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GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING LEAF AND STEM OF *MANILKARA ZAPOTA* L. AND ANTIOXIDANT AND ANTIDIABETIC ACTIVITY

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Abstract

Nanoparticles possess unique biological properties. Determination of *in vitro* antioxidant activity was carried out using DPPH assay and Reducing power assay and *in vitro* antidiabetic activity using Alpha amylase assay of AgNPs leaf and stem of *Manilkara zapota* L. Chacterization was carried out using UV-Vis spectroscopy and Scanning electron microscopy. In investigation it was found that AgNPs leaf and stem are stable and oval to spherical in shape. In DPPH assay IC_{50} value for leaf and stem was determined to be 92.68 ±0.004µg/ml and 250.06 ±0.007µg/ml respectively. In reducing power assay results shows that at reducing power increases with increase in concentration. In Alpha amylase inhibitory assay the IC_{50} value was determined to be 18.79±0.002 µg/ml for leaf; 51.78±0.003µg/ml for stem. Results revealed that the plant parts of *Manilkara zapota* L. have potent antioxidant and antidiabetic property.

Keywords: *Manilkara zapota* L., silver nanoparticles, antioxidant, antidiabetics.

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INTRODUCTION

Nanomedicine is the application of nanotechnology in the field of medicinal science. Silver nanoparticles (AgNPs) are commonly used nanomaterials, for pharmacological screening of various diseases using plant extraction. They are ecofriendly and non-toxic in nature. AgNPs are used for coating or embedding of bioactive compounds present in plant extract. The green silver nanoparticles can be used for delivery of therapeutic drug to cure diseases. Silver nanoparticles have different biomedical application. AgNPs are known for its antioxidant and antimicrobial properties [1]. Antioxidants inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and reactive oxygen species effects [2]. Diabetes type 2 is a metabolic disease characterized by hyperglycemia. Active compounds derived from herbs can be used as a source of new alpha amylase inhibitors. Alpha amylase inhibitors lower the level of postprandial hyperglycemia by controlling starch break down. Hence, retardation of starch digestion by inhibition of enzyme such as α-amylase would play a key role in the control of diabetes [3].

Synthetic antioxidants such as butylated hydroxylanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are toxic and carcinogenic [4] and the synthetic antidiabetic drugs acarbose, sulfonylureas and biguanide also have many side effects [5]. Recently, in research work it was observed that natural antioxidants and antidiabetic drug from herbal plants are safe and more reliable. *Manilkara zapota* L. is long - lived evergreen tree of family Sapotaceae. It is native to Central America, Mexico and West Indies. Stem contain various phytochemial constituent including gum, tannin and steroids. Leaf decoction used for treatment of fever, hemorrhage, wounds and ulcers [6] and also used to treat cough, cold, and diarrhoea [7]. The leaves were reported to be antibacterial, antioxidant, antidiabetic and hypocholesterolemic [8, 9].

MATERIAL AND METHODS

PLANT MATERIAL

Plant parts (Leaf and Stem) of *Manilkara zapota* L. were collected from district Jaipur, Rajasthan. It was authenticated as (RUBL 211588) by Herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

EXTRACTION AND SYNTHESIS OF AgNPs

Dried plant part material was extracted at temperature of 65º C for duration of 3-4 hours and then filtered through Whatman No.1 filter paper. The leaf and stem extract was mixed with1mM silver

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nitrate solution in 1: 9 [10]. The preparation was then incubated at room temperature for 24hours in dark. The color change of the plant part extracts to dark brown was checked.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Vis SPECTRA ANALYSIS

UV-Vis Analysis was done by using HR4000CG Spectrometer. Optical absorbance of the synthesized nanoparticles was monitored between the wavelength 200 - 1200 nm.

SCANNING ELECTRON MICROSCOPY (SEM)

The AgNPs solution was purified by repeated centrifugation at 5000rpm for 20 minutes, followed by re-dispersion of pellet of AgNPs into deionized water. Gold coated sample was loaded on SEM (Carl ZEISS EVOR -18) and operated at 20kv.

