Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(5): 50-54
Received: 22-07-2022
Accepted: 30-08-2022

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Synthesis of silver nanoparticles fabricated by leaf extract of *Caryota urens* and its antioxidant activity

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DOI: https://doi.org/10.22271/phyto.2022.v11.i5a.14514

Abstract
Many studies have been suggested that nanotechnology effects on various science fields. This study was instantiates leaf broth for synthesis of silver nanoparticles. The various physicochemical parameters *viz*., pH, incubation period, temperature, quality and quantity of leaf extracts and, silver nitrate concentration were influences synthesis of the phyto engineered nanoparticles. Secondary metabolites were present in leaf extract play an important role in the green reduction of the silver ions involved in the capping of the AgNPs during synthesis this study revealed from the FTIR spectroscopic studies. pH 10 influences the formation of brown color and bioreduction of Ag+ to Ag ions observed in surface Plasmon resonance bands developed by UV-Vis spectroscopic studies data revealed that 419 nm. The negative charged particles study revealed by zeta potential. The XRD study reveals that crystalline nature of the nanoparticles and, smallest size of the nanoparticles at highly purity at 2θ 38^0, lattice peak 111 and 15 nm. The peak was 1383 cm^{-1} mainly involving the aromatic ring involved in the capping of the AgNPs during phytoreduction. Atomic force microscopic and HR-TEM image reveal that spherical shape and size of the NPs. The size of the nanoparticles is 10-80 nm in scale. Formation of nanofringes describes the high stability in nature. The efficacy of silver nanoparticles on antioxidant activity eas determined by the DPPH assay at different concentrations *viz*., 12.5, 25, 50, 100, 200 and 400 µg/ml. This investigation revealed that the significant decrease the colour intensity of the reaction mixture, when compared to the standard ascorbic acid. The determined IC_{50} value of the silver nanoparticles was 50 µg/ml. These results were showed that a significant role in the increasing the radical scavenging activity.

Keywords: Silver nanoparticles, leaf extract, *C. urens*, DPPH

Introduction
Nanotechnology is growing rapidly research field in various science sectors. Nanotechnology measures the dimensions between 1-100 nm. Nanoparticles research increasing now days because of its reactivity due to enhanced solubility, greater proportion of surface atoms relative to the interior of a structure, unique magnetic/optical properties, electronic states, and catalytic reactivity that differ from equivalent bulk materials [1]. Many nobel metal nanoparticles have been synthesized among them, till today, Silver has received special focus in view of their distinctive, fascinating and extensive properties like semi-conductor, catalytic, piezoelectric, UV-shielding and chemical stability [2]. Bio-nanotechnology which integrates principles of biology with physic-chemical protocols to fabricate nanosize particles with specific functions [3]. The physico-chemical based synthesis of metallic nanoparticles are often toxic to environment, among many biological components that could participate in bio mineralization and be incorporated into bio-nanomaterials, proteins have been the subject of particular attention due to their nanoscale [4]. The green route for nanoparticles synthesis makes use of eco-friendly, non-toxic to the environment. Recently, the bio-fabrication of silver nanoparticles synthesized by extract of Banana peel extract [5], Cocoa pod husk [6], Cola nitidia [7], Amphora sps [8], Mari gold flower extract [9] have been documented.
Caryota urens L. was commonly called Fish tail palm and Jaggery Palm in English, Bagane mara in Kannada, Mari in Hindi. Tall unbranched palm with persistant scars of leaf bases. Leaves in terminal clusters, few, large, bi-pinnatisect, leaf-sheaths large, clasping basaly the trunk. Flower in much branched spadix of 8-10 ft. long, arise from the upper leaf-sheaths and then successively come downwards, monoecious. Fruits are globose, 1-2-seeded, pulp pungent; seeds with hard testa. Commonly found all over the state, usually along hill streams. Commonly open in evergreen and semi-evergreen forests, up to 1400 m.

The present work is an attempt to synthesise silver nanoparticles by leaf extract of *Caryota urens* L. As far as we know that, use of pathogenically synthesized AgNPs of *C. urenus* for this antioxidant activity.

