RESEARCH ARTICLE

Exploration of *In-vitro* Antidiabetic Activity of ZnO NPs and Ag NPs Synthesized using Methanolic Extracts of *Alpinia mutica* and *Tradescantia spathaeca* Leaves

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ABSTRACT

Diabetes might be cured with the use of medicinal herbs and environmentally friendly production of metallic nanoparticles (Ag NPs) and ZnO NPs. The methanolic leaf extracts of *Alpinia mutica* and *Tradescantia spathaeca* were used to synthesize silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs), respectively, for *in-vitro* evaluation.

Methanolic leaf extracts of *A. mutica* and *T. spathaeca* were used to create AgNPs and ZnO NPs under ambient conditions using ultrasound-assisted extraction (UAE). Their ability to block alpha- and beta-amylase confirmed the *in-vitro* antidiabetic efficacy of methanolic leaf extract of plant (MLEP), AgNPs, and ZnO NPs. In this study, α- amylase activity of ZnO and nanoparticles of silver produced from natural sources will be evaluated in an effort to lessen the toxicity and negative effects of the inhibitor used to treat diabetes. Antidiabetic action was especially impressive in the ZnO and silver nanoparticles produced using methanolic extracts of *A. mutica* and *T. spathaeca.* Because of their promising *in-vitro* antidiabetic action with alpha-amylase activity, MLEP of *A. mutica* and *T. spathaeca*, AgNPs, and ZnO NPs show promise for future medical uses.

Keywords: *Alpinia mutica*, Green synthesis, Phytochemical studies, Silver nanoparticles, *Tradescantia spathaeca*, Zinc oxide nanoparticles, *Alpha-amylase activity.*

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INTRODUCTION

Medicinal plants are a major source of drugs. Plants are used in health treatment by 80% of the global population[1]. Natural molecules may substitute synthetic components in food and pharmaceuticals, which have adverse effects.[2] Because of their free radical-scavenging abilities, therapeutic plants and their phytoconstituents are becoming more popular as natural sources.

Effectively, plants are providers of natural antioxidant molecules with various pharmacological actions and few to no adverse effects that defend against many illnesses and safeguard human health.[3-5] By preventing the spread of oxidative chain reactions, medicinal plant compounds delay the deterioration of lipids or additional molecules, hence the development of oxidative stress-related illness.[2]

Radiation, cigarette smoke, airborne hazardous chemicals, overnutrition, shifting dietary habits, and lack of physical exercise are all examples of exogenous sources of reactive oxygen compounds (ESROS), reactive nitrogen compounds (RNS), and free radicals in the body. A few examples of cardiovascular illnesses are heart failure with congestive systolic high blood pressure, chest pain, atherosclerosis, cerebral deficiency, vein insufficiency, and ventricular fibrillation, or VF. There are many different medicinal plants that contain powerful cardioactive glycosides and have good inotropic properties on the heart; some examples are *Digitalis*

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lanata, *D. purpurea* and *Apocynum cannabinum*, *Calotropis procera*, *Carissa spectabilis, Nerium oleander, Urginea rubra,* etc. Digitoxin, found in *Digitalis lanata* and *D. purpurea*, has been used to treat (CHF) congestive heart failure for decades. Cardiovascular disease is also warded off by elastin, laminin, collagen, and other antioxidant, extracellular, and long-lived proteins. Natural antioxidants like curcumin, baicalein, and resveratrol may scavenge free radicals and reduce the risk of atherosclerosis. *In-vitro* studies have shown that flavonoids such quercetin, morin, gossypetin, chrysin, myrecetin, rutin, which catechin and its byproducts, and some oligomeric proanthocyanidins inhibit LDL oxidation.[8]

Flavonoids from *Morus alba*, such as quercetin 3-(6-malonyglucoside), have been shown to boost LDL ability to resist oxidative modification, hence reducing the development of atherosclerotic lesions in mice lacking the LDL receptor.[9] In nanobiotechnology, the focus is on particles with dimensions between 1 to 100 nm. It has been shown that nanoparticles are used in various industries, including the ones dealing with medicine, engineering, pharmaceuticals, and textiles.[10–12] Antibacterial, antifungal, anticancer, catalytic, and electrical applications are just some of the many areas of research that have been conducted on metallic nanoparticles, particularly platinum, silver, and gold.[13] Silver nanoparticles' remarkable chemical and physical properties, particularly in electricity, thermal, catalytic, commercial, and medical fields, have recently attracted researchers.[14, 15]

