Research Article

ANTIOXIDANT, ANTIBACTERIAL, ANTIDIABETIC POTENTIAL AND GENOTOXICITY OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *Curcuma longa*: A NOVEL GREEN APPROACH

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ABSTRACT

In this paper, an aqueous fresh leaves extract of *Curcuma longa* was used for the green synthesis of silver nanoparticles (AgNPs) emerges as a cost-effective and eco-friendly approach. Characterization of nanoparticles was done using different methods, which include; ultraviolet-visible spectroscopy (UV-Vis), powder X-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray analysis (EDAX), dynamic light scattering (DLS) and antibacterial activity. UV-visible spectrum of the silver nanoparticles showed absorption peak at around 426 nm. Scanning electron microscope micrographs showed the formation of well-separated silver nanoparticles of size in the range of 90-111 nm. XRD study showed the particles to be crystalline in nature, with a face-centered cubic (fcc) structure. The size and stability were detected using DLS and zeta potential analysis. The antioxidant activities of silver nanoparticles were analyzed by the DPPH radical with IC_{50} 97.87 μ g/ml and standard while it was BHT= 60 μ g/ml, reducing power activity, hydroxyl and nitric oxide radical scavenging assays were analyzed. The synthesized nanoparticles having potent antibacterial activity against both Gram-positive and Gram negative bacteria's. The *in-vitro* antidiabetic activity was analyzed and it was effectively inhibited carbohydrate-hydrolyzing enzyme i.e. α -amylase with increasing of AgNPs concentration. SDS-PAGE analysis of capping protein around the silver nanoparticles was analyzed. The genotoxic effect of silver nanoparticles analyzed with different concentration (0.5-5 μ g/ μ l) against plasmid pBR322 DNA, and it was showed degradation of DNA. Hence, this AgNPs might be used as antibiotics and antidiabetic agent in future and can be used in many medicinal and technological applications due to non-toxic, cheap and eco-friendly.

Keywords: Green synthesis, AgNPs, Leaf extract, Antidiabetic activity, Antibacterial activity, Genotoxicity

INTRODUCTION

Nanoparticle (NPs) have been considered one of the most forefront materials in the recent decades. They have been reported to be the "material of the 21st century" because of their unique designs and property combinations compared with conventional materials ¹. There is wide range of applications of NPs such as human health appliances, industrial fields, medical applications, biomedical fields, engineering, electronics, and environmental studies ². Recently, enormous attention has been focused on the use of nanoparticles such as nanotubes, nanowires, fullerene derivatives, and quantum dots to create new types of analytical tools in the field of life science and biotechnology ³. Among all materials AgNPs are the most widely used and may be considered one of the most important, and they have become high demand material for consumer products ⁴. The silver nanoparticles are used in medicine, pharmacology, biotechnology, electronics, engineering energy and environmental remediation, because of their highly effective antibacterial activity both in solution and components ⁵. The AgNPs have gained popularity in industrial sectors including textiles, food, consumer products and medicine ⁶.

The increased usage of nanoparticles especially for medical purposes created a new challenge of synthesizing them by untraditional methods to overcome the undeniable disadvantages of the other physical and chemical methods ⁷. Plant mediated nanoparticles synthesis (Green Approach) is preferred as it is

clean, cost effective, nontoxic and safe for human therapeutic use ⁸. The characteristics of the nanoparticles obtained using various plant extracts are influenced by several factors such as temperature, time of reaction, and pH; however, the nature of biomolecules present in the plant extracts could be the most relevant factor in the bioprocess ⁹. Thus, the selected extract is of great importance, as it provides bioreductor agents in the synthesis, such as phenolic compounds, terpenoids, flavonoids, alkaloids, polysaccharides, proteins, enzymes, and amino acids ¹⁰.

