FREE RADICLE SCAVENGING ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT

Silver has been used since time immemorial in different chemical form to treat burns,wounds and several infections caused by pathogenic bacteria. The current study deals with the synthesis of silver nanoparticles using *Citrullus colocynthis* and evaluation of their antioxidant activity. Synthesized nanoparticles were characterized by using UV-Vis (Ultra Violet-visible) absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM) analysis and then the antioxidant activities are carried out by using DPPH method. The reaction mixture turned to a brownish gray color after 5 hrs of incubation and exhibits an absorbance peak around 450 nm characteristic of AgNPs. The approach of green synthesis seems to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.

KEYWORDS: *Citrullus colocynthis, Silver nanoparticles,* Ultra Violet-visible *absorption spectroscopy,* Fourier transform infrared spectroscopy, scanning electron microscope, antimicrobial activity.

INTRODUCTION

Nanotechnology finds extensive applications in nanomedicine, an emerging new field. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled dispersity and their potential use for human benefits. Nanoparticles can be synthesised by chemical and physical methods but these methods are quite expensive and toxic **(Putheti *et al*., 2008).** Traditionally, the chemical and physical methods used to synthesize silver nanoparticles are expensive and often raise questions of environmental risk because of involving the use of toxic, hazardous chemicals **Gnanajobitha *et al.*, 2010).** In recent years plant mediated biological synthesis of nano particles is gaining importance due to its simplicity and eco-friendliness**.** Several plants serve as potential biological materials for the synthesis of nanoparticles (**Thakkar *et al*., 2010)**

MATERIALS AND METHODS

Preparation of silver nanostructures

To synthesize different-sized AgNPs, the spherical AgNPs were prepared according to the literature procedure by Fang et al. (2005), by reducing aqueous $AgNO_3$ with sodium citrate at boiling temperature. In typical procedure, 50 ml of 0.001 M $AgNO_3$ was heated to boiling. To this solution, 5 ml of 1% trisodium citrate was added drop by drop. The solution was heated at boiling point under continuous stirring. The reaction was allowed to take place until the color changed to a yellow solution. The solution was then cooled to room temperature. The AgNPs in this solution were called citrate-AgNPs.

In vitro antioxidant activity of plant extract

Preparation of citrate-AgNPs

Different concentrations of citrate-AgNPs (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

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DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992). Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C} \right) \times 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

RESULTS AND DISCUSSION

S.No	Plant leaf extract+AgNo3	Color change		pH change		Color intensity	Time	Result
	Scientific name	Before	After	Before	After			
1	*Citrullus colocynthis*	Light Yellow	Brown	4.0	4.60	+++	20min	Positive

Table 1: Indication of Color Change in Synthesis of Silver Nano Particle (SNPs)

