In-vitro antidiabetic activity of *Plectranthusamboinicus* and preparation of gelatin conjugated nanoparticles

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Abstract

Background: By leveraging nanotechnology and exploring the potential of plants like Plectranthus amboinicus, it may be possible to meet the future global demand for cost-effective and safer bioactive molecules. The development of nanocarriers for drug delivery holds promise for improving therapeutic outcomes and addressing various healthcare challenges. These advancements can lead to early detection, improved treatment outcomes, and better disease management.

Objective:The aim of this study was to evaluate the in vitro antidiabetic activity of aqueous and ethanolic leaf extracts of Plectranthus amboinicus using α-amylase and α-glucosidase enzyme inhibitory assays. Additionally, the study aimed to prepare and characterize gelatin-conjugated nanoparticles using the Plectranthus amboinicus extract.

Materials and Methods:The leaves of Plectranthus amboinicus were dried in the shade and ground into a powder. Successive extractions were performed using different solvents, including methanol, chloroform, ethanol, and water. Various concentrations of acarbose, aqueous extract, and ethanolic extract of Plectranthus amboinicus were used in the study. The absorbance values were546 nm and 540 nm for α-glucosidase and α-amylase enzymes, respectively**.**For the preparation of gelatin-conjugated nanoparticles solvent evaporation method was employed. The

2153

Eur. Chem. Bull. 2023,12(8), 2153-2168

prepared nanoparticles were then characterized for particle size, zeta potential, polydispersity index, UV analysis, SEM, and FTIR.

Results: The results of the study confirmed the presence of various phytochemical. The extracts exhibited significant inhibition of α-amylase and α-glucosidase enzymes in a dose-dependent manner. The ethanolic extract showed higher inhibitory activity compared to the aqueous extract. The results showed that the gelatin conjugated nanoparticles of P. amboinicous extract were spherical in shape, desired particle size was 250nm and zeta potential ranging by-30 to -40, indicating the stability of the nanoparticlesThe FTIR spectraexhibited characteristic bands indicating the presence of specific functional groups.These results indicated that the plant extract contains functional groups that play a role in the reduction and stabilization of the biosynthesized gelatin nanoparticles.

Conclusion:These findings suggest that the aqueous and ethanolic extracts of Plectranthus amboinicus possess antidiabetic activity, and the prepared gelatin-conjugated nanoparticles show potential for drug delivery applications.

Keywords: *In-Vitro* Antidiabetic Activity; *Plectranthus amboinicus; α*-glucosidase; α-amylase; Acarbose; Zeta potential, Polydispersity- index;

1.INTRODUCTION

The human population has always been plagued by diseases that have adversely affected health and the well-being. The drastic change in the life style and advance technology has been associated with rise in non-communicable disorders. One common disease is diabetes mellitus that is giving rise to greater morbidity and mortality in both young and old.According to the International Diabetes Federation (IDF), approximately 415 million adults between the ages of 20 to 79 years had diabetes mellitus in 2015.[1]. DM is proving to be a global public health burden as this number is expected to rise to another 200 million by 2040.[1]. Herbaltherapies are proven safe and effective for healing diseases and have been the potential source for the development of new drugs [2,3]. More than 800 plants have been used as remedies for diabetes healing, and the most successful story in drug discovery [2,6]. Plectranthus amboinicus (Lour) Spreng belongs to family Lamiaceae, and is known as country borage in English[5].It is a large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose[6]. It is found throughout India, Ceylon, and Moluccas[7].Common names of *Plectranthus amboinicus* commonly known by differentnames at different places:

- ✓ Kannada- Dodda pathre. dodda pathre soppu
- ✓ Hindi -Patta ajavayin, Patharchur, Amroda, pathercheer

2154

Eur. Chem. Bull. 2023,12(8), 2153-2168

- ✓ English -Country borage, Indian borage, Indian mint
- ✓ Bengali -Amalkuchi
- ✓ Malayalam -Panikoorka
- ✓ Guajarati -Ovapan
- ✓ Marathi- Pan ova
- ✓ Sanskrit- Karpuravalli, Sugandhavalakam, Parnayavani
- ✓ Marathi -Pathurchur [14]