The green synthesis of silver nanoparticles using various medicinal plants leaf extracts including *Cestrum nocturnum* ¹¹, *Callophyllum tomentosum* ¹², *Lantana camara* L. ¹³, *Buddleja globosa* ¹⁴ and *Coriandrum sativum* ¹⁵ has been reported. By green technique for the synthesis of silver nanoparticles as an alternative to conventional methods. With these evidences, leaf extract of *Curcuma longa* (*C. longa*) belongs to the family *Zingiberaceae*, commonly known as curcumin is used as reductant and stabilizing agent for silver nanoparticles ¹⁶. The rhizome, the portion of the plant used medicinally as a yellow powder which is used as a flavor in many cuisines and as a medicines to treat many diseases particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage and colic or can be applied as an ointment to treat many skin diseases, and the active constituents of turmeric are the flavonoid curcumin (diferuloylmethane) and various volatile oils, including turmerone, atlantone, and zingiberone. Curcumin's ability to

inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth¹⁷.

The aim of the present study was to evaluate the use of leaf extract of *C. longa* as a reducing agent for silver nanoparticles formation. The synthesized AgNPs were then characterized by UV-Vis Spectroscopy, SEM, EDAX and XRD. Further, some biological activities including antioxidant, antibacterial, *in vitro* antidiabetic activities and genotoxicity of the synthesized silver nanoparticles were studied.

MATERIALS AND METHODS

Plant Collection and Authentication:

The *C. longa* leaves were collected from Chamara Nagar Dist, Karnataka. The plant specimen was authenticated by Prof. G. R. Shivamurthy, Retired Professor and Chairman in Botany, Departmental studies in Botany, Manasagangothri, University of Mysore, Mysuru.

Sample Preparation and Phytochemical Screening

The leaves of *C. longa* were weighed 10 gm and placed in the body tube of the Soxhlet extractor and successive solvent extraction was done for 16 hrs. Ethanol extract was concentrated in the rotary evaporator and the crude extract was used for further preliminary phytochemical analysis¹⁸. The phytochemical screening of fresh leaves extract of *C. longa* was performed as per previous procedures^{19,20}.

Chemicals and preparation of AgNO₃ solution

AR-grade silver nitrate (AgNO₃) was purchased from Finar Chemicals and fresh 0.01697 gm of AgNO₃ was dissolved in 100 ml triple distilled water (Millipore) to produce 1mM solution of AgNO₃. The Plasmid DNA was purchased from Medox Biotech Private Limited.

Green Synthesis of Silver Nanoparticles (AgNPs)

The green synthesis of AgNPs methodology was developed according to with a minor modifications²¹. Pre-collected fresh elder leaves of *C. longa* plant were washed thrice in sterile distilled water to remove adhering soil particles and salts. A fresh 10 gm of leaves were then cut in to small pieces and transferred to a beaker containing 100ml of distilled water and boiled for 20 minutes. The boiled extract was filtered through Whatman No.1 filter paper and the supernatant was used for further process. A fresh 10 ml *C. longa* aqueous extract was taken in a beaker placed on a magnetic stirrer with hot plate. To this 100 ml of 1 mM silver nitrate solution was added drop wise with constant stirring at 600 rpm. The color change of the solution was checked periodically. The color change of the solution from yellow to brown color indicates the formation of silver nanoparticles.

Characterization of Silver Nanoparticles(AgNPs)

The characterization of silver nanoparticles is important to understand and control nanoparticle synthesis and applications²². The formation of AgNPs was confirmed by sampling the reaction mixture at regular intervals and the absorption maximum was scanned by UV-Visible spectra, in a range of wavelength between 350 and 700 nm using HITACHI U-2900 Double beam spectrometer. The crystalline nature of the AgNPs were analyzed by using X-ray diffraction (XRD), and patterns of the silver nanoparticles were recorded using Smart Lab 3kW, Item (C/N) 2080B211, Rigaku Corporation Made in Japan. The size distribution and average size of the AgNPs was determined by

Differential light scattering (DLS) analysis, the size distribution and average size of the AgNPs were determined by DLS particle size analyzer (Microtrac.INC W3231 Made in USA) to estimate the average size distribution of the prepared particles. Elemental composition was determined by Energy dispersive X-RAY analysis. This analysis provides information regarding the presence of silver in the test sample. Scanning electron microscopy (SEM) analysis was performed (HITACHI S-3400N) to study the morphology of the AgNPs.

