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Research Article

Phyto-Fabrication, Characterization and Anti-Diabetic Activity of Silver Nanoparticles Using Costus Igneus (Insulin Plant) Leaf Extract

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder affecting millions worldwide, with projections indicating a rise in prevalence by 2045. To address this global health issue, recent research has turned to nanotechnology, particularly the use of silver nanoparticles (AgNPs) synthesized via green methods. This article highlights the biosynthesis of AgNPs using the leaf extract of Costus igneus, commonly known as the insulin plant, and evaluates their anti-diabetic potential both in vitro and in vivo.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of blood glucose, caused by the inability of the body to produce insulin or by the ineffective use of the insulin produced. According to International Diabetes Federation (IDF), globally, as of 2019, approximately 463 million people were living with diabetes, which rise to 700 million by 2045.In recent years, application of nanomaterial in antidiabetic studies has drawn attention due to their extraordinary features such as ultra-small size, ability to transport the therapeutic agent through the cell membrane at the target site and bio-adaptability. Green/biological synthesis (using plant, bacteria, algae and fungi) of AgNPs is preferred chemical and physical methods, since it is an environmentally friendly and cost-effective method without the use of high temperature, pressure, toxic chemicals. Plant (Phyto) mediated fabrication of AgNPs has gained popularity among the other green approaches since it is easily available in large quantities, contains secondary metabolites, and negligible cross-contamination between the plant extract. Hence in the present study, *Costus igneus* was selected for fabrication of AgNPs.

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Costus igneous (insulin plant) is used as an herbal cure for diabetes in India. Very few studies have been conducted on the biosynthesis of silver and zinc oxide nanoparticles using *Costus igneus* leaves extract and evaluation of their antioxidant, antimicrobial and anticancer activities. However, the anti-diabetic potential of AgNPs fabricated with *Costus igneus* leaves extract has so far not been reported.



Image of Costus igneous (Insulin) Plant.

Aqueous Extraction and synthesis of Costus igneous AgNPs.

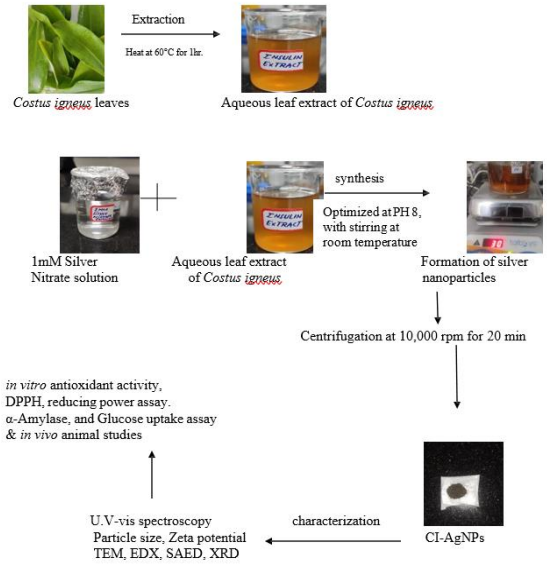


Figure No.5 Schematic illustration of the green synthesis of *Costus igneus* AgNPs

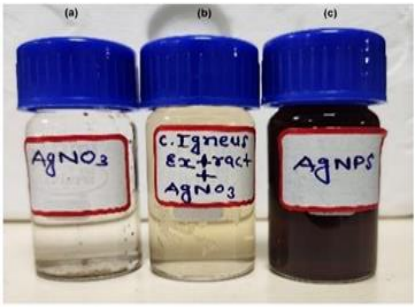
Results: -

Phytochemical analysis:

The results of qualitative screening of phytochemical constituents present in the aqueous extract of Costus igneus are shown phytochemical profile of aqueous leaf extract of Costus igneus revealed the presence of carbohydrates, proteins, steroids, glycosides, flavonoids, alkaloids, and tannins which may be responsible for the efficient capping and stabilization of nanoparticle.

