Supplementary information

Green synthesis of gold nanoparticles using an antiepileptic plant extract: *In-vitro* biological and photo-catalytic activities

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The protocols for phytochemical analysis of the methanolic leaves extract of *M. oleifera:*

The methodology undertaken for the phytochemical analysis are considered from earlier reports.[1-6] Typically, these tests can be performed as follows-

Test for alkaloids:

0.5 g of extract was mixed with to 10 mL acid alcohol, warmed and filtered. 2 mL dilute ammonia was added to 5 mL of the filtrate followed by 5 mL chloroform. The mixture was shaken gently to extract the alkaloidal base. 10 ml of acetic acid was used to extract the chloroform layer. Then, Mayer's reagent was added to one part of the extract and Draggendorff's reagent to the other part. The presence of alkaloids will be confirmed from the formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent).

Test for flavonoids:

Dilute ammonia (5 mL) was added to aqueous filtrate of the extract followed by 1 mL concentrated sulphuric acid. A yellow color will be observed that will disappear on standing, indicates the presence of flavonoids.

Test for terpenoids (Salkowski test):

2 mL chloroform was added to 0.5 g of the extract. A layer will be formed on adding concentrated sulphuric acid (3 mL) to the extract. A reddish-brown color will be observed at the interface if the terpenoids present.

Test for cardiac glycosides (Keller-Killiani test):

5 mL in water was added to 0.5 g of extract followed by 2 mL glacial acetic acid containing with one drop of ferric chloride solution. Then, 1 mL of concentrated sulphuric acid was added. A brown ring at the interface will indicate the presence of a deoxy sugar characteristic of cardenolides.

Test for tannins:

0.5 g of the extract was taken in 10 mL water in a test tube, boiled and then filtered. Then, 0.1 % ferric chloride was added dropwise and the presence of Tannins will be indicated by appearing brownish green or a blue-black color.

Detection of Phenols:

Ferric chloride test: 10 mg extracts was mixed with few drops of ferric chloride solution. Appearance of bluish black color indicates that the presence of phenol.

Detection of ascorbic acid:

1 mM ferric chloride and 1 mL of 0.5 mM potassium ferricyanide were mixed with 1 mL of the sample by vigorous stirring for 10 min. The presence of ascorbic acid will be observed from the blue colloidal solution.

Detection of Carbohydrates:

0.5 mg extracts were dissolved in 5 mL water and filtered. The filtrate was applied to test the presence of carbohydrates employing Fehling's solution resulting in red-brown precipitate.

Test for phlobotannins:

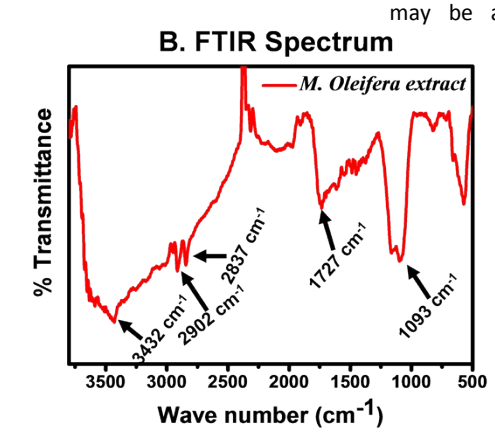
The sample was mixed with 1% aqueous hydrochloric acid and boiled. The formation of a red precipitate will act as evidence for the presence of phlobatinins.

Characterization of methanolic extract of leaves of *M. oleifera:*

The methanolic extract of the leaves of *M. oleifera* was employed to find out phytochemicals present using qualitative test following reported method. It has been discussed in the experimental section. From the analysis, the extract was found to be contained with alkaloids, flavonoids, terpenoids, polyphenols, glycoside etc as shown in Fig. S1 A below. The FTIR spectrum of the methanolic extract was also recorded (Fig. S1 B) and it was found to contain O-H bond with extended H-bonded interaction (3432 cm^{-1}). Also, it possesses asymmetric and symmetric C-H stretching vibration (2902 and 2837 cm^{-1}), C=O stretching linked with ester (1727 cm^{-1}), C-O stretching vibration (1093 cm^{-1}). The UV-Vis spectrum was taken for a particular concentration of *M. Oleifera* extract in methanol (Fig. S1 C). It has one strong peak near 220 nm arising from **π-π*** transition of C=O bond and two medium size peaks, one at 410 nm and one at 670 nm, may be appeared for **n-π*** electronic transition of O-H bond.