Materials and Method

Silver nitrate, DPPH, Ascorbic acid was procured from the Hi-media.

Preparation of Silver nitrate solution

0.001gm Silver nitrate was dissolved in 100 mili litre distilled water.

Preparation of leaf broth extract

Healthy fresh leaves was measured about 5 gms incised into very small pieces transferred into a beaker containing a 100 ml mili pore water and kept into the water bath boiled for 1 hr and temperature was about 80 °C. Cooled to room temperature and filtered through the what man filter paper no 1, filtered broth were covered with aluminum foil and kept in refrigerator at 4 °C until further use.

Synthesis of Silver nanoparticles

5 ml leaf extract was transferred into the 95 ml silver nitrate solution containing conical flask and incubated at room temperature after 15 mins color intensity was begins to changes pale brown to dark yellowish brown color.

DPPH Assay

Preparation of DPPH solution and test samples

4 mg of DPPH (Himedia) were dissolved in 100 ml of ethanol and stored in dark bottle. 1mg/ml of plant extract and synthesized silver nanopartilces were dissolved separately in ethanol.

DPPH radical-scavenging activity

Hydrogen-donating activity was measured by direct hydrogen donation to the DPPH radical explained by (Zhang *et al*., 2011; Azam Chahardoli *et al*., 2017) [11] with minor modification. Different concentrations (200, 400, 600, 800, 1000 µg/ml) of the test samples were taken into the test tubes and 3ml of DPPH added to each test tube. Reaction mixture was incubated in dark for 30 mins. At the end of reaction absorbance was measured at 517 nm against blank. DPPH in ethanol was used as control. Ascorbic acid as a standard solution. The percentages of inhibition of free radicals were determined by the following equation.

Percentage of inhibition= $(Ab_C - Ab_S / Ab_C) \times 100$

Result and Discussion

The plant mediated synthesis of silver nanoparticles were done by using aqueous extract of *C. urens*. The color of the reaction mixture was changes from pale brown to dark yellow brown colour. The color was stagnant and uniform solution more than 24 hrs indicates high stability. This may be due to the high surface Plasmon resonance effect and phytoreduction of $AgNO_3$ solution [10]. The silver nanoparticles produced a prominent sharp peak at 420 nm with pH 10 (fig.1). This observation was similar to various previous reports. However, 420 nm has considered as an ideal peak with various pH 8-10. Our results were also revealed that pH 10 play a vital role for the fabrication during synthesis of the AgNPs [11]. The Atomic force microscopy study reveals that the particles are monodispersed, and spherical in shape that the size ranges between 10-80 nm (fig. 3 A, B & C), B explains the 2D image of the particle height and, C explains the topography image of the nanoparticles. Topography image was explained that the particles distance from each other [12].

Fig1: A. Leaf extract B. AgNPs

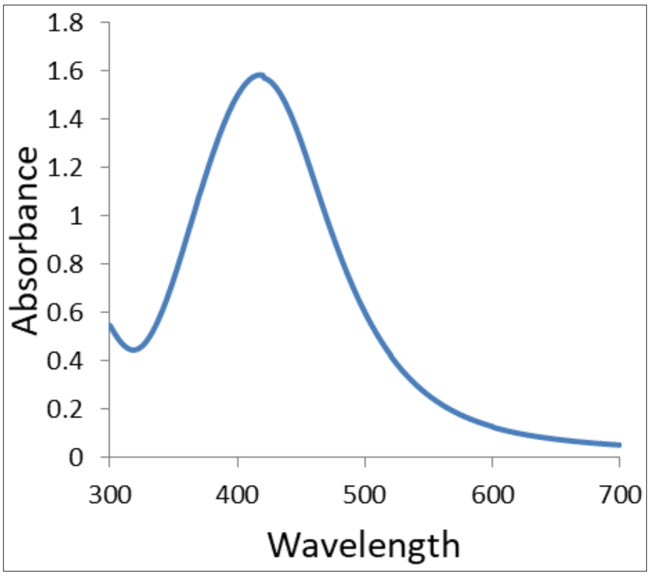

Fig 2: UV-v is absorption spectra of *Caryota urens* L.