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH RADICAL SCAVENGING ACTIVITY

DPPH (1-1-diphenyl-2picryl-hydrazyl) free radical scavenging activity was determined by the standard method [11, 12]. 160 µl of DPPH reagent was added with 1.0 ml of diluted test sample and standard. Incubate the reaction mixture for about 30 minutes in dark. Ascorbic acid was used as reference standard. Absorption of DPPH at its absorption maximum 517nm was measured.

The percentage inhibition is calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where, A control = Absorbance of control reaction and A sample = Absorbance of samples

REDUCING POWER ACTIVITY

Reducing power of plant sample was determined by standard method [13]. Each plant parts sample and standard solution were spiked with phosphate buffer (0.1M, 6.6 pH) to make upto 1ml. 0.5ml of 1% potassium ferricyanide solution was added. This mixture was kept at 50ºC on water bath. After cooling 0.5ml of 10 % trichloroacetic acid was added and centrifuged for 20 minutes at 3000 rpm. The upper layer was mixed with distill water and 0.5ml of 0.1% ferric chloride was finally added. Incubate the reaction mixture for 10 minutes. Ascorbic acid was used as reference standard. Absorption of reducing power was measured at 700nm.

***IN VITRO* ANTIDIABETIC ACTIVITY**

ALPHA AMYLASE INHIBITION ACTIVITY

The inhibition assay was performed using the chromogenic DNSA method [14]. The plant parts sample and standard solution was taken into different test tubes and incubated with 500µl of alpha

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amylase solution (0.001g of α amylase was dissolved in 100ml of 0.1M phosphate buffer having pH 6.9; with 6.7Mm sodium chloride). The solution was then incubated at 32°C for 10 minutes. Add 500µl of 1% starch solution to all the tubes, and then incubate at 32°C for 10 minutes. The reaction was stopped by adding 0.5 ml of DNS reagent and the reaction mixture was kept in boiling water bath at temperature 85-90°C for 5 minutes, cooled to room temperature. The solution was mixed with 5 ml distilled water. Maltose was used as reference standard. Read the absorbance of the sample mixture at 540 nm against blank.

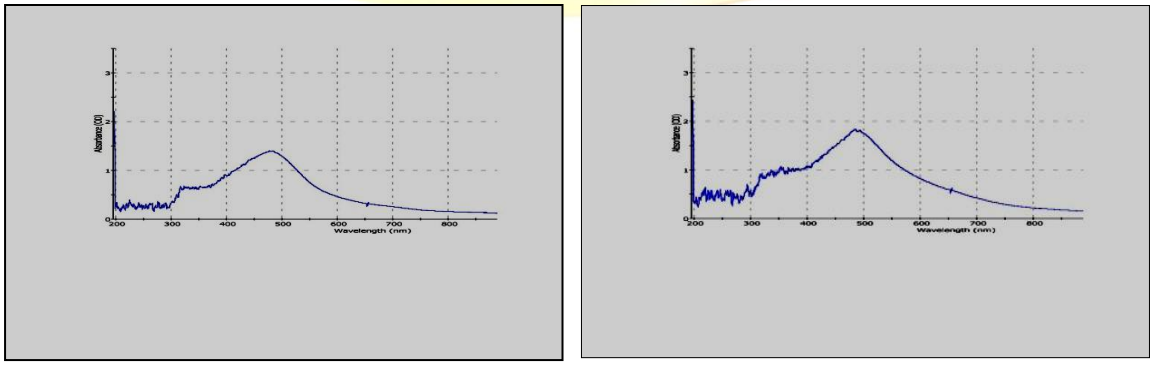
The percentage of inhibition is calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where, A control = Absorbance of control reaction and A test = Absorbance of the samples

RESULTS AND DISCUSSION

Bioreduction of Ag^{+} ions through plant part extract, played pivotal role in green synthesis of silver nanoparticles. Plants have polyphenols, flavonoids, alkaloids and other bioactive compounds which may reduce the silver ions to silver nanoparticles and acts as capping and stabilizing agent [15]. The green synthesis of silver nanoparticles of leaf and stem showed that shape and size of AgNPs of *Manilkara zapota* L. depends upon temperature and duration of time of extraction which also controls its stablity. In the UV-Vis Spectroscopy the absorption spectra of silver nanoparticles found maximum absorption in visible region. In the UV-Vis Spectroscopy the absorption spectra of silver nanoparticles shows absorbance peak at 485.47nm for leaf [Figure1 (a)] and 484.15 nm for stem. [Figure 1(b)]. In SEM analysis it was observed that AgNPs of leaf were found to be small and oval in shape [Figure 2(a)]; hexagonal and spherical in shape [Figure 2(b)].



