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# PREPARATION OF SILVER NANO PARTICLES FROM THE LEAF EXTRACT OF *ALSTONIA SCHOLARIS* AND EVALUATION OF ITS ANTI-DIABETIC ACTIVITY

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**ABSTRACT**

Alstoniascholarisis commonly known as Devil tree belonging to the familyApocynaceae. *Alstoniascholaris*species are used as medicinal agent for the treatment of various diseases. This study was conducted to develop an ecofriendly, cheap and effective procedure for the green synthesis of Silver Nanoparticles ($AgNP_s$) from *Alstoniascholaris*leaf aqueous extract (ASAE) and evaluating their *in-vitro* Anti-diabetic activity. Addition of ASAE to $AgNO_3$(1mM) solution kept on stirring at 60°C resulted in the formation of *Alstoniascholaris*aqueous extract loaded Nanoparticles (ASAENP). Synthesized Nanoparticles are subjected to characterization by various procedures. UV-Visible spectroscopy showed absorption peak at **440nm** which was present in ASAE and $AgNO_3$. FTIR spectroscopy revealed the absorption peaks of different functional groups involved in the formation of ASAENP. Scanning Electron Microscopy (SEM) revealed average size ranging from **72.9nm to 144.9nm** with an average size of **115.95nm** and are spherical in shape. Particle size was determined and its size was found to be **263.3 nm.** Stability of ASAENP was determined by Zeta Potential which was found to be **-61.3mV &**Electrophoretic Mobility mean was found to be -**0.000474cm²/Vs**. Successfully characterized ASAENP were evaluated for their *in-vitro* Anti-diabetic activities such asalpha-amylase activity and alpha-glucosidase activity. Acarbose was used as a standard.

**Keywords:** Alstoniascholaris, Silver nanoparticles, Diabetes, Acarbose and Alpha-amylase.

## INTRODUCTION

Diabetes mellitus (“diabetes” for short) is a chronic serious endocrine disorder caused by inherited (or) acquired deficiency in production of insulin by the pancreas (or) by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body’s systems, in particular the blood vessels and nerves. The pancreas produces little insulin or no insulin at all. Insulin is a naturally occurring hormone, produced by the beta cells of the pancreas, which helps the body use sugar for energy. The pancreas makes insulin, but the insulin made does not work as it should. This condition is called insulin resistance.[1]

### Types of Diabetes

### Type 1 Diabetes mellitus

Type 1 Diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin. Patients with type 1 diabetes have serum antibodies to several components of the islets of

Langerhans, including antibodies to insulin itself[13].

**Type 2 Diabetes mellitus**

Nine out of 10 people with diabetes have type 2. This type occurs most often in people who are over 40 years old but can occur even in childhood if there are risk factors present. Type 2 diabetes may be controlled with a combination of diet, weight management and exercise. However, treatment also may include oral glucose-lowering medications (taken by mouth) or insulin injections (shots).[14]

Many synthetic oral hypo glycaemic agents like sulphonylureas, biguanides, meglitinides derivatives and alpha glucosidase inhibitors are presently in use but they all have several side effects. India has more than 40 million diabetic individuals which represents nearly 20% of total diabetes population worldwide, therefore regulation of diabetes without any side effects is still a difficult task for health care researchers .Therefore in this investigation extract of leaves of *Alstoniascholaris* and silver nanoparticles has been chosen to study the effect in the enzyme activities which are involved in the carbohydrate metabolism.[1, 2, 13]

**PLANT INTRODUCTION**

Devils tree commonly known as *Alstoniascholaris* belonging to family Apocynaceae[4.] *Alstoniascholaris* are used as medicinal agent for the treatment of various diseases. Genus *Alstonia*is well known for its alkaloid contents. Plant contain Uvaol, betulin, oleanolic acid, ursolic; beta-amylin acetate, alpha –amyrin acetate; beta –sitosterol; stigma sterol, Squalene; beta- sitosterol – 3 beta glucopyranoside -6- O- fatty acid which has been reported for its antidiabetic activity. Study of antidiabeticactivity of medicinal plants is highly encouraged [in] order to discover new natural substances. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic [4, 5, 12].