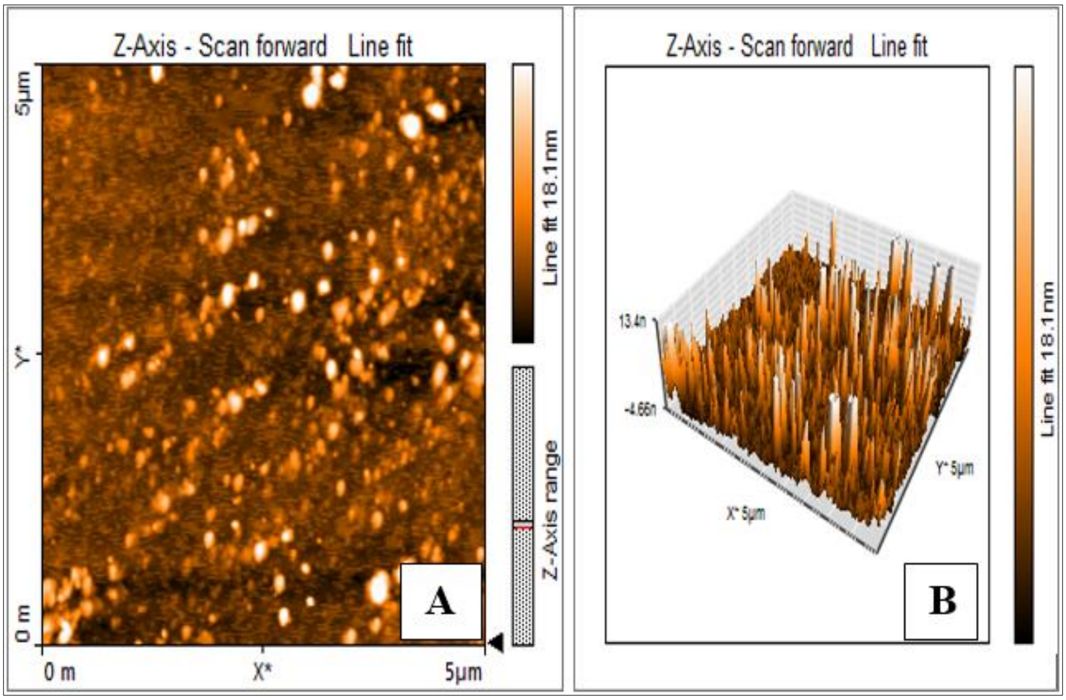

Fig 3: AFM Images of nanoparticles synthesized by *Caryota urens* **A**. Two dimensional image **B**. Three dimensional image

The XRD data of *C. urens* silver nanoparticles synthesized by leaf extract showed diffraction peaks at 38.860^0, 44.05^0, 65.14^0, 76.98^0 corresponding to (111), (200), (220), and (311) planes respectively (Fig 4). All the peaks in XRD data can be indexed to a face centred cubic structure of silver. It confirms the FCC crystalline nature of elemental silver and shape of the nanoparticles are spherical. Many unassigned peaks were observed which might have occurred due to the crystallization of the biogenic phases that remained attached on the surface of the synthesized nanoparticles [13]. FTIR Spectra of *C. urens* leaf extract are presented in (fig 5 & Table 1). The broad band at 3401 cm^{-1} due to the O-

