as shown in Table.6 and Fig .6 at 230–250 nm and a weaker peak at around 350 nm. The high UV absorption at $\sim 230\text{ nm}$ evidenced the presence of $\pi\rightarrow\pi^*$ transitions, usually in aromatic rings, and conjugated double bonds. It reflected the presence of alkaloids, flavonoids, and phenolic compounds, usual secondary metabolites of medicinal plants. The second peak at 350 nm implies $n\rightarrow\pi^*$ transitions, which are usually the result of carbonyl groups or conjugated ones, which means there are polyphenols or glycosidic compounds. The decline in absorbance at wavelengths greater than 400 nm and the visible region implies minimal pigmentation, which means that the extract is UV-active and can show antioxidant or pharmacological activities.

3.5.3. Scanning Electron Microscopy Analysis

Scanning Electron Microscope (SEM) micrograph Fig .7 of *Rauvolfia tetraphylla* leaf extract-mediated nanostructures are heterogeneous in nature, consisting of rod-like as well as needle-like structures, indicating that the biomolecules present in the leaves are efficient reducing and stabilizing agents. The nanostructures are also observed to have high density or aggregated, sharp tips, and a crystalline-like surface, indicating ideal nucleation as well as growth during the synthesis process. Some of the spherical and irregular particles were observed scattered among the large rods, exhibiting secondary particle growth or agglomeration. SEM parameters applied for the current study were 8.00 kV accelerating voltage and 13.0 mm working distance, and secondary electron detection mode was

utilized. And the morphology was a reflection of rich phytochemical composition of *Rauvolfia tetraphylla* leaf extract, which showed strong evidence of the presence of alkaloids, flavonoids, phenolic compounds and terpenoids, that helps in the anisotropic growth of nanorods. Uniform dispersion of nanorods indicates the potential of a green synthesized nanomaterials that has high surface area and stability, which are applicable for different applications in the biomedical and catalytic domain.

4. Conclusion

The current investigation results confirms that the aqueous leaf extract of *Rauvolfia tetraphylla* and its green synthesized silver nanoparticles were proven to be ideal alternative in the management of diabetes. Green synthesized silver nanoparticles showed enhanced effect in enzyme inhibition than its non-nanoparticle counterpart and the silver particles were characterised using FTIR, UV -VIS and SEM analysis, that confirmed the presence of essential plant metabolite required for maintaining the stability and availability. Silver nanoparticle tailored plant metabolite can act on multiple pathways simultaneously by offering synergistic effect, while synthetic drugs target any single specific mechanism also creates oxidative stress, cell damage, chronic side effects, affects body's natural metabolism and also fails to mimic physiology. Addressing this issue is a major concern in management postprandial glycemic index and the utilization of green synthesised *Rauvolfia tetraphylla* would offer ideal potent agent that can combat oxidative stress, mimic natural physiology, regenerate damaged cells, restore major organs architecture and environmentally safe.

Declarations

Ethical approval

This research does not require any ethical approval.

Consent to participate

This research does not involve any human participants or animals.

Consent to publish

The authors affirm that the research does not involve human participants or their personal data in any part of this research.

Clinical Registration & clinical trial number

Clinical Registration and clinical trial number is not applicable to this work.

Author Contributions

All authors contributed to the study conception and design. Material preparation, collection and analysis were performed by [G. Mythili], [K. Divyalakshmi] and [B. Lokeshwari] under supervision of [Archana

Kalimuthu] & [Gomathi M] commented on previous versions of the manuscript. The first draft of the manuscript was written by all authors [Archana Kalimuthu, G. Mythili, K. Divyalakshmi and B. Lokeshwari,]. All the authors read and worked meticulously to draft the final version of this manuscript.

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Competing Interest

The authors have no competing interest.

Availability of data and materials

All necessary data of this research has been enclosed with this manuscript.

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 22. Statements & Declarations

Tables

Table .1 Qualitative phytochemical analysis of *Rauvolfia tetraphylla*

S. No	Phytochemical Tests	Results
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Terpenoids	Positive
4	Saponins	Positive
5	Tannins	Positive
6	Phenolic Compounds	Positive
7	Glycosides	Positive
8	Steroids	Positive
9	Carbohydrates	Positive
10	Proteins/Amino Acids	Negative

The qualitative phytochemical analysis of *Rauvolfia tetraphylla* revealed the occurrence of rich content of phytochemicals of possible pharmacological importance. Such compounds are reported to exhibit medicinal activities like antimicrobial, anti-inflammatory, antioxidant, and cardiovascular effects. The protein and amino acid test was found to be negative for their presence or low concentration in the extract. The finding is consistent with previous reports on *Rauvolfia* species, validating its use as a traditional medicinal herb [18].

