Green Synthesis of *Crepidium acuminatum (Jeevak) Leaf* Extract Conjugated Gold and Silver nanoparticles

Soumen Patra, Sukhendu Kar, Sayan Das and Braja Gopal Bag*

Department of Chemistry and Chemical Technology, Vidyasagar University, Midnapore 721102, West Bengal, India
Email: brajagb@gmail.com

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The antioxidant activity of the leaf extract of *Crepidium acuminatum* (commonly known as Jeevak) has been studied against a long lived 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at room temperature. Green syntheses of Jeevak Leaf extract conjugated gold and silver nanoparticles at room temperature have been reported.

Crepidium acuminatum (Figure 1) commonly known as Jeevak is one of the eight members of the Astavarga plants used in the preparation of the Ayurvedic health tonic *Chyawanprash*.[1,2,3] Jeevak is a rare medicinal plant usually found in certain parts of Himalaya at an altitude of 1200 – 4000 m above the sea level. m.[4] It is a short-lived plant that grows in the month of May-June having a life span of 6-7 months. The tuber of the plant is usually used in the preparation of *Chyawanprash*. However, the leaves may also have useful medicinal properties. Herein we report the antioxidant activity of the leaf extract of Jeevak. Green synthesis of the Jeevak Leaf extract conjugated gold nanoparticles (JLAuNPs) and silver nanoparticles (JLAgNPs) are also reported.

The plant sample was collected from the Dhanoulti area of Himalaya during July-September and deposited at the herbarium of Patanjali Yogpeeth Haridwar. Each jeevak plant contains usually 2-4 leaves. The leaves are light green, acute with prominent veins, sessile or petioled, 8-12 cm long, ovate-lanceolate. The leaves whorls on the nodes are directly raised upwards, attenuate and angular. (Figure 1). The stem is covered by basal leaves forming a tubular structure. Fresh leaf sample (7.1 g) was chopped and then crushed using mortar and pestle and extracted with methanol via sonication for 20 min at 40 °C. This extract was centrifuged and preserved at 4 °C and used within four weeks for our studies.

Active oxygen species and free radicals have been recognized as one of the various causes of physiological disorders such as stress, age related diseases including cancer, tumor, etc.[5,6] Previous reports from our laboratory have shown that the pseudobulb of *Crepidium acuminatum* (Jeevak),[7] extract of *Roscoea purpurea* Sm. (Kakoli),[8,9] Rhizome Extract of *Polygonatum cirrhifolium* (Mahameda)[10] and, extracts of *Habenaria Edgeworthii* (*Vrddhi*)[11] and *Habenaria intermedia* (Rddhi)[12,13] are reach in antioxidants. Hence, it occurred to us that the leaf extract of Jeevak may also be rich in antioxidants. Indeed, when a methanolic solution of DPPH was treated with an increasing concentration of the leaf extract, decrease in intensity of the violet color of DPPH was observed (Figure 2) indicating antioxidant activity of the leaf extract. The percentage of radical scavenging activity was calculated to be 85%, 83%, 59%, 29% and 21% when the concentration of the leaf extract was 1200, 800, 400, 200 and 100 μg/mL respectively.

Figure 1: Photograph of *Crepidium acuminatum* taken at the Dhanoulti area of Himalaya, Uttarakhand, India, in July, 2016.

Gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in bio diagnostics, catalysis, pharmaceuticals, etc.[14,15,16,17,18] The AuNPs conjugated with non-toxic

biomolecules are preferable for many of such applications.[19] The green syntheses of AuNPs from the extracts of *Terminalia arjuna bark*,[20] *Azadirachta indica*,[21] *Saraca indica*,[22] *Acacia nilotica*,[23] *Punica granatum*,[24] *Ananas comosus (L.),*[25] *Ocimum sanctum* ,[26] have been reported.

Previously we have reported the green synthesis of gold nanoparticles using extracts Jeevak, Kakoli, Mahameda, Rddhi and and Vrddhi.[7,8,10,11,12] Hence it occurred to us that the leaf extract of Jeevak may be utilized for the green synthesis of AuNP conjugated with the leaf extract of Jeevak (JLAuNPs). For the green synthesis of gold nanoparticles, a fixed concentration (0.40 mM) of Au(III) was reacted with an increasing concentration of the leaf extract (50 μg/mL to 800 μg/mL).[27] Appearance of light pink to greyish brown color appeared at room temperature with 1 h indicated the formation of gold nanoparticles (JLAuNPs) (Figure 3A).

