Green Synthesis of *Roscoea purpurea* Sm. (Kakoli) Leaf Extract Conjugated Gold and Silver nanoparticles

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

*Kosceoa purpurea* Sm. (Figure 1) commonly known as Kakoli is one of the eight members of the Astavarga plants used in the preparation of the Ayurvedic health tonic *Chyawanprash*. Kakoli is a rare medicinal plant usually found in certain parts of Himalaya at an altitude of 1500-3100 m from Uttarakhand and Sikkim and Assam in grassy hillsides, ‘damp gullies and stony slopes’. It is a short-lived plant that grows in the month of May-June having a life span of 6-7 months. The rhizome 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 Kakoli. Green synthesis of the Kakoli Leaf extract conjugated gold nanoparticles (KLAuNPs) and silver nanoparticles (KLAGNPs) are also reported.

The plant sample was collected from the Dhanouli area of Himalaya during July-September, and deposited at the herbarium of Patanjali Yogpeeth Haridwar. Each kakoli plant contains usually 5-6 leaves. The leaves are 10-15 cm long, 2-3 cm wide, lanceolate having broad sheaths at flowering time (Figure 2). Fresh leaf sample (5.7 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 experiments.

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. Previous reports from our laboratory have shown that the pseudobulb of *Crepidium acuminatum* (Jeevak), Rhizome Extract of *Roscoea purpurea* Sm. (Kakoli), Rhizome Extract of *Polygonatum cirrhifolium* (Mahameda) and, Tuber Extracts of *Habenaria Edgeworthii* (Vrddhi) and *Habenaria intermedia* (Rdhdhi) are rich in antioxidants. Hence, it occurred to us that the leaf extract of Kakoli 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 92%, 92%, 89%, 72%, 61% and 43% when the concentration of the leaf extract was 120, 100, 80, 60, 40 and 20 μg/mL respectively.

Gold nanoparticles (AuNPs) with its unique optoelectronic and magnetic properties have found applications in biodiagnostics, catalysis, pharmaceuticals, etc. Previous reports from our laboratory have shown that the extracts of *Terminalia arjuna bark*, *Azadirachta indica*, *Saraca indica*, *Acacia nilotica*, *Punica granatum*, *Ananas comosus* (L.), *Ocimum sanctum*, have been reported. Previously we have reported the green synthesis of gold nanoparticles using extracts Jeevak, Kakoli, Mahameda, Rddhi and and Vrddhi. Hence it occurred to us that the leaf extract of Kakoli may be utilized for the green synthesis of AuNPs conjugated with the leaf extract of Kakoli (KLAuNPs). 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). Appearance of light pink to brown color appeared at room temperature within 1 h indicating the formation of gold nanoparticles (KLAuNPs) (Figure 3).
A surface plasmon band observed in the 520 -600 nm range by UV-Visible spectrophotometry (Figure 3) supported the formation of KLAuNPs. 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 530 nm due to surface Plasmon resonance (SPR) phenomenon of KLAuNPs. 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. 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 530 nm.

Figure 2: (i) Roscoea purpurea Sm. plant (ii) Mechanism of DPPH activity, (iii) plot of % DPPH radical scavenging by the methanol extract of leaf at 20, 40, 60, 80, 100, 120 µg/mL concentration, (iv) plot of UV-Vis spectra of the leaf extract upon addition of DPPH at varied concentration (v) Expanded spectra shown in (iv), (vi) corresponding vials.
Silver nanoparticles (AgNPs) have tremendous application for its antibacterial activities along with the applications in biomedicine, environment, catalysis, health care and, food and agriculture. Success in the synthesis of KLAuNPs inspired us to study the synthesis of Kakoli leaf extract conjugated silver nanoparticles (KLAgNPs). An aqueous solution of AgNO$_3$ (0.7 mM) was reacted with an increasing concentration of the leaf extract of Kakoli 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 4). With 400 $\mu$g/mL concentration of the leaf extract, $\lambda_{\text{max}}$ for KLAgNPs was 447 nm.

Figure 4: UV-Visible spectra (recorded with 10 mm path length cuvette) of (a) leaf extract (100 $\mu$g/mL) (b) AgNO$_3$ solution (0.7 mM), (c-f) KLAgNPs at 50, 100, 200 and 400 $\mu$g/mL concentration of the leaf extract. Inset: photograph of vials containing the above samples.

In conclusion, the antioxidant activity of the leaf extract of Roscoea purpurea Sm. (Kakoli) has been studied against the long lived 2,2-diphenylpicrylhydrazyl (DPPH) radical at room temperature. The phytochemicals present in the leaf extract of Kakoli have been utilized for the green synthesis of Kakoli leaf extract conjugated gold and silver nanoparticles at room temperature under very mild conditions without any additional stabilizing agents. As the leaf extract of Kakoli 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 leaf extract and the application of leaf-extract conjugated metal nanoparticles in medicine.

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Notes and References


25. Brief Experimental Procedure:

**Synthesis of KLAgNPs**: Synthesis of KLAgNPs in water medium was carried out in an identical method of KL AuNPs preparation keeping the concentrations of the leaf extract identical. Aliquots of AgNO₃ 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 AgNO₃ in the mixtures was 0.7 µg/mL in each vial (Figure 4).