Table .2 Antioxidant activity of *Rauvolfia tetraphylla* aqueoues leaf extract

Test Tubes	Plant Extract	Reagents	OD Value (695 nm)	% Inhibition
1	Control	5 ml	0.03	100
2	0.5 ml	5 ml	0.12	87.5
3	1 ml	5 ml	0.21	78.5
4	1.5 ml	5 ml	0.23	76.5
5	2 ml	5 ml	0.51	48.5
6	2.5 ml	5 ml	0.53	47

Table .3 Alpha amylase inhibition activity of *Rauvolfia tetraphylla* aqueous leaf extract

Test Tubes	Concentrations	OD Value (540 nm)	% Inhibition
1	Control	0.03	100
2	200 µl	0.10	90
3	400 µl	0.11	88.5
4	600 µl	0.13	87
5	800 µl	0.14	86
6	1000 µl	0.20	79.5

Table .4 Alpha amylase inhibition activity of green synthesized AgNPs

Test tubes	Concentrations	OD value (540 nm)	% Inhibition
1	Control	0.00	100
2	200	0.10	97.5
3	400	0.11	94
4	600	0.13	89.5
5	800	0.14	81.5
6	1000	0.20	78.5

Table .5 FT-IR Spectral Peaks and Functional Group Analysis of *Rauvolfia tetraphylla*

S. No	Peak Position (cm ⁻¹)	Functional Groups	Type of Vibration	Presence of Compounds
1	~3421 cm ⁻¹	-OH (Hydroxyl group)	O-H stretching (broad)	Alcohols, phenols, polyphenols, flavonoids
2	~2926 cm ⁻¹	-CH (Aliphatic C-H)	C-H stretching	Alkanes (lipids, fatty acids)
3	~2357 cm ⁻¹	-C≡C / CO ₂	Possibly atmospheric CO ₂	Trace contamination or environmental CO ₂
4	~1735 cm ⁻¹	-C=O (Carbonyl group)	C=O stretching	Aldehydes, ketones, esters, carboxylic acids
5	~1614 cm ⁻¹	C=C (Aromatic ring)	Aromatic C=C stretching	Flavonoids, alkaloids, phenolic compounds
6	~1411 cm ⁻¹	-CH ₂ / -CH ₃ (alkyl groups)	C-H bending	Aliphatic chains or methyl groups
7	~1242 cm ⁻¹	C-O-C or C-N	C-O or C-N stretching	Esters, ethers, and alkaloids
8	~1033 cm ⁻¹	C-N or C-O	C-N/C-O stretching	Amines or glycosides
9	~875, 790, 667 cm ⁻¹	Aromatic C-H (out of plane)	Aromatic bending vibrations	Aromatic compounds (mono- or di-substituted benzene rings)

Table .6 UV-Visible Spectral Analysis of *Rauvolfia tetraphylla*

S. No.	Peak Wavelength (nm)	Absorbance	Type of Transition	Functional Groups / Compounds
1	~230–250 nm	~2.5	$\pi \rightarrow \pi^*$ transition	Aromatic compounds, flavonoids, alkaloids (conjugated rings)
2	~350 nm	~0.55	$n \rightarrow \pi^*$ transition	Carbonyl groups, phenolic acids, and polyphenols

