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

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The antioxidant activity of the tuber 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. The phytochemicals present in the tuber extract have been utilized for the green synthesis of Rddhi tuber extract conjugated gold and silver nanoparticles at room temperature under very mild conditions.

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*. Herein we report the antioxidant activity of the tuber extract of Rddhi. Green synthesis of the tuber extract conjugated gold nanoparticles (RTAuNPs) and silver nanoparticles (RTAgNPs) 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 . The tubers of Rddhi were 2-3 cm long, 1.0 – 2.5 cm in diameter, oval or oblong shaped, 'covered with fine whitish hairs' (Figure 2). A fresh, raw sample of the tuber (5.7 g) was sliced and then crushed using mortar and pestle and extracted with methanol via sonication for 20 min at 35 °C. This extract was centrifuged and preserved at -20 °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] Rhizome Extract of *Roscoea purpurea* Sm. (Kakoli),[8] Rhizome Extract of *Polygonatum cirrhifolium* (Mahameda)[9] and Tuber Extract of *Habenaria Edgeworthii* (*Vrddhi*)[10] are reach in antioxidants. Hence, it occurred to us that the tuber extract of Rddhi may also be rich in antioxidants. Indeed, when a methanolic solution of DPPH was treated with an increasing concentration of the tuber extract, decrease in intensity of the violet color of DPPH was observed (Figure 2) indicating

Habenaria intermedia (Rddhi)

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

antioxidant activity of the tuber extract. The percentage of radical scavenging activity was calculated to be 90%, 60%, 37% and 17% when concentration of the tuber extract was 1200, 800, 400 and 100 μg/mL respectively.

Gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in biodiagnostics, catalysis, pharmaceuticals, etc.[11,12,13,14,15] The AuNPs conjugated with non-toxic biomolecules are preferable for many of such applications.[16] The green syntheses of AuNPs from the extracts of *Terminalia arjuna bark*,[17] *Azadirachta indica*,[18] *Saraca indica*,[19] *Acacia nilotica*,[20] *Punica granatum*,[21] *Ananas comosus (L.)*,[22] *Ocimum sanctum* ,[23] have been reported.

Previously we have reported the green synthesis of gold nanoparticles using extracts Jeevak, Kakoli, Mahameda and Vrddhi.[7,8,9,10] Hence it occurred to us the tuber extract of Rddhi may be utilized for the green synthesis of AuNP conjugated with the tuber extract of Rddhi (RTAuNPs). For the green synthesis of gold nanoparticles, a fixed concentration (0.40 mM) of Au(III) was reacted with an increasing concentration of the tuber extract (50 μg/mL to 800 μg/mL).[24] Appearance of light pink to greyish brown color appeared at room temperature with 2 h indicated the formation of gold nanoparticles (RTAuNPs) (Figure 3A).