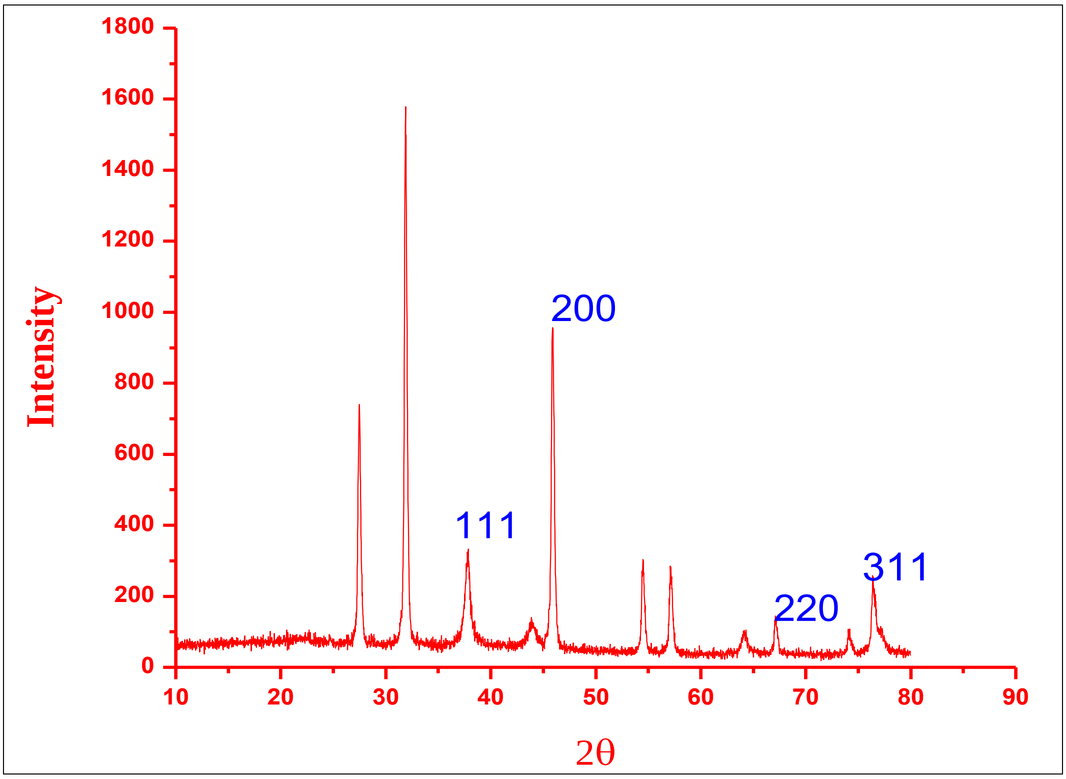

Fig 4: XRD of silver nanoparticles synthesized by leaf extract of *Caryota urens* L.