Figures

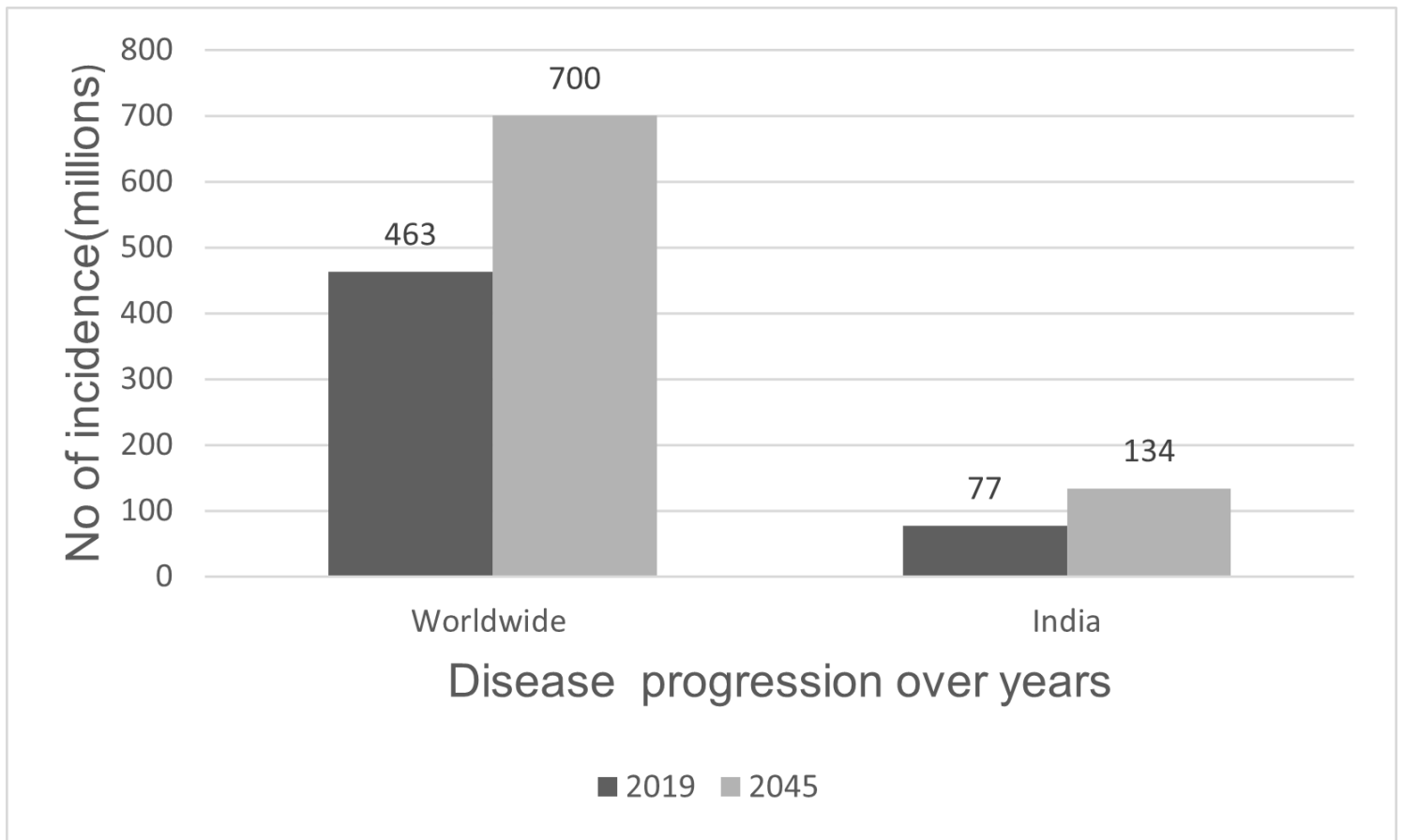


Figure 1

Global incidence and projection of diabetes mellitus incidence

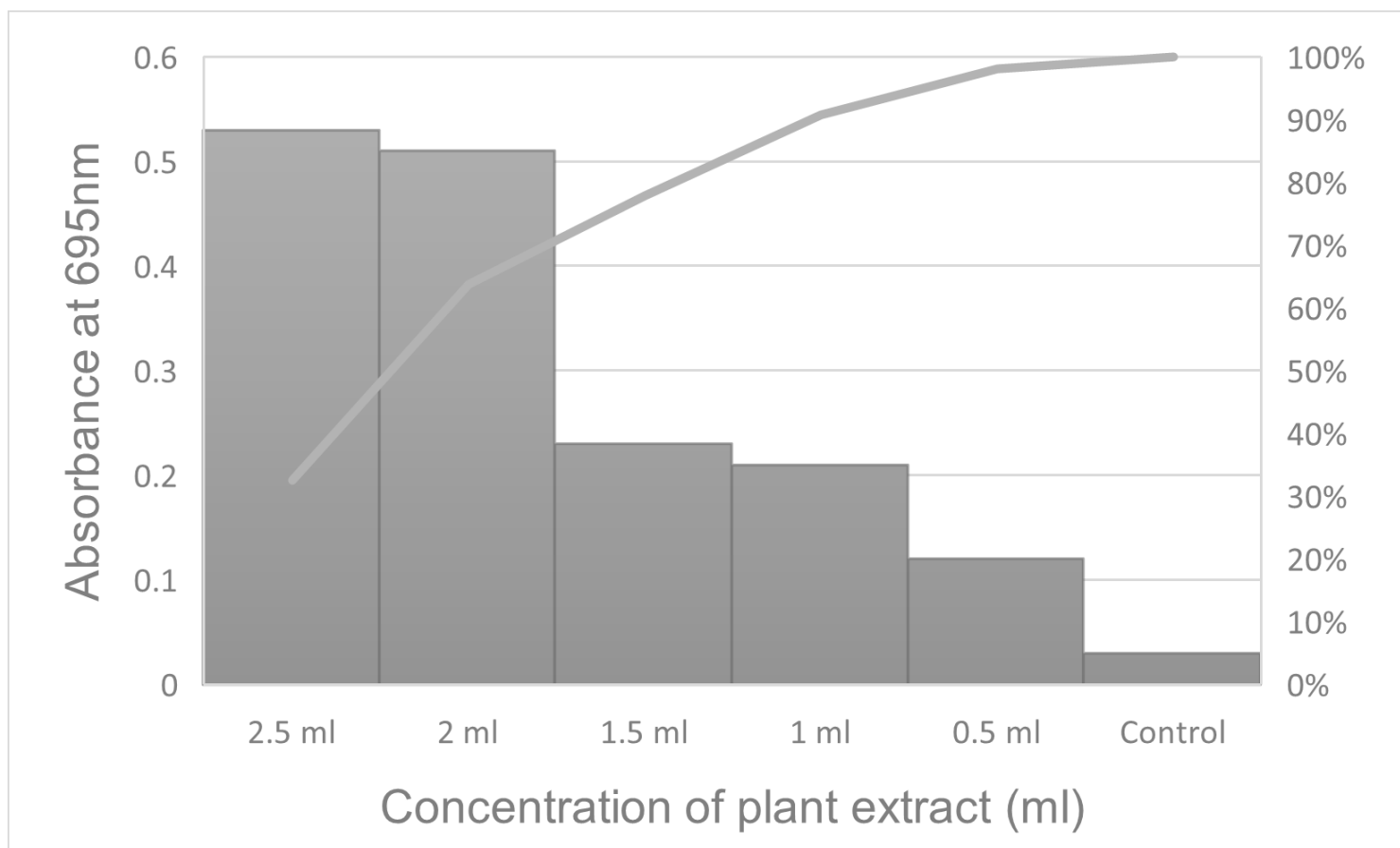


Figure 2

Antioxidant activity showing the %inhibition capacity of *Rauvolfia tetraphylla* aqueoues leaf extract. The antioxidant activity of *Rauvolfia tetraphylla* extract at different concentrations in a spectrophotometric assay at 695 nm. The blank control tube exhibited an optical density (OD) of 0.03 with 100% inhibition as the reference. When the concentration of the plant extract was raised from 0.5 ml to 2.5 ml, values of OD (from 0.12 to 0.53), signifying decreased scavenging efficiency, and percentage inhibition fell from 87.5% to 47%. This reveals that lower concentrations of the extract have higher antioxidant activity, perhaps because of bioactive entities such as phenolics and flavonoids having higher free radical scavenging activity. Results were compared with earlier research by [12], where *Rauvolfia tetraphylla* exhibited higher antioxidant activity and making *Rauvolfia tetraphylla* leaf extracts as a natural antioxidant source towards the optimization of concentration levels for optimal therapeutic efficiency.