IN VITRO ANTIOXIDANT ASSAYS

DPPH free radical scavenging assay

2, 2 diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging potential of the AgNPs was determined by the method of²³. Various concentrations (20, 40, 60, 80 and 100 µg/ml) of AgNPs. In the above samples, 1 mL of freshly prepared DPPH (0.1 mM) dissolved in methanol was added and vortexed thoroughly. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. The DPPH (containing no sample) was used as a control prepared using the same procedure. Standard Butylated hydroxytoluene (BHT) were taken in different test tubes as reference standard. The percentage inhibition vs. concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC₅₀ value. The inhibition percentage was calculated using the following formula.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging assay was determined by the method of²⁴. Various concentration (20, 40, 60, 80 and 100 µg/ml) of extract were added with 1 ml of iron ethylene diamine tetra acetic acid (EDTA) solution (0.13% ferrous ammonium sulphate and 0.26%, EDTA) 0.5 ml of EDTA solution (0.018%) and 1ml of DMSO (0.85 %v/v I 0.1 M phosphate buffer pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in water bath. After incubation the reaction was terminated by the addition of 1ml of ice cold TCA (17.5% w/v). 3 ml of Nash reagent was added and left at room temperature for 15 min. the reaction mixture without sample was as control. The identity of the color formed was measured spectrometrically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Reducing Power Capacity

Reducing power was estimated by the method of²⁵. Various concentrations of the extracts (20, 40, 60, 80 and 100 µg/ml) were prepared. To all the extracts in test tubes, 2.5 ml of sodium phosphate buffer was added followed by 2.5 ml of 1% potassium Ferricyanide [K₃Fe (CN)₆] solution. The content were vortexed well and then incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% TCA was added to all the tubes and centrifuged at 3000 g for 10 min. To 5 ml of the supernatant, 5 ml of deionized water was added. To this 1 ml of 1% ferric chloride was added to each test tube and incubated at 35°C for 10 min. The reducing power of the extract was linearly proportional to the concentration of the sample. Increasing absorbance of the reaction mixture indicated reducing power. Butylated hydroxyl toluene (BHT) was taken as

reference standard. The absorbance of these solutions was measured at 700 nm. Percentage of inhibition was calculated as follows.

$$\text{Percentage of reducing power activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Nitric Oxide Radical Scavenging Assay

Nitric Oxide radical scavenging assay was determined by the method of²⁶. Different concentrations of the both extracts (20, 40, 60, 80 and 100 µg/ml) were prepared. Then the volume is made up to 1ml using distilled water. To all extracts in test tubes, 1ml of Sodium nitroprusside was added followed by 1 ml of Griess reagent. The content was vortexed well and the incubated at room temperature 1 hour. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage of inhibition was calculated as follows.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Antibacterial Potential of Green Synthesized Silver Nanoparticles

Antibacterial activity of the synthesized silver nanoparticles was determined using agar disc diffusion assay method²⁷. This was confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone compared to know standard antibiotic (Ampicillin). The bactericidal activity of the AgNPs was evaluated against the clinical isolates *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus* obtained from the Postgraduate Department of Biochemistry, JSS College of Arts, Commerce and Science (Autonomous), Mysuru-570025, Karnataka, India. About 150 CFU/mL of inoculums was swabbed onto Nutrient Agar plates uniformly and allowed to dry in a sterile environment. Sterile disc of 6 mm (HIMEDIA) was loaded with different concentration of AgNPs (5, 10 and 15 µg/ml) solutions, and another disc was dipped in 1 µg/ml of antibiotic ampicillin was used as positive control. The plates were incubated at 26°C for 2 days to measure the zone of inhibition. The mean was calculated by performing the experiments in triplicates.

In vitro Antidiabetic Potential: α-Amylase

There has been growing interest in the study and usage of traditional plants for pharmaceutical and medical applications due to their low toxicity and economic viability. Carbohydrate digestive enzymes, mainly α-amylase are responsible for the breakdown of carbohydrates into monosaccharides for absorption. *C. longa* leaf extract formulations were shown to have effective antidiabetic activity. This indicates the potential usefulness of synthesized AgNPs to treat diabetics and could be considered an effective approach for diabetes care. The α-amylase assay mixture consisted of 0.5 mL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase (1U/mL) with AgNPs concentrations of 20–100 µg/mL pre incubated at 37°C for 20 min. After incubation, 250 µL of 1% starch solution in the above mentioned buffer was added to the tubes and incubated for 15 min at 37°C. The reaction was terminated by addition of 1 mL of Dinitrosalicylic acid reagent and heating in a boiling water bath for 10 min and then tubes were cooled, and absorbance measured at 540 nm²⁷.