(a) **(b)**

Fig.1: UV-Vis Spectra Analysis of AgNPs of leaf and stem of *Manilkara zapota* L.

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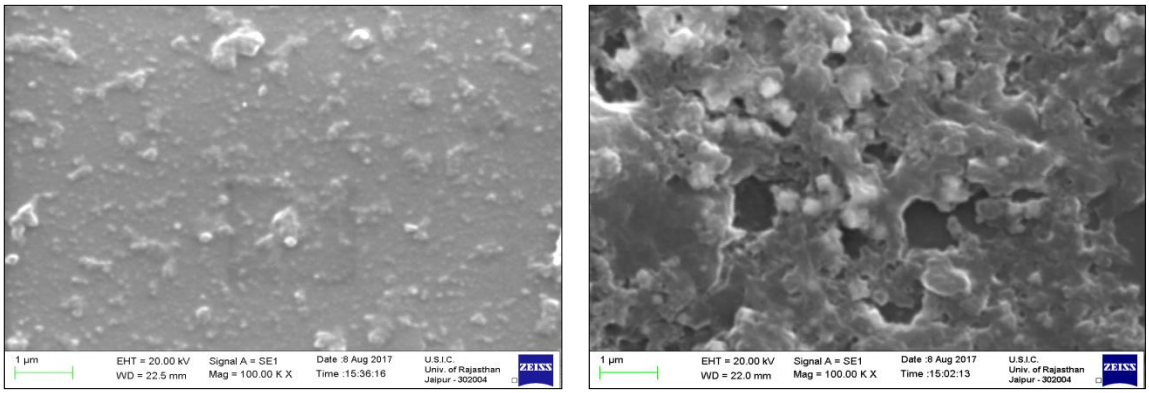

(a) **(b)**

Fig.2: Visualization of AgNPs of leaf and stem of *Manilkara zapota* L. using SEM

Table1: DPPH radical scavenging activity of AgNPs of leaf and stem of *Manilkara zapota* L.

Plant parts					Standard	
AgNPs of Leaves		AgNPs of Stem			Ascorbic acid	
Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition		Concentration (µg/ml)	% Inhibition
20 µg/ml	34.65±0.02	50 µg/ml	15.46±0.004		5	19.77±0.05
50 µg/ml	47.11±0.04	100 µg/ml	25.10±0.06		10	28.67±0.03
100 µg/ml	52.50±0.01	200 µg/ml	47.30±0.03		20	36.81±0.01
200 µg/ml	68.40±0.02	300 µg/ml	62.30±0.05		50	49.90±0.04
300 µg/ml	76.50±0.05	400 µg/ml	75.77±0.01		100	58.15±0.02
400 µg/ml	86.90±0.01	500 µg/ml	81.55±0.02		200	67.83±0.04
IC_{50} = 92.68 µg/ml		IC_{50} = 250.06 µg/ml			IC_{50} = 93.55 µg/ml	

Mean ± Standard Deviation

Table 2: Reducing Power activity of AgNPs of leaf and stem of *Manilkara zapota* L.

Plant parts					Standard	
AgNPs of Leaves		AgNPs of Stem			Ascorbic acid	
Concentration (µg/ml)	Reducing activity (OD at 700 nm)	Concentration (µg/ml)	Reducing activity (OD at 700 nm)		Concentration (µg/ml)	Reducing activity (OD at 700 nm)
0.2 µg/ml	0.630±0.03	0.2 µg/ml	0.198±0.01		0.2 µg/ml	0.580±0.01
0.5µg/ml	0.977±0.01	0.5 µg/ml	0.588±0.04		0.5 µg/ml	0.587±0.01
1 µg/ml	1.203±0.01	1 µg/ml	1.302±0.01		1 µg/ml	1.759±0.02
2 µg/ml	1.366±0.01	2 µg/ml	1.486±0.02		2 µg/ml	1.988±0.013
5 µg/ml	2.013±0.02	5 µg/ml	1.520±0.01		5 µg/ml	2.059±0.02
10 µg/ml	2.174±0.01	10 µg/ml	1.655±0.01		10 µg/ml	2.238±0.01

Mean ± Standard Deviation

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Table 3: Alpha amylase Inhibition activity of AgNPs of leaf and stem of *Manilkara zapota* L.