The leaves also possess many medicinal uses like rheumatism and flatulence, cold, asthma, constipation, headache, antimicrobial, anti-inflammatory, antitumor, wound healing, anti-epileptic, larvicidal, antioxidant and analgesic activities, cough, fever, and skin disease. Upon literature review it was found that the plant contains butylanisode, β-caryophyllene, quercetin, ursolic acids, triterpenic acids, α-pinene, β-pinene, thymol, eugenol, carvacrol, 1,8-cineole, β-phellandrene,*p*-cymene, salvigenin, crisimaritin and chrysoeriol[8-13].The focus of our study on investigating the antidiabetic activity of Plectranthus amboinicus and developing gelatin-conjugated nanoparticles aligns with the growing need to address the global burden of diabetes mellitus. Diabetes is a prevalent chronic disease that requires effective management to improve patients' quality of life.The wide range of medicinal uses attributed to Plectranthus amboinicus leaves, including their antidiabetic potential, provides a strong rationale for exploring their efficacy in managing diabetes. By evaluating the antidiabetic activity of the aqueous and ethanolic extracts of Plectranthus amboinicus leaves using α-amylase and α-glucosidase enzyme inhibitory assays, our study aimed to assess the ability of these extracts to regulate blood sugar levels by inhibiting key enzymes involved in carbohydrate digestion and glucose absorption.In addition to evaluating the antidiabetic activity of the plant extracts, our study also focused on the preparation and characterization of gelatin-conjugated nanoparticles. These nanoparticles can serve as drug delivery systems, potentially enhancing the bioavailability and controlled release of bioactive compounds found in Plectranthus amboinicus. The use of gelatin as a carrier offers advantages such as biocompatibility, biodegradability, and the ability to stabilize and protect the encapsulated bioactive molecules.Characterization techniques such as particle size analysis, polydispersity index, zeta potential measurement, SEM imaging, and FTIR spectroscopy were employed to assess the physical properties, stability, and composition of the gelatin-conjugated nanoparticles. These characterization steps provide crucial information to ensure the suitability of the nanoparticles for drug delivery applications and confirm the successful encapsulation of the bioactive compounds.By combining the investigation of the antidiabetic activity of Plectranthus amboinicus extracts and the development of gelatin-conjugated nanoparticles, our study aimed to contribute to the understanding of this plant's potential as a therapeutic agent for diabetes mellitus. The utilization of nanotechnology-based drug delivery systems can also provide insights into improving the efficacy and delivery of bioactive compounds, potentially enhancing treatment outcomes for diabetes and other related conditions.

2.MATERIALS AND METHODS

Plant collection and authentication

The leaves of Plectranthus amboinicus (Lour) Spreng used in the study were collected from the fields of Mysore, Karnataka, India. To ensure their authenticity and proper identification, the plant material was verified by Dr. Madhava Chetty, a plant taxonomist (IAAT:357) from the Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.Preserving voucher specimens is a standard practice in botanical research, enabling researchers to validate the authenticity of the plant material used in their studies and facilitating future reference.

Preparation of extracts

The shade-dried leaf powder of Plectranthus amboinicus was subjected to extraction using two different solvents: ethanol and distilled water. The extraction process was performed using the Soxhlet apparatus for ethanol extraction and the decoction method for water extraction. For the ethanol extraction, 100 ml of ethanol was added to the leaf powder in the Soxhlet apparatus. The ethanol extract was then collected, and the solvent was removed under reduced pressure using a rotary vacuum evaporator. The resulting dried residue was stored in a desiccator for future use.

Phytochemical investigation

Phytochemical investigations of the Plectranthus amboinicus leaf extracts revealed the presence of various bioactive compounds, including flavonoids, tannins, glycosides, phenols, carbohydrates, and proteins. These compounds are known for their potential therapeutic properties and contribute to the medicinal value of the plant.The percentage yield obtained from the extraction process was 10.1% for the alcoholic extract and 9.3% for the aqueous extract. This indicates the efficiency of the extraction process in obtaining a considerable number of bioactive compounds from the plant material.To ensure the preservation of the extracts and their stability, they were stored in a refrigerator until further use. This step helps maintain the integrity and potency of the extracts for future experiments and analyses.

Inhibition of α-amylase Enzyme

In the study, different concentrations of the Plectranthus amboinicus leaf extract were prepared in DMSO (2-10 mg/ml). A series of assays were performed to evaluate the inhibitory activity of the extract against α-amylase enzyme.The assay procedure involved the following steps200 µl of the extract at each concentration was mixed with 200 µl of α-amylase solution (2 units/ml). The mixture was incubated for 10 minutes at 30°C.Then, 200 µl of 1% starch solution was added to the mixture and further incubated for 15 minutes.The reaction was terminated by

2156

Eur. Chem. Bull. 2023,12(8), 2153-2168

adding 200 µl of DNSA reagent (di-nitro salicylic acid). The mixture was boiled for 10 minutes in a water bath.After cooling, 5 ml of distilled water was added.The absorbance of the solution was measured at 540 nm using a spectrophotometer. For comparison, a control sample without the plant extract was included. Additionally, a sample blank without the α-amylase enzyme was prepared.The amount of reducing sugar released during the enzymatic reaction was estimated by comparing the results with a glucose standard graph. The percentage inhibition of α-amylase activity was calculated using the formula:

% inhibitory activity = (Ac-As)/Ac × 100

Where Ac is the absorbance of the control sample and As is the absorbance of the sample with the plant extract.

