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Bio synthesis, characterization and activity studies of Ag nanoparticles, by (*Costus igneus*) insulin plant extract

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ABSTRACT

Nanotechnology is receiving much importance in the present century due to its capability of modulating metals in to their nanoparticles. Research in nanotechnology highlights the possibility of their green chemistry pathways to produce important nanomaterials. This report, first of its kind focuses on the biological synthesis of silver nano-particles using Costus Igneus extract and its antidiabetic, antibacterial and antifungal activities. Characterization of newly synthesized silver nanoparticles was made using TEM and XRD studies. Costus Igneus mediated nanoparticles showed high antidiabetic activity, showed maximum amylase inhibition concentration of 87% at 100µg. The silver nanoparticles from Costus Igneus extract showed good antidiabetic activity than plant extract themselves.

Key words: Nanomedicine: silver nano-particles: Antidiabetic activity.

INTRODUCTION

The plant *Costus igneus* belongs to the family Costaceae, which is found in tropical Africa, Asia, Australia, and North, Central and South America. In India, it is cultivated in coastal area, Uttar Kannada district of Karnataka. In this area, people take traditionally 2-3 leaves of this plant twice a day for the management of diabetes. It is a prostrate growing plant with spreading, rooting stems. Its leaves are slender and lance shaped with toothed, scalloped or lobed margins. They are grayish green stained with red purple above and darker purple beneath. The tiny white flowers grow intermittently through out the year. This plant reaches a height of 6-inches and have an indefinite spread [1].

With the development of nanotechnology, controllable synthesis of noble metal Nanoparticles (NPs) attracted much attention due to their potential applications in many areas [2]. Especially,

they have been extensively exploited for use in biomedical areas, such as targeted drug delivery [3], imaging [4], sensing [5] and antimicrobial [6]. Among these metal NPs, Ag NPs are of particular interest due to their strong and wide-spectrum antimicrobial activities [7-10], which might act as a novel bactericide to solve the serious antibiotic resistance problem. Ag NPs can be synthesized by traditionally chemical and physical methods. However, these methods strongly depend on severe reaction conditions, for example, aggressive agents (sodium borohydride, hydrazinium hydroxide, cetyltriethylammonium bromide), harmful solvent system to environment and ecology, higher temperature and higher pressure, and so on. To pursue a healthy life and space, it is imperative to develop a clean synthetic approach (“green chemistry”) to obtain nanomaterials targeted on different applications, especially in biomedical fields.

An environmentally acceptable solvent system, eco-friendly reducing and capping agents are considered to be three essential elements for a completely “green” synthesis [11]. Bio templated strategies might be alternative to achieve the three standards. [12,13] which are based on biological molecules, microorganism or plant extract for synthesis of NPs, such as Au NPs [14], Ag NPs [15] and other functional materials [16, 17]. There are many biologically active components in the extracts of fresh plant, such as proteins, polysaccharides, vitamins, polyphenols and so on. On one hand, some molecules can act as electron shuttles in metal reduction; On the other hand, some constituents are responsible for the capping of resulting NPs. As a result, not only the aggregation of NPs could be effectively avoided, but also the post-surface modification of NPS might be easily completed. Additionally, green plants are reproducible sources which fit for the requirements of sustainable development. Meanwhile, S. Shiv Shankar [18] and co-workers have demonstrated that use of plants extract outweigh microorganisms in the biosynthesis of metal NPs. Therefore, the approach based on green plants extract is an intrinsically green alternative to NPs synthesis. In recent years, some environmentally friendly methods based on plants extracts are largely explored. For example, Mallikarjuna [11] and co-workers generated Ag and Pd NPs at room temperature employing coffee and tea extract. The single-pot method used no surfactant, capping agent, and/or template, which is an intrinsically green approach. Stimulated by these novel synthesis ideas, herein, we attempted to use *Costus igneus* extract to synthesize Ag NPs. *Costus igneus* leaf contains many biological activity components, including steroids and many organic compounds. The pharmacological activities of *Costus igneus* was studied in vivo animals (in most cases the total leaf extract was used) including anti-diabetic activity [19] antibacterial effects [20]. In this paper, we report a low-cost, convenient, green synthetic approach to obtain large quantities of AgNPs by reduction of silver ions with *Costus Ingneus* extract at room temperature. The material exhibited excellent antidiabetic, antifungal and antibacterial characteristics.

MATERIALS AND METHODS

2.1 *Costus Ingneus* Extract Preparation

A 30 g portion of thoroughly washed *Costus Ingneus* (insulin plant) leaves were finely cut and boiled in 100 mL of sterile distilled water. The resulting extract was used for further experiments. Fig. 1 is the optical picture of *Costus Ingneus* used in our experiment.



Figure 1. The optical picture of Costus Ingneus

2.2 Synthesis of Costus Ingneus Ag NPs

For the synthesis of the Ag NPs Costus Ingneus, we adopted previous report [21] with slight modification Typically, 5 mL 10^{-2} M AgNO₃ solution was added to 5 mL of Costus extract. The AgNO₃ reduced Costus Ingneus solution was centrifuged at 4000rpm for 25min individually. The deposited residue was dried and subjected to TEM and XRD studies.