A. Phytochemical Analysis

Serial Number	Components	Moringa oleifera
1.	Alkaloids	+
2.	Flavonoids	+
3.	Terpenoids	+
4.	Cardiac glycoside	–
5.	Phenolic compounds	+
6.	Ascorbic acid	–
7.	Carbohydrate	–
8.	Glycoside	+
9.	Phlobatannins	–
10.	Tannins	+



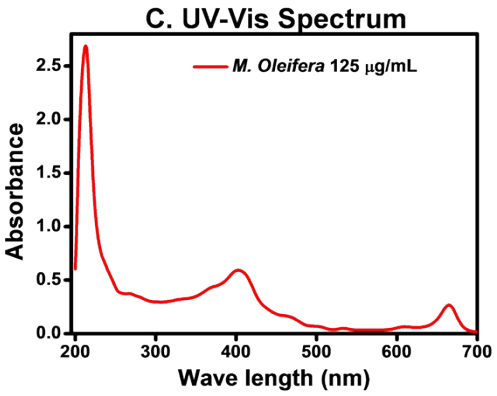


Figure S1: A) Phytochemical analysis table, B) FTIR spectrum and C) UV-Vis spectrum (with methanol as blank) of *M. Oleifera* methanolic leaves extract.

High resolution TEM image and particle size distribution:

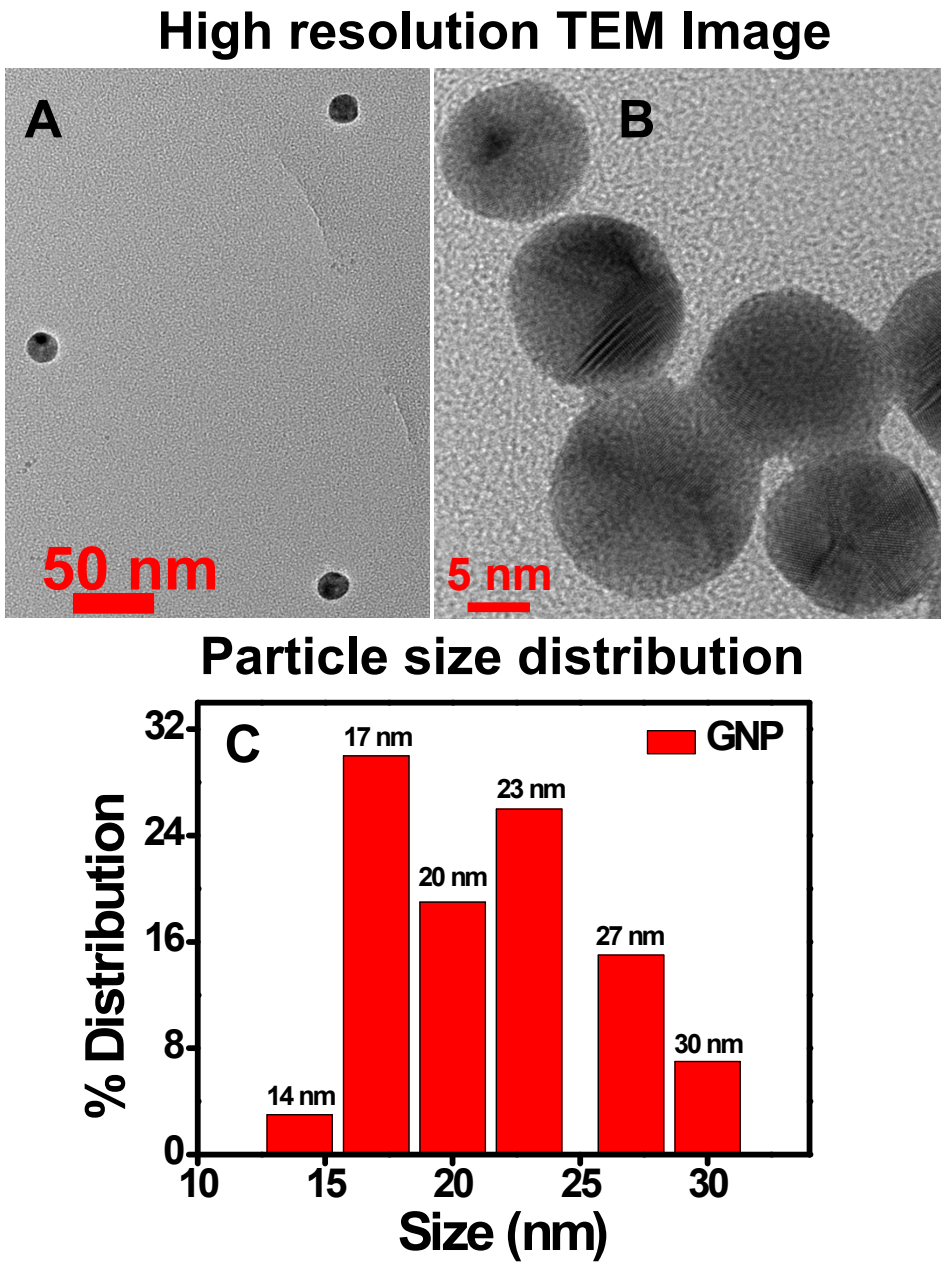


Figure S2: High resolution TEM images of A) gnp-citrate B) GNP and C) Particle size distribution of GNP from TEM image

SEM analysis and SEM EDAX elemental mapping DATA for gnp-citrate and GNP:

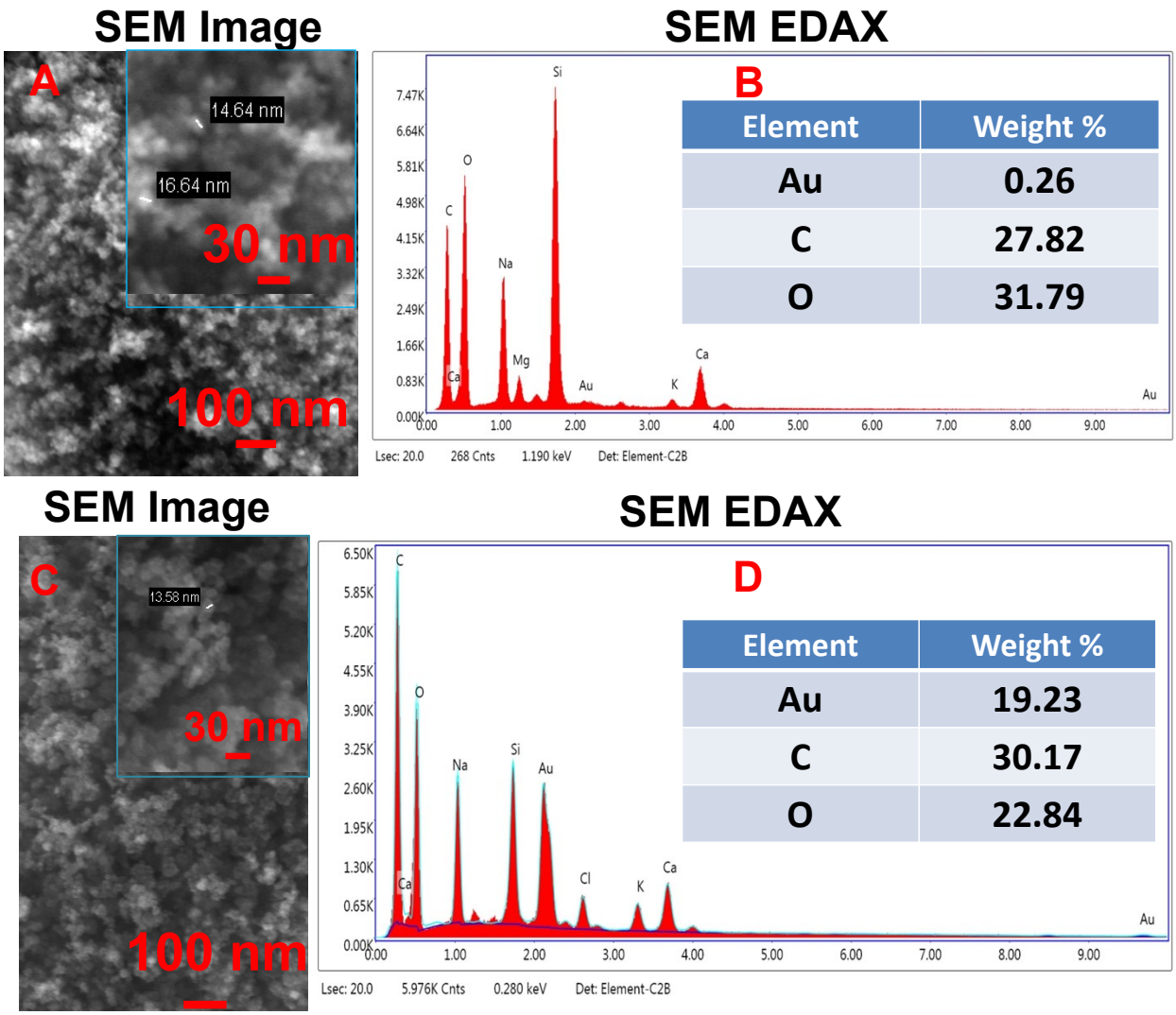


Figure S3: The SEM images (high resolution images at inset) and SEM EDAX results including elemental % for gnp-citrate (Fig. S3 A and Fig. S3 B) and GNP (Fig. S3 C and Fig. S3 D) respectively.

Stability and band gap with time of citrate stabilized and *M. oleifera* stabilized gold nanoparticles:

<u>At 15 days:</u>

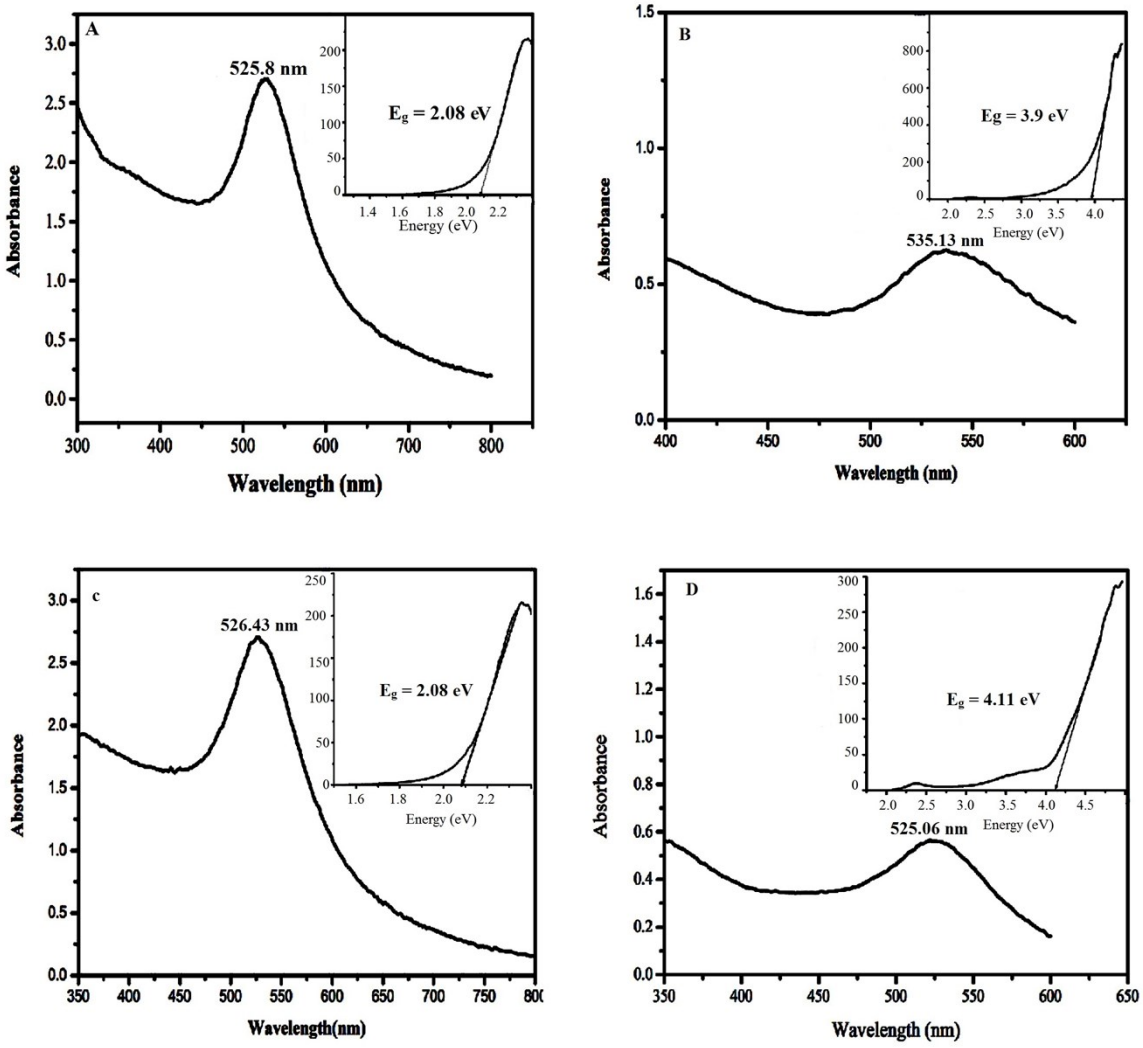


Figure S4. UV-Vis spectra of AuNP, after 15 days interval of the synthesis, stabilized by **(A)** citrate **(B)** immature leaf **(C)** mature leaf **(D)** old age leaf (Inset: *Tauc plot* to determine direct band gap energy of respective samples).

At 30 days:

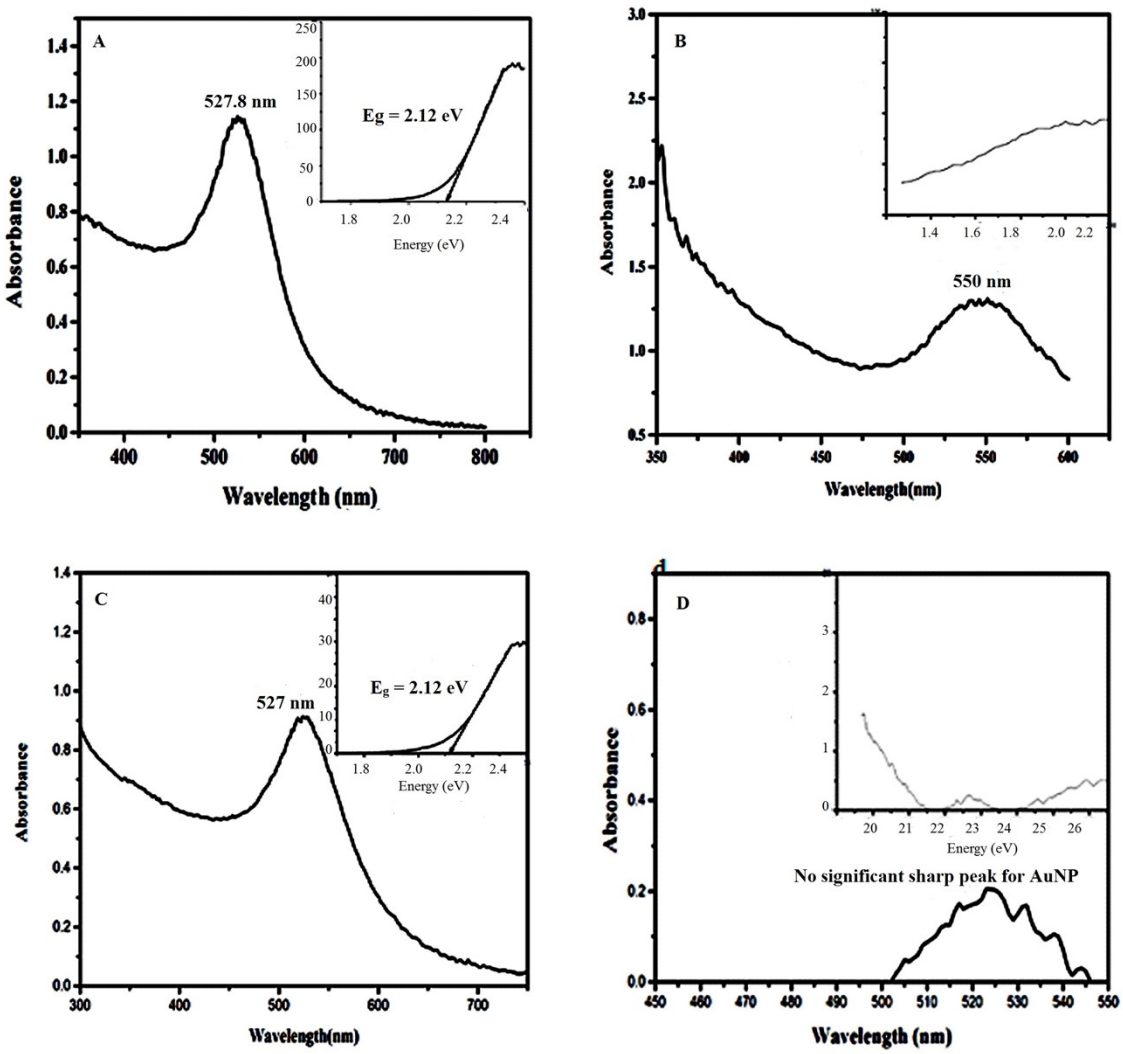


Figure S5. UV-Vis spectra of AuNP, after 30 days interval of the synthesis, stabilized by **(A)** citrate **(B)** immature leaf **(C)** mature leaf **(D)** old age leaf (Inset: *Tauc plot* to determine direct band gap energy of respective samples)

Comparative surface plasmon bands for GNP with respect to time:

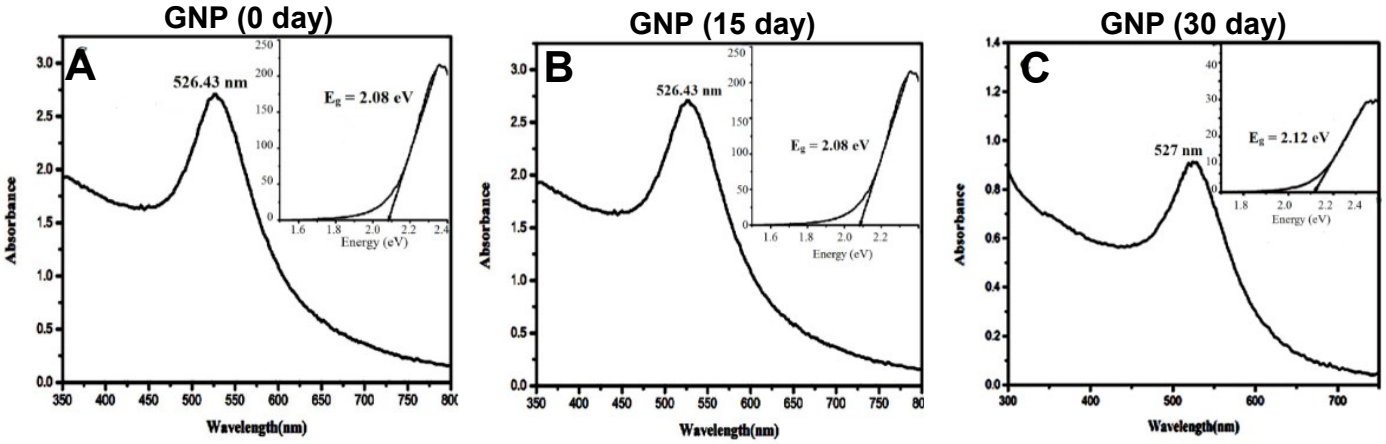


Figure S6: The surface plasmon band of GNP after time **(A)** 0 days and **(B)** 15 days and (C) 30 days

Data on pH variation and stability of green gold nanoparticles (GNP):

To get an idea of pH dependency on the stability of gold nanoparticles, an experiment was performed taking similar reaction condition as described in experimental section of main manuscript. 4 mL of the *M. oliefera* leaf extract was added to the 20 mL gold chloride ($HAuCl_4.3H_2O$) solution having pH 3.4, 6 and 9.6. The mixture was kept under continuous stirring in a magnetic stirrer at temperature of 75^0 C till the color of the mixture becomes reddish brown. Formation of gold nanoparticles was confirmed taking UV-Vis spectra of the respective samples monitoring the surface Plasmon peak (525-545 nm wavelength) of gold nanoparticles as shown in Fig. S(3-5). Stability was revealed analyzing their UV-Vis spectra just after synthesis and after 22 days.