Analysis of Capping Protein around the Silver Nanoparticles

The capping of protein is done according to the method of²⁸ with minor modification. In order to isolate the protein(s) bound to the surface of silver nanoparticles, the particles were washed with sterile distilled water and boiled with 1% Sodium dodecyl sulfate (SDS) for 10 min. Electrophoresis was performed in 12% SDS-Polyacrylamide gel at a constant voltage of 70 V for 2 hrs. And the gel was stained with Coomassie Brilliant blue dye and observed.

Genotoxic Effect of Silver Nanoparticles against Plasmid DNA

Genotoxic potential of the silver nanoparticles was tested against plasmid pBR322 according to the method of²⁸ with minor modification. The plasmid was purchased from Medox Biotech private limited. 0.5µg of plasmid was incubated with 0.5, 1.2, 2.5, 3.5, 5 µg of silver nanoparticle (in a total volume of 100 µl solution) in 1 mM Tris (pH=7.8) for a period of 2 h at 37°C. In control set only plasmid is taken without nanoparticle solution. Products were run on 1.2% agarose gel in 1X TAE buffer at 70 V for 1 hr and visualized by ethidium bromide staining using agarose gel electrophoresis.

Statistical analysis

The results were expressed as mean ± SD of three independent experiments (P<0.01). IC50 values were calculated from DPPH assay and subjected to statistical analysis.

RESULTS

Green Synthesis of AgNPs and UV-Vis absorption spectroscopy analysis

In this study, silver nanoparticles (AgNPs) synthesis by reducing Ag⁺ ions present in the aqueous solution of silver nitrate with the help of turmeric leaves (*Curcuma longa*) was investigated. The color change from pale yellow to dark brown indicated the formation of AgNPs is shown in (Fig.1). The UV-Vis spectra of the AgNPs band occur near 426 nm due to excitation of surface plasmon vibrations in nanoparticles is shown in (Fig.2) indicating the formation of AgNPs and reduction of silver ions by active molecules present in the leaf extract. In accordance with previous literature studies were also reported by many researchers as one of the most important features in the optical absorbance spectra of metal nano particles in surface plasmon band, which is due to collective electron oscillation around the surface plasmon resonance mode of the particles¹⁸. The brown colour confirms that it was due to the reduction of Ag⁺ which indicates the formation of AgNPs.

Time kinetics of synthesized Silver Nanoparticle at different stirring time intervals

The stirring time effect on the green synthesis of silver nanoparticles is shown in the (Fig.3). The color intensity and monodispersity increased with stirring time. The appearance of surface plasmon resonance peak (SPR) at a wavelength range of 400-450 nm corresponds to AgNPs which absorb radiations intensely at wavelength of 426 nm.

Dynamic light scattering (DLS) measurement

The size distribution histogram of dynamic light scattering (DLS) between 100-365 nm indicates that the size of these silver nanoparticles with a positive polarity favoring the drug targeting

and it has shown in (Fig.4). Zeta potential analysis has shown the positive polarity of particle favouring the drug targeting.

X-ray diffraction (XRD) spectrum measurement

The green synthesized AgNPs were further confirmed by the characteristic peaks observed in X-ray diffraction (XRD) is shown in (Fig.5). AgNPs were in the form of 2 θ values of at 27.83°, 31.94°, 37.91° & 46.39° indicated the (2.23098), (2.775), (2.35099) & (1.96458) reflections of metallic silver. A sharp and strong diffraction peak centered at 31.94° was appeared, which can be indexed to the (2.775). The sharp peaks clearly indicate the synthesized AgNPs are crystalline in nature, with a face-centered cubic (fcc) structure. Other peaks suggested that the crystallization of bioorganic phase occurs on the surface of the silver nanoparticles.