Plant parts					Standard	
AgNPs of Leaves		AgNPs of Stem			Maltose	
Concentration (µg/ml)	% Inhibition	Concentration (µg/ml)	% Inhibition		Concentration (µg/ml)	% Inhibition
10 µg/ml	46.18±0.02	10 µg/ml	35.36±0.02		10 µg/ml	11.78±0.02
20 µg/ml	50.64±0.04	20 µg/ml	35.95±0.04		20 µg/ml	13.69±0.04
30 µg/ml	54.78±0.01	30 µg/ml	39.81±0.01		30 µg/ml	18.15±0.01
40 µg/ml	58.56±0.02	40 µg/ml	43.32±0.02		40 µg/ml	39.87±0.02
50 µg/ml	59.92±0.05	50 µg/ml	47.78±0.05		50 µg/ml	47.10±0.05
60 µg/ml	63.38±0.01	60 µg/ml	52.86±0.01		60 µg/ml	51.86±0.01
70µg/ml	67.20±0.01	70 µg/ml	57.97±0.01		70 µg/ml	55.97±0.01
80 µg/ml	71.02±0.02	80 µg/ml	63.06±0.02		80 µg/ml	62.10±0.02
90 µg/ml	74.21±0.03	90 µg/ml	65.61±0.03		90 µg/ml	76.50±0.03
100 µg/ml	78.67±0.01	100 µg/ml	72.03±0.01		100 µg/ml	82.50±0.01
IC_{50} = 18.79µg/ml		IC_{50} = 51.78 µg/ml			IC_{50} = 59.94 µg/ml	

Mean ± Standard Deviation

In reaction when antioxidants react with DPPH, it becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as result of it the absorbance of sample decreased from the DPPH [16]. The AgNPs of leaf and stem both shows good results in comparsion with standard (ascorbic acid) in DPPH Assay. In Table 1 DPPH radical scavenging activity of AgNPs of leaf and stem extract; IC_{50} value was determined to be 92.68 ±0.004 µg/ml for leaf and 250.06 ±0.007 µg/ml for stem. Ascorbic acid was used as reference standard and IC_{50} was determined to be 93.55 ±0.004 µg/ml. In Table 2 Reducing power was determined at 700 O.D. at various concentrations of silver nanoparticles of leaf and stem extract. Reducing power assay shows that generally reducing power increase with increase in concentration of AgNPs of leaf and stem extract. Diabetes mellitus and control of blood glucose level is facilitated by enteric enzymes including α amylase. Alpha amylase hydrolyses alpha-bonds of large alpha linked polysaccharide such as starch. The inhibition of alpha enzyme delay carbohydrate digestion and can control reduction in glucose absorption rate [17]. Alpha amylase inhibitory activity is activated by AgNPs of leaf and stem extract showing their antidiabetic property at lower concentration than standard (maltose). In Table 3 Alpha amylase activity shows IC_{50} values to be 18.79±0.002 µg/ml for leaf; 51.78±0.003 µg/ml for stem and 59.94±0.005 µg/ml for maltose (standard).

CONCLUSION

In current research work, it was observed that natural antioxidants drug from herbal plants are safe without any side effects. Antioxidant-based drug formulations are used for the prevention

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and treatment of complex diseases like atherosclerosis, diabetes and cancer. AgNPs of *Manilkara zapota* L. are stable and cost effective. It could inhibit the production of free radicals, reduce oxidative stress and also inhibit alpha amylase enzyme, thus lowering postprandial glucose and control diabetics. This revealed that the plant parts of *Manilkara zapota* L. have potent antioxidant and antidiabetic property.

STATISTICAL ANALYSIS

All the analysis was done in triplicate and results are expressed as ± mean S.D. The IC_{50} values were determined using Graphpad scientific software. p- Value < 0.001 was considered to be statistically significant.

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