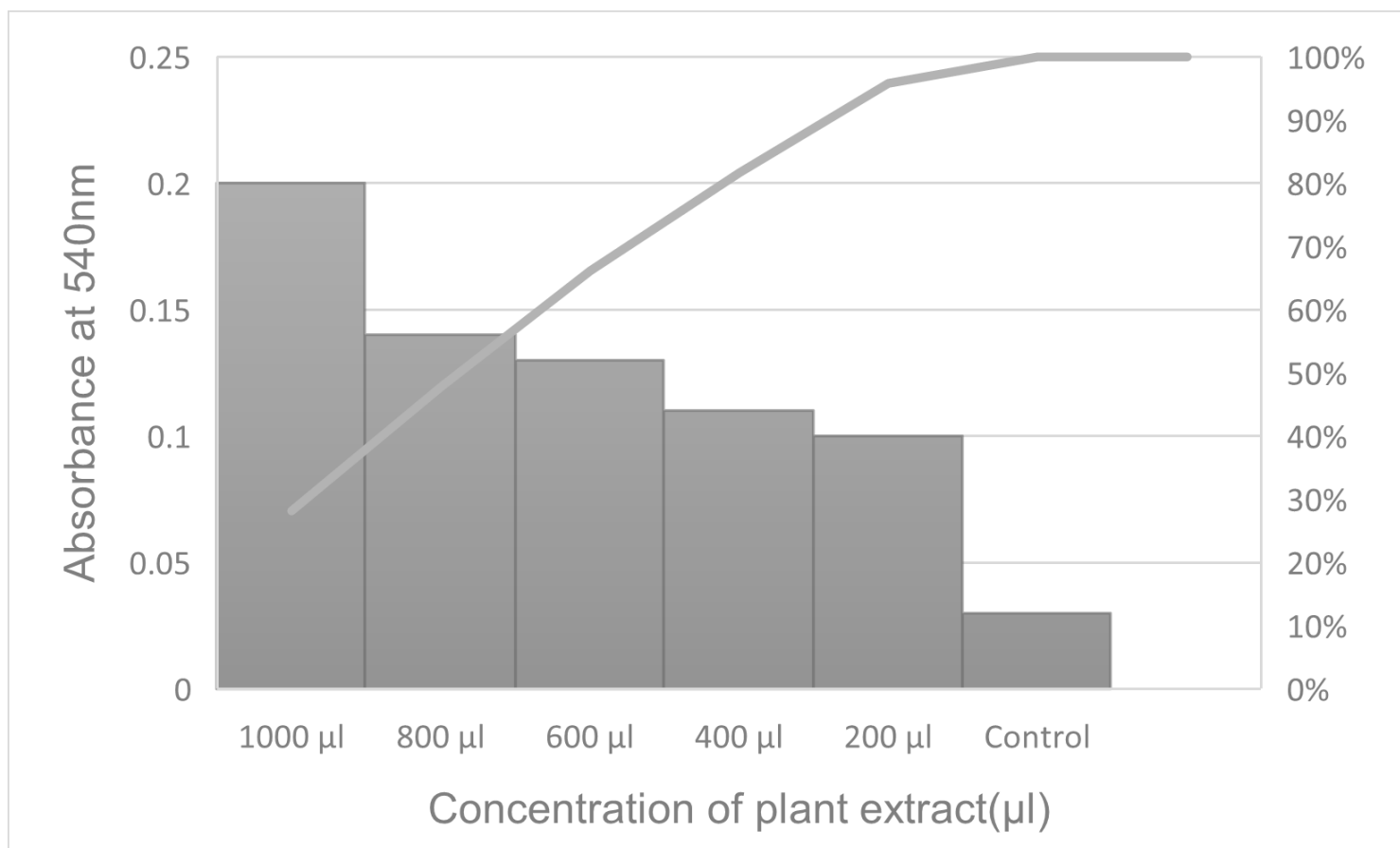


Figure 3

Alpha amylase inhibition activity of *Rauvolfia tetraphylla* aqueous leaf extract. Represents the inhibition assay of *Rauvolfia tetraphylla* extract of different concentrations, spectrophotometrically calculated at 540 nm. The control (no extract) had an OD reading of 0.03, with 100% inhibition considered as the control. As the extraction concentration was raised from 200 μl to 1000 μl, the readings of OD grew cumulatively (0.10 to 0.20), while the percentage inhibition fell (90% to 79.5%), indicative of concentration-dependent reduction in α-amylase inhibitory activity. It suggests that the lower extracts of the plant might be more effective in inhibiting α-amylase, an enzyme linked with carbohydrate metabolism and diabetes management. The flavonoids that have been reported to have enzyme-inhibitory activity as reported in [19]. The finding indicates *Rauvolfia tetraphylla* has promising antidiabetic active components.