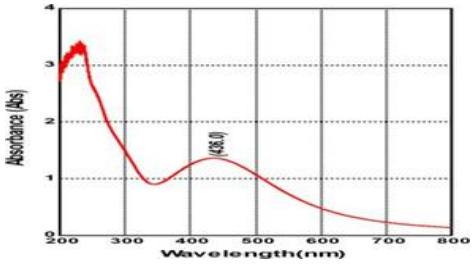
Visual observation:

The formation of CI-AgNPs was confirmed by observing the colour change of the colloidal solution from light yellow to dark brown.



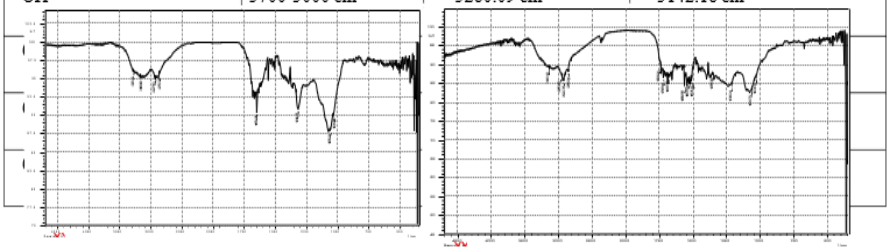
UV-Vis spectral analysis:

The UV-Vis spectrum of colloidal solution of CI-AgNPs showed an absorption peak at 436nm.



Fourier transform infrared analysis (FTIR):

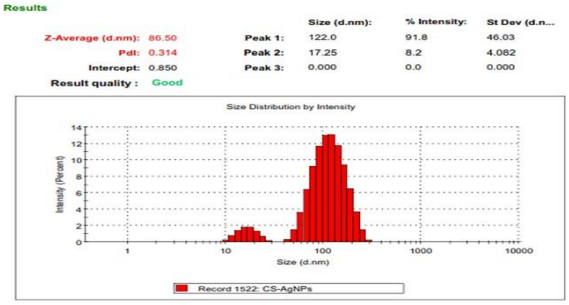
Functional Groups	Standard IR Peaks	Observed IR Peaks of AECI	Observed IR Peaks of AECI-AgNPs
OH	3700-3000 cm^{-1}	3280.09 cm^{-1}	3142.18 cm^{-1}

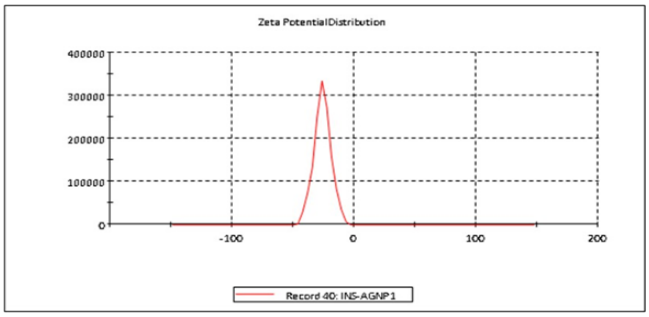


A. Fourier transform infrared analysis of AECI Extract. B. Fourier transform infrared spectra of CI-AgNPs

Particle size and Zeta potential:

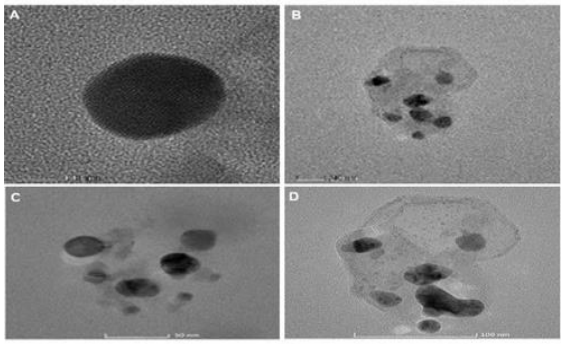
The biosynthesized silver nanoparticles have a particle size of 86.50nm and Zeta potential of -25.8 mV.