AgNPs and ZnONPs may be used in medical equipment coating, sensors, medication delivery, pharmaceutics, orthopedics, and cancer.[16] Due to their low surface area-to-volume ratio, metallic nanoparticles may be altered physically, biologically, and chemically[17]. Research shows silver nanoparticles from *Hypnea valentiae* have excellent antioxidant, antimicrobial, and anticancer properties.[18]

High blood glucose levels characterize diabetes.[19] Most symptoms include increased hunger, thirst, and urination. Untreated diabetes may cause chronic renal failure, stroke, cancer, neuropathy, visual loss, and cardiovascular disease.[20] According to estimates, roughly 25% of the world's population has diabetes mellitus, affecting individuals in industrialized and developing nations.[21] Most antidiabetic medications lower blood glucose, including sulphonylurea, the enzyme alpha inhibitors, biguanides, and thiazolidinediones, sulfonylurea depolarizes cell membranes, allowing calcium ions to enter beta cells and increase insulin release from secretory granules.[22]

Despite their long history, antidiabetic medicines have adverse effects such as weight gain, intestinal disruption, acidosis of the intestines, liver damage, and increasing drug resistance and toxicity.[23,24] Studies have reported the use of nanotechnology is used to treat diabetes. Nanoparticles may be employed to make accurate glucose sensors.[25,26] Nanomedicine can detect blood glucose variations and autonomously release insulin to maintain normal levels.[27] Nanoparticulate cancer medications like abraxane, onivyde, and doxil may increase drug delivery to target locations.[28,29] This project will conjugate AgNPs and ZnONPs with commercial medicines to deliver treatments to the target region and boost biological efficacy.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$), zinc nitrate hexahydrate (ZNH), and NaOH were procured from Sigma–Aldrich, India. The Hyderabad, India-based Plant Research Centre gathered and verified disease-free fresh leaves of *A. mutica* (AM) and *T. spathaeca* (TS). The gathered leaves were cleaned twice with double-distilled water and once with running tap water. Then, 20 g of dried leaves were cooked with 100 mL of double-distilled water for 20 minutes at 60°C to produce a light-yellow solution. The solution was passed through filters using Whatman No.1 paper filters and refrigerated after cool to ambient temperature. These extracts of plants and chemical techniques synthesized $AgNO_3$ and ZnO nanoparticles.

Fabrication of NPs of AM and TS

Biological fabrication of Ag nanoparticles

Simple green synthesis yielded Ag NPs. A magnetic stirrer was used to add 10 mL of the filtered AM and TS medication solution gradually to 45 mL of the 1 mM $AgNO_3$ solution in conical flasks. The cylindrical flasks were vigorously shaken for the next 0, 12, and 24 hours to produce Ag NPs. The solution became dark brown after 12 to 24 hours in conical flasks at room temperature. The colored solution underwent centrifuging for twenty minutes at 5000 rpm. The residue was the only thing left behind after the supernatant was removed. Sterile distillation water was employed to clean and dry the residual residue.[30]

Biological fabrication of ZnO nanoparticles

In 25 mL of plant extract and roughly 0.1 M hexahydrate of zinc nitrate were stirred vigorously for two hours. The produced dirty-colored precipitate was allowed to cool for 24 hours after the reaction. The precipitate was centrifuged at 6000 rpm for 15 minutes to separate it from the reaction solution. The precipitate was then repeatedly washed in deionized water to eliminate impurities, and the dry product was then dried at 80°C. The as-prepared powdered sample was heated to 350°C in a muffle furnace for three hours, and 5 L of ZnO NPs solutions was then poured over a copper grid that had been dusted with carbon and cooled further before being brought to a scanning electron microscope.[31]

In-vitro Antidiabetic Activity

The activity of α -amylase inhibition

The DNS technique evaluated the inhibitory activity against *α -amylase*. Silver nanoparticles at various concentrations (20–100 g/mL) were made from a conventional phosphate buffer solution at 1-mg/mL concentration. In 250 µL of samples (2 units/mL) were incubated at 27°C for 10 minutes with 250 µL of an amylase solution. After another 10 minutes of incubation, 250 µL of a starch solution (1% was added). Dinitro-salicylic acid (DNS) (from 0.5 mL), a colorant, was used to halt the

reaction, after which the fluid was heated over a water bath, boiling for 10 minutes.

