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Synthesis of silver nanoparticles using *Cassia auriculata* leaves extracts and their potential antidiabetic activity

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Abstract
The green biosynthesis of silver nanoparticles against diabetes was carried out using leaf extract of *Cassia auriculata* (L.). The aqueous silver ions are reduced when exposed to the leaves broth resulting in a green syntheis of silver nanoparticles. Studies on the *in vitro* antidiabetic activity Posses shown that particles have high levels of α-amylase and α-glucocidase inhibitory activity. It was concluded that green biosynthesized silver nanoparticles could be used as a positive phyto- drug to treatment of diabetes.

Keywords: *C. auriculata*, AgNPs, antidiabetic, α-amlase, α-glucosidase

Introduction

Diabetes is a chronic disease, caused by a combination of hereditary and environmental sources that cause abnormal high blood sugar levels (Yanling *et al* 2014) [1]. Diabetes is a major health problem in developed and undeveloped countries (Frank B Hu 2011) [2]. Although there are many therapies available to treat diabetes, they do not completely cure the disease and cause numerous side effects. Many plants and vegetables have been evaluated and confirmed for their antidiabetic activities in animal models that propose that the world is looking for new anti-diabetic agents from plant sources with fewer side effects (Khan *et al* 2012) [3]. Identification of an inhibitor for carbohydrate-hydrolyzing enzymes, namely amylase and alpha-glucosidase, is one of the most significant therapies for decrease the high blood sugar level, by inhibiting the growth of glucose (Baldea *et al* 2010) [4]. Numerous researches have been done to identify substances that inhibit carbohydrate-hydrolyzing enzymes.

Advances in nanotechnology have lead to the synthesis of silver nanoparticles from natural materials, which act as an effective inhibitor of α-glucocidase and α-amylase enzyme to treat diabetes(Balan *et al* 2016) [5]. Manymore Indian medicinal plants have been used to synthesize silver nanoparticles (Ahmed *et al* 2016; Senthil Kumar and Sivakumar 2014; Sivakumar and Gajalakshmi 2014; Senthil Kumar *et al*., 2015; Sivakumar *et al*., 2015; Senthil Kumar *et al*., 2016; Senthil Kumar *et al*., 2017; Sivakumar 2019; Jothi *et al*., 2019; Sivakumar and Deepa, 2020; Angelin *et al*., 2020) [6-16], but only a few number these products have been used to be antidiabetic agents. The plant-based green biosynthesis of (green synthesis) nanoparticles is nontoxic, cost effective, ecofriendly and safe for human therapeutic use.

Cassia airiculata is a important herb that is broadly distributed in India, Sri Lanka, Malaysia, Indonesia, Japan, Vietnam and Australia, tropical Africa and the southwestern parts of the People's Republic of China. *C. auriculata* is a powerful antidiabetic plant and is used in folk, Ayurvedic and homeopathic remedies. *C. auriculata* is used in the treatment of eye complaints, asthma, snakebite, urinary problems, family planning, piles, stomach problems, chronic cough, colic pain, respiratory disorders, heart disease, constipation, dyspepsia, hepatosplenomegally and hemorrhoids. Moreover, it has antimicrobial, antihypercholesterolemic, sweet suppressant properties, anti-inflammatory and also acts as a caterpillar. *C. auriculata* has been reported to have antibacterial and antimicrobial activity (Parkas 2006) [17].

In the current research work, an attempt has been made to synthesized silver nanoparticles using the leaf extract of *Cassia auriculata*. The characterization was done using the various spectral analysis. The synthesized silver nanoparticles were analyzed for their antidiabetic activity.

Materials and Methods

Collection and preparation of leaves materials

The leaves of the plant namely *Cassia auriculata* is collected from Elusempon, Villupuram District, Tamil Nadu, India. The freshly collected leaves were washed with sterile double distilled water and air dried then grind with the help of mortar and pestle. The leaves were grind until it reaches to a paste form and then the juice of extracts was filtered using Whatmann no.1 filter paper under separating funnel.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized by adding 90ml of 0.1mM aqueous $AgNO_3$ silver nitrate solution added with 10 ml of *Cassia auriculata* leaves extracts. The synthesis was carried out in a dark condition to minimize the photo activation of silver nitrate. The formation of silver nanoparticles was indicated by the color change. After the synthesis, the reaction mixture was shaken thoroughly and kept into the oven for 7days. The pellets containing silver nanoparticles was collected and dried at room temperature. The dried powder was used for further analysis.

