Prayogik Rasayan

Study of Antioxidant Property of the Endosperm Extract of *Borassus flabellifer* (Taal) and its use in the green synthesis of Gold nanoparticles

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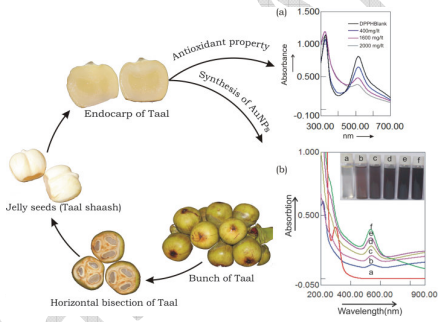
Abstract

The antioxidant activity of the endosperm extract of *Borassus flabellifer* (commonly known as Taal) has been studied against a long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the endosperm 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, X-Ray diffraction and FTIR studies and a mechanism for the synthesis of stabilized gold nanoparticles has been proposed.



Keywords: Antioxidant, DPPH, *Borassus flabellifer* (*Taal*), polyphenols, gold nanoparticles

1. Introduction

The past decade has witnessed a tremendous academic and industrial research activities in the areas of nanoscience and nanotechnology.[1,2,3,4,5] Among various metal nanoparticles, gold nanoparticles (AuNPs) are of special interest due to their fascinating optical properties and diversified applications in catalysis, chemical sensing, imaging, drug delivery, etc. The colloidal AuNPs in aqueous medium, stabilized with non-toxic biomolecules, are required for many of its applications. Plant extract mediated solution phase synthesis of AuNPs involving reduction of Au(III) to Au(0) by the reducing phytochemicals present in the plant extracts has gained profound significance in recent years over other synthetic methods, because of the renewable and biocompatible nature of the plant extracts, eco-friendly aqueous medium and mild reaction conditions. Additionally, as the plant extract itself acts as a stabilizer, this method is more advantageous over other synthetic methods. The extracts of *Mimusops elengi* bark,[6] *Acacia nilotica* leaf,[7] *Abroma augusta* Linn bark,[8] *Lantana camara* leaf,[9] *Breynia rhamnoides*,[10] *Saraca indica bark*,[11] *Piper betle*,[12] Green coconut shell,[13] *Ocimum sanctum* stem,[14] *Azadirachta indica* bark,[15] etc., have been utilized for the synthesis of AuNPs. While studying the antioxidant activities of locally grown food items surrounding Midnapore town of West Bengal, India, it occurred to us that the reducing phytochemicals present in the endosperm *Borassus flabellifer* can be utilized for the green synthesis of AuNPs.

Borassus flabellifer (commonly called Taal) is a well known tree throughout India. The leaves of Taal are used for making fans, hats, umbrellas, mats, etc. by the common people. In Indonesia, the leaves of Taal are used in the ancient culture of paper making, known as "lontar". Various sweet dishes are prepared from the yellow viscous fluid of ripened fruits. The sugary sap obtained from young inflorescences are concentrated to yield crude sugar called jiggery which is also used for sweetening different types of foods. Anti-inflammatory activities of the ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) have been demonstrated.[16] The tender endosperm of *Borassus flabellifer* (called Taal sash) appears as a soft gel-like material and is consumed during summer as a seasonal food. Herein we report the antioxidant activities of the ethanol extract of endosperm of *Borassus flabellifer* by % radical scavenging activities of DPPH and its utilization in the green synthesis of AuNPs. The synthesized colloidal AuNPs have been characterized by surface Plasmon resonance spectroscopy, high-resolution transmission electron microscopy (HRTEM) and X-ray diffraction analysis. The stabilized AuNPs have been utilized as a catalyst for the reduction of 4-nitrophenol to 4-aminophenol in aqueous medium at room temperature.

2. Material and Methods

Plant Material: Tender Taal was collected from Midnapore region of West Bengal in the month of May, 2015. Fruit of this plant (endosperm) was used for the study of anti-oxidant property and synthesis of AuNPs at room temperature.

Preparation of endosperm extract of Borassus flabellifer (Taal)*:* Endosperm of Taal (5.84 g) was suspended in ethanol (6 mL) in a test tube, sonicated in an ultrasonicator bath for 45 min and then centrifuged for 10 mins to obtain a clear supernatant. To know the concentration of the endosperm extract, an aliquot of the clear supernatant (3 mL) was taken in a round bottom flask and the volatiles were removed under reduced pressure to afford a sticky solid (0.095 g). Thus the concentration of the endosperm extract was 31.66 g L^{-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.

