Prayogik Rasayan

Study of Antioxidant Property of the Rhizome Extract of *Polygonatum cirrhifolium* (*Mahameda*) and its use in the green synthesis of Gold nanoparticles

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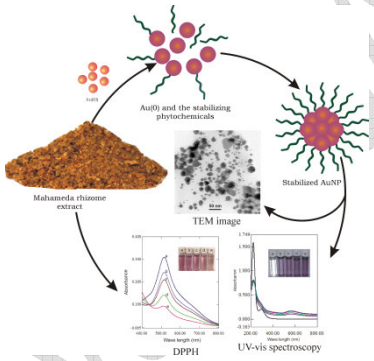
Abstract

The antioxidant efficacy of the rhizome extract of *Polygonatum cirrhifolium* (*Mahameda*) has been studied against a stable 2, 2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The chemical constituents present in the rhizome extract have been utilized for the one step synthesis of stable gold nanoparticles at room temperature under very mild conditions. The synthesized gold nanoparticles were well characterized by surface plasmon resonance spectroscopy, high resolution transmission electron microscopy, x-ray diffraction and FTIR studies and a mechanism for the synthesis and stabilization of gold nanoparticles has been described.



Keywords: Antioxidant, DPPH, *P.cirrhifolium* (Mahameda), rhizome, phytochemicals, AuNPs

1. Introduction

Ayurveda, the science of long life and healing is eternal and it has been practiced in India since the prehistoric period. The Samhitas of Sushruta (600 BCE) and Charaka (600 BCE) are the conveyor of this eternal tradition.[1] In early days, this science was orally discussed and verbally transmitted from one generation to another. However, due to lack of proper documentation, true identification of the medicinal plants became illusory and difficult. This was also the case with Astavarga plants. Astavarga, an important ingredient of the Ayurvedic health tonic Chyavanprash, was formulated by the reputed Ayurvedic wonder healers Ashwini Kumars for the rejuvenation of the old, frail and emaciated body of Rishi Chyavan. It is a set of eight medicinal plants namely *Kakoli, Kshrikakoli, Jeevak, Rishvok, Meda, Mahameda*, *Riddhi* and *Vriddhi* that grow in small patches in particular ecological environments in Himalaya at the elevation of 1200 – 4000 m from the sea level. Miraculously, the ill health of Rishi Chyavan was rejuvenated and he got back his youth. Since then the Ayurvedic medicine became an important and demanding health tonic for the Kings and the rich people. As the plants grow naturally in specific ecological environments in the remote areas of Himalaya, in spite of their
immense cell regeneration and immune strengthening properties, their botanical description and classification remained a mystery. However, recent investigations by a group of scientists and sages have led to the identification, botanical description and classification of the eight Astavarga plants.[2] Antioxidant importance Here in, we report the results of our investigations on studies of the antioxidant property of the rhizome extract of *Polygonatum cirrhifolium (Mahameda).* The rhizome 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) and x-ray diffraction (XRD) studies.

2. Materials and Methods

2.1 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.

2.2 Plant Material

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

2.3 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.

2.4 Preparation of methanol extract of rhizome of Mahameda

Air dried rhizome part of *Mahameda* was finely powdered using a grinder. Finely powdered rhizome of *Mahameda* (5.41 g) was suspended in methanol (75 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 gray colored foamy solid (1.90 g). The rhizome extract (0.0103 g) was suspended in methanol (10 mL) and sonicated in an ultra sonicator bath for 10 minutes to get a semi transparent solution (1030 □g/mL).

2.5 Preparation of ethanol extract of rhizome of Mahameda

Air dried rhizome part of Mahameda was finely powdered using a grinder. Powdered rhizome of Mahameda (6.5 g) was suspended in ethanol (50 mL) and refluxed with magnetic stirring for 5 h, cooled at room temperature and then filtered (sintered glass funnel). Volatiles of the filtrate were removed under reduced pressure to afford a foamy solid (0.73 g). The rhizome extract (23 mg) was suspended in ethanol (10 mL) and sonicated in an ultra sonicator bath for 10 minutes to get a semi transparent solution (2300 μg/mL).

2.6 DPPH assay

A semi transparent solution of the rhizome extract of Mahameda (1030 □g/mL) was diluted with methanol to prepare a series of the extract; methanolic solution of DPPH (0.04 mL, 5.58 mM) was added to each solution of the extract and the volume was made upto 4 mL having final concentrations of rhizome extract as 20, 40, 60, 80, 100, 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 rhizome 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.

2.7 Synthesis of gold nanoparticle

For the synthesis of AuNPs, ethanolic extract of the rhizome was prepared (as the methanol extract described previously). A stock solution of the extract was prepared in water (1600 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 600, 800, 1000, 1200 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 rhizome extract were mixed together.

