Study of Antioxidant Property of the Tuber Extract of Habenaria Edgeworthii (*Vrddhi*) and its use in the green synthesis of Gold nanoparticles

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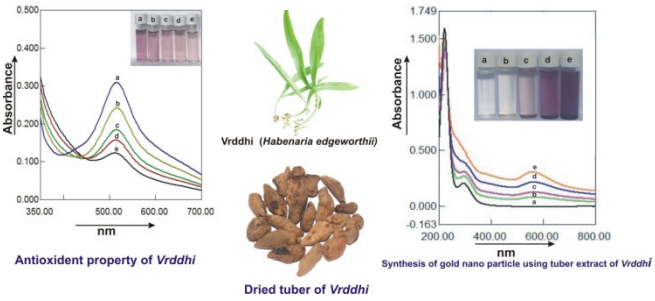
Abstract



The antioxidant activity of the tuber extract of Habenaria edgeworthii (commonly known as Vrddhi) has been studied against a long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the tuber extract have been utilized for the synthesis of stable gold nanoparticles at room temperature under very mild conditions. The synthesized gold nanoparticles were characterized by Surface Plasmon Resonance spectroscopy, High resolution transmission electron microscopy studies and a mechanism for the synthesis of stabilized gold nanoparticles has been proposed.

Keywords: Antioxidant, DPPH, *Habenaria edgeworthii* (*Vrddhi*), tuber, polyphenols, gold nanoparticle.

1. Introduction

Thousands of years ago, an Ayurvedic health tonic, formulated by Ayurvedic wonder healers Ashwini Kumars could rejuvenate the ill, feeble and emaciated body of Rishi Chyavan and he got back his youth. Since then this Ayurvedic formulation has been known as *Chyavanprash* and it became an important and demanding health tonic for the Kings and the rich people. It contained a set of eight medicinal plants namely *Kakoli, Kshrikakoli, Jeevak, Rishvok, Meda, Mahameda, Riddhi* and *Vrddhi* collectively known as Astavarga plants that grow in small patches in particular ecological environments of Himalaya at the elevation of 1200 – 4000 m from the sea level. However, in early days, the knowledge of Ayurveda (a Sanskrit word, '*Ayus*' means life and '*Veda*' means science or knowledge) used to be taught orally through the lineages of sages (Rishis) that involved the use the inherent principles of nature to maintain and prolong the life of a person via restoration of a balance among body, mind and spirit.[1] But, due to the lack of proper documentation and also because of the fact that the plants grow in the remote regions of Himalaya, the identification of the plants became illusory and difficult. However, recent investigations by a group of scientists and sages have led to the identification, botanical description and classification of all the eight Astavarga plants. Thorough literature search by us have revealed that there is no report of the active chemical constituents of most of the

Astavarga plants. Herein we report the results of our investigations on studies of the antioxidant property of the tuber (thick root) extract of *Habenaria Edgeworthii (vrddhi).* The tuber extract has also been utilized for the green synthesis of gold nanoparticles (AuNPs) under very mild conditions without any additional stabilizing or capping agents. The stabilized gold nanoparticles have been characterized by Surface Plasmon Resonance (SPR) spectroscopy, High Resolution Transmission Electron Microscopy (HRTEM) studies.

2. Material and Methods

Plant Material: The plant *Vrddhi* was collected from Dhanolti region of Himalaya, identified by a group of scientists and Baidyas from Patanjali Yogpeeth, Haridwar and deposited in their herbarium. Pseudobulb of this plant was dried in air and used for the study of anti-oxidant property and synthesis of AuNPs at room temperature.

Chemical: DPPH was purchased from Sigma-Aldrich. $HAuCl_4$ was purchased from SRL. Ferric chloride ($FeCl_3$) was procured from Himedia. All chemicals were analytical grade and used without further purification. Double distilled water was used for the experiment.

Au (III) solution: $HAuCl_4$ was purchased from SRL (Sisco Research Laboratory) and used without further purification. $HAuCl_4$ (35.4 mg) was dissolved in distilled water (10 mL) to obtain a 10.42 mM Au(III) stock solution.