Invitro antidiabetic activity

α-Amylase inhibition activity

The α -amylase inhibition activity was tested by using the DNS method (Ali *et al*., 2006) [18]. Various concentrations (10–50lg/ml) of silver nanoparticles were prepared from 1mg/ml standard solution of phosphate buffer. The samples (250µl) were incubated with 250µl of α -amylase solution (2 units/ml) for 10 min at 27°C temperature. Then 250µl of starch solution (1%) was added and incubated for another 10min. Dinitrosalicylic acid color reagent (0.5 ml) was used to stop the reaction and the mixture was heated in boiling water bath for 10 min. Then it was allowed to cool and was diluted by adding distilled water (5ml). The blank was prepared by replacing the enzyme with buffer for each set of concentration of test sample. The control was maintained without the addition of sample which represented 100% enzyme activity. The absorbance of the colour solution was measured at 540 nm. Acarbose was used as the positive control.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the silver nanoparticles was determined by assessment of the 4-nitrophenol released from p-nitrophenyl α-D glucopyranoside. The various concentrations (10–50 lg/ml) of sample (0.2 ml) was added to the assay mixtures containing 0.3ml of 10mM p-nitrophenyl α–D glucopyranoside, 1.0 ml of potassium phosphate (0.1M, pH:6.8), 0.2 ml of enzyme solution and incubated for 30 min at 37°C. The addition of 100mM sodium carbonate (2.0ml) completed the reaction. The liberated p-nitrophenol was assessed by determining the absorbance at 400nm using spectrophotometer. A positive control (Acarbose) was used to compare the inhibitory activity of silver nanoparticles. Percentage of inhibition was considered to express the α - glucosidase inhibitory activity (Shinde *et al*., 2008)[19].

Statistical analysis

The statistical analysis was carried out using SPSS/20 software. Experimental data were expressed as mean±standard deviation and obtained from biological and technical three replicates of each experiment.

Results and Discussion

***In vitro* antidiabetic activity**

α-Amylase inhibition activity

α -Amylase is an key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the greatest approaches to lower the blood sugar levels. Amylase inhibitors or starch blockers prevented the body from absorbing starch dietary. Therefore, it can reduce the increase in blood sugar level through carbohydrate consumption (Tamil *et al*., 2010)[20]. Green synthesized silver nanoparticles have been reported to act as amylase inhibitors to lower blood sugar levels (Saratale*et al*., 2018)[21]. The silver nanoparticles synthesized from *Cassia auriculata* indicated the higher level of α -amylase inhibition activity than the acarbose in all the tested concentration (Figure.1). As the concentration of silver nanoparticles increased, the percentage of inhibition also increased in a dose-dependent manner. Similarly, silver nanoparticles synthesized from other medicinal plants have been exposed to α -amylase inhibition activity (Abideen and Sankar 2015) [22].