2.8 Characterization

HRTEM images of AuNPs were recorded in JEOL JEM-2100 instrument. X-ray diffraction (XRD) patterns of the stabilized AuNPs were recorded Rigaku Miniflex II diffractometer with Cu-Kα radiation (λ= 1.54 Å). Mass spectra were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV-visible spectrophotometry of the samples was carried out in Shimadzu 1601 spectrophotometer. FTIR spectra of samples were recorded using a Perkin Elmer FTIR Spectrum Two model using KBr pellet.

3. Results and Discussion

Mahameda is a tall, perennial herb of 30-120 cm in height found in Himalayas at an elevation of 1500-3300 m. *Mahameda* rhizome is look like ginger, 1-2 cm in diameter and white or dull white in color. It is particularly useful in cough, leprosy, skin diseases, fever, sexual debility and other seminal disorders, etc. Chemical constituents extracted from *Mahameda* rhizome showed fungicidal activities. Mass spectral studies in our laboratory showed the presence of several polyphenolic compounds including flavanoids along with steroids and other plant secondary metabolites (supporting information Figure S1) in the methanol extract of the air dried rhizome. Evidence for the presence of phenolic compounds was also obtained from a positive ferric chloride test. As the phenolic compounds are well known as antioxidant, we resorted to test the antioxidant activity of the rhizome extract against a long lived 2, 2-diphenylpicrylhydrazyl (DPPH) radical at room temperature.[3]

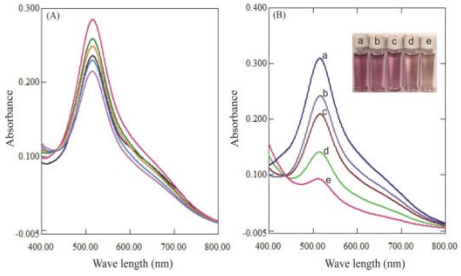


Figure 1: *Antioxidant activity studies of the rhizome extract of Mahameda: (A) UV-visible spectra of methanol extract in presence of DPPH (B) UV-visible spectra of ethanol extract in the presence of DPPH. Inset of B: photographs of the vials containing DPPH and ethanol extract of rhizome.*

3.1 Determination of Antioxidant activity by DPPH Assay

The 2, 2-diphenylpicrylhydrazyl (DPPH) assay is a widely used tool in plant biochemistry to investigate the free radicals scavenging activity of plant constituents. The free radical scavenging activity of the methanolic and ethanolic extract of rhizome of *Mahameda* was tested against DPPH

following a procedure described in the literature.[4] Antioxidants react with DPPH and convert it to 1-1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the plant extract. The reducing ability of antioxidants towards DPPH radical can be evaluated by monitoring the decrease in the absorbance intensity at 517 nm in the UV-visible spectroscopy (Figure 1).

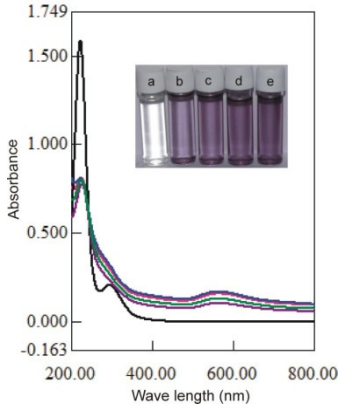


Figure 2: UV-visible spectra of stabilized AuNPs at various concentration of Mahameda rhizome extract. Inset: Photograph of the vials containing the aforementioned mixtures (after 15 h of mixing).

The decrease in the absorption intensity of DPPH takes place because of the reaction between antioxidant present in the rhizome extract of *Mahameda* and DPPH radical progresses, results in the hydrogen transfer from antioxidant molecule (A-H) to DPPH radical. % radical scavenging activity was calculated to be 29.62 when concentration of the rhizome extract is 120 □g/mL.

3.2 Synthesis of gold nanoparticles using the rhizome extract of Mahameda

Gold, the commonly used ornamental and coinage metal at the bulk scale has become an area of tremendous research interest at the nano scale 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.[5,6,7,8,9] The AuNPs dispersed in water and stabilized with non-toxic biomolecules are preferable for many of such applications to avoid any undesired environmental effects.[10] The green syntheses of AuNPs from the extracts of *Azadirachta indica* bark,[11] *Acacia nilotica*,[12] *Punica granatum*,[13] *Saraca indica* bark,[14] *Ananas comosus (L.)*,[15] *Terminalia arjuna bark*,[16] *Ocimum sanctum stem*,[17] have recently been reported. The polyphenolic compounds along with other easily oxidizable phytochemicals present in the plant extracts were capable of forming AuNPs from Au (III) and then stabilize them in aqueous medium. As the rhizome extract of *Mahameda* 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. To test this, we treated the rhizome extract of *Mahameda* with gold colloid Ethanolic extract of *Mahameda* rhizome was utilized for the synthesis of AuNPs at room temperature. The UV-visible spectrum of stabilized AuNPs at various concentration of rhizome extract is given in Figure 3. The charge transfer interaction between the metal and chloro ligands leads to the formation of two peaks at 220 and 290 nm in the UV-visible spectrum of $HAuCl_4$ solution. But interestingly, on addition of stabilized AuNPs 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 plant extract a blue shift of the SPR band was observed due to the formation of small sized AuNPs.