At pH 3.4:

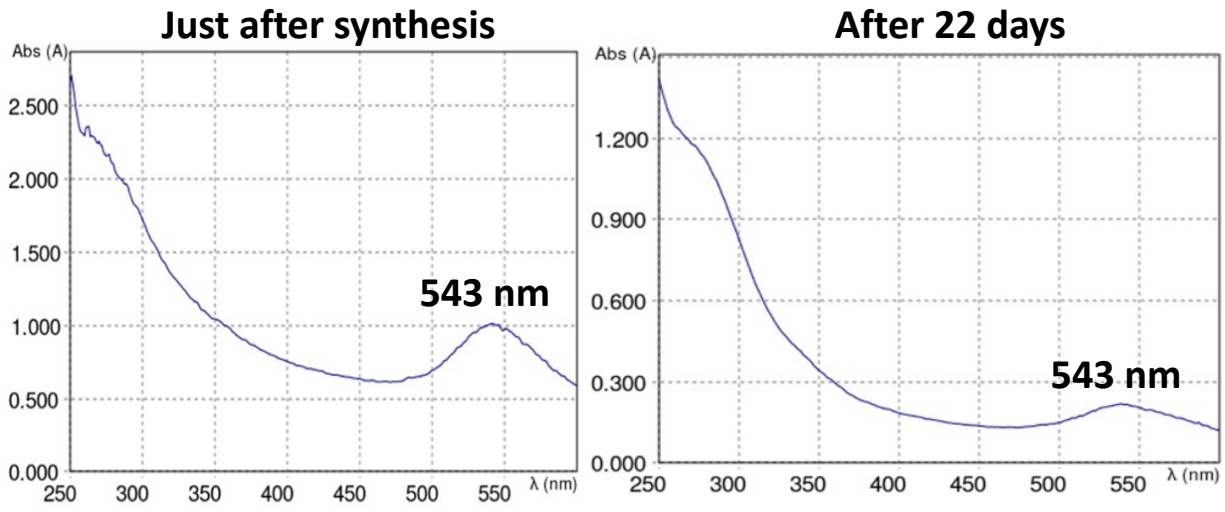


Figure S7: UV-Vis spectra of GNP at pH 3.4 keeping other parameter same.

At pH 6:

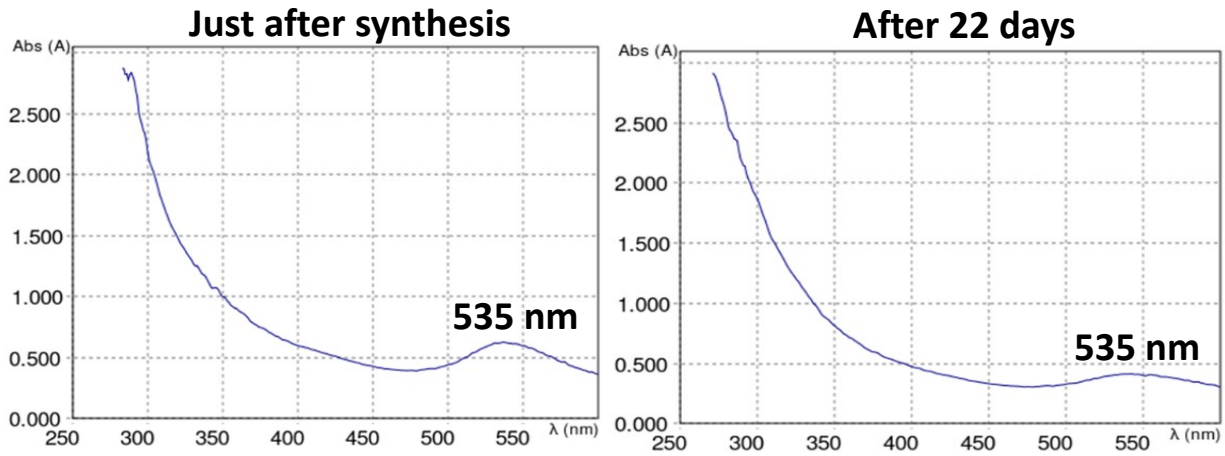


Figure S8: UV-Vis spectra of GNP at pH 6 keeping other parameter same.

