Antioxidant Property of the Leaf Extract of *Habenaria intermedia* (Rddhi) and its use in the Green Synthesis of Leaf Extract Conjugated Gold and Silver nanoparticles

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The antioxidant activity of the leaf extract of *Habenaria intermedia* (commonly known as Rddhi) has been studied against a long lived 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at room temperature. Green synthesis of Rddhi leaf extract conjugated gold and silver nanoparticles at room temperature has been reported.

Habenaria intermedia (Figure 1) commonly known as Rddhi is one of the eight members of the Astavarga plants used in the preparation of the Ayurvedic health tonic *Chyawanprash*.[1,2,3] It is a rare medicinal plant usually found in certain parts of Himalaya at an altitude of 1500-2500 m from Himachalpradesh to Uttarakhand and Sikkim and Jammu and Kashmir.[4] Rddhi is a short-lived plant that grows in the month of May-June having a life span of 5-6 months. The tuber of the plant is usually used in the preparation of *Chyawanprash*. However, the leaves may also have medicinal properties. Herein we report the antioxidant activity of the leaf extract of Rddhi. Green synthesis of the leaf extract conjugated gold nanoparticles (RLAuNPs) and silver nanoparticles (RLAgNPs) 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 plant contain usually 3-5 leaves. The leaves of Rddhi are 'ovate or oblong or ovate-lancelate, scattered, 5-8 cm long, sheathing, acuminate, base rounded or cordate, 5-7 nerved' (Figure 2).[4] A fresh, raw sample of the matured leaves (3.4 g) was sliced 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] extracts of *Roscoea purpurea* Sm. (Kakoli),[8,9] Rhizome Extract of *Polygonatum cirrhifolium* (Mahameda)[10] and Tuber Extracts of *Habenaria Edgeworthii* (*Vrddhi*)[11] and *Habenaria intermedia* (*Rddhi*)[12] are rich in antioxidants. Hence, it occurred to us that the leaf extract of Rddhi 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 90%, 60%, 37% and 17% when concentration of the leaf extract was 1200, 800, 400 and 100 µg/mL respectively.

Figure 1: Photograph of *Habenaria intermedia* taken on July 16, 2016 at Dhanoulti, Himalaya, India.

Gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in biodiagnostics, catalysis, pharmaceuticals, etc.[13,14,15,16,17] The AuNPs conjugated with non-toxic biomolecules are preferable for many of such applications.[18] The green syntheses of AuNPs from the extracts of *Terminalia arjuna bark*,[19] *Azadirachta indica*,[20] *Saraca indica*,[21] *Acacia nilotica*,[22] *Punica granatum*,[23] *Ananas comosus (L.)*,[24] *Ocimum sanctum* ,[25] have been reported.

