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Research Article

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Synthesis of silver nanoparticles using *Momordica charantia* and its applications

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ABSTRACT

In the present study, silver nanoparticles were synthesized by biological methods using Momordica charantia extract. UV–Visible absorption spectroscopy, Fourier Transfer Infra Red (FTIR) and Scanning Electron Microscope (SEM) were used for characterization of synthesized silver nanoparticles. Diabetes is the most common metabolic disorder in human and it is characterized by hyperglycemia, due to relative or absolute lack of insulin. The approaches of nanoscale and the percentage of atoms at the surface of a material becomes significant. Silver nanoparticles were screened for inhibitory activity of alpha amylase enzyme. The synthesized Silver Nanoparticles showed significant effect on Antidiabetic activity. Acarbose was used as a standard drug. The IC_{50} *value for Bittergourd extract was found to be 49.86µg/ml where as for acarbose it is 88.3µg/ml.*

Key words: Diabetics, *Momordica charanti,* Nanoparticles

INTRODUCTION

Nanotechnology is a broad-based science involving manipulation of atoms, electrons, protons and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in medicine, engineering, agriculture, surface science, marine science, and geology. It involves in the dimensions at nanoscale size ranging up to 100nm [1].Nanoparticle has potential applications in various fields such as healthcare, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics, and space industries[2]. It also have been achieved extensively in the drug delivery system for the treatments of cancer[3],diabetes[4], allergy[5], infection [6]and inflammation [7].

Nanoparticles are grouped into organic, inorganic, metal and semi-conductor nanoparticles due to their superior material properties. There are many ways to synthesize nanoparticles such as solid reaction, co-precipitation, chemical reaction, and solgel method, microwave irradiation etc. The various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots(CdS, ZnS), among these Silver nanoparticles are known as excellent antimicrobial agents, and therefore they could be used as alternative disinfectant agents. On the other hand, released silver nanoparticles could pose a threat to naturally occurring microorganisms.

In recent years Green synthesis provides an advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis. This technique eliminates the use of energy, high pressure, temperature and toxic chemicals[8,9].As plant medicated nanoparticles preparation is easy to handle, safe and economical [10]. It finds more advantages over chemical and physical method. In biological method plants have been used for the synthesis of nanoparticles were coated by the plant extract which has medical benefits and can be used as drug and cosmetic applications[11]. Plants used for green synthesis of AgNPs like *Acalypha indica* leaf, *Coriandrum sativum, Sorbus aucuparia* leaf, *Gliricidia sepium, Rose* leaf, *Cinnamomum camphora, Aloevera and Neem, Camellia sinensis, Magnoliakobus and Diopyroskaki* leaf, *Geranium* leaf etc.All the parts of the plant like leaf, stem, flower, seed and skin of the fruits were used earlier for the synthesis of AgNPs. In this report, *Momordica charantia* belonging to the family of Cucurbetaceae commonly known as Bitter gourd, which includes melons, squashes and gourds found throughout India[12,13] and has been used as a traditional medicine to cure various diseases. The plant mainly confirms the presence of phytochemicals such as steroids flavonoids, alkaloids, terpenoids, proteins, phenols and anthraquiones which are already reported[14].

The current investigation focuses on the extract of *Bittergourd* used to synthesize AgNPs using different experimental conditions and thereby enhancing the importance of plant sources and implementing green chemistry for the future research.

EXPERIMENTAL SECTION

Preparation Of *Bittergourd Juice* Extract

Bitter gourd is purchased from a local super market. About 500 grams of Bitter gourd is taken and cut into small slices. The samples were crushed in a mixer and the extract was taken for experiments. The extract was centrifuged at 5000 rpm for 15mins. The supernatant was collected and 100 ml of supernatant was taken in a conical flask.

Synthesis Of AgNps

One mM of Silver nitrate was dissolved in 100ml of distilled water and it was added to the sample. Brown color change was observed after 15mins.The spectrum were taken after 48 Hrs of Silver nitrate addition between 300nm to 600nm for the confirmation of AgNPs formation.