A surface plasmon band observed in the 500-700 nm range by UV-Visible spectrophotometry (Figure 3) supported the formation of AuNPs. In the UV-visible spectrum of Au(III) solution, two peaks were observed at 220 and 290 nm due to 'charge transfer interaction between the metal and chloro ligands'. With increasing concentration of the leaf extract, decrease in intensity of these two peaks were observed with

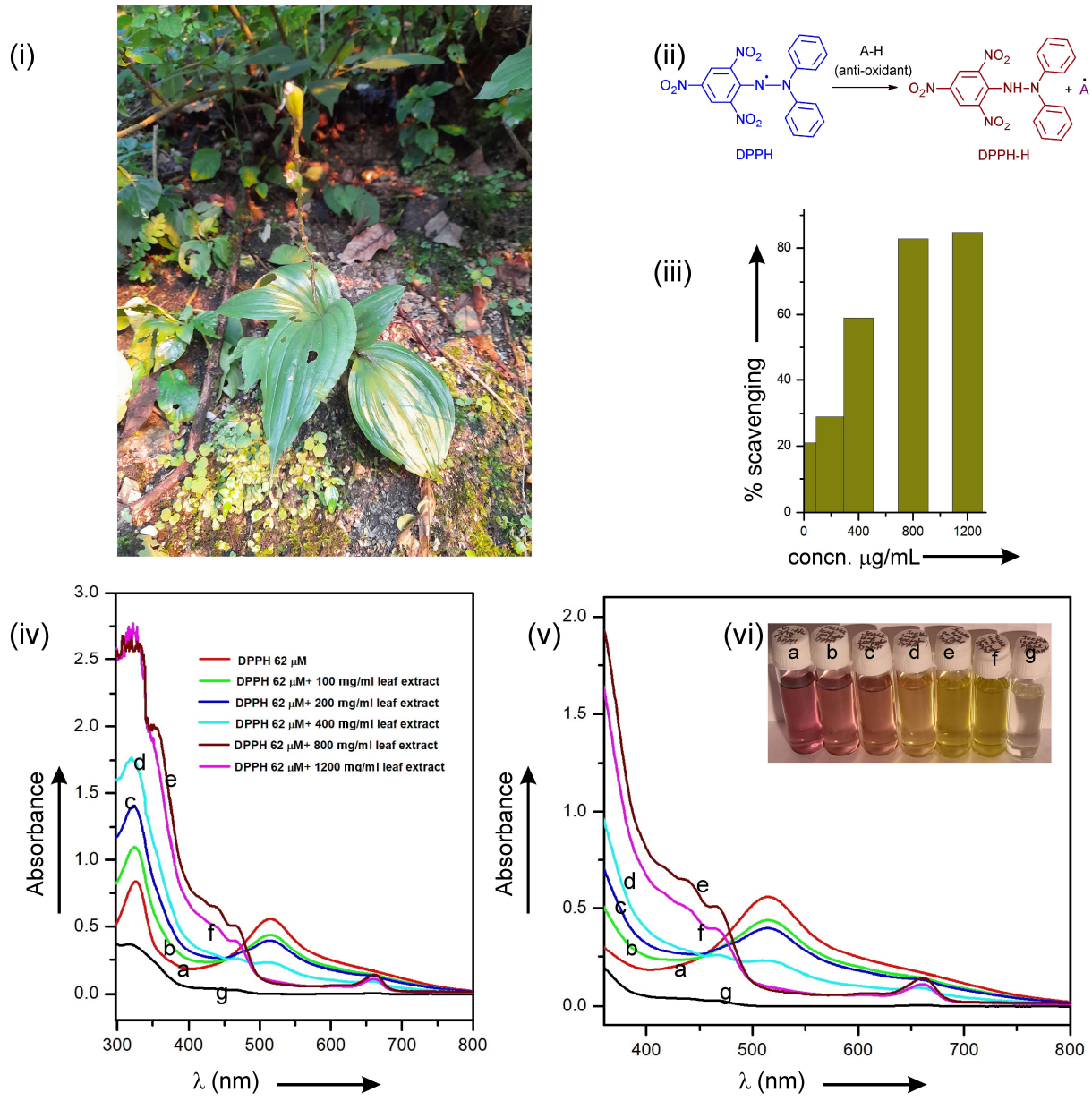

Figure 2: (i) *Crepidium acuminatum* plant, (ii) Mechanisam of DPPH activity (iii) plot of % DPPH radical scavenging by the methanol extract of leaf at, 100, 200, 400, 800, 1200 μg/mL (iv) plot of UV-Vis spectra of the leaf extract upon addition of DPPH at varied concentration, (v) concentration,zoomed spectra shown in (iv); (vi) corresponding vials