After cooling, it was diluted by adding 5 mL of distilled water. The enzyme was replaced with a buffer for each test sample concentration to create a blank. Control was kept constant without including a sample that showed 100% enzyme activity. Acarbose was employed as a positive control. The absorbance of the colored solution at 540 nm was measured using a spectrophotometer, and the technique was used to compute the percentage of inhibition[32-34] and formula is (Absorbance $_{Control}$ − Absorbance $_{Test}$) / (Absorbance $_{Control}$) ×100.

RESULTS

Fabrication of Nanoparticles of AM

Biological fabrication of Ag nanoparticles

Green synthesis created silver nanoparticles. In 45 mL of a 1 mM $AgNO_3$ solution was put into conical flasks with a magnetic stirrer, and 10 mL of filter AM medicine solution was added dropwise. Ag NPs were then produced by vigorously agitating the conical flasks for 0, 12, and 24 hours. After 12 to 24 hours of room-temperature incubation in conical flasks, the solution turned from pale yellow to deeper brown (Figures 1 and 2). Centrifuging the fully colored solution was carried out for 20 minutes at 5000 rpm. Only the residue remained after the supernatant was eliminated. Finally, the residual residue was cleaned and dried using sterile distilled water.

Biological fabrication of ZnO nanoparticles

For two hours, vigorous stirring was used to combine 25 mL of the extract with approximately 0.1 M zinc nitrate hexahydrate. After the reaction, a 24 hours cooling period was given to the dirty-colored precipitate that had developed. Centrifuging the precipitate at 6000 rpm for 15 minutes separated the reaction solution from the precipitate. Next, contaminants were removed by repeatedly washing the precipitate in deionized water, and the dried product was then baked at 80°C. The powder as-prepared sample was oxidized at 350°C in a muffle furnace for three hours, and 5 µL of ZnONPs solution was then deposited on a copper grid coated in carbon and cooled further before being transported to the scanning electron microscope (Figure 3).