To assess the activity of the extract, a standard graph was prepared using different concentrations of acarbose (5-50 µg/ml) as a reference.

Inhibition of α-glucosidases Enzyme

In the study, different concentrations (2-10 mg/ml) of the Plectranthus amboinicus leaf extract were prepared in 0.1M phosphate buffer (pH 6.8). The α-glucosidase enzyme solution (1 U/ml) was added to each extract concentration (125 µl) and incubated for 20 minutes at 37°C.After the initial incubation, the reaction was initiated by adding 20 µl of 1M pNPG (p-nitrophenyl-α-D-glucopyranoside) substrate. The mixture was then further incubated for 30 minutes.To terminate the reaction, 50 µl of 0.1 N Na_2CO_3 was added, and the final absorbance of the mixture was measured at 405 nm using a spectrophotometer.A control sample without the plant extract was included to compare the results. Acarbose, a known α-glucosidase inhibitor, was used as a positive control at varying concentrations (5-50 µg/ml).The activity of the α-glucosidase enzyme is determined by the formation of p-nitrophenol from the substrate. One unit of the enzyme is defined as the amount of enzyme required to produce one µmol of p-nitrophenol per minute under the assay conditions.The inhibitory concentration (IC50) value, which indicates the concentration of the inhibitor required to inhibit 50% of the enzyme activity, was determined. It was calculated by plotting the percentage inhibition versus the logarithm of the inhibitor concentration and performing logarithmic regression analysis on the mean inhibitory values.

Preparation of PA extract loaded gelatin nanoparticles

The preparation of nanoparticles of Plectranthus amboinicus extract with a polymer was done using the solvent evaporation method.The required quantity of Plectranthus amboinicus (PA) dry extract was dissolved in 10 ml of ethanol using sonication at 20 watts for 60 seconds. This solution served as the organic phase.The organic phase was added slowly dropwise into an aqueous phase containing gelatin and tween 80. The aqueous phase was continuously stirred at 1000 rpm using a magnetic stirrer. After 1 hour of stirring, a 0.01% glutaraldehyde solution was added to cross-link the gelatin in the mixture. The stirring was continued for 7 hours at room temperature to allow solvent evaporation and the formation of nanoparticles. A blank

2157

nanoparticle formulation was also prepared using only the polymer and surfactant, without the extract, for comparative studies.The nanoparticles were separated from the solution by centrifugation for 30 minutes. The resulting pellet was then re-suspended in Milli Q water and washed three times with water to remove any impurities.The suspension of nanoparticles was dried using a lyophilizer, which is a freeze-drying process, to remove the water content. The dried nanoparticles were stored at 40°C until further use.By following this method, nanoparticles loaded with the Plectranthus amboinicus extract were successfully prepared, allowing for potential applications in various fields.

Characterization of Nanoparticles

Nanoparticles are typically characterized using advanced microscopic techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to determine their size, morphology, and surface charge. These characteristics play a crucial role in the physical stability and in vivo behavior of nanoparticles. SEM allows for the visualization of the surface of nanoparticles, providing information about their overall shape and morphology. This information can be valuable in assessing potential toxicity and understanding the interactions of nanoparticles with biological systems. TEM provides higher resolution and can be used to visualize the internal structure of nanoparticles. It allows for the examination of individual nanoparticles and provides detailed information about their size, shape, and distribution. The average particle diameter and size distribution of nanoparticles are important parameters that impact their behavior and functionality. Size affects their stability, drug loading capacity, release kinetics, and in vivo distribution. A narrow size distribution is desirable for consistent and predictable performance.The surface charge of nanoparticles, determined by their zeta potential, is another critical factor. It affects their stability in solution, ability to be re-dispersed, and interactions with cells and tissues in vivo. The surface charge can influence the biodistribution, cellular uptake, and therapeutic efficacy of nanoparticles.Overall, the characterization of nanoparticles using advanced microscopic techniques provides crucial information about their physical properties, which is essential for optimizing their design and understanding their behavior in biological systems.

Characterization of prepared nanoparticles

Particle size and Polydispersity Index

The dynamic light scattering (DLS) technique, performed using the Malvern Zeta sizer instrument, was used to analyze the average particle size (PS), polydispersity index (PDI), and electrokinetic potential of the nanoparticle formulation. DLS is a commonly used method to measure the size distribution of particles in a suspension or solution.To prepare the sample for DLS analysis, a prescribed amount of the nanoparticle formulation was dispersed in a generous amount of Milli-Q water. This step helps to prevent particle aggregation or blockage during the measurement. The sample was then vortexed for 5 minutes to ensure proper dispersion.