2.3 Transmission Electron Microscopy (TEM) measurement and X-Ray study

TEM samples of the NPs synthesized using the Costus Ingneus extract were prepared by placing drops of the reaction mixture over carbon-coated copper grids and allowing the solvent to evaporate. Then TEM measurements were performed on a JEOL model 1200EX instrument operated at an accelerating voltage of 80 kV. The crystalline nature of AgNPs Costus Ingneus hybrids was confirmed from the X-ray diffraction analysis and TEM measurements

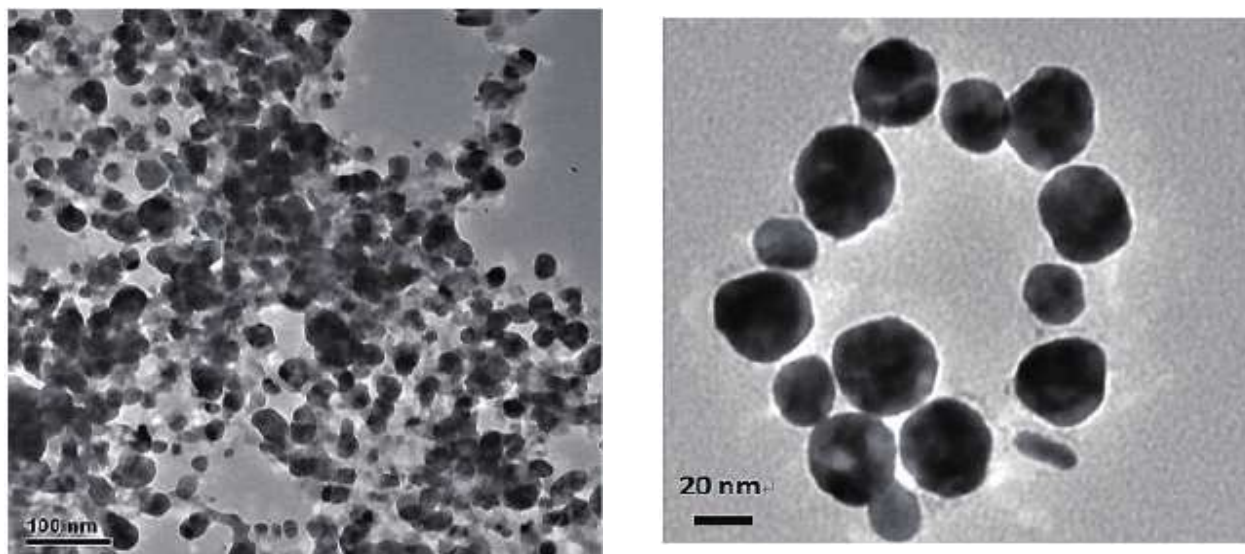
RESULTS AND DISCUSSION

3.1 Characterization of AgNPs at Costus Ingneus hybrids by TEM

Fig.2(a,b) shows the TEM image of as-synthesized AgNPs at Costus Ingneus hybrids, revealing that the NPs are predominantly spherical in the size range of 20 nm. The NPs could be well dispersed in water, and be stable for at least three months.

3.2. X-ray diffraction (XRD) study

The crystalline nature of Ag NPs was confirmed by the XRD analysis as shown in Fig. 3. The diffraction peaks at $<46.18^\circ$ correspond to the (200) facets [22] of the fcc crystal structure. The peak corresponding to the (200) plane is more intense than the other planes, suggesting that the (200) plane is the predominant orientation [22].



(A) (B)
Fig. 2 TEM image of AgNPs at Costus Ingneus: (A) lower magnification scopes, (B) At higher magnification

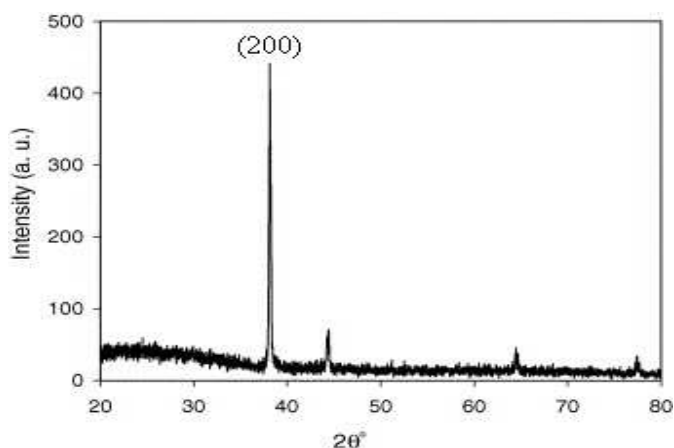


Figure. 3 XRD pattern of AgNPs at Costus Ingneus hybrids

3.3 Antidiabetic (Invitro) Studies

AMYLASE INHIBITION ASSAY

The inhibition assay was performed using the chromogenic DNSA method [23, 24]. The total assay mixture composed of 1400 μ l of 0.05 M sodium phosphate buffer (pH 6.9), 50 μ l of amylase (Diastase procured from HiMedia, Mumbai, Cat No.RM 638) and extracts at concentration 100, 250 and 500 μ g were incubated at 37°C for 10 min. After pre-incubation, 500 μ l of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 ml DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. The maltose liberated was

determined by the help of standard maltose curve (Fig 4.) and activities were calculated according to the following formula

$$\text{Activity} = \frac{\text{Conc. of Maltose liberated X ml of enzyme used}}{\text{Mol. wt of maltose X incubation time (min)}} \times \text{X dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition (Fig 5). This was calculated according to the following formula and is shown in Table 1.