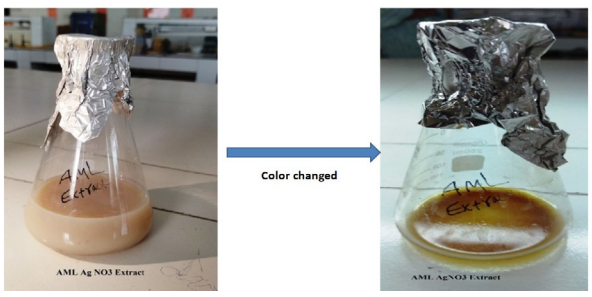

Figure 1: Biosynthesis of nanoparticles of Ag Nanoparticles

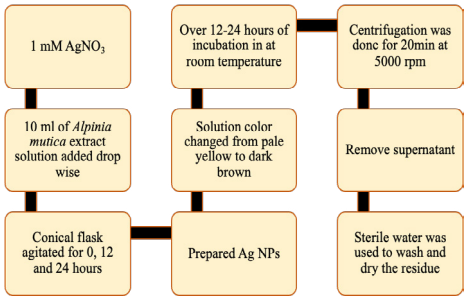

Figure 2: Biological fabrication of Ag NPs from AM extract

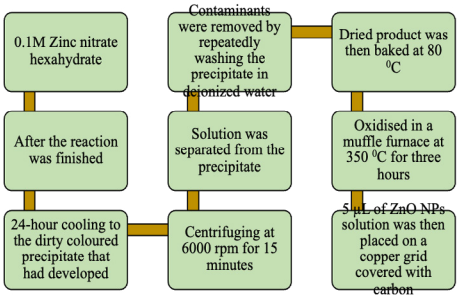

Figure 3: Biological fabrication of ZnO NPs from AM extract

Biosynthesis of Nanoparticles of TS

Biological fabrication of Ag nanoparticles

Ag nanoparticles were created utilizing a simplified green synthesis method. A dropwise addition of 10 mL of filtered TS drug solution was made after 45 mL of a 1 mM AgNO3 solution had been introduced to conical flasks using a magnetic stirrer. The conical flasks were aggressively shaken for 0, 12, and 24 hours to make Ag NPs. The solution became dark brown after 12 to 24 hours in conical flasks at room temperature (Figures 4 and 5). On the fully colored solution, centrifuging was carried out for 20 minutes at 5000 rpm. Just the residue was preserved after the supernatant was removed. The last step was to wash and dry the remaining residue with sterile distilled water.

Biological fabrication of ZnO nanoparticles

After the reaction, a 24-hour cooling period was given to the dirty-colored precipitate that had developed. Centrifuging the precipitate at 6000 rpm for 15 minutes separated the reaction solution from the precipitate. Next, contaminants were removed by repeatedly washing the precipitate in deionized water, and the dried product was then baked at 80°C. The as-prepared powdered sample was oxidized for three hours at 350°C in a muffle furnace, and 5 liters of the ZnONPs solution was then deposited on a copper grid coated in carbon and coated and dried subsequently before being transported to a scanning electron microscope (Figure 6).

In-vitro Antidiabetic Activity

α-Amylase inhibition activity

The *in-vitro* antidiabetic activity of alpha-amylase inhibition AMLE AgNPs and AMLE ZnONPs showed similar activity compared with standard drug acarbose, and percentage

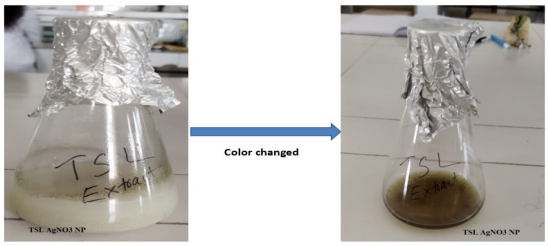

Figure 4: Biosynthesis of Ag Nanoparticles

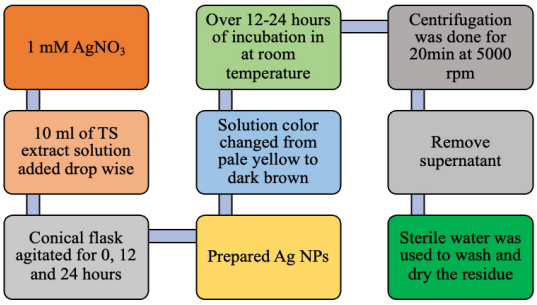

Figure 5: Biological fabrication of Ag nanoparticles from TS extract

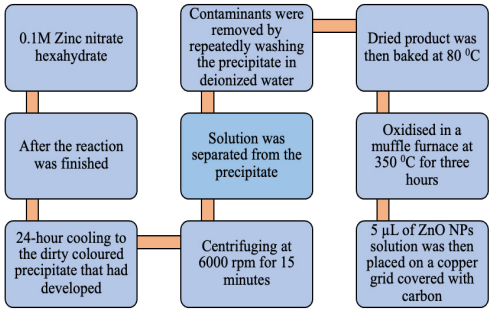

Figure 6: Biological fabrication of ZnO NPs from TS extract

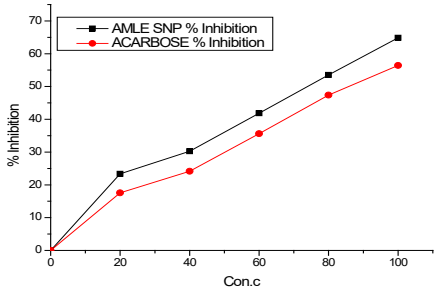

Figure 7: α-Amylase inhibition activity of AM Ag NPs

Table 1: α-Amylase inhibition activity of AMLE SNP

Conc'n (µg/mL)	*AMLE SNP %Inhibition*	*ACARBOSE % Inhibition*
0	0	0
20	23.36	17.57
40	30.26	24.14
60	41.85	35.6
80	53.51	47.39
100	64.84	56.44
IC_{50} Values	73.72	87.26