2158

Eur. Chem. Bull. 2023,12(8), 2153-2168

After sample preparation, the DLS measurement was carried out at a temperature of 25°C. The sample was evaluated in triplicate for a duration of 1 minute. During the measurement, the instrument analyzes the fluctuations in the intensity of scattered light caused by the Brownian motion of particles, providing information about their size distribution.The average particle size (PS) obtained from DLS represents the mean diameter of the nanoparticles in the formulation. The polydispersity index (PDI) is a measure of the width of the particle size distribution. A lower PDI value indicates a narrower and more uniform size distribution.

The electrokinetic potential, also known as zeta potential, provides information about the surface charge of the nanoparticles. It is a measure of the electric potential difference between the particle surface and the surrounding liquid. The zeta potential helps to assess the stability of the nanoparticle formulation, as particles with high absolute zeta potential values are less likely to aggregate or undergo flocculation.By performing DLS analysis using the Malvern Zeta sizer instrument, the average particle size, polydispersity index, and zeta potential of the nanoparticle formulation can be determined, providing important insights into their physical properties and stability.

Scanning electron microscopy

The morphology of the PA-5 formulation was examined using a scanning electron microscope (SEM) model Philips XL 30, located in Hillsboro, USA. To prepare the sample for SEM analysis, PA-5 powder was applied to a double-sided tape. The sample was then coated with a thin layer (30 nm) of gold using a coating instrument. This gold coating helps to enhance the sample's conductivity and improve imaging quality during SEM observation.After the gold coating, the sample was subjected to a 2-minute period of vacuum (10-6 Pa) to remove any excess debris or contaminants. The SEM observations were conducted using a 15 kV accelerating voltage. The accelerating voltage determines the energy of the electron beam used in the microscope, which affects the resolution and contrast of the obtained images.During SEM analysis, the sample is bombarded with a focused electron beam, and the resulting interactions between the electrons and the sample's surface produce various signals, such as secondary electrons, backscattered electrons, and characteristic X-rays. These signals are detected and used to generate high-resolution images of the sample's surface.By examining the PA-5 formulation using SEM,detailed information about the surface morphology, shape, and structure of the nanoparticles was obtained. SEM images provide valuable insights into the size, distribution, and overall appearance of the particles, helping to assess their physical characteristics and potential toxicity.

2159

Eur. Chem. Bull. 2023,12(8), 2153-2168

UV-Visible analysis

UV-visible analysis was performed using a UV-visible spectrophotometer in the wavelength range of 200-800 nm. The UV-vis spectrum of the PA leaf extract did not exhibit any distinct peak in the measured range. This absence of a peak suggests the absence of surface plasmon resonance, which is typically associated with the collective oscillation of surface electrons in nanoparticles. On the other hand, the UV-vis spectrum of the PA-loaded gelatin nanoparticles (NPs) showed a single surface plasmon resonance (SPR) band. The maximum wavelength (λmax) of this band was observed at around 400 nm-450 nm (specifically, 428 nm). This characteristic peak indicated the occurrence of surface plasmon resonance and confirmed the successful formation of nanoparticles.The presence of a surface plasmon resonance peak in the UV-vis spectrum of the PA-loaded gelatin nanoparticles suggests the presence of nanoparticles with specific size and shape. This optical analysis provides important information about the formation and properties of the nanoparticles, aiding in their characterization and further understanding of their behavior and potential applications.

Fourier Transform Infrared Spectroscopy (FT-IR)

The drug-polymer interactions were investigated using FTIR spectroscopy. Both the pure drug (PA extract) and the excipients were subjected to FTIR studies. The measurements were performed at ambient temperature using an IR spectrophotometer (Perkin Elmer Instruments, North Billerica, MA, USA). The FTIR spectra were obtained qualitatively to analyze the pattern of peaks and for comparative purposes.To obtain the FTIR spectra, KBr discs were prepared by mixing the sample (PA extract or PA-5 formulation) with potassium bromide (KBr) and compressing it into a disc. The spectra were then recorded in the range of 400-4000 cm-1, which is the typical range for FTIR analysis.By comparing the FTIR spectra of the PA extract and the PA-5 formulation, any changes in the pattern of peaks or the appearance of new peaks can indicate interactions between the drug and the polymer. These interactions can provide insights into the compatibility and stability of the drug-polymer system, which is important for the formulation and development of effective drug delivery systems.

Statistical Analysis

The determinations were conducted in triplicate, and the values obtained are expressed as the mean ± standard error of the mean (SEM). This indicates the average value of the measurements along with the measure of the variability or precision of the results.

To determine the IC50 value, regression analysis was performed. This involves plotting the percentage inhibition (or activity) against the logarithm of the inhibitor concentration. By fitting a regression line to the data points, the concentration of the inhibitor that corresponds to 50% inhibition can be determined. This concentration is referred to as the IC50 value and is a measure of the potency or effectiveness of the inhibitor in inhibiting the target enzyme or activity.