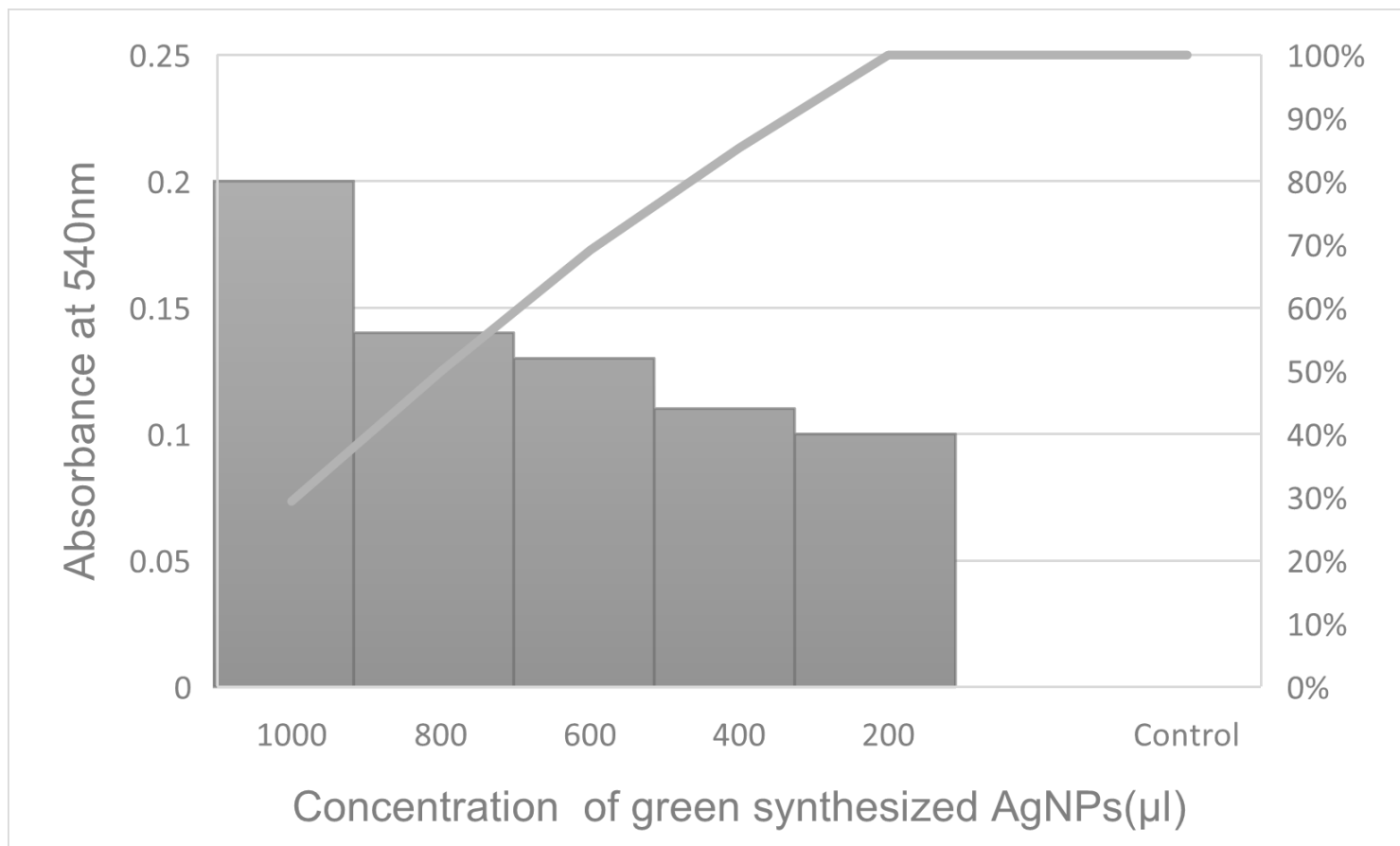


Figure 4

Alpha amylase inhibition activity of green synthesized AgNPs. Illustrates the α -amylase inhibition potential of silver nanoparticles (AgNPs) at different concentrations, spectrophotometrically determined at 540 nm. The control tube containing no AgNPs exhibited total inhibition (100%) with an OD value of 0.00, which was taken as the reference. With increasing concentrations of AgNPs from 200 to 1000 μ l, OD values increased (0.10 to 0.20), but percentage inhibition levels reduced (97.5% to 78.5%), which suggests a concentration-dependent reduction in inhibitory efficiency against enzymes. It means that reduced concentrations of AgNPs have greater α -amylase inhibitory action in diabetes through the inhibition of carbohydrate digestion. The results are consistent with the existing research by [20], who showed the bioactive activity of silver nanoparticles derived from plant sources for their use in biomedical and therapeutic applications.