Au (III) solution*:* $HAuCl_4$ was purchased from SRL (Sisco Research Laboratory) and used without further purification. $HAuCl_4$ (44 mg) was dissolved in distilled water (10 mL) to obtain a 11.17 mM Au(III) stock solution.

DPPH assay: A semi transparent solution of the endosperm extract of Taal (31.66 g L^{-1}) was diluted with ethanol to prepare a series of the extracts; methanolic solution of DPPH (0.04 mL, 5.58 mM) was added to each solution of the extract and the volume was made up to 4 mL having final concentrations of the endosperm of Taal extract as 400, 800, 1200, 1600, 2000 mg L^{-1}. All the solutions were mixed thoroughly and then allowed to stand in the dark for 45 min 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 endosperm extract of Taal was observed when compared with a control solution of DPPH in ethanol 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 endosperm of Taal was prepared. A stock solution of the extract was prepared in ethanol (31.66 gL^{-1}) and diluted with double distilled water to prepare a series of the solutions, aliquots of Au (III) (0.2 mL, 11.17 mM each) were added drop-wise to the extract to prepare the stabilized AuNPs colloids where the concentration of the extract varied from 1000, 2000, 4000, 6000, and 8000 mg L^{-1} and the concentration of Au (III) was fixed at 0.56 mM. UV-visible spectrophotometry of the gold colloids was carried out after 15 h of $HAuCl_4$ and endosperm extract of Taal were mixed together.

Characterization: HRTEM images of AuNPs were recorded in JEOL JEM-2100 instrument. X-ray diffraction (XRD) patterns of the stabilized AuNPs were recorded Bruker-D_8 Advanced with Cu-Kα radiation (λ= 1.54 Å). Mass spectra were recorded in Shimadzu GCMS QP 2100 Plus instrument. UV-Visible spectrophotometry was carried out in Shimadzu 1601 spectrophotometer. FTIR spectra of samples were analyzed using a Perkin Elmer FTIR Spectrum Two model using KBr pellet. DLS Study was carried out by using Malvern Zetasizer Nano series (Model-Nano ZS90) to know the stability and size distribution of AuNPs.

3. Results and Discussion

Borassus flabellifer (known as Taal in Bengali and Tari in Hindi) is a robust tree and available throughout India. It belongs to the family of Arecacese. The endosperm of the tender fruits are soft jelly-like, juicy, translucent (Figure 1) and sweetish in taste. 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 ethanol extract of the Taal. Evidence for the presence of phenolic compounds was also obtained from a positive ferric chloride test. As the phenolic compounds have antioxidant properties, we resorted to test the antioxidant activity of the extract against a long lived 2, 2-diphenylpicrylhydrazyl (DPPH) radical at room temperature.[17]

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. Antioxidants react with DPPH and convert it to 1-1-diphenyl-2-picryl hydrazine. The degree of decolorization indicates the scavenging potential of the endosperm 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). The decrease in the absorption intensity of DPPH takes place because of the reaction between antioxidant present in the endosperm

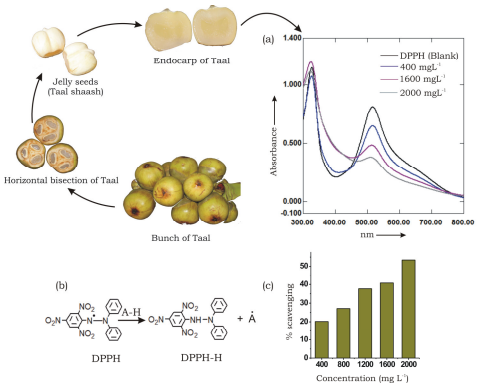


Figure 1: *Antioxidant activity studies of the endosperm extract of Taal: (a) UV-visible spectra of DPPH and DPPH + ethanol extract, (b) Reaction scheme showing quenching of DPPH radical by the antioxidant (A-H); (c) plot of % DPPH radical scavenging by the endosperm extract of Taal.*

extract of Taal and DPPH radical progresses, results in the hydrogen donation from antioxidant molecule (A-H) to DPPH radical. % radical scavenging activity was calculated to be 53.44 when concentration of the endosperm extract of Taal is 2000 mg L^{-1}.