The IC50 value provides valuable information about the concentration at which the inhibitor can exert its desired effect, such as inhibiting the α-amylase or α-glucosidase enzyme activity in this case. It serves as an important parameter for comparing the inhibitory potential of different compounds or extracts and can be used to evaluate the efficacy of potential antidiabetic agents.

RESULTS

α -amylase Inhibitory Activity and α-glucosidase Inhibitory Activity

Aqueous extract of 10, 20,30,40,50 μg doses inhibits the α-amylase enzyme by 16.22%, 22. 63%, 30.20%, 41.30%, 54. 64 % respectively.

Ethanolic extract of 10, 20,30,40,50μg doses inhibits the α-amylase enzyme by 10.78%, 20.81%, 29.14%, 43.33%, 49.41%, respectively.

Acarbose of 10, 20,30,40,50μg doses inhibits the α-amylase enzyme by 10.99%., 15.11%, 22.09% 33.08%, 40.69% respectively.
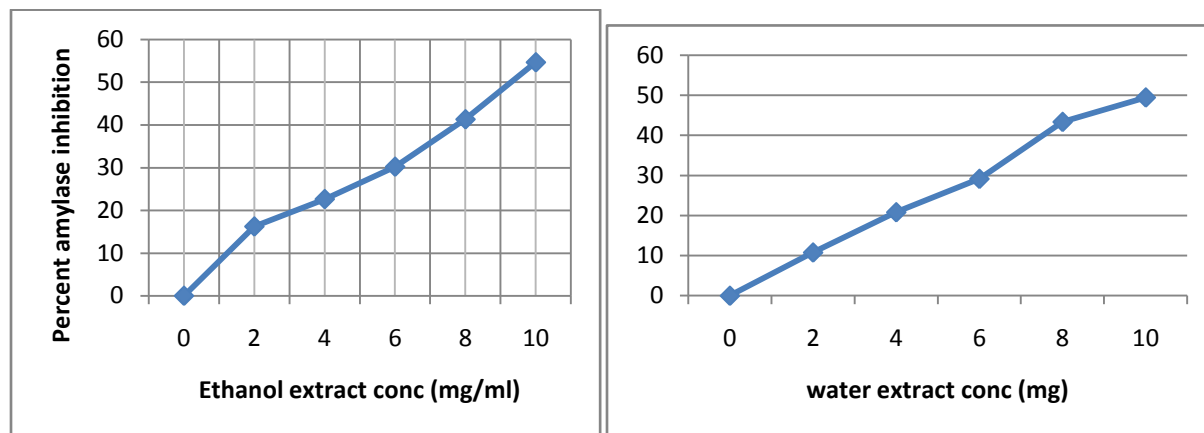

Fig (1,2)

Alpha -glucosidase Inhibitory Activity

Aqueous extract of 5, 10,15,20,25, μg doses inhibits the α-glucosidase enzyme by 12.24%, 25.52 %, 34.15%, 42.12 %, and 60.72%, respectively.

Ethanolic extract of 100, 200, 300, 400, 500 μg doses inhibits the α-glucosidase enzyme by 9.30%,18.79%,28.94%,39.18%, 43.55%, 50.38%

Acarbose of 100, 200, 300, 400, 500 μg doses inhibits the α-glucosidase enzyme by 14.50%., 20.69%, 31.52%, 44.38%, 56.86%, respectively (Figure 2). Dose-dependent % inhibition of α-glucosidase enzyme is observed with the both extracts
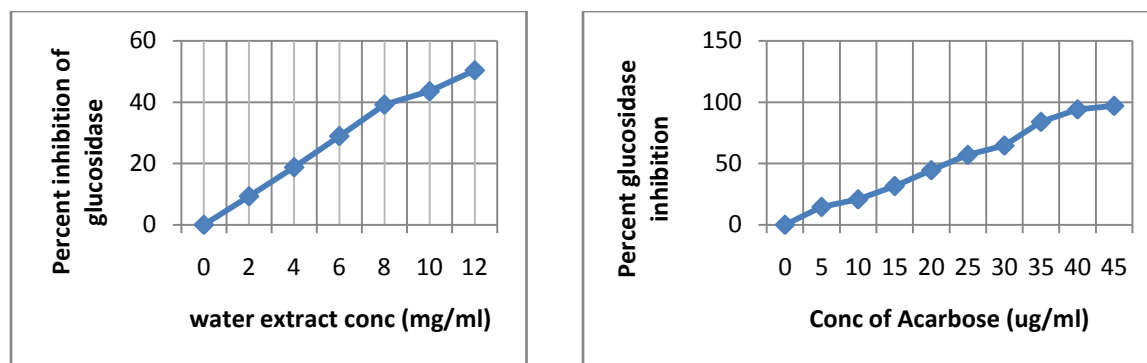
Fig (3,4)

Particle size and Polydispersity Index

Among all the formulations, PA-5 formulation found to be optimized and shown desired particle size, zeta potential and PDI. PA-5 formulation was further studied for various evaluation parameters.