$$\% \text{ inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \times 100$$

From all above data the Ag sample shows significant amylase inhibition Hence synthesized nano-particals shows very good antidiabetic activity. (Table 1).

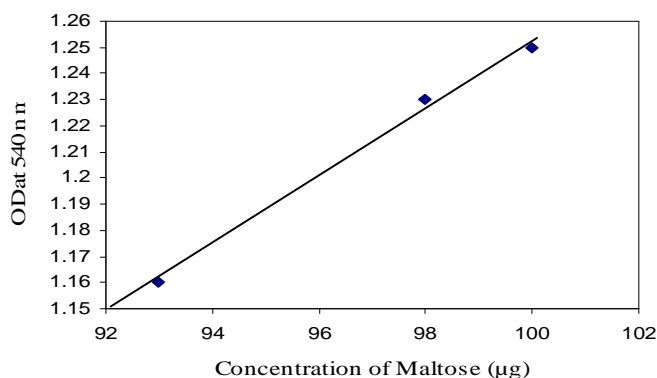


Fig 4. Standard Maltose Curve

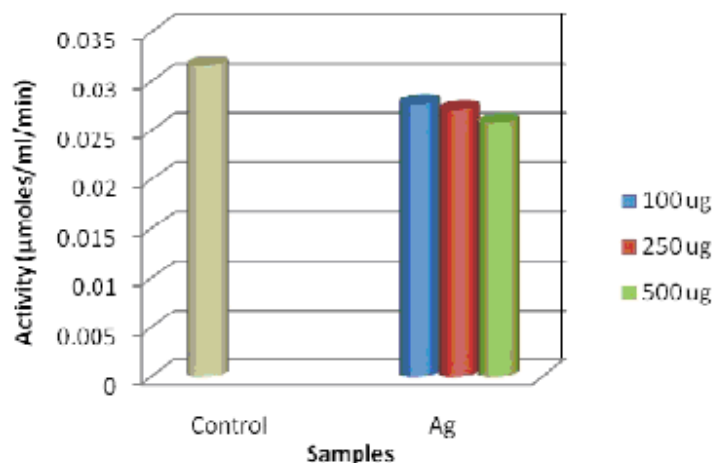


Figure 5. The graph showing the comparative analysis

Table 1. showing the assay of the data

Sample	O D at 540nm	Concentration of maltose liberated (μg)	Activity ($\mu\text{moles/ml/min}$)	% Activity
Control	1.43	114	0.03316	100
Ag 100 μg	1.25	100	0.0277	87.66
Ag 250 μg	1.24	98	0.0271	85.76
Ag 500 μg	1.16	93	0.0258	81.65

3.4 In vitro antibacterial and antifungal assay

The biological activities of synthesized nano-particles have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods respectively. The antibacterial and antifungal activities were done at 200 and 500 $\mu\text{g/mL}$ concentrations in DMF solvent by using three bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*) and antifungal activities (*Aspergillus niger* and *Candida albicans*) by minimum inhibitory concentration method. These bacterial strains were incubated for 24 h at 37 °C and fungal strains were incubated for 48 h at 37 °C. Standard antibacterial (Gentamycin) and antifungal drugs (Amphotericin) were used for comparison under similar conditions. Antibacterial and antifungal activities of prepared AgNPs at *Costus Ingeus* is shown in Table 2.

Table 2

Sample	*Growth Inhibition against Bacteria in mm			*Growth Inhibition against Fungi in mm	
	<i>E.coli</i>	<i>B.Subtilis</i>	<i>S. Aureus</i>	<i>A.niger</i>	<i>C.albicans</i>
AgNPs	0.3 (2)	1 (1)	1(1)	Nil	0.5 (0.5)
Gentamycin	3.1(25)	2.5(25)	3.4(<25)	-	-
Amphotericin	-	-	-	0.7(100)	1.5(50)

*MIC values are given in brackets.

CONCLUSION

Our results demonstrate the ability of the *Costus Ingeus* on synthesizing silver nanoparticles and their antidiabetic, antimicrobial and antifungal activity which represent a significant advancement in the nanomaterial with realistic implications. The green chemistry approach addressed in the present work on the synthesis of silver nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

The silver nanoparticles from *Costus Ingeus* extract showed good antidiabetic activity than plant extract themselves.

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