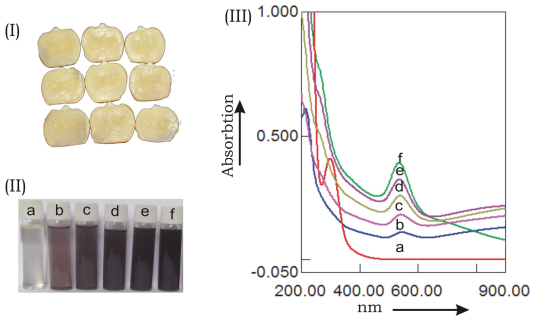


Figure 2: *(I) Endosperm of Taal used for the synthesis of AuNPs; (II) Vials containing Au(III)/AuNPs at various concentration of the extract: (a) $HAuCl_4$ (0.58 mM), (b-f) AuNPs at 1000, 2000, 4000, 6000, and 8000 mgL^{-1} concentrations of the endosperm extract of Taal ; (III) UV-Vis spectra of the aforementioned mixtures (after 15 h of mixing).*

3.2 Synthesis of gold nanoparticles using the endosperm extract of Taal:

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.18,19,20,21,22 The AuNPs dispersed in water and stabilized with non-toxic biomolecules are preferable for many of such applications to avoid any undesired environmental effects.23 The green syntheses of AuNPs from the extracts of *Mimusops elengi* bark,6*Acacia nilotica* leaf,24 *Abroma augusta* Linn bark,25 *Lantana camara* leaf,26 *Breynia rhamnoides*, *Saraca indica bark*, *Piper betle*, Green coconut shell,27 *Ocimum sanctum* stem, *Azadirachta indica* bark,28have 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 endosperm extract of Taal 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 ethanol extract of endosperm of Taal was employed for the synthesis of AuNPs at room temperature. The UV-visible spectrum of stabilized AuNPs at various concentrations of the endosperm of Taal extract is given in (Figure 2). 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 HAuCl4 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 546 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.

3.3 FTIR, HRTEM, XRD and DLS studies

The broad peak around 3400 cm-1 observed in the FTIR spectrum of the endosperm extract of Taal 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 endosperm extract of Taal stabilized AuNPs, the peak in this region became narrower probably due to the interaction of the OH groups with AuNPs (supporting information Figure S2).

The size distribution, shape and morphology of the AuNPs formed at different concentration of the endosperm extract of Taal were studied by high resolution transmission electron microscopy (HRTEM) (Figure 3). Mostly spherical shaped AuNPs were observed along with some triangular and pentagonal shaped AuNPs. The average size of the AuNPs formed at 8000 mg L^{-1} concentration of the plant extract was 38.15 nm (calculated from 119 particles, Figure 3c). The AuNPs were held inside the organic matrix derived from the endosperm of Taal extract.

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) (311) and (222) at 2θ = 38.24°, 44.54°, 64.82° , 77.66° and 81.87° 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 3d)

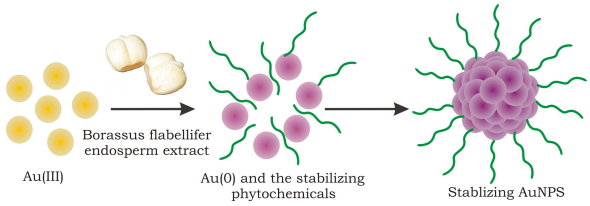


Figure 3: *(a) & (b) Tem image (c) Histogram (average diameter 38.15 nm) (d) XRD (e) EDX (f) SAED of stabilized AuNPs.*

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle which indicates the information about the surface charge of the particles. In our analysis Zeta

potential of AuNPs (synthesized with 2000 mg L^{-1} of the extract) was -12.2 mV and the average particles size of AuNPs formed was 56.52 nm determined by dynamic light scattering shown in (supporting information Figure S3).

3.4 Mechanism of the formation of Stabilized AuNPs

The endosperm extract of Taal is rich in different types of plant secondary metabolites such as polyphenols including flavanoids, steroids, etc. Evidence for the presence of polyphenolic compounds was obtained from the ferric chloride test (supporting information). Mass spectral analysis of the endosperm extract of Taal was carried out by us supported the presence of the several polyphenolic compounds (supporting information Figure S1) such as gallic acid (M^+ 171), quercetin (M^+ 302), □-sitosterols (M^+ 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 4). Polyphenolic compounds along with other easily oxidizable phytochemicals present in the endosperm extract of Taal 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 endosperm extract of Taal.