At pH 9.6:

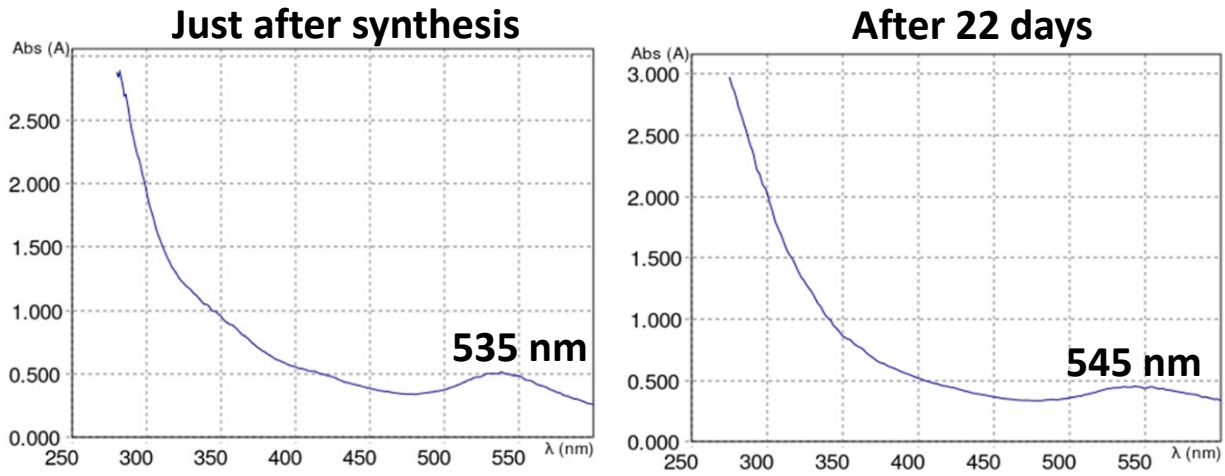


Figure S9: UV-Vis spectra of GNP at pH 9.6 keeping other parameter same.

From the figures shown above (Fig. S3-S5), it can be inferred that the green gold nanoparticles are stable at pH 6 (Fig. S8) and 9.6 (Fig. S9) upto 22 days compare to pH 3.4 (Fig. S7). Between these two pH, stability of nanoparticles is more at pH 6 as after 22 days, the surface Plasmon peak is red shifted for pH 9.6 system (Fig. S9) which may be due to agglomeration.

Surface charge measurement of synthesized gold nanoparticles (gnp-citrate and GNP):

Surface charge on the prepared gold nanoparticles was determined by zeta potential value from DLS experiment. It was found that with change in stabilizing agent on gold nanoparticles, surface charge got changed. Citrate stabilized gold nanoparticles was found to have zeta potential value of -31 mV (Fig. S10 A) while green gold nanoparticles have -15.7 mV (Fig. S10 B).

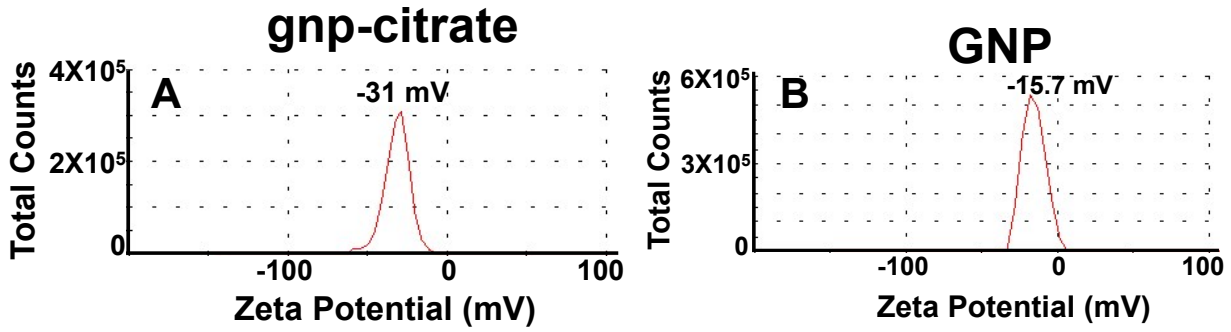


Figure S10: Zeta potential value of **(A)** gnp-citrate and **(B)** GNP

References:

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