Original Article

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 by using alpha amylase assay of AgNPs leaf and stem of Manilkara zapota L. Characterization was carried out using UV-Vis spectroscopy and scanning electron microscopy. In investigation it was found that AgNPs leaf and stem are stable and round to spherical in shape. In DPPH assay IC50 value for leaf and stem was determined to be 124.62±0.007 μg/ml and 183.43±0.007 μg/ml respectively. In reducing power assay results shows that at 10 μg/ml concentration of test sample reducing power is 74.64% for leaf, 69.88% for stem and 71.28% for ascorbic acid. In alpha amylase inhibitory assay the IC50 value was determined to be 25.94±0.002 μg/ml for leaf, 51.78±0.003 μg/ml for stem. Results revealed that the plant parts of Manilkara zapota L. have potent antioxidant and antidiabetic property.

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 extract. 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 hydroxyanisole (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 phytochemical 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 hypcholesterolemic [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 and filtered through Whatman No.1 filter paper. The leaf and stem extract was mixed with 1mM silver nitrate solution in 1:9. The preparation was then incubated at room temperature for 24 hours in dark. The color change of the plant part extracts to dark brown was checked [10].
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-2-picryl-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:

\[ \text{Percentage inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \]

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 10 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 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.

RESULTS AND DISCUSSION

Bioruduction 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]. 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 484.15nm for stem. [Figure1(b)]. In SEM analysis it was observed that AgNPs of leaf were found to be small and round in shape and particles were found to be in range between 94.2-207.2nm for leaf [Figure 2(a)]; hexagonal and spherical in shape and 254.3-452.1 nm size for stem [Figure 2(b)].

In reaction when antioxidants react with DPPH, it becomes paired off in the presence of a hydrogen donor and is reduced to 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 comparison with standard (ascorbic acid) in DPPH Assay. In Figure 3 DPPH radical scavenging activity of AgNPs of leaf and stem extract; IC50 value was determined to be 124.62+0.007 μg/ml for leaf and 183.43+0.007 μg/ml for stem. Acsorbic acid was used as reference standard and IC50 was determined to be 114.69+0.004 μg/ml. In Table 2 reducing power assay shows that generally reducing power increase with increase in concentration of AgNPs of leaf and stem extract. At 10 μg/ml concentration reducing power was determined to be 74.64% for leaf, 69.88% for stem and 71.28% for ascorbic acid.

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 Figure 4 Alpha amylase activity shows IC50 values to be 25.94+0.002 μg/ml for leaf and 51.78+0.003μg/ml for stem and 62.35+0.005 μg/ml for maltose (standard).
CONCLUSION

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 IC50 values were determined from linear regression analysis using Graphpad scientific software. p-value < 0.05, 0.001 were considered to be statistically significant.

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