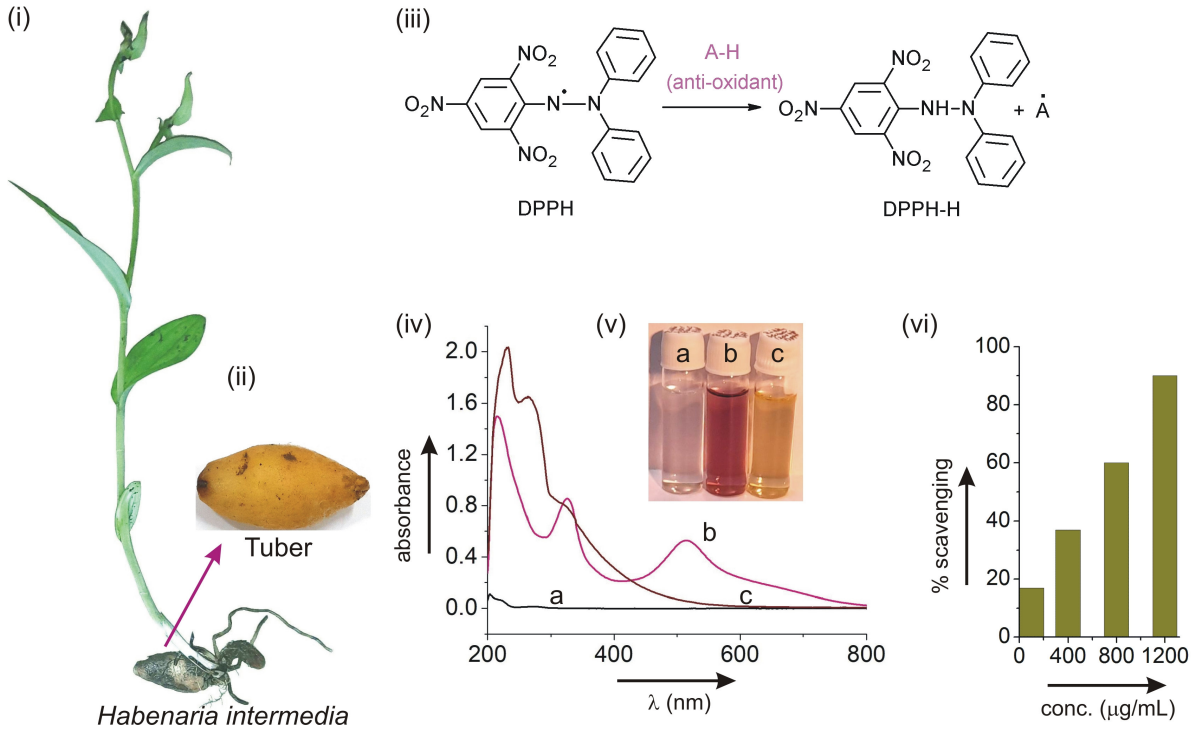

Figure 2: (i) *Habenaria intermedia* plant (ii) Tuber of *Habenaria intermedia* (iii) Mechanisam of DPPH activity, (iv) anti-oxidant property of tuber extract (a) Extract, (b) DPPH, (c) DPPH + extract (v) Inset: corresponding vials (vi) plot of % DPPH radical scavenging by the methanol extract of tuber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

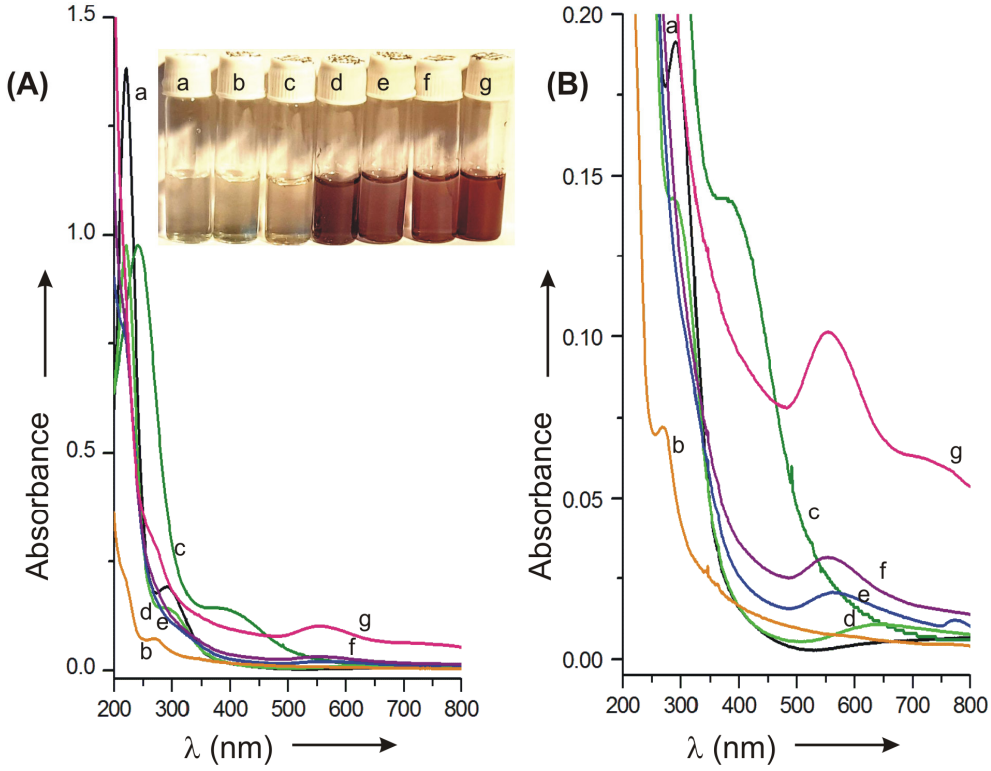

Figure 3: (A) UV-Visible spectra (recorded in a 2 mm path length cell) of (a) $HAuCl_4$ solution (0.4 mM), (b) tuber extract (400 μg/mL), (c-g) RTAuNPs at 50, 100, 200, 400 and 800 μg/mL concentration of the tuber extract. Inset: photograph of vials containing the above samples. (B) zoomed UV-Visible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

