# Green Synthesis of *Habenaria edgeworthii* (Vrddhi) Leaf Extract Conjugated Gold and Silver nanoparticles

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

*Habenaria edgeworthii* (Figure 1) commonly known as Vrddhi is one of the eight members of the Astavarga plants used in the preparation of the Ayurvedic health tonic *Chyawanprash*.[1,2,3] Vrddhi is a rare medicinal plant usually found in certain parts of Himalaya at an altitude of 2500-3000 m 'in the north-western parts of Jammu and Kashmir, Himachalpradesh and Uttarakhand'.[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 Vrddhi. Green synthesis of the Vrddhi Leaf extract conjugated gold nanoparticles (VLAuNPs) and silver nanoparticles (VLAgNPs) 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 Vrddhi plant contains usually 2-4 leaves. The leaves are 6-10 cm long, 1-2 cm wide, ovate to ovare-lanceolate. (Figure 2). Fresh leaf sample (6.7 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 Vrddhi 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 65%, 51%, 50%, 45%, 14% and 7% when the concentration of the leaf extract was 120, 100, 80, 60, 40 and 20 µg/mL respectively.

Figure 1: Photograph of *Habenaria edgeworthii* Taken at the Dhanoulti area of Himalaya, Uttarakhand, India

Gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in biodiagnostics, 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 Vrddhi may be utilized for the green synthesis of AuNP conjugated with the leaf extract of Vrddhi (VLAuNPs). 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 (VLAuNPs) (Figure 3A).

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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 550-600 nm due to surface Plasmon resonance (SPR) phenomenon of VLAuNPs. 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, $\lambda_{max}$ was 540 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.[28] Success in the synthesis of VLAuNPs inspired us to study the synthesis of Vrddhi leaf extract conjugated silver nanoparticles (VLAgNPs). An aqueous solution of $AgNO_3$ (0.7 mM) was reacted with an increasing concentration of the leaf extract of Vrddhi at room temperature. Observation of light pink color within 1 h indicated with formation of silver nanoparticles. Observation of broad surface plasmon resonance band in the 300-600 nm range indicated the formation of silver nanoparticles (Figure

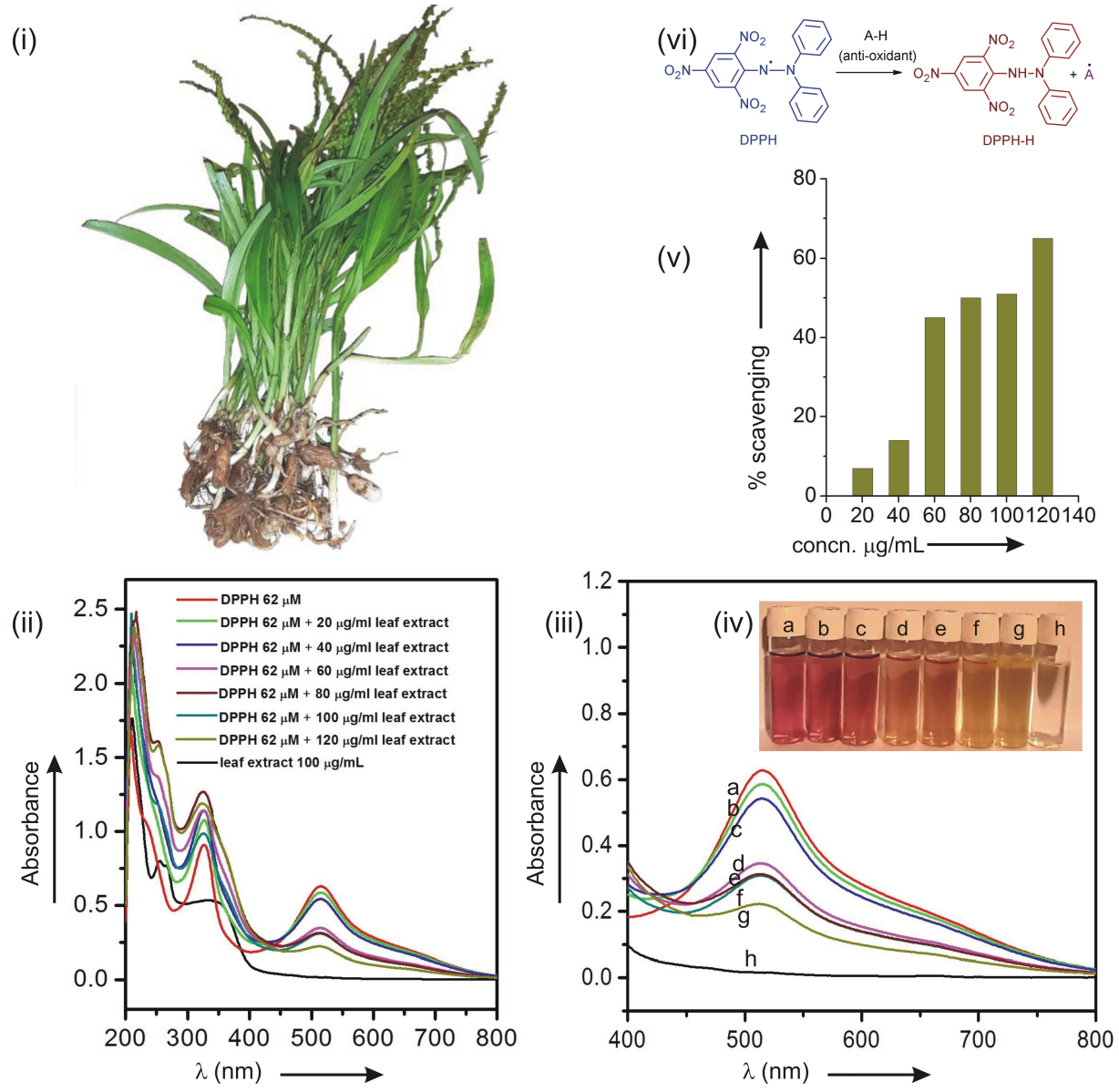


Figure 2: (i) *Habenaria edgeworthii* plant, (ii) plot of UV-Vis spectra of the leaf extract upon addition of DPPH at varied concentration, (iii) zoomed spectra shown in (ii); (iv) corresponding vials, (v) plot of % DPPH radical scavenging by the methanol extract of leaf at, 20, 40, 60, 80, 100, 120 µg/mL concentration, (vi) Mechanisam of DPPH activity,