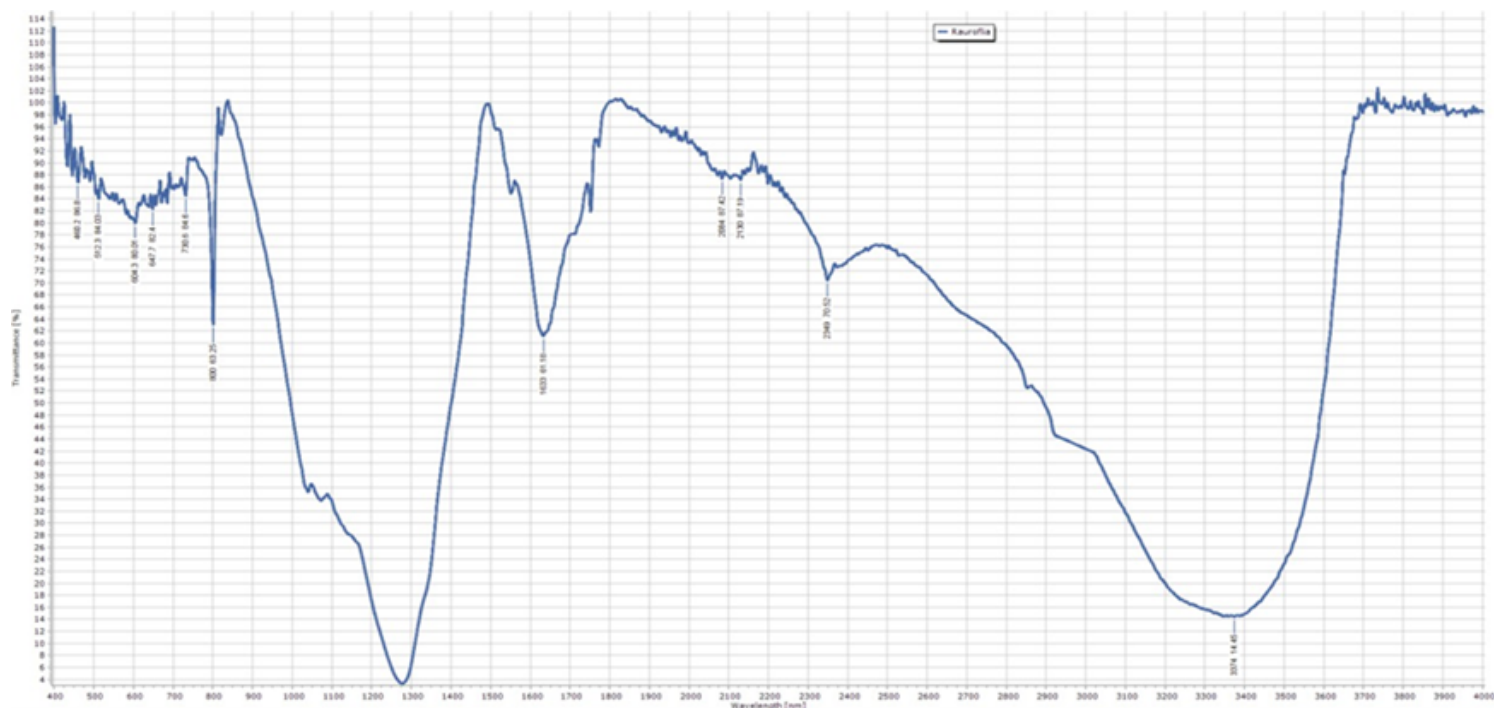


Figure 5

Fourier Transform–Infrared Spectrophotometer analysis. This provides FT-IR spectral data of *Rauvolfia tetraphylla* with major functional groups and their respective vibrational modes. The broad band at $\sim 3421\text{ cm}^{-1}$ indicates the presence of hydroxyl (-OH) groups, typical of alcohols, phenols, and flavonoids, well recognized to be linked with antioxidant activity. Aliphatic C–H stretching at $\sim 2926\text{ cm}^{-1}$ indicates the presence of lipids or fatty acids, and a strong carbonyl (C=O) band at $\sim 1735\text{ cm}^{-1}$ suggests the presence of aldehydes, ketones, or esters, which are compounds commonly linked with bioactivity. Aromatic C=C stretching at $\sim 1614\text{ cm}^{-1}$ and out-of-plane bending vibrations ($\sim 875\text{--}667\text{ cm}^{-1}$) also indicate the presence of flavonoids and alkaloids, by the medicinal nature of the plant. Appearance of C–O/C–N stretching ($\sim 1242\text{ cm}^{-1}$ and $\sim 1033\text{ cm}^{-1}$) indicates potential glycosides or alkaloids, in keeping with its richness in phytochemicals. The results in previous studies on bioactive plant constituents, e.g., those of [11], highlighted the role of functional groups in antioxidant activity. FT-IR analysis thereby detects *Rauvolfia tetraphylla* as a phytochemical reservoir of rich phytochemicals with therapeutic significance.