Preparation of tuber extract of Habenaria Edgeworthii (*Vrddhi*)*:* Air dried root of *Habenaria edgeworthii* (*Vrddhi*) was finely powdered using a grinder. Finely powdered tuber of vrddhi (6.5 g) was suspended in ethanol (50 mL) and refluxed with magnetic stirring for 7 h, cooled at room temperature and then filtered (sintered glass funnel). Volatiles of the filtrate were removed under reduced pressure to afford a sticky solid (64 mg). The tuber extract (64 mg) was suspended in methanol (10 mL) and sonicated in an ultra sonicator bath for 10 min to get a semi transparent solution (6.4 mg/mL).

DPPH assay: A semi transparent solution of the tuber extract of *Vrddhi* (6.4 mg/mL) was diluted with ethanol to prepare a series of the extract; methanolic solution of DPPH (0.08 mL, 5.58 mM) was added to each solution of the extract and the volume was made upto 4 mL having final concentrations of the tuber extract as30, 60, 90, 120 □g/mL. All the solutions were mixed thoroughly and then allowed to stand in the dark for 1 hour at room temperature. The UV-visible spectrum of the colored solution was measured and the absorbance at 517 nm was noted. Reduction in absorption intensity of DPPH in the solutions containing the tuber extract was observed when compared with a control solution of DPPH in methanol at the same concentration. % scavenging was calculated using the following formula. % DPPH radical scavenging activity = (Control OD – Sample OD /Control OD) X 100.

Synthesis of nanoparticles: For the synthesis of AuNPs, ethanolic extract of the tuber was prepared. A stock solution of the extract was prepared in water (1009.52 mgL^{-1}) and diluted to prepare a series of the solutions, aliquots of Au (III) (0.2 mL, 10.42 mM each) were added drop-wise to the extract to prepare the stabilized AuNPs colloids where the concentration of the extract varied from 100, 200, 400, 600 mgL^{-1} and the concentration of Au(III) was fixed at 0.42 mM. UV-visible spectrophotometry of the gold colloids was carried out after 15 h of $HAuCl_4$ and tuber extract were mixed together.

Characterization: HRTEM images of AuNPs were recorded in JEOL JEM-2100 instrument. Mass spectra were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV-Visible spectrophotometry was carried out in Shimadzu 1601 spectrophotometer.

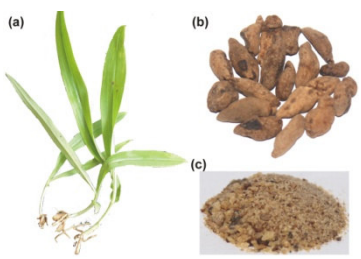


Figure 1: Photographs of *Vrddhi* (a) the whole plant, (b) dried tuber and (c) the crushed tuber.

3. Results and Discussion

Vrddhi, a terrestrial tuberous orchid, growing upto 75 cm in height, is found across the Himalayas at an altitude 2500 – 3000 m above sea level (Figure 1). Tuberous roots are small, ellipsoid, usually 1-2.5 cm long, 0.5 - 1 cm in diameter, white inside and fleshy (Figure 1b). It is used for the treatment of asthma, fever, skin diseases, leprosy, blood disorders, burning sensation, general debility, etc. Mass spectral studies of the methanol extract of the tubers carried out in our laboratory showed the presence of several polyphenolic compounds including flavanoids along with steroids and other plant secondary metabolites (supporting information Figure S1) (Figure 1c). Evidence for the presence of phenolic compounds was also obtained from a positive ferric chloride test. As the phenolic compounds have antioxidant properties, we tested the antioxidant activity of the ethanol extract of the dried tubers against a long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature.[2]

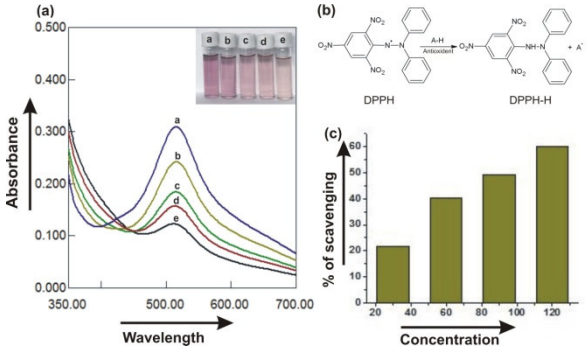


Figure 2: Antioxidant activity studies of the tuber extract of *Vrddhi*: (i) UV-visible spectra of (a) DPPH, (b -e) DPPH + with ethanol extract of tuber 30, 60, 90, 120 µgmL^{-1}., Inset: photographs of the vials containing the respective solutions; (ii) reaction scheme showing quenching of DPPH radical by the antioxidant (A-H); (iii) plot of % DPPH radical scavenging by the ethanol extract of tuber.