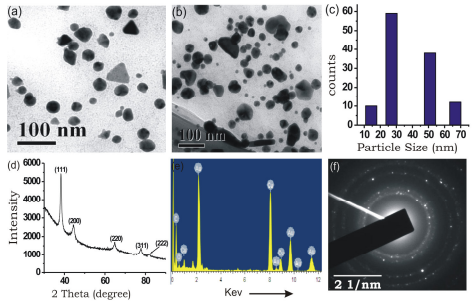


Figure 4*: Mechanism of formation and stabilization of AuNPs by phytochemicals present in the endosperm extract of Taal.*

3.5 Application of AuNPs in catalysis

AuNPs with very high surface to volume ratio has recently been utilized as a catalyst for various kinds of chemical transformations (Zhang et al. 2012, Wunder et al. 2011). To test whether the endosperm extract of Taal derived colloidal AuNPs can be utilized as a catalyst; we chose the sodium borohydride reduction of 4- nitrophenol to 4- aminophenol as a model reaction (Figure 5-II-a). On treatment of an aqueous solution of 4-nitrophenol (0.2 ml of 0.05 mM) with sodium borohydride (3.6 ml 0f 16.5 mM) at room temperature, the absorption band of 4-nitrophenol at 317 nm shifted to 401 nm due to the formation of 4-nitrophenolate anion (Figure 5-II-b). Though the reduction of 4-nitrophenol to 4-aminophenol by sodium borohydride is a thermodynamically favorable reaction (E_0 for 4-nitrophenol/4-aminophenol -0.76 and for H_3BO_3/BH_4^- -1.33 V), no reduction of the nitro group took place even on standing the mixture at room temperature for several days due to very high kinetic barrier of the reduction reaction. Interestingly, the reduction was complete in several minutes in the presence of the freshly prepared endosperm extract of Taal derived AuNPs. The progress of the reduction reaction was monitored spectrophotometrically. Using the UV-visible data at different time intervals, the catalytic rate constant (k) for the reduction reaction was calculated using different volume of stabilized AuNPs. When freshly prepared colloidal AuNPs (0.2 mL, synthesized with 500 mgL^{-1} endosperm extract) was used for the reduction of 4-nitrophenol (0.05 mM, 4mL) containing aqueous sodium borohydride (16.5 mM) then the peak at 401 nm arising due to 4-nitrophenolate anion (Figure 5-II-b) started disappearing with time with concomitant appearance of a new peak at 307 nm indicating the formation of 4-aminophenol. The reaction was complete in 7 minutes (Figure 5-II-c) and the apparent catalytic rate constant was calculated to be 0.505 min^{-1}.This apparent catalytic rate constant was comparable to the value observed by us and others.[6-9, 11, 13-15].

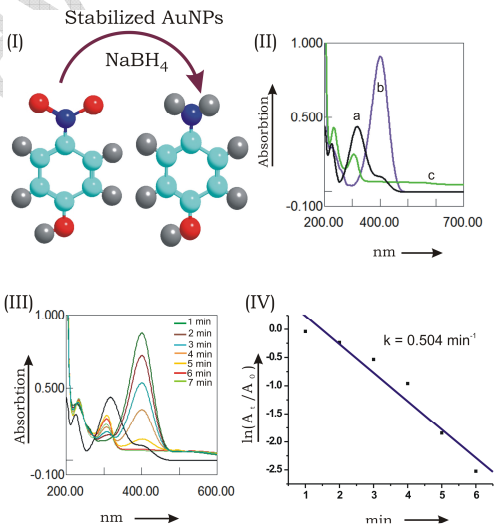


Figure 5: *(I) schematic representation of the catalytic reduction of 4-nitrophenolate ion to 4-aminophenolate ion; (II) UV–visible spectra of a) 4-nitrophenol (0.05 mM), b) 4-nitrophenol in the presence of added sodium borohydride (16.5 mM), c) after complete reduction using colloidal AuNPs as catalyst; (III) UV-visible absorption spectra of the reaction mixtures at various time intervals using freshly prepared colloidal AuNPs (0.2 mL containing 500 mg L^{-1} endosperm extract of Taal) (IV) Plot of ln A vs. time (min) for the determination of rate constant*

4. Conclusion

Evidence for the presence of antioxidants including polyphenols has been obtained in the endosperm extract of *Borassus flabellifer (commonly known as Taal).* The

antioxidant activity of the endosperm extract has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the endosperm extract have been utilized for the synthesis of gold nanoparticles at room temperature under very mild conditions without any additional stabilizing agents. A mechanism for the synthesis of the gold nanoparticles has also been proposed. According to our knowledge, this is the first report of the study of antioxidant property of the endosperm extract of *Taal* and its utilization in the green synthesis of gold nanoparticles. The catalytic activity has been studied by Using 0.5 ml (500 mg L^{-1}) freshly prepared gold Nano particles catalytic reduction of 4- nitro phenol to 4-amino phenol occurs within 7 minute. As the endosperm extract of *Taal* has tremendous medicinal significance, the studies described will be useful in biomedical applications as well as nanoscience and nanobiotechnology.

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