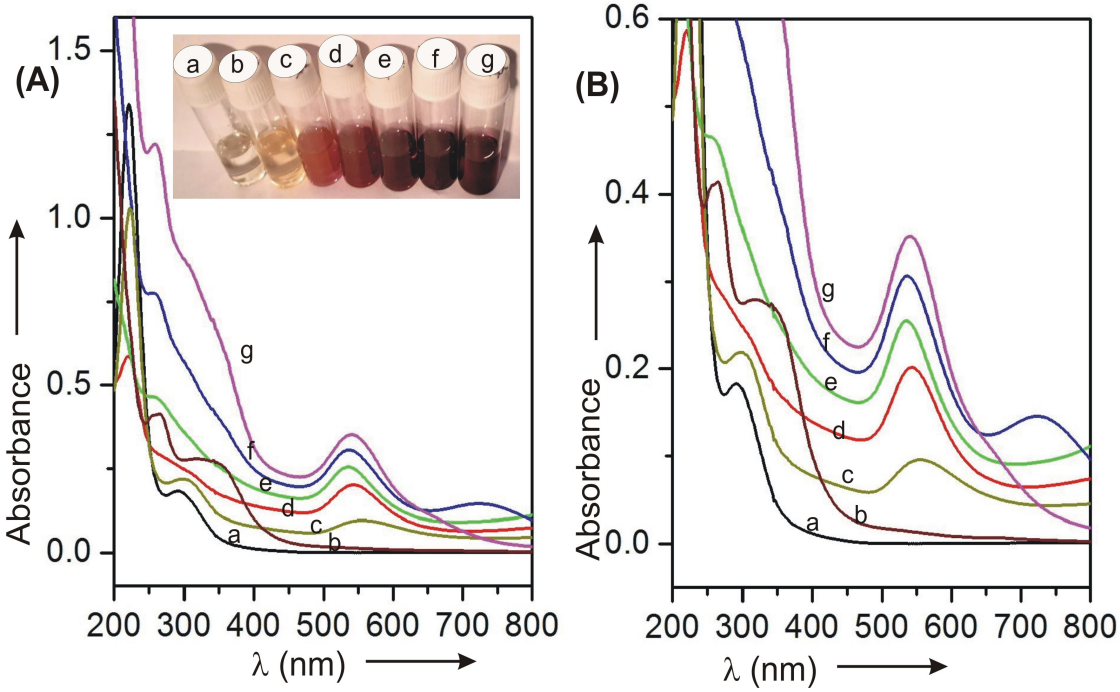


Figure 3: (A) UV-Visible spectra (recorded in a 2 mm path length cell) of (a) $HAuCl_4$ solution (0.4 mM), (b) leaf extract (100 µg/mL), (c-g) VLAuNPs at 50, 100, 200, 400 and 800 µg/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples. (B) zoomed UV-Visible spectra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

4). With 400 µg/mL concentration of the leaf extract, $\lambda_{max}$ for VLAgNPs was 450 nm.

In conclusion, the antioxidant activity of the leaf extract of *Habenaria edgeworthii* (Vrddhi) 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.

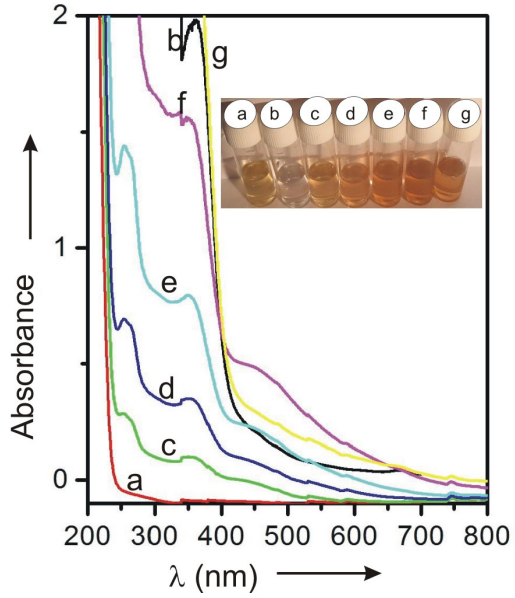


Figure 4: UV-Visible spectra (recorded with 10 mm path length cuvette) of (a) leaf extract (100 µg/mL) (b) $AgNO_3$ solution (0.7 mM), (c-f) VLAgNPs at 50, 100, 200 and 400 µg/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples.

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27. *Brief Experimental Procedure*:

    **Synthesis of VLAuNPs**: Synthesis of VLAuNPs was carried out following the procedure as described previously.[12] A stock solution of the methanolic extract of leaf of Vrddhi was prepared (6900 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 VLAgNPs**: Synthesis of VLAgNPs in water medium was carried out in an identical method of VLAuNPs preparation keeping the concentrations of the leaf 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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