3.1 Determination of Antioxidant activity by DPPH Assay

The 2,2-diphenylpicrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The free radical scavenging activity of the methanol and ethanol extracts of tubers of *Vrddhi* was tested against DPPH according to the procedure described in the literature.[3] Antioxidants with active hydrogen react with DPPH radical and convert it to 1-1-diphenyl-2-picryl hydrazine. The radical scavenging potential of the extract is indicated by the degree of discoloration. Evaluation of the reducing ability of antioxidants present in the extract towards DPPH radical can be carried out by monitoring the decrease in the absorbance intensity at 517 nm in the UV-visible spectroscopy (Figure 2). The decrease in the absorption intensity of DPPH takes place because of the reaction between antioxidant (A-H) present in the root extract and DPPH radical (Figure 2b). % radical scavenging activity was calculated to be 60.19 when concentration of the tuber extract is 120 □g/mL.

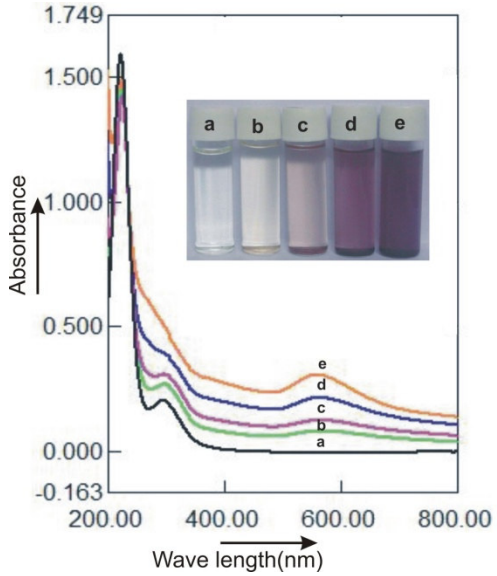


Figure 3: UV-Vis spectra of (a) $HAuCl_4$ (0.42 mM), (b-e) AuNP's at 100, 200, 400, 600 mgL^{-1} concentrations of ethanol extract of *Vrddhi* respectively. Inset: Photograph of the vials containing the aforementioned mixtures (after 15 h of mixing).

3.2 Synthesis of gold nanoparticles using the tuber extract of *Vrddhi*

Nanoscience and nanotechnology is an increasingly growing field of research. Whereas

atoms are of sub-nano dimensions, scientists have prepared many inorganic nanoparticles having several elemental metals, metal oxides, metal sulfides, metal halides, etc. mostly during the last two decades with excellent control over size, shape and composition.[4] Among various metal nanoparticles, studies on gold nanoparticles has become an area of tremendous research interest during the last two decades because the gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in nanobiodiagnostics, pharmaceuticals, catalysis, etc.[5,6,7,8] Colloidal AuNPs dispersed in water and stabilized with medicinally active phytochemicals are preferable for many of such applications.[9] Green syntheses of AuNPs from the extracts of the various parts of *Ananas comosus (L.),*[10] *Azadirachta indica*,[11] *Acacia nilotica*,[12] Mimusops elengi,[13] *Ocimum sanctum*,[14] *Punica granatum*,[15] *Saraca indica*,[16] *Terminalia arjuna*,[17] have recently been reported. Easily oxidizable phytochemicals including polyphenols present in the plant extracts were capable of forming AuNPs from Au(III) and then stabilize them in aqueous medium. As the tuber extract of *Vrddhi* is rich in polyphenolic compounds along with other easily oxidizable compounds, it occurred to us that it can also be utilized for the efficient synthesis of AuNPs. The UV-visible spectrum of stabilized AuNPs at various concentration of ethanol extract of tuber of *Vrddhi* is given in Figure 3. The UV-visible spectrum of $HAuCl_4$ solution showed two peaks at 220 and 290 nm due to charge transfer interaction between the metal and chloro ligands. But interestingly, on addition of $HAuCl_4$ solution to the tuber extracts, the intensities of these two peaks reduced and concomitantly a new peak appeared in the region of 550 nm due to surface Plasmon resonance (SPR) phenomenon of AuNPs. With increasing the concentration of the tuber extract of *Vrddhi,* a blue shift of the SPR band was observed due to the formation of small sized AuNPs.