Characterization

The UV-Visible spectral measurements were used to confirm the formation of silver nanoparticles by using Shimadzu – UV1800 spectrophotometer instrument in the range between 300-600nm [16].FTIR experiment were carried out to determine the biomolecules present in the sample extract responsible for the reduction of Ag ions with 400 -4000 $cm^{-1}$of spectral range. The sample was centrifuged at 9000rpm for15min,then dried and ground with KBr to form a pellet and analyzed on ThermoNicolet iS5 model. The SEM analysis was carried out to determine the morphology and the mean particle size of nanoparticles. The sample were prepared on a carbon coated grid by just dipping a very small amount of the prepared AgNPs on the grid, by using blotting paper the extra solution was isolated, then the sample were allowed to dry for SEM analysis using JEOL-JSM-5610LV with resolution of 1µm at 15kV with 27mm.

RESULTS AND DISCUSSION

UV-Vis Analysis

The reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The optical property of AgNPs was determined by UV-Visible spectrophotometer. After the addition of Silver nitrate to the plant extract, the spectrum were taken at 48Hrs between 300 nm to 600 nm. The AgNPs has sharp absorbance with highest peak at 422nm and progressively decreased while nanometer increased [17].

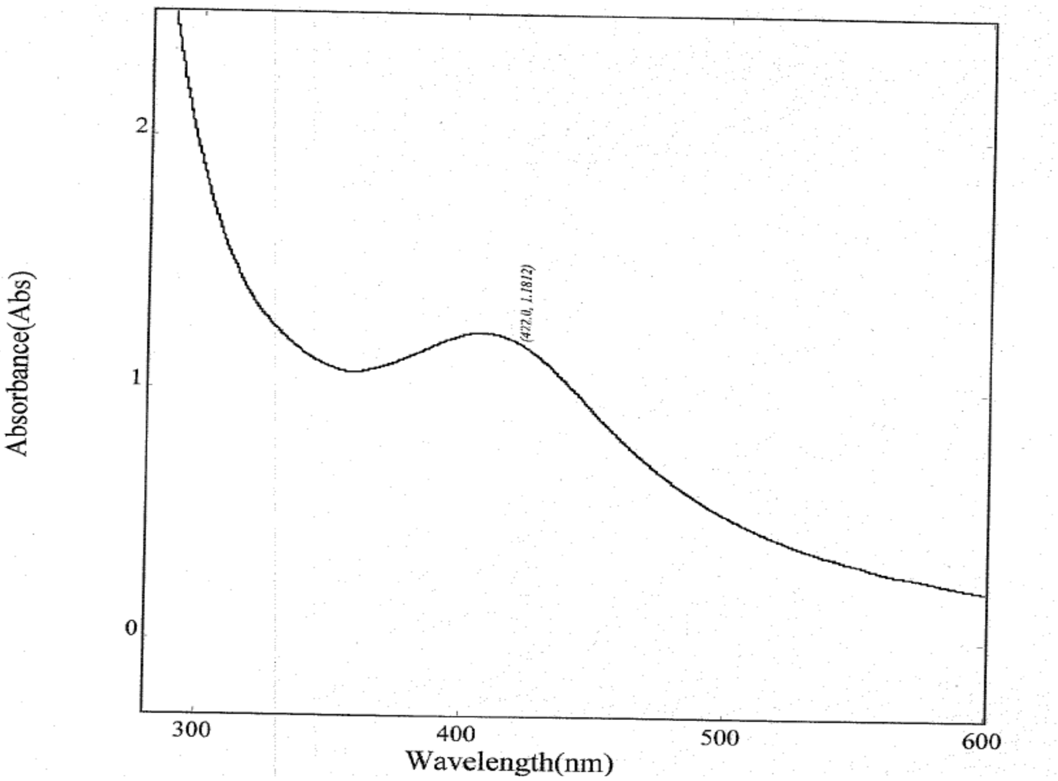

Fig.2: Uv-visible spectroscopic image

SEM Analysis

The morphological features of synthesized silver nanoparticles from Bittergourd extract was studied by Scanning Electron Microscope. The figure shown the high density of AgNPs synthesized using the extract of *Momordica charantia* has confirmed the presence of AgNPs. The electrostatic interactions such as hydrogen bond , bio-organic bond and capping molecules are the reason for biosynthesis of silver nanoparticles [18]. The Synthesized nanoparticles were spherical and rod shape. Bittergourd mediated silver nanoparticles in the average range of 600nm. The size was more than the desired size as a result of proteins which were bound in the surface of the nanoparticles. The shape may vary due to the concentration profile [19].