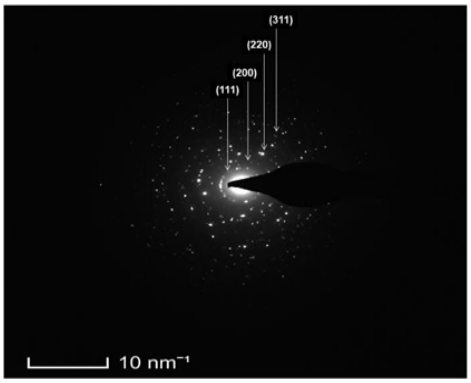
Transmission electron microscopy (TEM):

The silver nanoparticles were found to be roughly textured and grossly spherical in shape and the average particle size was found to be 86.50nm



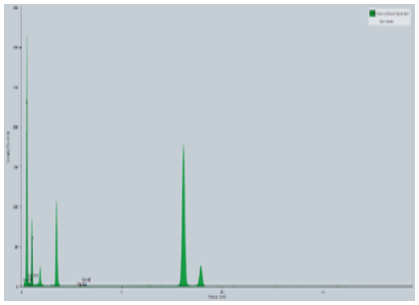
Energy dispersive x-ray spectroscopy (EDX):

The EDX spectrum of AECI-AgNPs shows a signal peak at 3 keV which is typically for elemental silver.



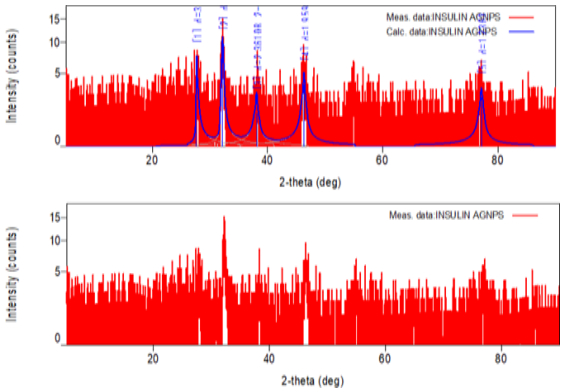
Selected area electron diffraction (SAED):

The SAED pattern was used to determine the crystalline structure of silver nanoparticles synthesized using AECI as reducing agent the image revealed the ring patterns appeared with light spots on the dark field.



X-ray diffraction analysis (XRD):

The XRD pattern of AECI-AgNPs showed the Bragg's diffraction peaks at 38.08°, 46.29°, and 77.08° respectively corresponding to (111), (200), and (300) planes of the face centered cubic lattice. The XRD analysis data conformed the SAED results.



3. In Vitro Anti-Diabetic Activity*:

α-Amylase Inhibition Assay*: The AgNPs exhibited significant inhibition of α-amylase, an enzyme involved in carbohydrate digestion, with an IC50 value of 105.10 µg/ml, making them potentially effective for diabetes management.

Glucose Uptake Assay: The glucose uptake by yeast cells was higher in the presence of AgNPs (78.04%) compared to the aqueous extract alone (49.78%), demonstrating their potential in regulating blood glucose levels.

***In-vivo* antidiabetic activity was assessed in STZ induced rats:**

Effect of AE-CI, CI-AgNPs on lipid profiles (TC, TG, LDL and HDL)

Groups	Treatments	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
I	Normal control	68.33 ± 2.76	81.1 ± 4.15	54.04 ± 3.67	33.7 ± 2.19
II	Diabetic control	102.1 ± 5.03	161.4 ± 4.87	39.11 ± 1.83	115.5 ± 3.97
III	Diabetic + Glimepiride (15 µg/kg b.w)	75.19 ± 5.80	90.64 ± 4.41	51.14 ± 2.13	44.79 ± 2.47
IV	Diabetic + AE-CI (100 mg/kg b.w)	89.42 ± 4.29	107.6 ± 3.01	43.77 ± 1.34	65.56 ± 3.13
V	Diabetic + CI-AgNPs (10 mg/kg b.w)	81.66 ± 5.94	96.23 ± 4.13	49.7 ± 2.50	57.66 ± 2.81

The *in-vivo* antidiabetic activity in STZ induced diabetic rats proved that AECI and CI-AgNPs improved the diabetic dyslipidemia.