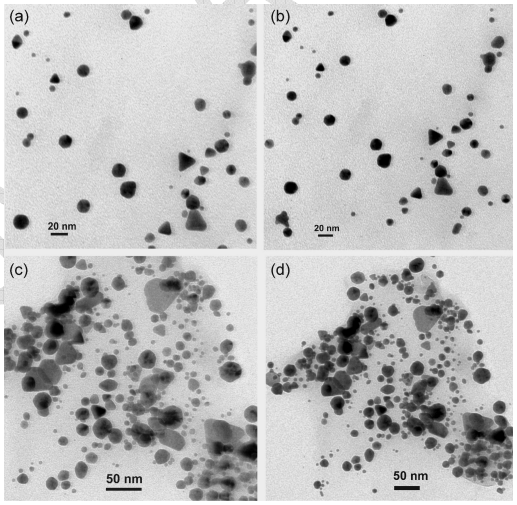


Figure 3: *HRTEM images of rhizome extract stabilized AuNPs.*

3.3 HRTEM, FTIR and XRD studies

The size distribution, shape and morphology of the AuNPs formed at different concentration of the rhizome extract of *Mahameda* were studied by high resolution transmission electron microscopy (HRTEM) (Figure 4). Mostly spherical shaped AuNPs were observed along with some tri angular, and pentagonal shaped AuNPs. The average size of the AuNPs formed at 1200 mgL^{-1} concentration of the plant extract was 10.7 nm (calculated from 75 particles, Figure 4f). The AuNPs were held inside the organic matrix derived from the rhizome extract of *Mahameda*.

The colloidal AuNPs samples were coated over a glass plate, the volatiles were removed and x-ray diffraction analysis of the AuNPs was carried out. The reflections of the planes (111), (200), (220) and (311) at 2θ = 38.24°, 44.54°, 64.82° and 77.66° respectively resembled the characteristic

reflections of crystalline metallic face centered cubic Au (JCPDS file no. 04-0784). The comparatively greater peak intensity of the (111) plane indicated the predominant orientation of the (111) plane.

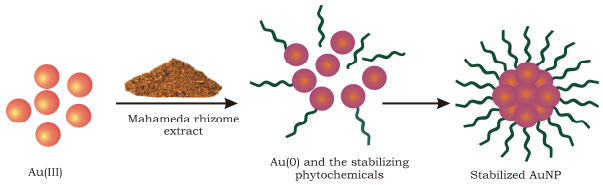


Figure 4: *Mechanism of formation and stabilization of AuNPs by phytochemicals present in the rhizome extract of Mahameda.*

The broad peak around 3400 cm^{-1} observed in the FTIR spectrum of the rhizome extract was due to the stretching vibration of the phenolic hydroxyl group (-OH) present in it. The broadness of the peak may be due to intermolecular H-bonding. However, in the FTIR spectrum of the rhizome extract stabilized AuNPs, the peak in this region became narrower probably due to the interaction of the -OH groups with AuNPs

3.4 Mechanism of the formation of Stabilized AuNPs

The rhizome extract of *Mahameda* is rich in various plant phytochemicals such as polyphenols including flavanoids, steroids, etc. DPPH study and positive ferric chloride test also support the presence of polyphenolic compounds in the rhizome part of this plant (supporting information). Mass spectral analysis of the rhizome extract carried out by us supported the presence of the several polyphenolic compounds (supporting information Figure S1) such as gallic acid (m/z = 171), quercetin (m/z = 302), □-sitosterols (m/z = 414) or their analogues. A schematic representation of the possible mechanism for the formation of AuNPs and their stabilization by the phytochemicals present in the extract is shown in Figure 5. Polyphenolic compounds along with other easily oxidizable phytochemicals present in the rhizome extract can reduce Au (III) to Au (0) with concomitant oxidation of the phytochemicals to a higher oxidation state. Collision of the neighboring Au (0) atoms with each other leads 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 rhizome extract of *Mahameda.*

4. Conclusion

Evidence for the presence of antioxidants including polyphenols has been obtained in the alcoholic extract of *P. cirrhifolium (Mahameda)* rhizome. The antioxidant efficacy of the rhizome extract has been examined against the long lived 2, 2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The studies revealed that ethanolic extract of *Mahameda* rhizome are highly efficient as antioxidant compare to methanolic extract. The phytochemicals present in the ethanolic extract of *Mahameda* rhizome have been utilized for the one step synthesis of gold nanoparticles at room temperature under very mild conditions without any additional stabilizing agents. A plausible mechanism for the synthesis of the gold nanoparticles has also been demonstrated. According to our knowledge, this is the first report of the study of antioxidant property of the rhizome extract of *Mahameda* and its utilization in the green synthesis of gold nanoparticles. As the rhizome extract of *Mahameda* has tremendous medicinal significance, the studies described here will be useful in biomedical applications as well as nanoscience and nanobiotechnology.

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