Color intensity: +++ = very dark color

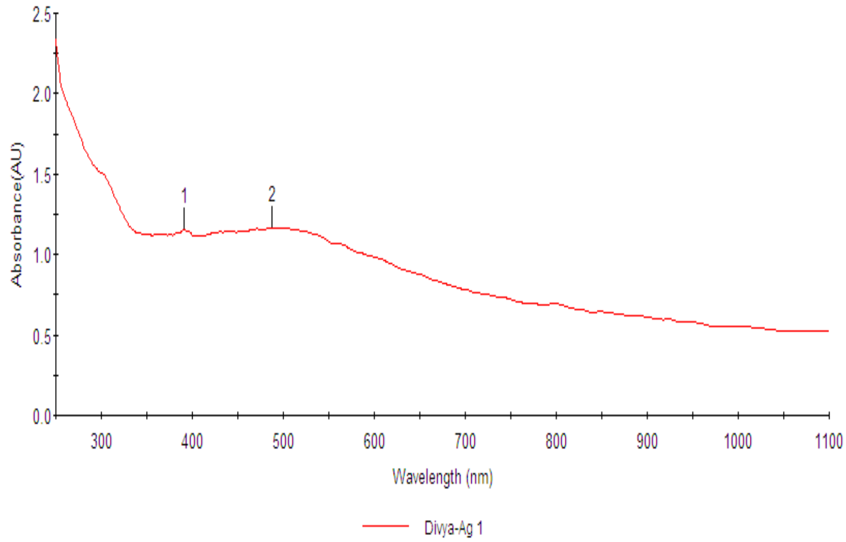

Fig:1 UV- VIS ANALYSIS OF FRUIT EXTRACT OF of *Citrullus colocynthis*

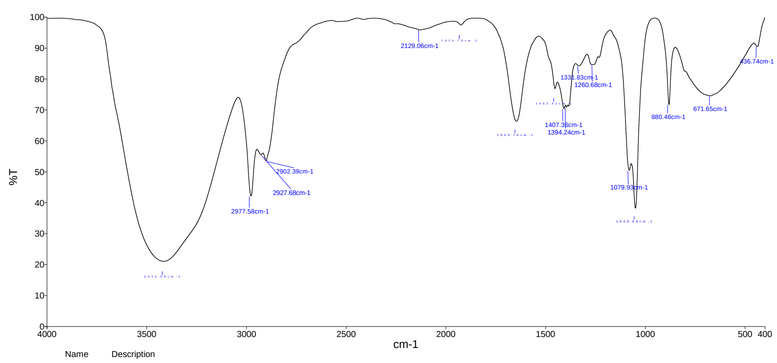

Fig:2 FT IR ANALYSIS OF FRUIT EXTRACT OF of *Citrullus colocynthis*

Table 2- % of DPPH Radical scavenging activity of AgNPs at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
DPPH	18.5±1.2	33.10±2.3	58.10±4.13	81.28±5.75	33.91
Standard (Ascorbic acid)	25.0±2.04	60.26±4.90	87.98±7.11	98.34±7.94	50.47

Values were expressed as Mean ± SD for triplicates

SNPs Synthesis:

In the present study SNPs were synthesized by using extract of *Citrullus colocynthis* rapidly within 15 min of incubation period and brown colour was developed by addition of Silver Nitrate. The time duration of change in colour and thickness of the colour varies from plant to plant. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H+ ions to reduce the silver. It is well known that SNPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol.

Detection and Characterization of Phyto Silver Nanoparticles

Visual Observation: After treatment of *Citrullus colocynthis* extract with AgNO3, the colour change of the reaction mixture was visually observed. The time taken for the reaction mixture to change colour was noted.

It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nano particles (**Thirumurugan *et al.*, 2010).**

UV- Vis- Spectroscopy

The reduction of silver metal ions to silver nanoparticles was preliminarily analysed using UV-Vis Spectrophotometer between 300-700nm. This analysis showed an absorbance peak at 420 nm which was specific for Ag nanoparticles.

UV–visible spectroscopy is an important technique to determine the formation and stability of metal .Nanoparticle in aqueous solution. The reaction mixture changes the colour by adding various concentrations of metal ions. These color changes arise because of the excitation of surface plasmon vibrations in the silver Nanoparticle **(Thakkar, *et al*.2008)**. It shows yellowish to dark brown in colour . The dark brown colour of silver colloid is accepted to surface plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromagnetic field .

FTIR analysis:

FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Ag nanoparticle synthesized using *Citrullus colocynthis* fruit extract. This spectrum shows lot of absorption bands indicates the presence of active functional groups in the synthesized silver Nanoparticles. The intensity peaks are slightly increased for the period of silver nanoparticle synthesis like 3432,2398, 2390, 1124 cm-1 as well as some intensity peaks decreased like 1045, 2080, and 2359 cm-1. Fig shows the band at 3429 correspond to N-H, O-H Stretching vibrations of Silver, amide, alcohol and H–bonded to phenols. The peak at 1637 indicate to C=C, C=O stretching vibrations to alkenes and amide. The peak at 1382 represents to C-H in plane bend to alkenes. The peak at 595 corresponds to C- Cl, C-Br stretching vibrations to alkyl halides. The band at 2080 corresponds to C-N stretching vibration. The weak band at 1045 indicates C-O, C-N stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids, ethers, esters and aliphatic amines in the seed extract. The presence of active functional groups in seed extract results in the swift reduction of silver ions to silver Nanoparticle. To obtain good signal to noise ratio of silver nanoparticle were taken in the range 500–3400 cm−1.

The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity.Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions**(Wang *et al*., (2008).**

SEM

The SEM image showing the high intensity of silver nanoparticles synthesized by *Citrullus colocynthis fruit* extract further confirmed the development of silver nanostructures. SEM provided further insight into the morphology and size details of the silver nanoparticles. SEM analysis showed the particle size of about 10 µm as well the crystal structure of the nanoparticles. The silver nanoparticles synthesized via green route are highly toxic to multidrug resistant bacteria hence has a great potential in Biomedical applications. The present study showed a simple, rapid, economical route to synthesized silver nanoparticles. Application of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications makes this method potentially exciting for the large scale synthesis of other inorganic materials (nano-materials).

Diphenyl Picryl Hydrazine Assay (DPPH)

Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on Citrate silver nanoparticles (AgNPs), food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). DPPH radical scavenging activity of Citrate silver nanoparticles (AgNPs) and standard as ascorbic acid are presented in table 1 and Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.*, 2003). The half inhibition concentration (IC_{50}) of ascorbic acid and Citrate silver nanoparticles (AgNPs) were 51.22μg ml^{-1} and 34.91 μg ml^{-1} respectively**.** The Citrate silver nanoparticles (AgNPs) exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

CONCLUSION

On the basis of the results of this study, it clearly indicates that AgNPs had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, Total antioxidant assay, superoxide anion scavenging and metal chelators. From our results, the antioxidant activity of AgNPs was concentration dependent. The AgNPs could exhibit antioxidant properties higher than comparable to commercial synthetic antioxidants as ascorbic acid. From the above assays, the possible mechanism of antioxidant activity of AgNPs includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.

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