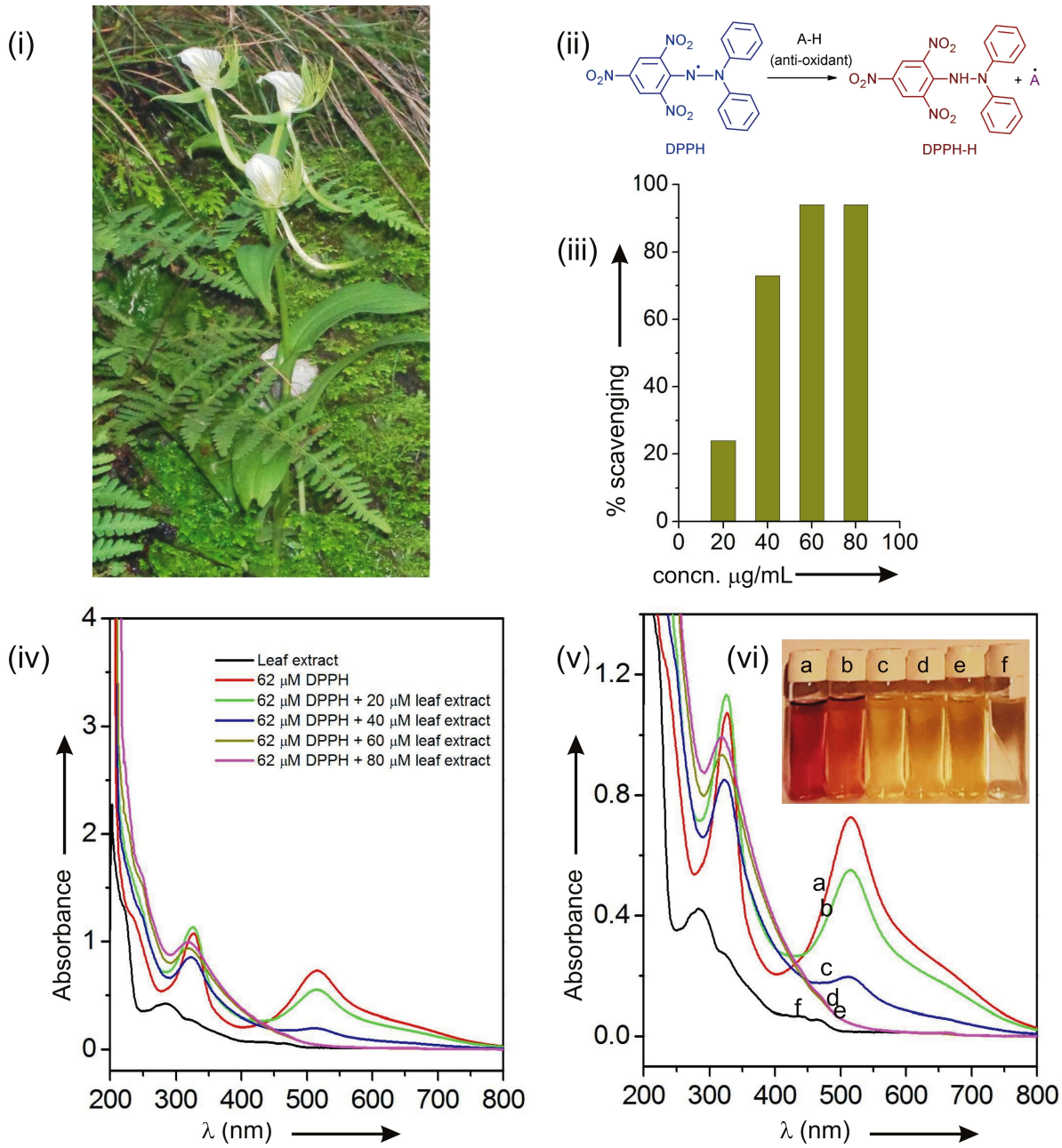

Figure 2: (i) *Habenaria intermedia* plant (ii) Leaf of *Habenaria intermedia* (iii) Mechanisam of DPPH activity, (iv) anti-oxidant property of leaf extract (a) Extract, (b) DPPH, (c) DPPH + extract (v) Inset: corresponding vials (vi) plot of % DPPH radical scavenging by the methanol extract of leaf at 100, 400, 800, 1200 μg/mL concentration.

Previously we have reported the green synthesis of gold nanoparticles using extracts Jeevak, Kakoli, Mahameda and Vrddhi.[7,8,10,11] Hence it occurred to us the leaf extract of Rddhi may be utilized for the green synthesis of AuNP conjugated with the leaf extract of Rddhi (RLAuNPs). 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).[26] Appearance of light pink to dark brown color appeared at room temperature with 2 h indicating the formation of gold nanoparticles (RLAuNPs) (Figure 3A).

A surface plasmon band observed in the 520 -600 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 concomitant formation of a new band around 550 nm due to surface Plasmon resonance (SPR) phenomenon of RLAuNPs. With increasing the concentration of the leaf extract a blue shift of the SPR band was observed due to the