Distinctive property of nanoparticles is their large surface area to volume ratio. Silver, gold, palladium and platinum are the metals used in the synthesis but silver is superior among all the metals. Physical and chemical methods are used for the synthesis of nanoparticles. Green synthesis is another approach for synthesis of nanoparticles. Research increased currently because of safety, effectiveness and low cost. This innovative technique involves the reduction of Ag+ to Ago and stabilisation of AgNPs by bio molecules which are present in plant extract[11].Based on these benefits, present study was designed to use ASAE for the synthesis of silver nanoparticles and further evaluating their in-vitro anti-diabetic activity.

**MATERIALS AND METHODS**

**Plant material collection and extraction**

Leaves of Alstonia plant were collected from in and around areas of Sanga Reddy Dist. in Dec 2018. Plant material was authenticated with reference No.BSI/DRC/2018-19/Tech, by Scientist & HOO, P.V.Prasanna at Botanical Survey of India, Hyderabad, Telangana, India. PRIP/PCOG/18-19/003 is the reference no. given to herbarium and it is stored in the dept. of Pharmacognosy, Pulla Reddy Institute of Pharmacy, Hyderabad, India. Freshly collected leaves were washed with sterile water and shade dried for 10 days, and grinded to form a coarse powder. 20gm of powder with 200 ml of double distilled water (DDW)were taken and was kept for Soxhlet extraction for about 9-10 cycles. After cooling to room temperature, mixture was filtered with whatman filter paper no.1.Obtainedfiltrate was concentrated under Rota evaporator and later it was stored in refrigerator at $4^0$C for further use.

**CHEMICALS USED FOR ANTI-DIABETIC ACTIVITY**

Acarbose, Alpha-amylase, Alpha-glucosidase, Phosphate buffer, Sodium chloride, 3,5-Dinitro salicylic acid, Yeast extract, Disodium hydrogen phosphate, Potassium sodium L(α)tartarate, Sodium hydroxide pellets, Disodium hydroxide orthophosphate anhydrous, Starch soluble extra pure and Silver nitrate.

**Synthesis of $AgNo_3$ and loading of silver nanoparticles from ASAE (ASAENP)**

0.068mg of silver nitrate is dissolved in380ml of double distilled water (DDW) to get 1mM solution. The solution was kept on magnetic stirrer for 24 hrs in dark place for continuous stirring. To this add 20ml of ASAE solution slowly and the reaction was carried out in a conical flask on stirring at a temperature of 60°c for 8 hrs .Change in the colour of solution specifies the formation of ASAENP.

Now pour the above solution in a china dish and heating is continued until it gets evaporated. Dry the above solution in hot air oven at 60°c to obtain the dry powder form of ASAENP.Collect and store.

**Characterization**

**UV-Visible spectroscopy and Visual identification of ASAENP**

Colour of ASAE and AgNO3 solution was taken as control and change in the colour of reaction mixture after addition of ASAE to $AgNO_3$ solution is the first indication for the formation of ASAENP. UV-Visible spectroscopy was conducted in a range of 200-800nm by using UV-VIS Spectrophotometer (UV3000, LBINDIA) model No. 18-1885-01-0115.

**FTIR spectroscopy of ASAE and ASAENP**

In order to determine the involvement of functional groups in the formation of ASAENP, FTIR spectroscopy (SHIMADZU) was conducted in the range of 4000 to 500 $cm^{-1}$. ASAENP synthesized also contains bio molecules which are not capped on nanoparticles; they are removed by dissolving in DDW and centrifuged at 5000rpm for 15 min. Procedure is repeated for 3 times and final pellet obtained is dried in at $60^0$C in hot air oven and used for characterization**.**

**SEM studies of ASAENP**

Size, shape and morphology of ASAENP was determined by using SEM (ZEISS) operated at accelerating voltage 10.00kV, magnification 50.00 KX, working distance 8.5mm. Minute quantity of ASAENP was dropped on copper grid coated with carbon, extra solution was removed by using blotting paper and gird was used for analysis.

**Zeta potential and dynamic light scattering (DLS) analysis of ASAENP**

Dimensions of solid particles is determined by light scattering method operated at electrode voltage -3.4 V, conductivity -0.199 mS/cm, scattering angle 90, viscosity of the dispersion medium 1.100 mPas, along with count rate 205 kCPS. The temperature was maintained at $25.1^0$c

**RESULTS AND DISCUSSION**

**Visual identification and UV-visible spectroscopy of ASANP**

UV visible spectroscopy was employed to understand the formation of ASAENP is change of colour of $AgNO_3$ solution from colourless to dark brown. Excitation of surface Plasmon resonance and reduction of Ag+ to $Ag^0$ are the two major causes for the change of colour of solution (Figure 1). Temperature, time and stirring accelerated the reaction. UV spectra of ASAE, silver nitrate and ASAENP are presented in figure 2. Many studies reported the characteristic peak of SNPs in the range of 400- 450 nm which was absent in ASAE and $AgNO_3$, where as a peak at 440 nm was successfully appeared in ASAENP spectrum.