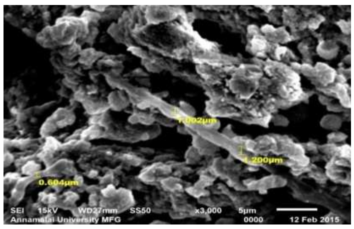

Fig.2: SEM analysis of Bittergourd mediated AgNPs

FTIR

FTIR measurement was done to identify the biomolecules and functional groups present in the *Momordica charantia* extract. The aqueous extract of Bittergourd shown peaks at $1630cm^{-1}$,$1529cm^{-1}$,$1445cm^{-1}$,$1383cm^{-1}$,$3404cm^{-1}$.The broad peak at $3404cm^{-1}$ which shown the presence OH phenolic compound is mainly responsible for bioreduction of Ag ions. The absorption peak at around 485cm-1 can be assigned as Secondary amide N-H wagging, likewise the peak at 1030cm-1 as phosphorus ester P-OH stretching,1445cm-1 as Nitrate NO2 symmetric stretching,1529cm-1 as Aliphatic nitro compound NO2 symmetric,1630cm-1 as Primary amide NH2 bending-amide II band. Particularly the extract of Bitter gourd contained the chemical constituents of phenolic compounds, Alkaloids, flavonoids, steroids , glycosides, reducing sugar, saponins are acting as a capping and reducing agents [20]. The reduction of silver ions to silver nanoparticles was confirmed from the peak value of Bitter gourd extract (1630 cm^{-1} to 1529 cm^{-1}).