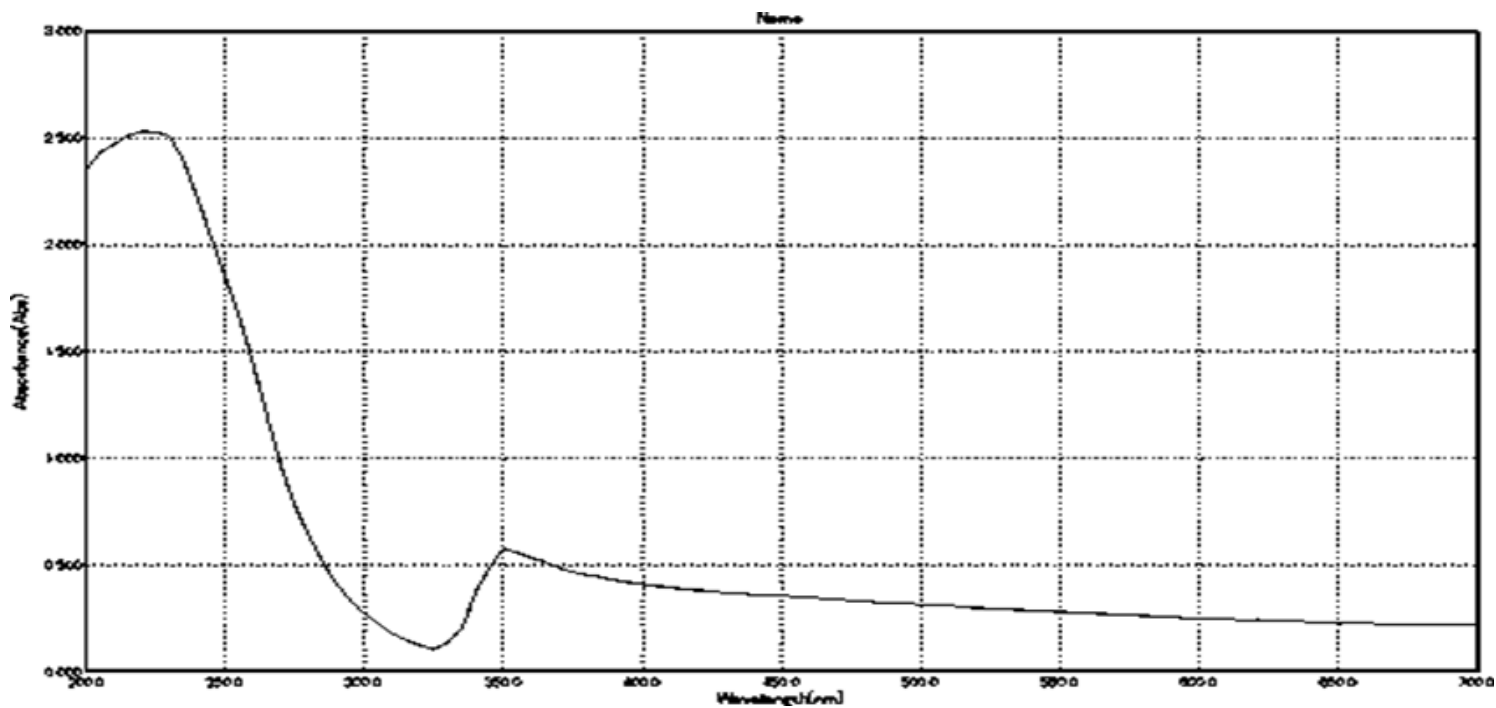


Figure 6

UV-Vis Spectroscopy

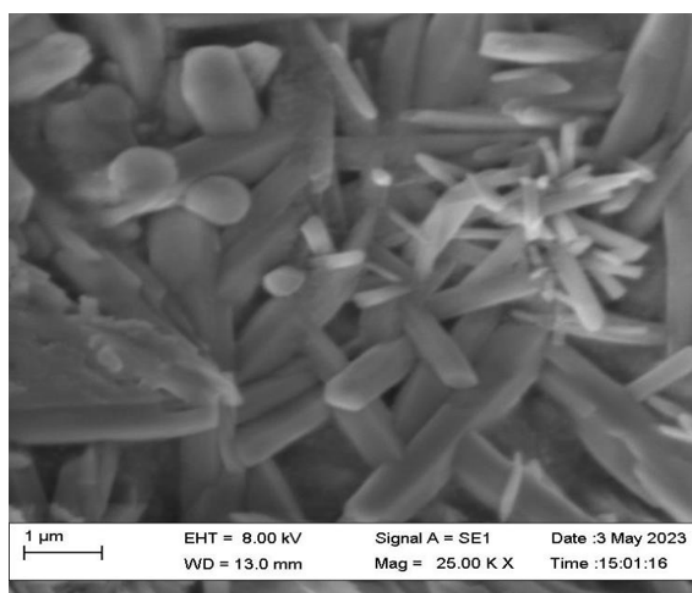
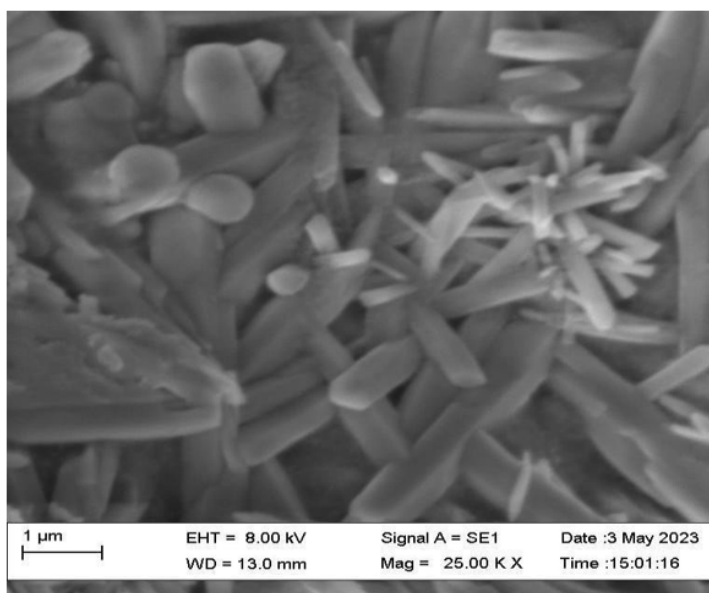


Figure 7

Scanning Electron Microscopic analysis Shows UV-Visible spectral scanning of *Rauvolfia tetraphylla*, having two different absorption bands, providing details about its phytochemical make-up. The initial peak of absorption at ~230–250 nm (maximum absorbance ~2.5) signifies a $\pi \rightarrow \pi^*$ electronic transition characteristic of conjugated aromatic structures found in flavonoids, alkaloids, and other polyphenolic compounds. The second max at ~350 nm (lower absorbance ~0.55) is an $n \rightarrow \pi^*$

transition, which typically represents the carbonyl groups of phenolic acids and other polyphenolic compounds. The transitions also affirm the presence of bioactive constituents with significant antioxidant as well as medicinal activities, as seen from the traditional applications of the plant. The findings are in agreement with spectroscopic theory used in phytochemical characterization, such as in nanoparticle biosynthesis [21], wherein UV-Vis spectroscopy was used in the quantification of bioactive compounds in green-synthesized nanoparticles. The analysis also shows *Rauvolfia tetraphylla* to be a source of therapeutic phytochemicals for their isolation and pharmacological applications.