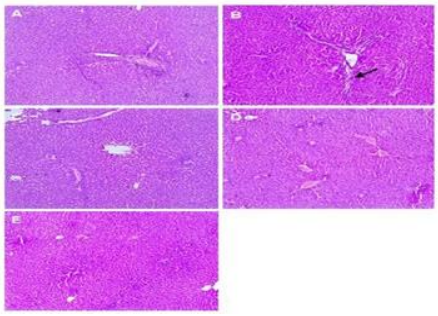
Effect of AE-CI, CI-AgNPs on body weight and blood glucose in streptozotocin induced diabetic rats.

Groups	Body weight				Blood glucose			
	1day	7thday	14th day	21thday	1day	7th day	14thday	21th day
Normal control	185.87± 45.42	190.91± 91	194.81± 30.50	197.72 ± 42.77	107.33± 3.21	103.94± 2.68	100.36± 2.60	109.49± 1.72

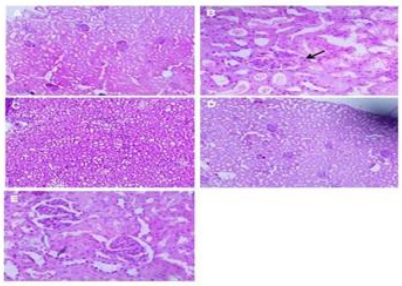
Diabetic control	197.17± 38.21	178.07± 30.06	163.46± 17.90	154.03 ± 10.58	374.41± 11.03	333.68± 48.23	310.23± 24.91.	286.39± 24.25
Std. Glimepiride	194.40± 39.93	187.48± 31.21	182.96± 8.81	178.70 ± 13.25	356.14± 8.32	244.72± 29.08	195.64± 12.00	163.30± 8.83
Diabetic+ AE-CI(100 mg/kg b.w)	206.03± 49.64	200.05± 26.38	193.85± 76.00	186.19 ± 56.71	366.24± 8.53	265.37± 40.72	237.24± 54.66	204.38± 25.37
Diabetic+ C.I-AgNPs (10mg/kg b.w)	184.41± 13.34	181.53± 16.47	177.03± 33.15	173.33 ± 20.14	360.84± 13.98	248.87± 26	225.94± 49.41	177.49± 32.80

Histopathology Examination:-

Figure No.20. The liver section of histopathology (A) Group I – Normal control (B) Group II- Diabetic control group (C) Group III- Glimepiride treated group (D) Group IV- AE-CI treated group (E) Group V-. CI-AgNPs treated group. Arrows indicate fatty liver central venous congestion along with chronic cholangitis.

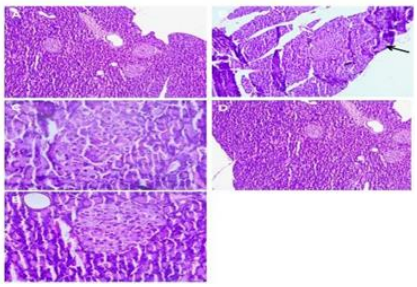


The kidney section of histopathology:a(A) Group I – Normal control (B) Group II- Diabetic control group (C) Group III- Glimepiride treated group (D) Group IV- AE-CI treated group (E) Group V-. CI-AgNPs treated group. Arrows indicate few glomeruli show mild mesangial widening, thickening of basement membrane and mild interstitial nephritis.



The pancreas section of histopathology (A) Group I – Normal control

(B) Group II- Diabetic control group (C) Group III- Glimepiride treated group (D) Group IV- AE-CI treated group (E) Group V-. CI-AgNPs treated group. Arrows indicate reduction in number of islets of Langerhans with islets showing in distinct borders.



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