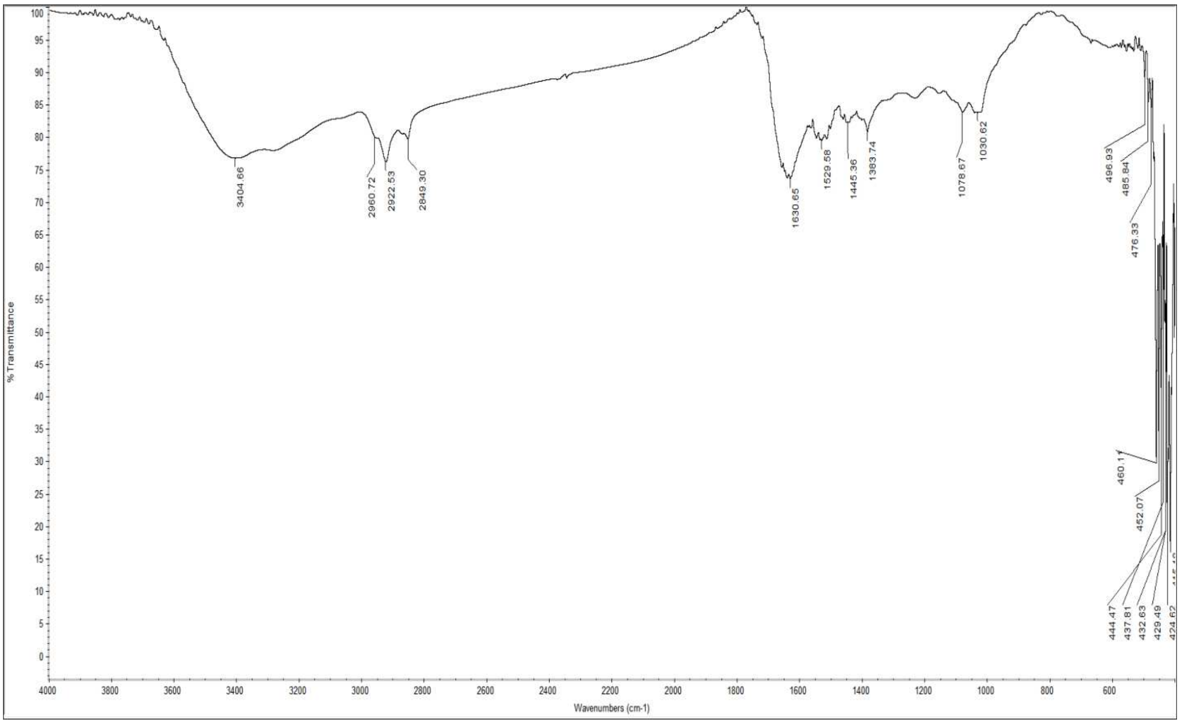

Fig.3: FTIR analysis of Bittergourd AgNPs

Bioassay for α-Amylase Inhibition

Alpha Amylase enzyme aids in the breakdown of starch to maltose. It hydrolyzes the bonds between glucose repeats . Before glucose is transported from the gut to the blood. Bittergourd reduces the amount of glucose that is released into the blood thereby inhibiting the enzymes alpha amylase and alpha glucosidase that break down disaccharides into two monosaccharides. In this study, focused on inhibition of α-amylase enzyme was done using silver nanoparticles synthesized from *Momordica charantia* to evaluate their potential in hypoglycaemic effects. The enzyme solution was prepared by mixing 0.0253g of (α-amylase in 100ml of cold distilled water). A starch solution (0.5% w/v) was obtained by stirring 0.1g of potato starch (Sigma) in 25ml of 20mM sodium phosphate buffer along with 6.7mM sodium chloride, pH 6.9 at 65°C for 15min. Silver nanoparticles of *Momordica charantia* extract is dissolved in citrate buffer to give a final concentration from 10µg/ml to 80 µg/ml. The colorimetric reagent was prepared by mixing a sodium potassium tartrate solution (12.0g of sodium potassium tartrate with 2M NaOH) and 96mM of 3,5-dinitrosalicylic acid solution. Both control and Bitter gourd extracts were added with starch solution and left to react with (α-amylase solution under an alkaline condition at 25°C). The reaction was measured over 3min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid[21]. This reaction (corresponding to colour change from orange- yellow to red) is detectable at 540nm. In the presence of an alpha-amylase inhibitors less maltose would be produced and the absorbance value would be decreased. Preliminary experiments were carried out to establish optimal conditions.

$$\text{Inhibition activity (\%)} = \frac{\text{Abs(control)- Abs (extract) x 100}}{\text{Abs (control)}}$$

The IC50 values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the Bittergourd juice extracts were determined by performing the assay as above with varying concentrations of extracts ranging from 10-80 µg/ml. The IC50 values were determined from plot of percent inhibition versus inhibitor concentration by linear regression analysis[22].

Positive control :

Acarbose is an anti-diabetic drug used to treat Type-2 diabetes mellitus. It was used as a positive control. It is an inhibitor of alpha glucosidase, an enteric enzyme that releases glucose from larger carbohydrates [23,24].

Table 1: Alpha amylase inhibitory effects of Acarbose[24]

Concentration of Acarbose (µg/ml)	% Inhibition
10	18.67
20	22.39
40	28.99
60	38.24
80	47.34
100	58.21