SEM analysis

The morphology (viz shape and size) of the silver nanoparticles studied under SEM (Scanning Electron Microscope) revealed that the nanoparticles were mostly spherical in shape and polydisperse in nature (Fig.4). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. Thus scanning electron microscope gave descriptions of shape, size and structure of the biosynthesized AgNPs in detail.

Energy Dispersive X-Ray (EDX) spectra analysis

Energy Dispersive Analysis of X-ray (EDAX) gives qualitative as well as quantitative status of the elements that me involved in the formation of AgNPs. Data illustrated in (Fig.7) and the peak in silver region at 3KeV (130.5 in mass) which is a typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance. Further additional signals from C, N, O and Cl atoms are also recorded.

Antioxidant Potential of AgNPs

Antioxidants are micro-constituents that can be act as a scavenger of reactive oxygen species (ROS) by terminating the oxidizing chain reaction. ROS play a fundamental role in the pathogenesis of a variety of degenerative conditions including cardiovascular diseases and carcinogenesis. Free radicals are highly reactive species but play an effective role in the pathogenesis of acute inflammation. Antioxidants play a vital role in biological system function by scavenging fatal free radicals and their potential application in the therapy of many diseases caused by inflammation and oxidative stress²⁹. The DPPH assay are widely used to evaluate the radical-scavenging ability of green synthesized silver nanoparticles. In the present study, DPPH, a stable free radical with a characteristic absorption at 517nm was used to study the radical-scavenging effects. The DPPH scavenging assay exhibited effective inhibition activity of AgNPs when compared with the standard BHT (butylated hydroxytoluene). The antioxidant activity (DPPH approach) suggest that, *C. longa* leaf extract of IC₅₀ value was (97.87 µg/ml) which was compared with standard BHT (60 µg/ml), The *C. longa* leaf extract percent of inhibition was higher to the standard. The previous study reveals that, *Hydnocarpus pentandra* leaf extract AgNPs showed potent antioxidant activity as compared to ascorbic acid was used a standard drug³⁰, for nitric oxide radical scavenging activity suggest that, *C. longa* leaf extract (78.88 µg/ml) when compared with standard BHT (63.23 µg/ml) and it is revealed with previous studies suggest that potent nitric oxide radical scavenging activity³¹, for hydroxy radical scavenging activity for leaf extract of *C. longa* (61.97 µg/ml) and compared

with standard BHT is (97.27 µg/ml) having potent hydroxyl radical scavenging activity, when it was revealed with previous reports suggest that potent hydroxyl radical scavenging activity³². The green synthesized AgNPs of leaf extract of *C. longa* was dose dependent in reducing power, by increasing the concentration of AgNPs consistently increased the reducing power activity. The AgNPs have shown almost equal reducing power activity *C. longa* (67.90 µg/ml) of standard BHT (54.71 µg/ml) and is shown in (Fig.8). The reducing power activity was due to presence of phytoconstituents present in the leaf extract³³.

Evaluation of the antibacterial potential

The antibacterial potential of the green synthesized *C. longa* leaf extract having AgNPs has potent antibacterial activity against both Gram-negative and Gram-positive human pathogens. AgNPs displayed antibacterial activity against Gram positive and Gram negative bacteria, with varying degrees, as suggested by the diameter of inhibition zone, while *C. longa* leaf extract show low antibacterial activity compare to AgNPs (Fig.9 & Table.1). The results showed that AgNPs are effective antibacterial activity against *Escherichia coli* (Gram negative), *Bacillus subtilis* (Gram positive), *Bacillus cereus* (Gram positive), *Salmonella typhi* (Gram negative), *Staphylococcus aureus* (Gram positive)³⁴.

Carbohydrate Hydrolyzing Enzyme Inhibition Assay (α -Amylase Assay)

AgNPs synthesized from leaf extract of *C. longa* were effectively inhibited carbohydrate-hydrolyzing enzyme i.e. α -amylase. With increasing of AgNPs concentration, the enzymatic activity level was significantly induced. The EC₅₀ values for AgNPs inhibition of α -amylase were *C. longa* is 61.97µg/ml respectively is shown in (Fig.10) and it was revealed with previous literature for green synthesis of silver nanoparticles using *Lonicera japonica* leaves extract having potent *in vitro* antidiabetic activity were reported³⁵.