3.3 HRTEM studies

The size distribution, shape and morphology of the AuNPs formed at different concentration of the ethanol extract of tuber of *Vrddhi* were studied by high resolution transmission electron microscopy (HRTEM) (Figure 4). Trigonal, tetragonal, pentagonal, hexagonal along with spherical shaped AuNPs were observed. The average size of the AuNPs formed at 1200 mgL^{-1} concentration of the plant extract was 15.2 nm (calculated from 75 particles, Figure 4d). The AuNPs were held inside the organic matrix derived from the tuber extract of *Vrddhi*.

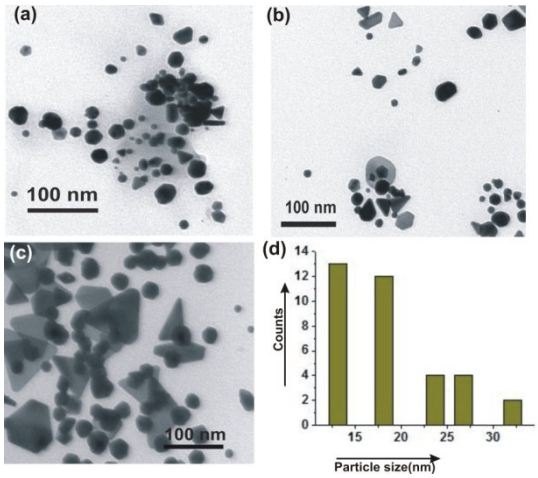


Figure 4: (a-c) HRTEM images of AuNPs; (d) histograms of AuNPs**.**

3.4 Mechanism of the formation of Stabilized AuNPs

The ethanol extract of tuber of *Vrddhi* is rich in polyphenolic compounds including flavanoids along with steroids and other plant secondary metabolites.. Evidence for the presence of polyphenolic compounds was obtained from the ferric chloride test (supporting information). Mass spectral analysis of the ethanol extract of tuber of Vrddhi carried out by us supported the presence of the several polyphenolic compounds (supporting information Figure S1) such as pinocembrine (M^+ 256), cinnamic acid (M^+ = 149) □-sitosterols (M^+ 414) or their analogues. A possible mechanism for the formation of AuNPs and their stabilization by the phytochemicals present in the extract is shown schematically in Figure 5. Polyphenolic compounds along with other easily oxidizable phytochemicals present in the tuber extract can reduce Au (III) to Au (0) with

concomitant oxidation of the phytochemicals to a higher oxidation state. Neighboring Au(0) atoms can collide with each other leading to the formation of the AuNPs. The AuNPs can be stabilized by the polyphenolic compounds, quinones as well as the other coordinating phytochemicals present in the tuber extract.

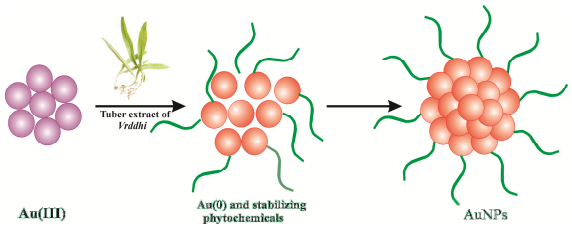


Figure 5: Mechanism of formation and stabilization of AuNPs by phytochemicals present in the ethanol extract of tuber of *Vrddhi*

4. Conclusion

Evidence for the presence of antioxidants including polyphenols has been obtained in the tuber extract of Habenaria edgeworthii *(Vrddhi)*. The antioxidant activity of the ethanol extract of tuber of *Vrddhi* has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the tuber extract have been utilized for the synthesis of gold nanoparticles at room temperature under very mild conditions without any additional stabilizing or capping agents. Mechanism for the synthesis of the gold nanoparticles from Au(III) has also been proposed. According to our knowledge, this is the first report of the study of antioxidant property of the tuber extract of *Vrddhi* and its utilization in the green synthesis of gold nanoparticles. As the tuber extract of *Vrddhi* has tremendous medicinal significance, the studies described will be useful in biomedical applications as well as nanoscience and nanobiotechnology.

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