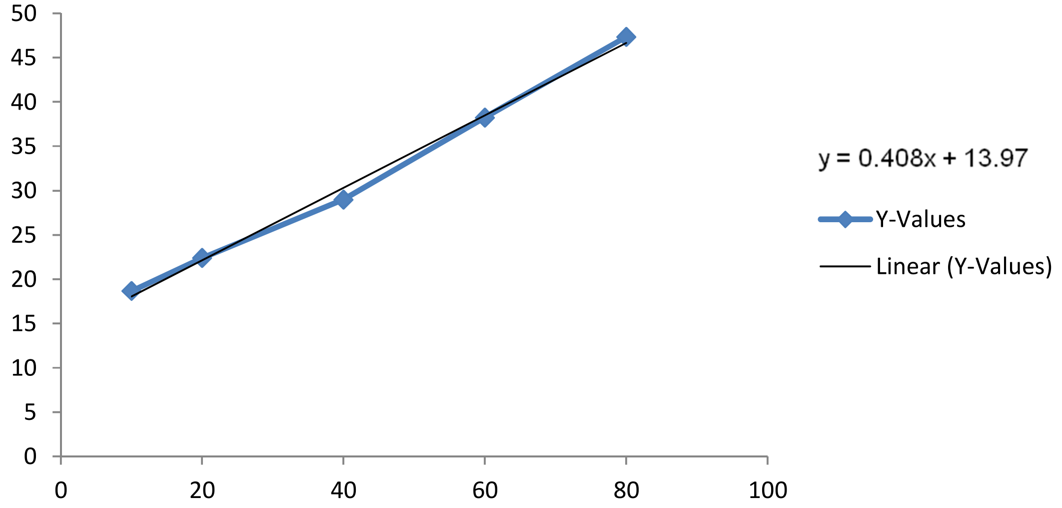

Fig. 4: IC_{50} value of acarbose against α-amylase Enzyme

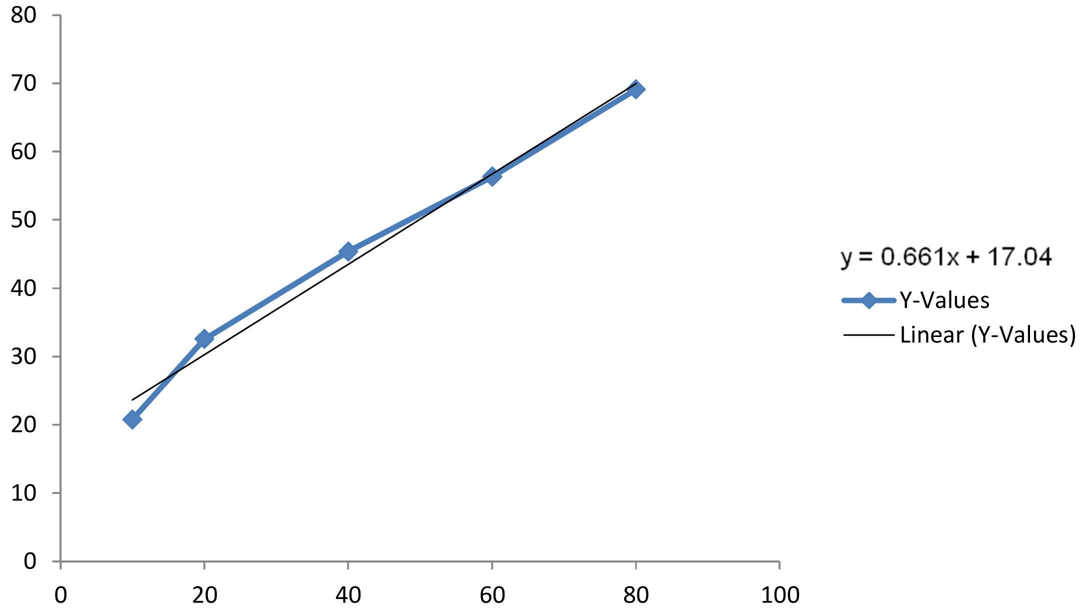

Fig.5: IC_{50} value of Bittergourd mediated Silver nanoparticles against alpha amylase enzyme

Table 2: Alpha amylase inhibitory effects of Momordic acharantia

Concentration of Bitter gourd AgNp (μg/ml)	% Inhibition
10	20.76
20	32.55
40	45.35
60	56.32
80	69.10

CONCLUSION

Silver Nanoparticles were successfully prepared by Green synthesis method using the juice extract of *Momordica charantia*. On exposure to darkness, reduced the 1mM of $AgNO_3$ solution leads to the formation of silver nanoparticles. The change green to brown indicated the formation of Silver nanoparticles. The formation of Silver nanoparticles and the concentration of Bittergourd extract required to produce Bittergourd Mediated Silver nanoparticles with no aggregation was found out by UV-Visible spectroscopic analysis. SEM results showed that the particles were spherical and rod shape.The Silver Nanoparticles exhibited various physio chemical and biological properties. Bioassay method was done to check the antidiabetic property of Bittergourd mediated silver nanoparticles. It showed the better significant effect on inhibition of alpha amylase. The IC50 value of bittergourd silver Nanoparticle was found to be 49.86µg/ml. Thus, it showed the appreciable inhibition towards an enzyme amylase.

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