Analysis of Capping Protein around the Silver Nanoparticles

SDS-PAGE analysis of capping protein around the silver nanoparticles synthesized from leaf extract of *C. longa* ; Lane 1, molecular size marker; lane 2, nanoparticle (10 µg/ml); lane 3, nanoparticle (20 µg/ml); lane 4, nanoparticle (30 µg/ml); lane 5, nanoparticles (40 µg/ml) were shown in (Fig.11). There is strong protein band (28 kDa) observed in leaf extract of *C. longa*, which is mainly involved in the capping of protein and strongly it provides stability for bioreduction of silver nitrate to silver nanoparticles.

Genotoxic Effect of Silver Nanoparticles against Plasmid DNA

Agarose gel electrophoresis of plasmid pBR322 treated with different concentrations of silver nanoparticles (µg/100µl) synthesized from leaf extract of *C. longa*; Lane 1, control plasmid without silver nanoparticles showing only supercoiled plasmid band that moves ahead of relaxed circular and linear plasmid; lane 2, 0.5 µg of nanoparticle showing disappearance of the supercoiled plasmid band and appearance of relaxed circular and linear plasmid bands along with smaller fragmented DNA; Lane 3, plasmid incubated with 1.2 µg nanoparticles; lane 4, plasmid incubated with 2.5 µg nanoparticle; lane 5 plasmid incubated with 3.5 µg nanoparticle showing gradual degradation of the fragmented DNA bands; and lane 6, plasmid incubated with 5 µg nanoparticles showing more degradation of DNA is shown in (Fig. 12).

Table 1: Effect of Silver Nanoparticle against Selected Human Pathogenic Bacteria

Sl. No.	Name of the Microorganism	Zone of inhibition in mm (<i>Curcuma longa</i>)				
		Ampicillin (1µg/ml)	AgNO ₃ (5µg/ml)	AgNPs (5µg/ml)	AgNPs (10µg/ml)	AgNPs (15µg/ml)
1	<i>Staphylococcus aureus</i>	10±0.50	4.5±0.50	5±0.50	5.5±0.50	6.5±0.50
2	<i>Bacillus subtilis</i>	8±0.50	3±0.50	4±0.50	5±0.50	6±0.50
3	<i>Bacillus cereus</i>	10±0.50	5±0.50	5.5±0.50	5.5±0.50	6±0.50
4	<i>Escherichia coli</i>	8±0.50	4±0.50	5±0.50	6±0.50	7±0.50
5	<i>Salmonella typhi</i>	10.1±0.50	5±0.50	6±0.50	6.5±0.50	7±0.50

Values are average of triplicates, ± indicates standard error

Fig. 1: Visual observation of A) 1 mM AgNO₃ solution B) before bio-reduction and C) AgNO₃ after bio-reduction

Fig. 2: UV-Vis absorption spectrum of synthesized AgNPs

Fig.3: Time kinetics of synthesized Silver Nanoparticle at different stirring time Intervals

Fig.4: Dynamic light scattering (DLS) measurement

Fig. 5: XRD pattern of biosynthesized silver nanoparticles using leaf extract

Fig.6: SEM and EDAX analysis of synthesized AgNPs using *Curcuma longa* leaf extract

Figure.7: Energy Dispersive X-Ray (EDX) spectra of nanoparticle synthesized from *Curcuma longa* leaf extract.