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 tuber 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 RTAuNPs. With increasing the concentration of the tuber extract a blue shift of the SPR band was observed due to the formation of smaller sized AuNPs.[7,8,9] The gradual upward shifting of the baseline with increasing concentration of the tuber extract may be attributed to absorptions of the phytochemicals. With 800 µg/mL concentration of the tuber extract, λ_{max} was 556 nm.

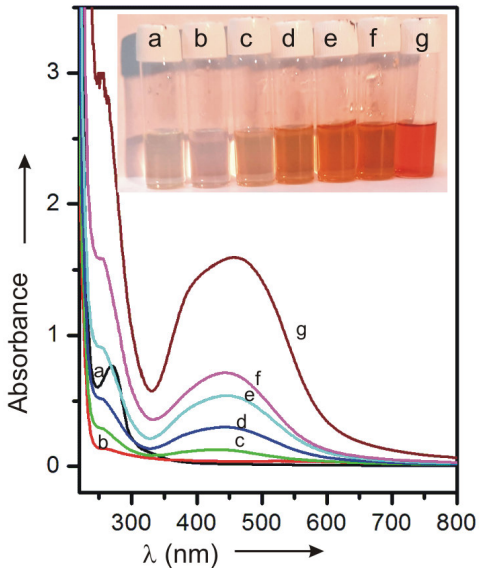

Figure 4: UV-Visible spectra (recorded with 10 mm path length cuvette) of (a) tuber extract (100 mg/mL) (b) $AgNO_3$ solution (0.7 mM), (c-g) RTAgNPs at 50, 100, 200, 400 and 800 µg/mL concentration of the tuber extract. Inset: photograph of vials containing the above samples.

Silver nanoparticles (AgNPs) have tremendous application for its antibacterial activities along with the applications in biomedicine, environment, catalysis, health care and, food and agriculture.[25] Success in the synthesis of RTAuNPs inspired us to study the synthesis of Rddhi Tuber extract conjugated silver nanoparticles (RTAgNPs). An aqueous solution of $AgNO_3$ (0.7 mM) was reacted with an increasing concentration of the tuber 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 800 µg/mL concentration of the tuber extract, λ_{max} for AgNPs was 461 nm.

In conclusion, the antioxidant activity of the tuber 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 tuber extract of Rddhi have been utilized for the green synthesis of Rddhi tuber extract conjugated gold and silver nanoparticles at room temperature under very mild conditions without any additional stabilizing agents. As the tuber extract of *Habenaria intermedia* has tremendous medicinal significance, the studies described will be useful in biomedical applications as well as nanoscience and nanobiotechnology. Current studies in our laboratory are in progress to find out the chemical composition of the tuber extract and the application of tuber-extract conjugated metal nanoparticles in medicine.

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24. *Brief Experimental Procedure*:

Synthesis of RTAuNPs: For the synthesis of RTAuNPs, methanolic extract of tuber of Rddhi was prepared (7600 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 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 3A). The mixtures were shaken by gentle hand shaing and then allowed to stand at room temperature at around 30 °C. Appearance of pink color appeared within an hour and the color intensified on standing the solution. UV-visible spectrophotometry of the gold colloids were carried out after 3 h of addition of $HAuCl_4$ using 2 mm path length cuvette.

Synthesis of RTAgNPs: Synthesis of RTAgNPs in water medium was carried out in an identical method of RTAuNPs preparation keeping the concentrations of the tuber extract identical. 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. Appearance of light yellowish color appeared within 15 h and the color intensified on standing the solution under sun shine. The UV-visible spectra were recorded using 10 mm pathlength cuvette (Figure 4).

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