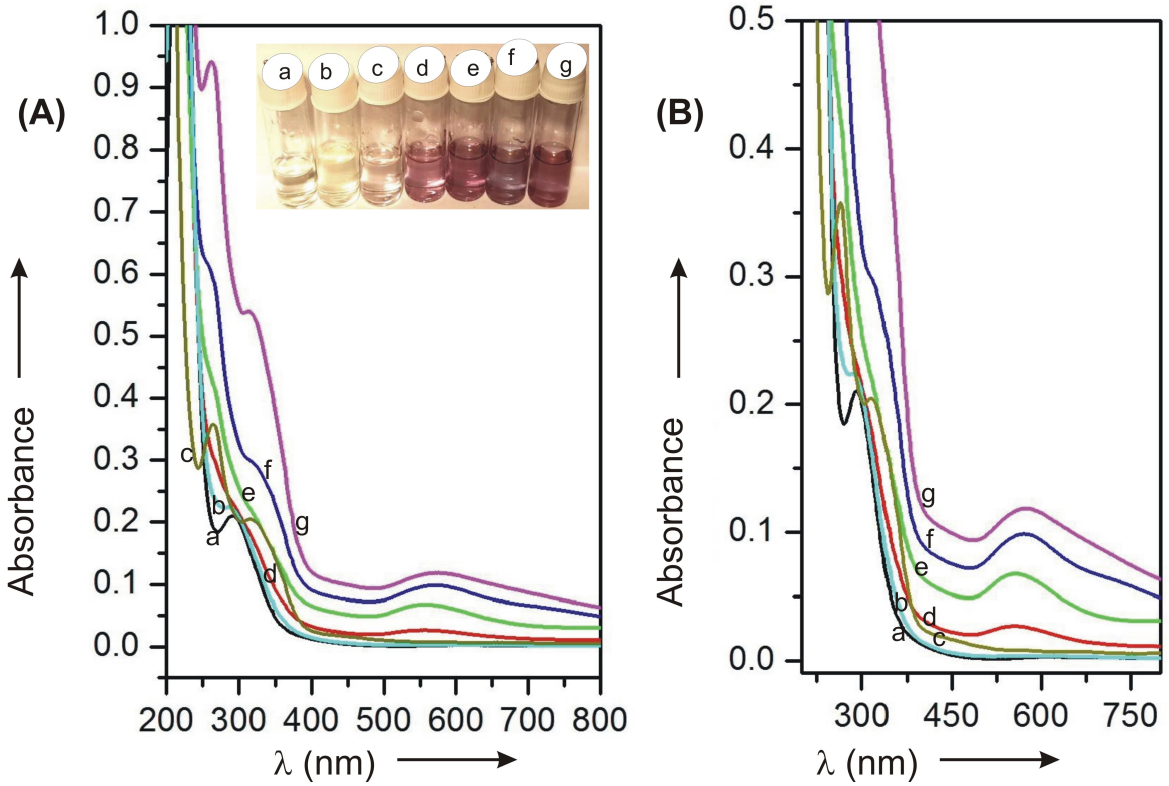

Figure 3: (A) UV-Visible spectra (recorded in a 2 mm path length cell) of (a) $HAuCl_4$ solution (0.4 mM), (b) leaf extract (100 μg/mL), (c-g) VLAuNPs at 50, 100, 200, 400 and 800 μg/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples. (B) zoomed UV-Visible spectra of set (A)

concomitant formation of a new band around 550-650 nm due to surface Plasmon resonance (SPR) phenomenon of JLAuNPs. With increasing the concentration of the leaf extract a blue shift of the SPR band was observed due to the formation of smaller sized AuNPs.[7,8,10] The gradual upward shifting of the baseline with increasing concentration of the leaf extract may be attributed to absorptions of the phytochemicals. With 800 μg/mL concentration of the leaf extract, λ_{max} was 573 nm.

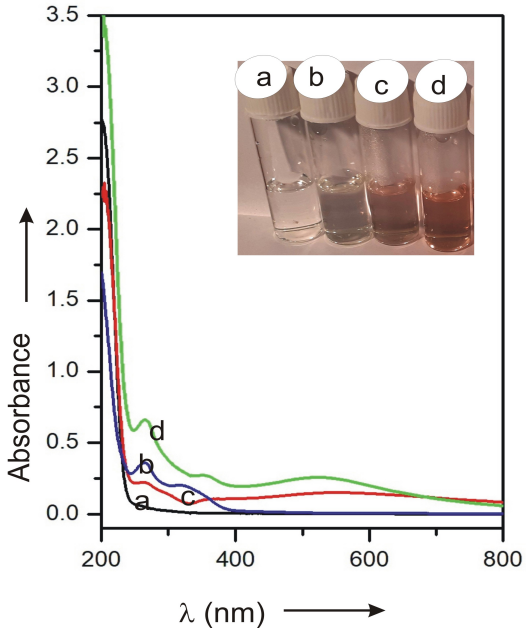

Figure 4: UV-Visible spectra (recorded with 2 mm path length cuvette) of (a) $AgNO_3$ solution (0.7 mM) (b) leaf extract (100 μg/mL), (c-d) JLAgNPs at 50, 100 μg/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples.