Table 1

S. No	Formulation	Particle Size (nm)	Zeta Potential (mV)	PDI	
1.	PA-1	100	-10.5	0.45	
2.	PA-2	154	-12.8	0.56	
3.	PA-3	124	-14.6	1.4	
4.	PA-4	224	-15.8	1.6	
5.	**PA-5***	**252**	**- 40.5**	**0.04**	
6.	PA-6	485	-22.8	1.1	
7.	PA-7	1318	-15.9	2.2	
8.	PA-8	1254	-18.2	1.8	
9.	PA-9	1485	-19.5	1.6	

2162

Eur. Chem. Bull. 2023,12(8), 2153-2168

		--	-32.5	2.2	
10.	BNP (Blank Nanoparticles)				

*- Optimized/Best formulation.

Scanning electron microscopy:

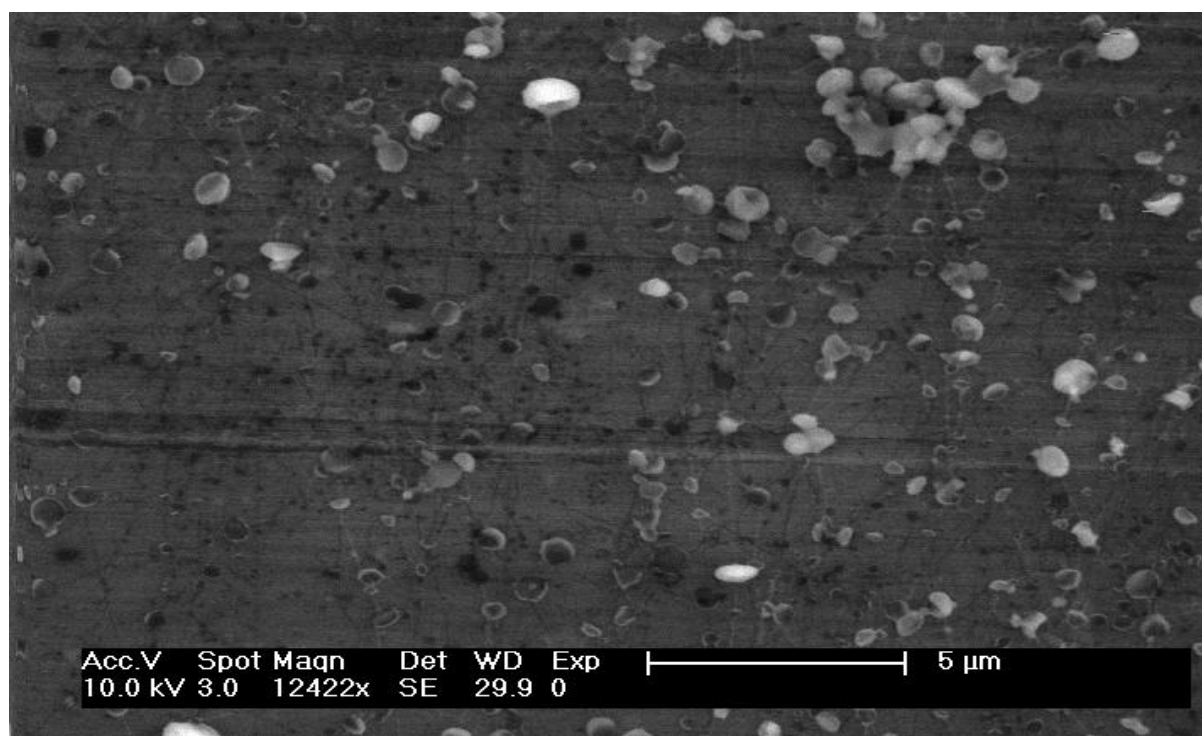SEM images shown the almost spherical shape of the PA-5 formulation.

Fig 5
Scanning electron microscopy images of PA-5 formulation at various magnifications

UV-Visible analysis

The UV-vis spectrum of PA leaf extract did not show a peak, this band can be attributed to the surface plasmon resonance due to collective oscillation of surface electrons. whereas that