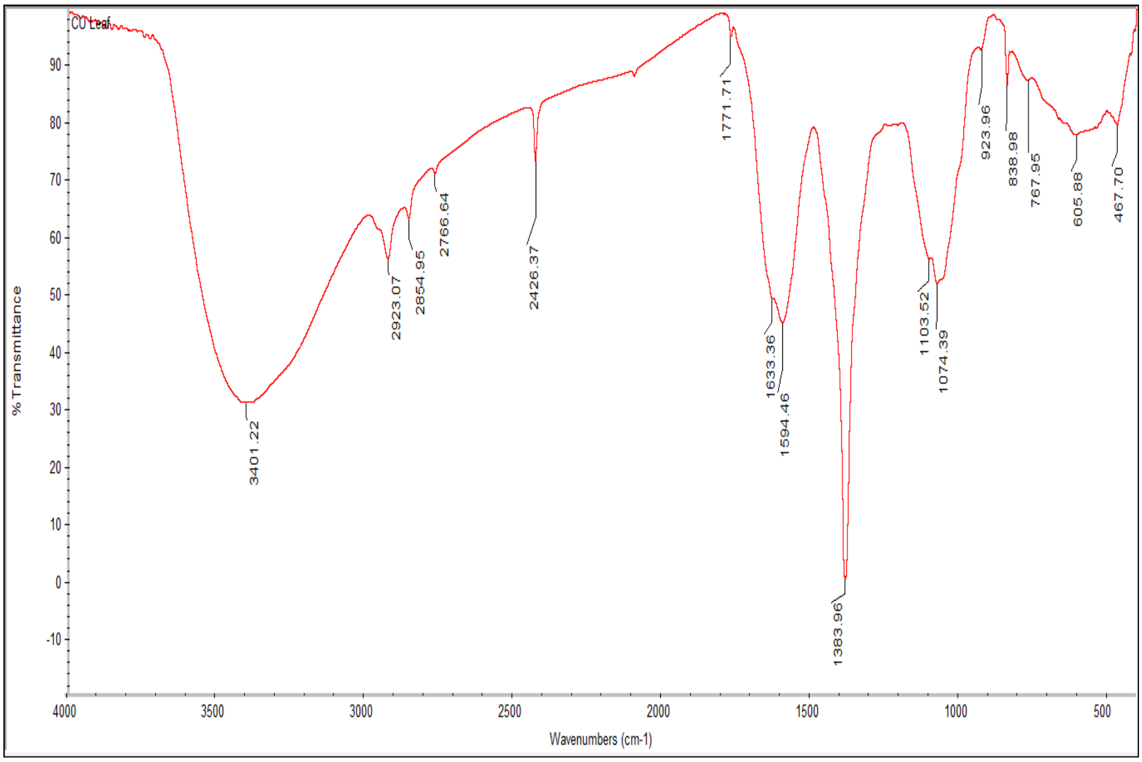

Fig 5: FTIR spectra of nanoparticles synthesized by *Caryota urens* L.

H group of alcohols, the band at 2923 was due to the O-H stretch of carboxylic acid and 2854 cm^{-1} attributed to the methylene symmetric vibrations. 1594 cm^{-1} band assigned to the –N-H- bending of primary amines, the band at 1383 cm^{-1} due to the aromatic rings and band 838 cm^{-1} for the bending of C-H groups [14]. The HR-TEM image of the silver nanoparticles synthesized by leaf extract of *Caryota urens* were showed in the fig 6. The High Resolution- Transmission Electronic Microscopic studies were read at the scale between 10 and 20 nm. Clearly demonstrates the capping of the silver nanoparticles. Presence of the nanofringes and disappearance of the nanofringes was demonstrated in the fig. B of the AgNPs synthesized by *C. urens* aqueous leaf extract. The fig C shows the phytochemicals of the plant extract were involved in the capping of the nanoparticles.

Table 1: FTIR functional group of silver nanoparticles synthesized by *Caryota urens* L.

Absorption peak in Cm^{-1}	Functional groups
3401.22	O-H group of alcohol
2923.07	CH_3 methylene vibration mode
2854.95	CH_3 methylene antisymmetric vibration mode
2766.64	C-H stretching of alkene
2426.37	O=C=O string
1594.46	-N-H bending of primary amines
1383.96	C-O aromatic ring
838.98	C-H group of phenyl ring

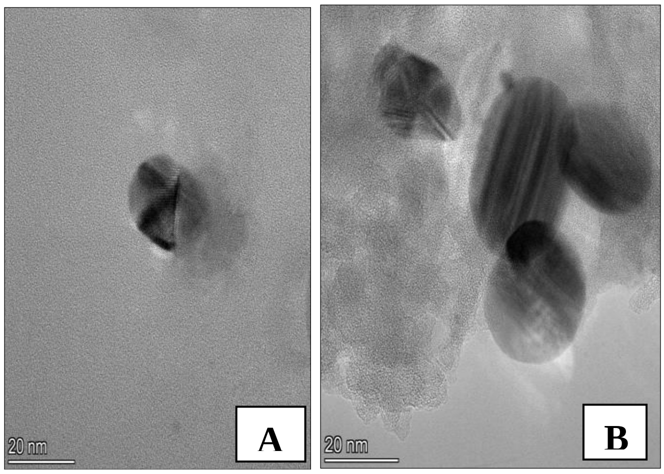

Fig 6: HR-TEM images of nanoparticles synthesized by *Caryota urens* L.

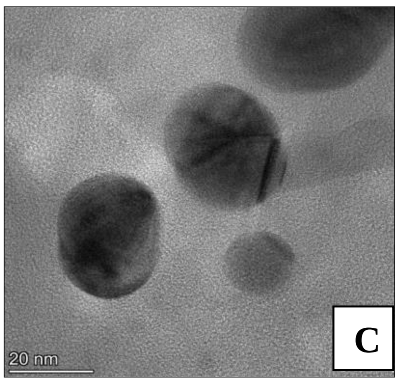

Fig 6: C. Capping of the nanoparticles at 20nm scale.