**FTIR spectroscopy of ASAE and ASAENP**

**FTIR** spectroscopy was carried out to determine different functional groups involved in the formation of AgNPs depicted in Figure 3. Different absorption peaks and their corresponding functional groups of ASAE and the shifts in ASAENP are discussed in table 1. Proteins, polysaccharides and enzymes present in the extract contains –OH group which undergoes stretching vibrations and produces peak at 3446.5 in ASAE, which is shifted to 3436.2 in ASAENP indicating their role in the formation of SNPs. FTIR spectroscopy proved the capping of functional groups of different chemical constituents present in ASAE on AgNPs. ASAE absorption peaks at 1073, 1397, and 1635 and ASAENP absorption peaks at 1248.7, 1270.5 and 1628 corresponds to carbon skeleton. ASAE peaks at 758.6, 780.6 and ASAENP absorption peaks at

1071.8 corresponds to flavonoids like structure.

**SEM (SCANNING ELECTRON MICROSCOPY) STUDIES OF ASAENP**

Morphology of surface of ASAENP is presented in figure numbers, 4, 5, 6, 7, 8 and 9.

Silver nanoparticles (AgNPs) identified by SEM analysis have a size ranging from 72.9 um

to 144.9 um with an average size of 115.95 um and are spherical in shape. Thus SEM results strongly confirm the role of ASAE as reducing and capping agent in the synthesis of ASAENP**.**

**Zeta potential and dynamic light scattering (DLS) analysis of ASAENP**

Zeta potential was conducted to determine the stability of ASAENP. Particle size distribution of ASAENP was estimated by DLS measurements. The modes of cumulative histogramic operations were depicted in fig no.10. From the result, the diameter of ASAENP was found to be **263.3 nm** and the average mean was found to be **278.4 nm.**

Mean zeta potential was found to be **-61.3mV.**andelectrophoretic mobility mean was found to be **0.000474 cm2/Vs** Negative value indicates the capping of constituents present in ASAE on surface of ASAENP. Moreover, negative charge also proves the stability and thus preventing them from agglomeration.

**IN-VITRO ANTI DIABETIC ACTIVITY**

**Effect of Acarbose and ASAENP on α-amylase activity**

Various concentrations (2 to 10mg/L) of Acarbose and ASAENP were prepared by using DDW. 100µL of each of the concentrations was taken in a test tube; to this add 500µL of 20mM sodium phosphate buffer with 6mM NaCl pH 6.9 containing porcine pancreatic α-amylase (0.5 mg/mL). Mixture was incubated for 10min at 25 °C. 500µL of 1% starch solution prepared in 20mM sodium phosphate buffer with 6mM NaCl pH 6.9 was added to each test tube. Reaction mixture was again incubated at 25 °C for 10 minutes. 1ml of 3,5-dinitro salicylic acid was added to stop the reaction; mixture was further incubated for 5 min in boiling water bath. Cooled to room temperature and 10ml of DDW was added and absorbance was measured by using UV-Visible spectrophotometer at 540 nm. Without Acarbose and ASAENP, a complete reaction mixture was taken control.

Inhibitory activity of α-amylase was calculated by using the formula:

**% inhibition =**

**(Acontrol – Asample / Acontrol) × 100**

**Effect of Acarbose and ASAENP on α-glucosidase activity**

Various concentrations (2 to 10mg/L) of Acarbose and ASAENP were prepared by using DDW. 20 µL of each of the concentration was taken in a test tube. To this add 100 µL of α-glucosidase (16.9 U/ml) solution in 0.1M phosphate buffer pH 6.9. Incubate at 250 C for 10 minutes and then add 50 µL of 5mM p-nitrophenyl-α-D-glucopyranoside solution prepared in 0.1M phosphate buffer pH 6.9. Reaction mixture was incubated at 250 C for 5 minutes and absorbance was measured by using UV-Visible spectrophotometer at 405 nm. Control contains complete reaction mixture without Acarbose or ASAENP. All the tests are carried out in triplicates.