2163

Eur. Chem. Bull. 2023,12(8), 2153-2168

of PA loaded gelatin NPs showed a single surface Plasmon's resonance (SPR) band with a maximum wavelength (λmax) at 400 nm–450 nm (428 nm), which indicated the formation of NPs.
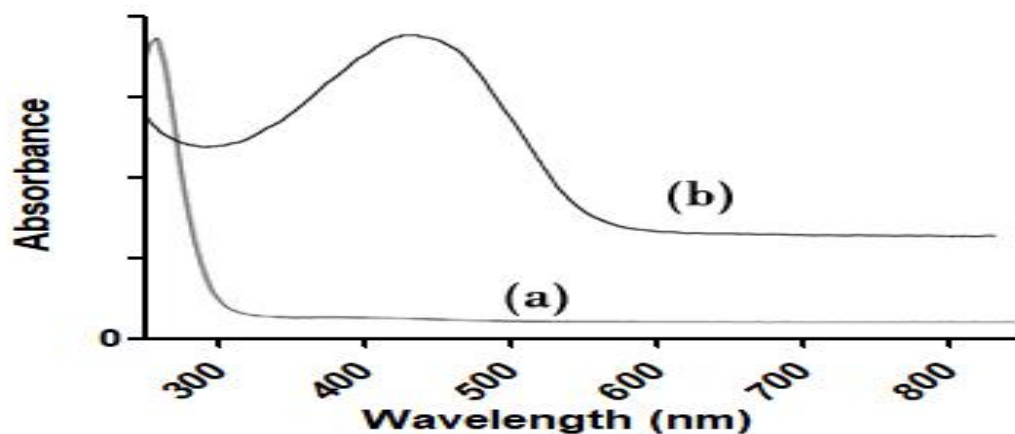

Fig 6

UV Visible spectra of (a) Pure PA and (b) PA-5 formulation**.**

Construction of standard calibration curve:

Table 2

Sl.No.	Conc.(ug/ml)	Absorbance
1	0	0
2	2	0.07
3	4	0.157
4	6	0.272
5	8	0.371
6	10	0.481
7	12	0.561
8	14	0.651

2164

Eur. Chem. Bull. 2023,12(8), 2153-2168
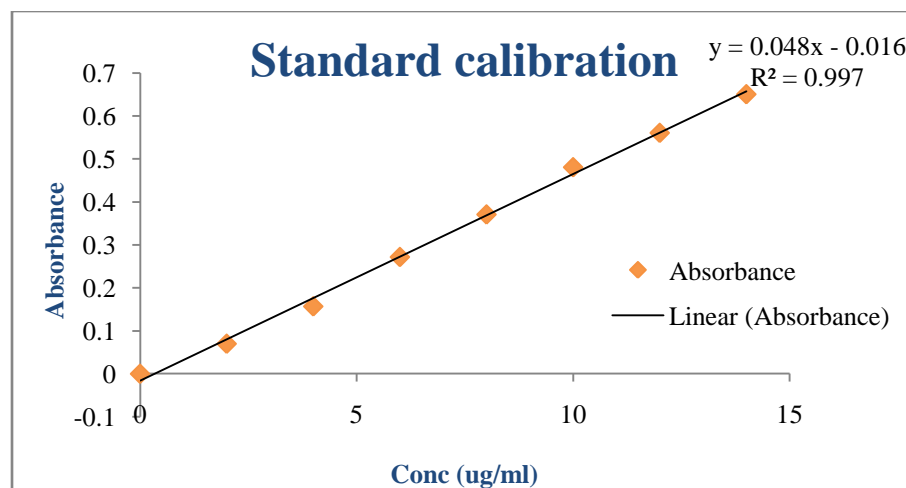
Fig 7

Fourier Transform Infrared Spectroscopy (FT-IR)

Fig 8

FTIR spectra of PA extract.