In-vitro antioxidant activity of silver nanoparticles synthesized by leaf extracts of *C. urens*, leaf extract and control ascorbic acid were studied at different concentrations *viz*; (12.5, 25, 50,100,200 and 400 µg/ml). The results of radical scavenging activity of DPPH assay was presented in the fig. 7. The IC_{50} of DPPH assay was leaf extract, silver nanoparticles and Ascorbic acid is, 133.5, 50, 6 µg/ml. The mean result of standard deviation Standard deviation and standard error by using SPSS 2.0 software in the Table 2.of ascorbic acid is 0.306±0.176, 0.206±0.166, 0.200±0.115, 0.265±0.153, 0.450±0.260, 0.578±0.136, leaf extract is, 0.427±0.135, 0.462±0.133, 0.341±0.276, 0.376±0.221, 0.329±0.019, 0.384±0.190 and silver nanoparticles are, 0.211±0.0122, 0.325±0.0188, 0.266±0.015, 0.444±0.025, 0.314±0.018 and 0.351±0.020 respectively [10, 11].

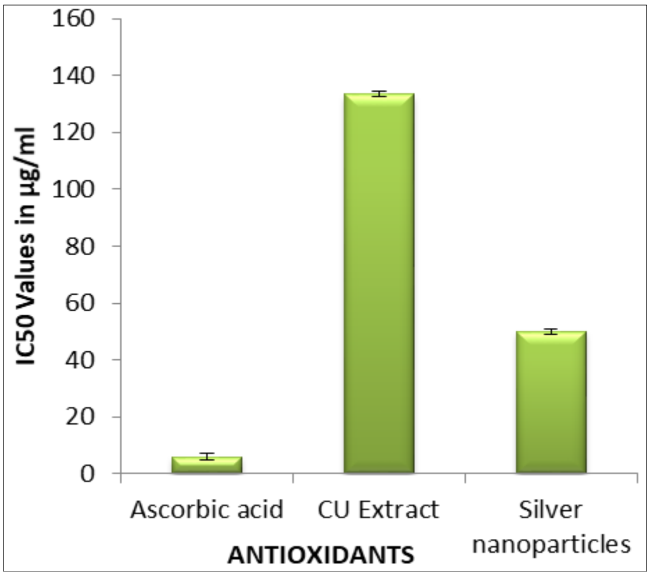

Fig 7: Antioxidant activity of silver nanoparticles synthesized by *C. urens*

Table 2: Percentage of radical scavenging activity of silver nanoparticles synthesized by leaf extract of *C.urenus* represented in the SD±SE.

Concentration in µg/ml	Ascorbic acid	Aqueous leaf extract	Silver nanoparticles
12.5	0.306±0.176	0.427±0.135	0.211±0.012
25	0.206±0.166	0.462±0.133	0.325±0.018
50	0.200±0.115	0.341±0.276	0.266±0.015
100	0.265±0.153	0.376±0.221	0.444±0.025
200	0.450±0.260	0.329±0.019	0.314±0.018
400	0.578±0.136	0.384±0.190	0.351±0.020

Conclusion
This study shows that using an aqueous extract of *Leea macrophylla* leaf to synthesise silver nanoparticles is a green, environmentally friendly method. These nanoparticles had a spherical form and were formed of nano crystals. When compared to leaf extract, silver nanoparticles have the strongest antioxidant activity at lower doses. Its antioxidant qualities may potentially increase its usefulness in biomedicine.

Acknowledgement
Authors were thankful Chairman, P. G. Department of Botany, Karnatak University, Dharwad. University Scientific Instrumentation Centre, Karnatak University, Dharwad. MIT, Manipal and IIT Chennai, for providing XRD and HR-TEM facility. Author Tejashwini Doddamani acknowledges the University Research Scholarship and NFPWD, New Delhi for providing financial facility.

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