Silver nanoparticles (AgNPs) have tremendous application for its antibacterial activities along with the applications in biomedicine, environment, catalysis, health care and, food and agriculture.[28] Success in the synthesis of JLAuNPs inspired us to study the synthesis of Jeevak leaf extract conjugated silver nanoparticles (JLAgNPs). An aqueous solution of $AgNO_3$ (0.7 mM) was reacted with an increasing concentration of the leaf extract of Jeevak at room temperature. Observation of light pink color within 20 min indicated with formation of silver nanoparticles. Observation of broad surface plasmon resonance band in the 400-700 nm range indicated the formation of silver nanoparticles (Figure 4). With 100 μg/mL concentration of the leaf extract, λ_{max} for JLAgNPs was 520 nm.

Atomic force microscopy (AFM) studies were carried out to investigate the morphology of Jeevak leaf extract conjugated gold and silver nanoparticles. When air dried samples of JLAuNPs and JLAgNPs were analylzed by AFM (Figure 5), mostly spherical shaped polydisperse nanoparticles were observed.

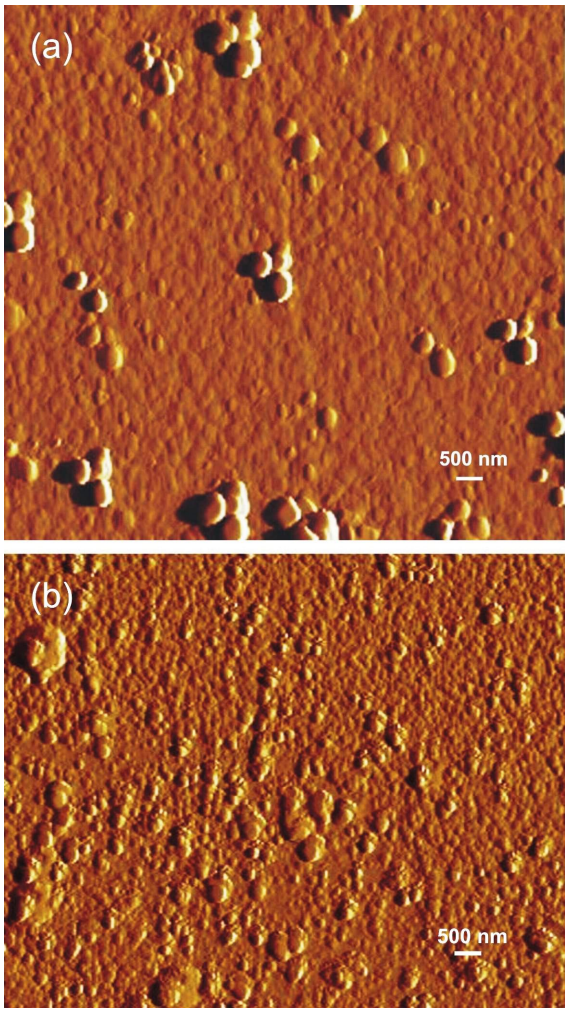

Figure 5: AFM images of (a) JLAuNPs having Leevak leaf extract concentration of 400 μg/mL, (b) JLAgNPs having the concentration of 400 μg/mL

In conclusion, the antioxidant activity of the leaf extract of *Crepidium acuminatum* (Jeevak) has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the leaf extract of Jeevak have been utilized for the green synthesis of Jeevak leaf extract conjugated gold and silver nanoparticles at room temperature under very mild conditions without any additional stabilizing agents. Current studies in our laboratory are in progress to find out the chemical composition of the leaf extract and the application of leaf-extract conjugated metal nanoparticles in medicine.

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27. *Brief Experimental Procedure*:

Synthesis of JLAuNPs: Synthesis of JLAuNPs was carried out following the procedure as described previously.[7] A stock solution of the methanolic extract of leaf of Jeevak was prepared (9200 μg/mL, as described previously). The stock solution of the extract was diluted in vials of capacity 4 mL (Figure 3A) to prepare a series of the solutions in water. Aliquots of Au (III) (80 μL, 10.0 mM each) were added drop-wise to the extract solution so that the final volume becomes 2 mL and the final concentration of the leaf extract varies from 50, 100, 200, 400, 800 μg/mL. The concentration of Au(III) was fixed at 0.40 mM in each vial (Figure 3).

Synthesis of JLAgNPs: Synthesis of JLAgNPs in water medium was carried out in an identical method of JLAuNPs preparation keeping the concentrations of the leaf extract identical.[12] Aliquots of $AgNO_3$ solution (57 μL, 14.0 mM) in water were added to each of the vials of capacity of 4 mL. The final volume of the mixtures was 2 mL each and the final concentration of $AgNO_3$ in the mixtures was 0.4 μg/mL in each vial (Figure 4).

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