Inhibitory activity was expressed as percentage inhibition and determined by using the formula:

**% inhibition =**

**(Acontrol – Asample / Acontrol) × 100**

**CONCLUSION**

The nanoparticles of aqueous leaf extract of Alstoniascholaris demonstrated the anti-diabetic property by inhibiting the alpha amylase and alpha glucosidase enzymes at various concentrations.

Administration of silver nanoparticles is beneficial in normalizing the alterations in carbohydrate mechanism during diabetes. With view of the result obtained from the study presents, the green synthesis as safe, cheaper for the production of nanoparticles. Various methods of characterization proved the formation of ASAENP. UV-Visible spectroscopy showed the peak at 440 nm. FTIR identifies the different functional groups

capping on ASAENP. SEM and DLS determine the morphology as well as its particle size. Stability is proved by Zeta potential. The data obtained in this study proves the inhibitory potential of ASAENP. However, further in depth studies are required to be conducted in terms of in-vivo procedures to develop ASAENP as a potent contender with high therapeutic efficacy and low side effects for the treatment of diabetes.

**ACKNOWLEDGEMENT**

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**Table 1: FTIR peak values and corresponding functional groups of ASAE and ASAENP**

| S.No | Peak in ASAE($cm^{-1}$) | Peak in ASAENP ($cm^{-1}$) | Corresponding functional groups |
|---|---|---|---|
| 1. | 3446.5 | 3436.2 | Stretching vibrations of -OH group of phenolic acid compounds and carbohydrates with -H bonding. |
| 2. | 1635.1 | 1628.7 | Stretching vibrations of C=C of alkenes |
| 3. | 1397.4 | 1384.2&1489.4 | Stretching vibrations of C-O group of Carboxylic acids. |
| 4. | 1073.9 | 1041.8&1248.7 | Stretching vibrations of C-N group of amines |
| 5. | 927.3 | ------ | Stretching vibrations of C-H group of alkenes |
| 6. | 2094.2 | ------ | Stretching vibrations of N=C=S of iso-thiocyanate |
| 7. | 58.6&780.6 | ----- | Bending vibration of O-H group of polyphenol |
| 8. | 681.9 | 693.9 | Stretching vibrations of C-Br |
| 9. | 585.1 | 596.5 | Stretching vibrations of C-H group of aldehydes |
| 10. | 572.6 | 568.7 | Bending vibrations of C-H group of alkanes |

**Table 2: Effect of Acarbose onalpha-amylase**

| Concentration | Acarbose | ASAENP |
|---|---|---|
| 2mg | 133.0672 | 123.90398 |
| 4mg | 158.5651 | 129.88 |
| 6mg | 164.5412 | 134.6608 |
| 8mg | 170.5172 | 136.2544 |
| 10mg | 174.5013 | 141.0353 |

**Table 3: Effect of Acarbose onalpha-Glucosidase**

| Concentrations | Acarbose | ASAENP |
|---|---|---|
| 2mg | 90.6384921 | 94.91779 |
| 4mg | 91.5296462 | 93.5725 |
| 6mg | 94.3198804 | 93.47285 |
| 8mg | 96.2132536 | 93.32337 |
| 10mg | 97.1599402 | 90.63279 |

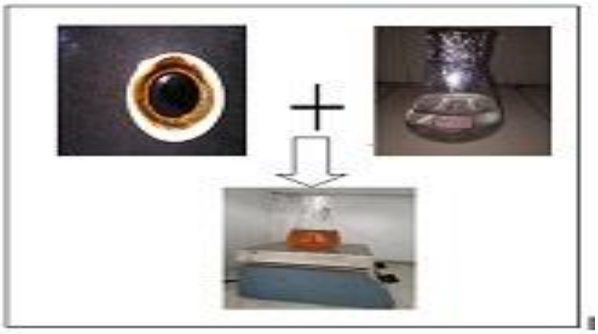

**Fig. 1: ASSE which is light brown in colour when added in $AgNO_3$ solution 1mM it forms dark coloured ASAENP. Change in colour indicates formation of nanoparticles**