2165

Eur. Chem. Bull. 2023,12(8), 2153-2168

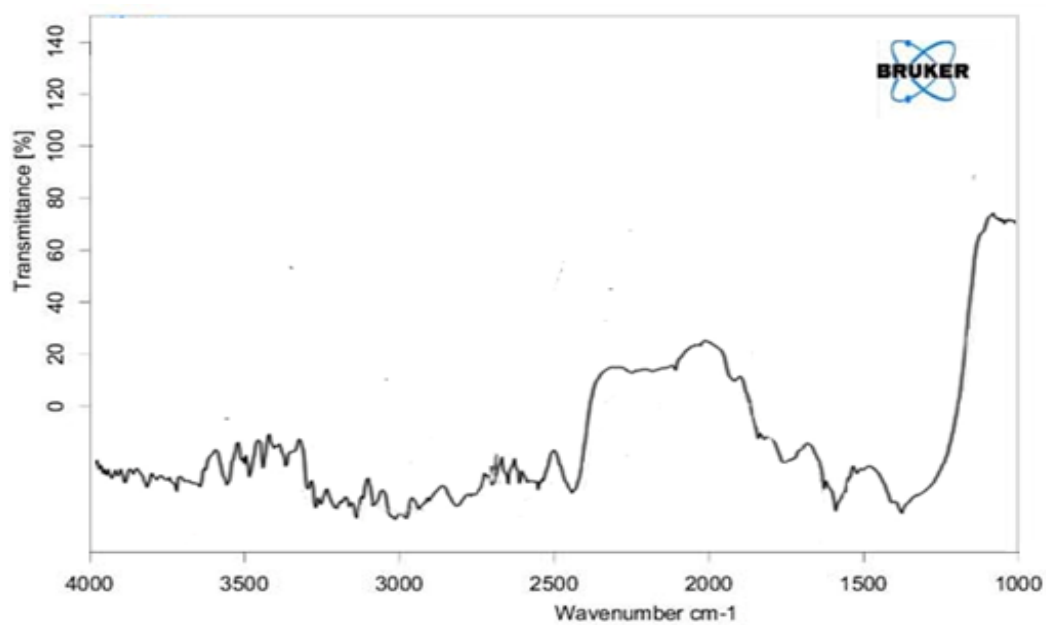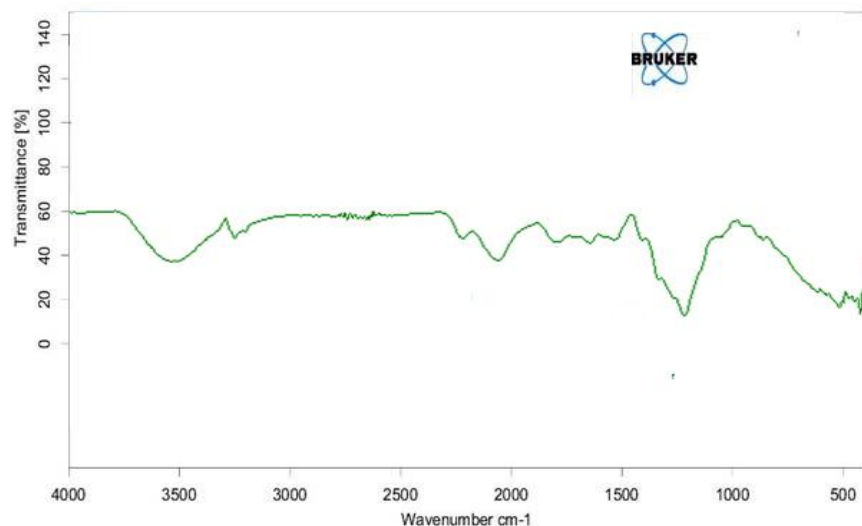**Fig 8**

FTIR spectra of PA-5 formulation

DISCUSSION

The present research describes the extraction process and characterization of Plectranthus amboinicus leaves extract.The higher alcohol and water-soluble extractive values suggest good solubility of active components in these solvents. The presence of inorganic residues in the extract was indicated by the higher total ash value. Characteristic fluorescence observed in different solvents could signify the presence of specific compounds. Phytochemical investigations of the Plectranthus amboinicus leaves extract revealed the presence of various bioactive compounds including flavonoids, tannins, glycosides, phenols, carbohydrates, and proteins. These compounds are known for their diverse therapeutic properties. The extract exhibited inhibitory activity against α-amylase and α-glucosidase enzymes involved in carbohydrate digestion and absorption. The ethanol extract showed better inhibitory action, suggesting its potential as an antidiabetic agent.Gelatin-encapsulated Plectranthus amboinicus nanoparticles were prepared using the solvent evaporation method, and different formulations were characterized. The nanoparticles had an average particle size below 250 nm, suitable for drug delivery applications. The zeta potential values ranging from -30 to -40 indicated nanoparticle stability. Among the formulations, PA-5 exhibited a smaller particle size and higher entrapment efficiency, making it a favorable candidate for further studies. Scanning electron microscopy (SEM) images confirmed the spherical shape of the nanoparticles.The Fourier-transform infrared (FT-IR) spectra of PA-5 exhibited characteristic bands corresponding to the Plectranthus amboinicus leaf extract, indicating the presence of specific functional groups such as -OH, -CHO, C=O, NH2, and C=N. However, the signals were relatively weak, suggesting that the components of the Plectranthus amboinicus extract had reacted to form gelatin nanoparticles. These results indicate that the plant extract contains functional groups that play a role in the reduction and stabilization of the biosynthesized gelatin nanoparticles.

2166

Eur. Chem. Bull. 2023,12(8), 2153-2168

CONCLUSION

Herbal medicine offers a holistic approach and has the potential to target multiple aspects of type 2 diabetes mellitus (T2DM), making it a promising complementary and alternative treatment option. However, further research is necessary to bridge the gaps in understanding herbal medicine. Medicinal plants are generally more affordable and have fewer side effects compared to synthetic drugs, showing efficacy in the treatment of diabetes mellitus.Nanotechnology has emerged as a beneficial strategy for herbal drugs, as it improves solubility, bioavailability, and pharmacological activity. In summary, this study sheds light on the potential of nanoparticulate systems as a novel therapeutic approach for the treatment of diabetes and related physiological disorders. Further exploration and research in this field can lead to advancements in nanotechnology-based drug delivery systems for herbal medicine.

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2167

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2168

Eur. Chem. Bull. 2023,12(8), 2153-2168