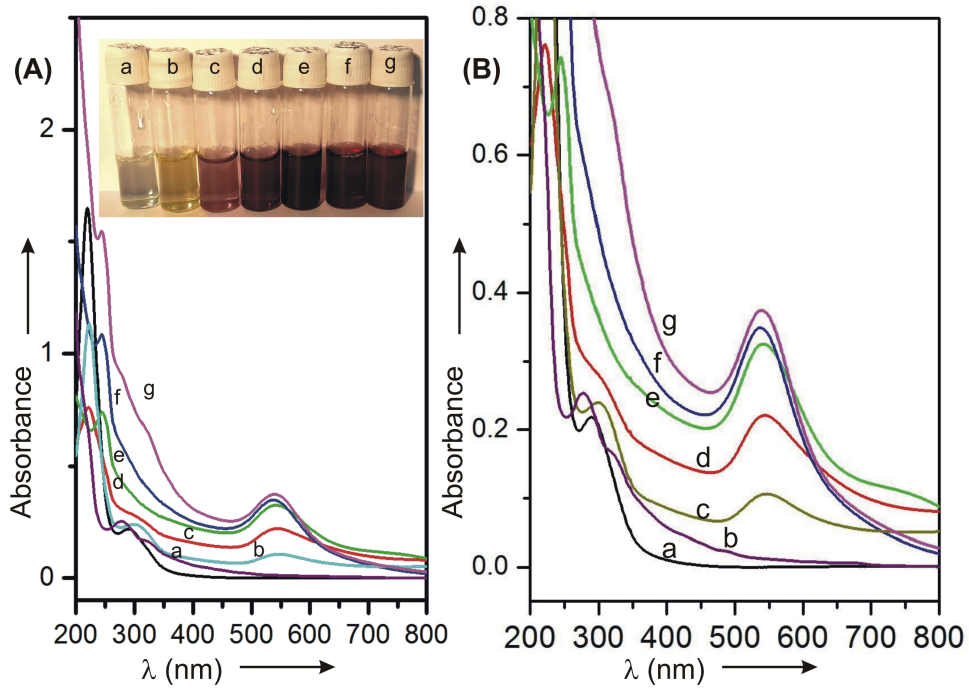

Figure 3: (A) UV-Visible spectra (recorded in a 2 mm path length cell) of (a) $HAuCl_4$ solution (0.4 mM), (b) leaf extract (400 µg/mL), (c-g) RLAuNPs at 50, 100, 200, 400 and 800 µg/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples. (B) zoomed UV-Visible spectra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

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 539 nm.

Silver nanoparticles (AgNPs) have tremendous application for its antibacterial activities along with the applications in biomedicine, environment, catalysis, health care and, food and agriculture.[27] Success in the synthesis of RLAuNPs inspired us to study the synthesis of Rddhi Leaf extract conjugated silver nanoparticles (RLAgNPs). An

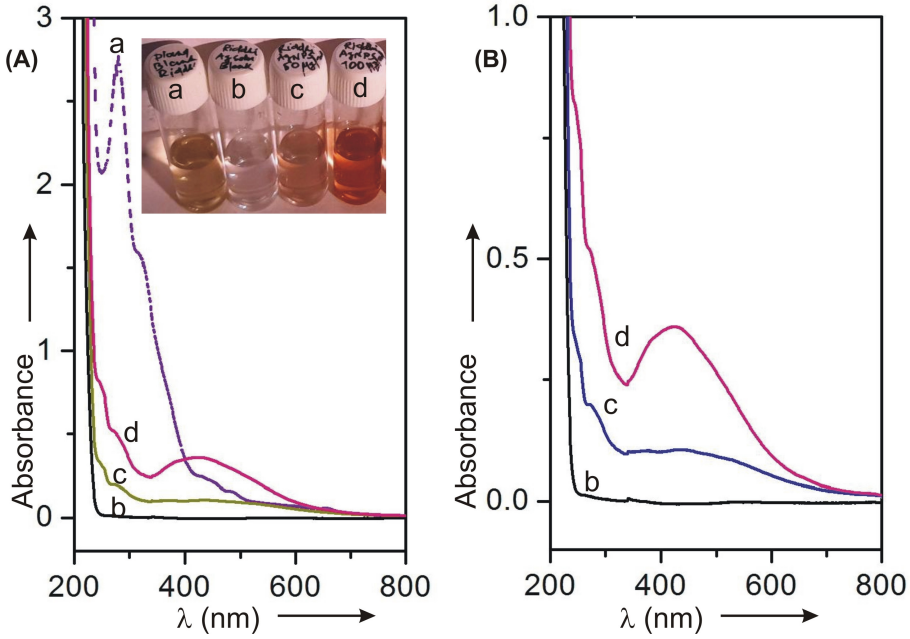

Figure 4: (A) UV-Visible spectra (recorded with 10 mm path length cuvette) of (a) leaf extract (100 µg/mL) (b) $AgNO_3$ solution (0.7 mM), (c-g) RLAgNPs at 50 and 100 µg/mL concentration of the tuber extract. Inset: photograph of vials containing the above samples. (B) zoomed (A) (without the spectrum of the leaf extract, for clarity).

aqueous solution of $AgNO_3$ (0.7 mM) was reacted with an increasing concentration of the leaf extract of Rddhi at room temperature. Observation of light pink color within 15 h indicated with formation of silver nanoparticles. The color was intensified on standing the mixture under sun shine for 15 min. Observation of broad surface plasmon resonance band in the 300-600 nm range indicated the formation of silver nanoparticles (Figure 4). With 100 µg/mL concentration of the leaf extract, λ_{max} for AgNPs was 424 nm.

In conclusion, the antioxidant activity of the leaf extract of *Habenaria intermedia (Rddhi)* has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the leaf extract of Rddhi have been utilized for the green synthesis of Rddhi 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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26. *Brief Experimental Procedure*:

    Synthesis of RLAuNPs: Synthesis of RLAuNPs was carried out following the procedure as described previously.[12] A stock solution of methanolic extract of leaf of Rddhi was prepared (8100 µg/mL). The stock solution 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 tuber 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 RLAgNPs: Synthesis of RLAgNPs in water medium was carried out in an identical method of RLAuNPs preparation keeping the concentrations of the tuber extract identical.[12] Aliquots of $AgNO_3$ solution (100 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 0.7 µg/mL in each vial (Figure 4).
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