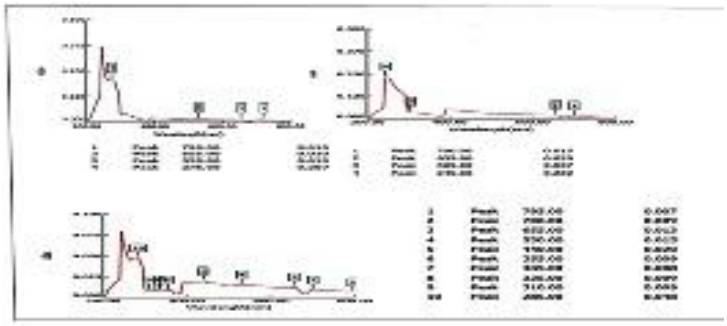

**Fig. 2: UV spectra of$AgNO_3$, ASAE and ASAENP**

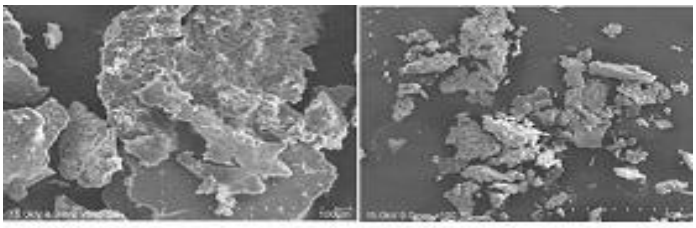

**Fig. 3: FTIR spectra of ASAE and ASAENP**

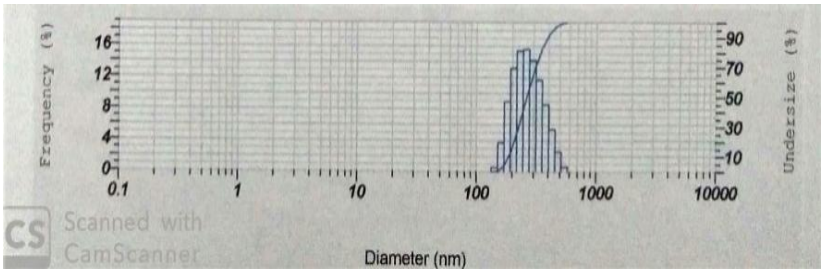


**Fig. 4: Histogramic study indicating particle size distribution pattern**

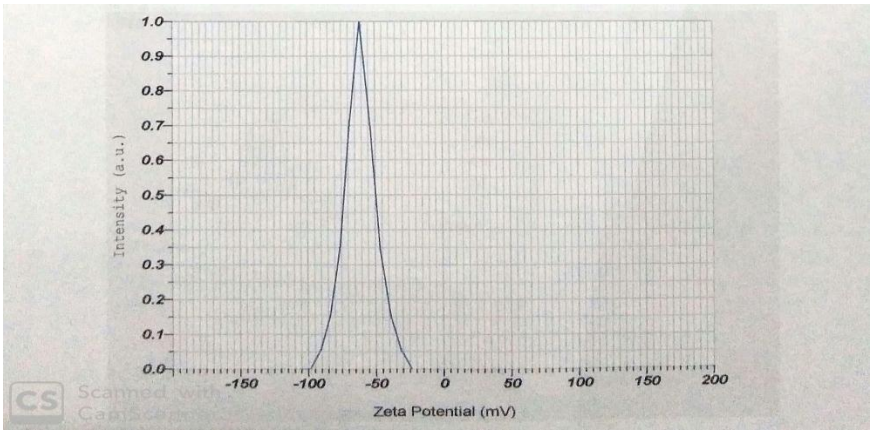


**Fig. 5: ZETA Potential Analysis**


**Fig. 6: Graphical representation of effect of Acarbose onalpha-amylase**

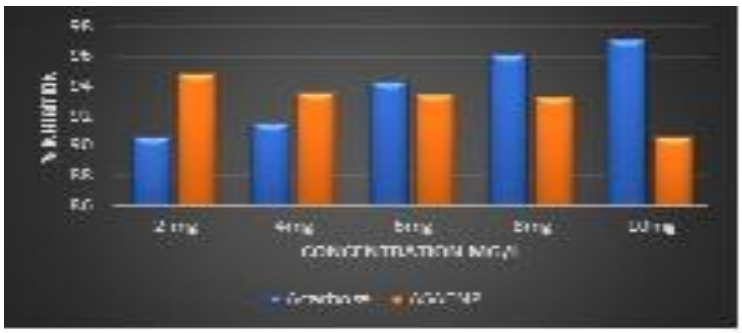

**Fig. 7: Graphical representation of effect of Acarbose onalpha-Glucosidase**

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