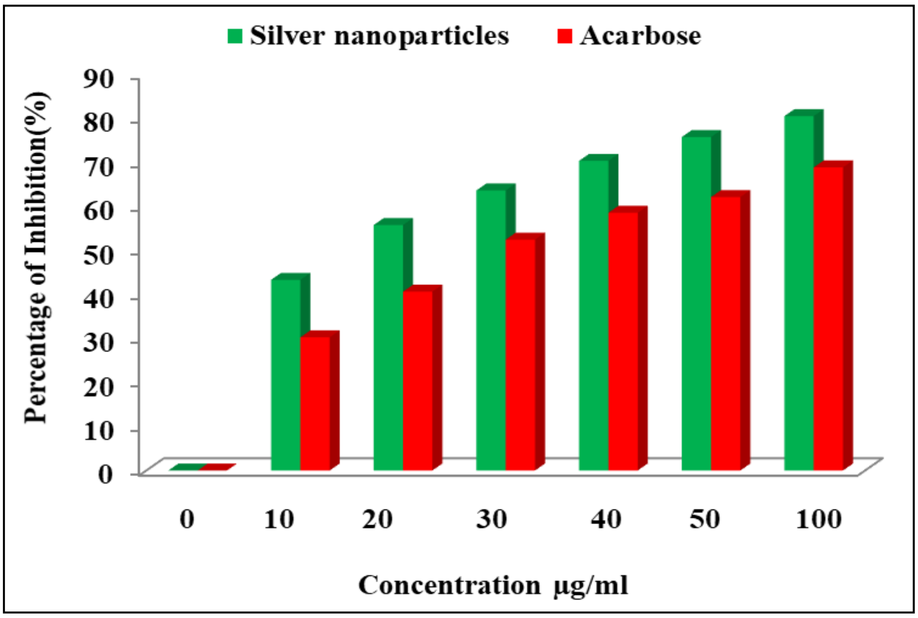

Fig 1: α -amylase inhibition activity of silver nanoparticles from C. auriculata leaves extract (Silver nanoparticle was compared with acarbose).

α -Glucosidase inhibitory activity

α -Glucocidase is an vital enzyme involved in carbohydrate metabolism by catalyzing the cleavage of oligosaccharides and disaccharides into monosaccharides (Chen and Guo, 2017) [23]. Studies exhibit that inhibition of α-glucosidase can prolong the digestion and absorption of carbohydrates, thus reduced the blood glucose levels (Khan*et al*., 2014) [24]. The silver nanoparticles synthesized from *Cassia auriculata* exhibit a-glucosidase inhibitory activity in a concentration dependent manner. When the concentration of silver nanoparticles increased, the inhibitory activity also increased along with acarbose (Figure. 2). Therefore, it can be used as α- glucosidase preventive agent to treat diabetes. In the same way, the silver nanoparticles synthesized from other medicinal plants were also showed α-glucosidase inhibition activity (Malaperma *et al*., 2017) [25].

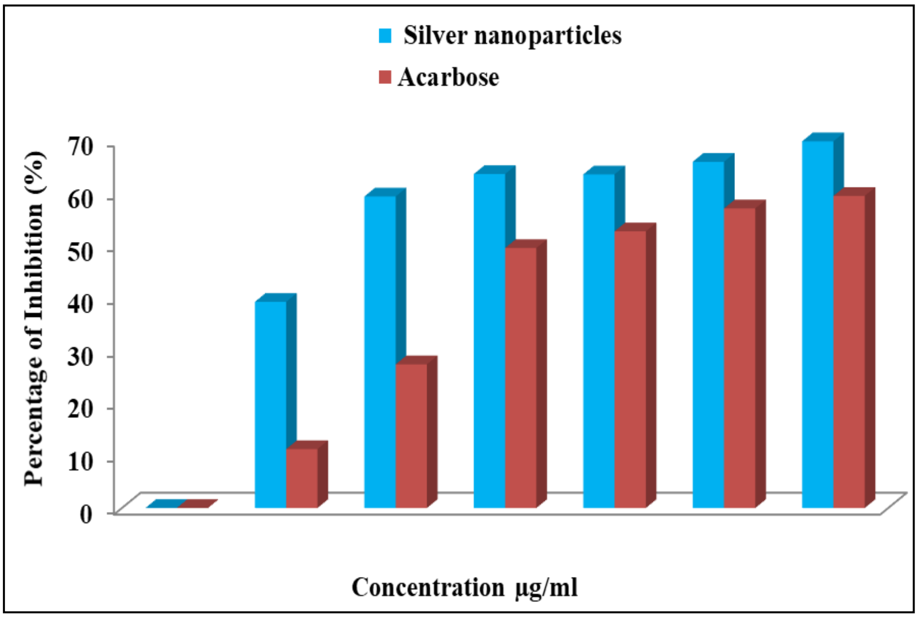

Fig 2: α -Glucosidase inhibition activity of silver nanoparticles from C. auriculata leaves extract (Silver nanoparticle was compared with acarbose).

Conclusion

The present study focused on Green synthesis of silver nanoparticles by using *Cassia auriculata* and evaluation of its antidiabetic activity by inhibiting the carbohydrate hydrolysing enzymes. The synthesized silver nanoparticles exhibit the higher level of antidiabetic activity by inhibiting the carbohydrate metabolizing enzymes such asα-amylase and α-glucosidase. Hence, the synthesized nanoparticles can be a good therapeutic agent to manage diabetes by inhibition the carbohydrate hydrolyzing enzymes. Further studies are needed to confirm its precise mechanism in animal and human samples to prescribe this product as a pharmacological agent for the treatment of diabetes.

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