Table 2: α-Amylase inhibition activity of AM ZnO NPs

Conc'n (µg/mL)	*AMLE ZnO NP %Inhibition*	*Acarbose %Inhibition*
0	0	0
20	23.36	17.57
40	29.94	24.14
60	41.59	35.6
80	53.96	47.39
100	64.97	56.44
IC_{50} Values	73.49	87.26

Table 3: *α-Amylase* inhibition activity of TS SNPs

Conc'n (µg/mL)	*TS SNPs %Inhibition*	*Acarbose %Inhibition*
0	0	0
20	23	17.57
40	30.33	24.14
60	41.66	35.6
80	53.57	47.39
100	64.64	56.44
IC_{50} Values	73.77	87.26

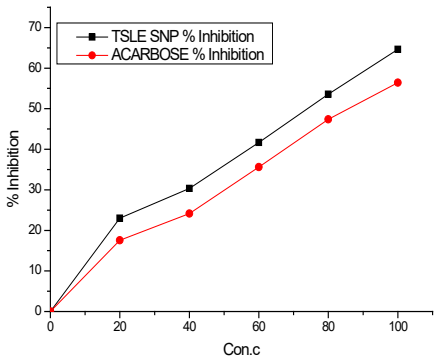

Figure 9: *α-amylase* inhibition activity of TS Ag NPs

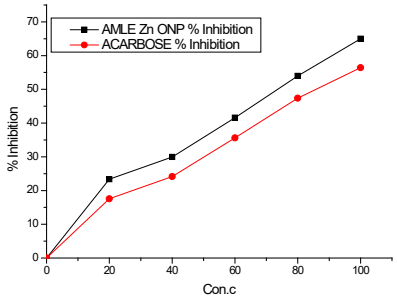

Figure 8: *α-Amylase* inhibition activity of AM ZnO NPs

inhibition of *alpha-amylase* resulted in being similar to acarbose. If IC_{50} values are less than 100 µg/mL, they contain more potent activity, and IC_{50} values of AMLE AgNPs 73.72 µg/mL, AMLE ZnONP 73.49 µg/mL and acarbose were 87.26 µg/mL, here calculations kept in Tables 1 and 2, Figures 7 and 8.

Table 4: *α-amylase* inhibition activity of TS ZnO NPs

Conc'n (µg/mL)	*TS ZnO NPs %Inhibition*	*ACARBOSE %Inhibition*
0	0	0
20	23.26	17.57
40	30.07	24.14
60	41.66	35.6
80	53.25	47.39
100	64.77	56.44
IC_{50} Values	73.93	87.26

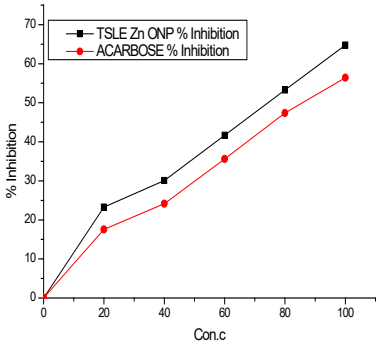

Figure 10: *α-amylase* inhibition activity of TS ZnO NPs

In-vitro Antidiabetic Activity

α-Amylase inhibition activity

The *in-vitro* antidiabetic activity of alpha-amylase inhibition TS Ag NPs and TS ZnO NPs showed similar activity compared with standard drug acarbose, and percentage inhibition of alpha-amylase resulted in being similar to acarbose. If IC_{50} values are less than 100 µg/mL, they contain more potent activity, and IC_{50} values of TS SNPs (silver nanoparticles) 73.77 µg/mL, TS ZnO NPs 73.93 µg/mL, and ascarbose were 87.26 µg/mL, here calculations kept in Tables 3 and 4 and Figures 9 and 10.

CONCLUSIONS

Using silver and zinc oxide nanoparticles, a sustainable, affordable, and easy-to-use "green synthesis" technique produced *A. mutica* and *T. spathacea* extracts from methanol. These nanomaterials showed promising *in-vitro* antidiabetic activity against an *alpha-amylase* inhibitor. These results support the widespread application of these nanoparticles in the pharmaceutical and nanomedical industries. More in-depth research on these materials, including *in-vivo* studies, can pave the way for exciting advances in nanomedicine.

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