Fig. 8: *In vitro* antioxidant activities

Fig.9: Evaluation of the antibacterial potential

Fig.10: Carbohydrate Hydrolyzing Enzyme Inhibition Assay (α-Amylase Assay)

Fig.11: Analysis of Capping Protein around the Silver Nanoparticles

Fig.12: Genotoxic Effect of Silver Nanoparticles against Plasmid DNA

DISCUSSION

The present work explains the green synthesis of AgNPs using leaf extract of *C. longa*. When the extract was added to the silver nitrate solution the color of the solution was changed from light yellow to dark brown color. The dark brown color of solution confirmed the bioreduction of silver nitrate into the AgNPs. Further, the formation of AgNPs was confirmed by the UV-Vis spectrophotometer (λ , 300-700 nm). The absorbance band at 426 nm was recorded due to excitation of surface plasmon resonance (SPR) vibrations in nanoparticles indicating the formation of AgNPs and reduction of silver ions by active molecules present in the leaf extract. In accordance with previous literature studies were also reported by many researchers as one of the most

important features in the optical absorbance spectra of metal nano particles in surface plasmon band, which is due to collective electron oscillation around the surface mode of the particles¹⁸. The presence of single surface plasmon resonance band in the absorption spectra of the produced AgNPs gives an indication to their spherical shape³⁶. The green synthesis of AgNPs becomes most popular because of no use of toxic chemicals, cheap, environmental eco-friendly and suitable for pharmacological and biomedical applications³⁷. The DLS analysis for *C. longa* the diameter of AgNPs were 365 nm with a positive polarity favoring the drug targeting. The XRD results confirmed the formation of crystalline AgNPs (Fig.5). The SEM results were confirmed AgNPs was spherical in shape and AgNPs are variable in size and maximum 90-111 nm. The EDAX results confirmed the presence

of elemental silver and EDAX signal between the 3KeV confirmed the elemental silver. The bioactive compounds of functional groups are responsible for the capping of silver nanoparticles and stable in size ²⁸. The DPPH, reducing power activity, hydroxyl radicals and nitric oxide radicals methods were confirmed the green synthesized silver nanoparticles has potent antioxidant activity (Fig.8).

The results of antibacterial activity were confirmed the silver nanoparticles and silver nitrates have potent antibacterial activity. The bactericidal activity of the green synthesized leaf extract of *C. longa* AgNPs has potent antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *E.coli* and *Salmonella typhi* were shown in (Fig.9 & Table. 1) and it was compared with previous reports ³⁸ having potent antibacterial activity against Gram positive and Gram negative bacteria, with varying degrees, as suggested by the diameter of inhibition zone, while synthesized leaf extract of *C. longa* AgNPs show high antibacterial activity compare to AgNO₃. The carbohydrate hydrolyzing enzyme inhibition assay (α -Amylase Assay) were analyzed. The AgNPs synthesized from *C. longa* was effectively inhibited carbohydrate-hydrolyzing enzyme i.e. α -amylase with increasing of AgNPs concentration, the enzymatic activity level was significantly induced. The SDS-PAGE analysis of capping protein around the silver nanoparticles synthesized from leaf extract of *C. longa* were shown in (Fig.11). There is strong protein band (28 kDa) observed in leaf extract of *C. longa*, which is mainly involved in the capping of protein and bioreduction of silver nitrate to silver nanoparticles. The Genotoxic effect of silver nanoparticles against plasmid pBR322 DNA was analyzed using agarose gel electrophoresis reacted with different concentrations of silver nanoparticles (μ g/100 μ l) synthesized from leaf extract of *C. longa* and it was showing more degradation of DNA (Fig. 12).

CONCLUSION

The present work describe the formation of AgNPs with the help of leaf extract of *C. longa*. The leaf extract of *C. longa* have bioactive compounds which are responsible for the bio-reduction and capping of silver nitrate into silver nanoparticles. The capping agent provides stability to the AgNPs. The green synthesized AgNPs has antioxidant, hydroxy radical, nitric oxide radical and reducing power activity. The activity occurs due to the presence of functional groups on the surface of AgNPs. The green synthesized AgNPs have strong antibacterial activity against selected human pathogenic bacteria's, and more potent *in vitro* antidiabetic activity to the small size and presence of bio-reduction capping agents. The Genotoxicity effect of silver nanoparticles against plasmid pBR322 DNA was analyzed using agarose gel electrophoresis reacted with different concentrations of silver nanoparticles synthesized using leaf extract of *C. longa* and it was showing more degradation of DNA. These AgNPs might be used as antibiotics and antidiabetic agent in future due to non-toxic, cheap, eco-friendly. Hence, this method can be employed in large-scale nanoparticles synthesis and can be used in many medicinal and technological applications

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