Size-dependent synthesis of gold nanoparticles and its peroxidase-like activity for the colorimetric detection of glutathione from human blood serum

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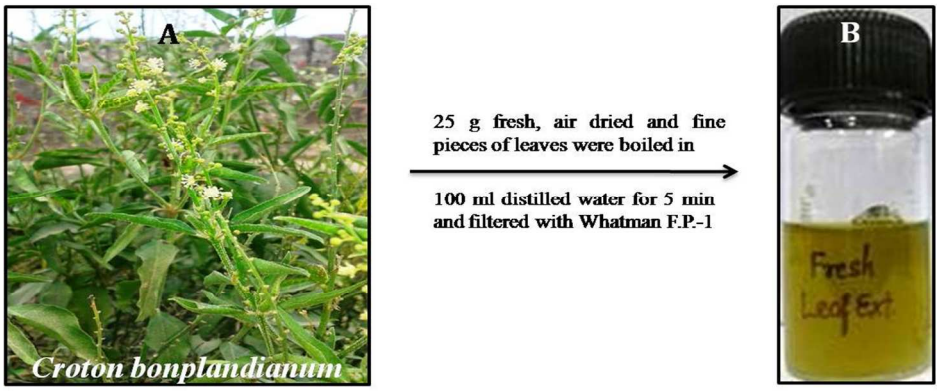
Preparation of leaf extract

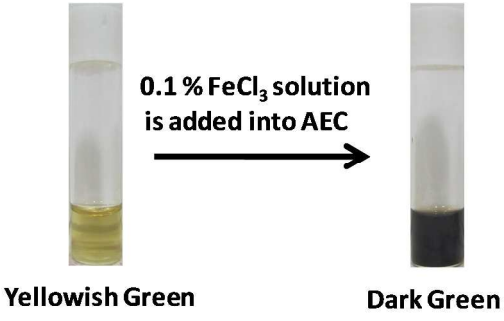


Scheme Supporting Information 1. (A) *Croton bonplandinum* plant **(B)** Aqueous extract of *Croton bonplandinum* (AEC)

Confirmation and quantification of polyphenolic

Ferric chloride Test

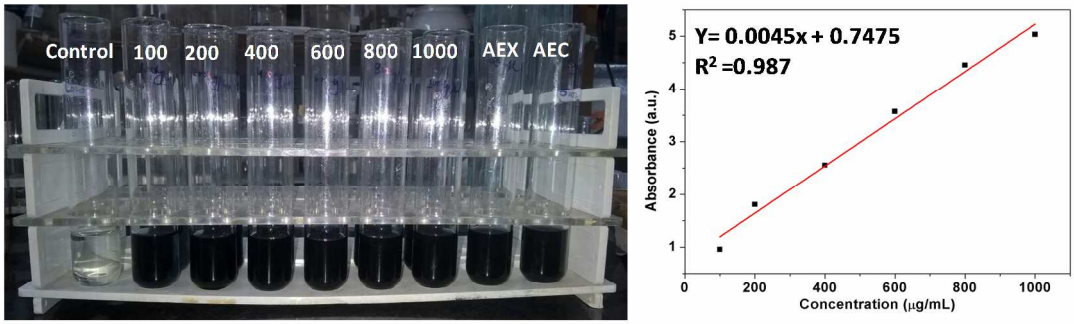
The presence of polyphenolic compound was confirmed by performing Ferric Chloride test. For this 0.1% $FeCl_3$ solution was added into the AEC which resulted into a sudden color change form light yellow to dark green color. This change in color confirmed the presence of polyphenolic compound.



Scheme Supporting Information 2. Scheme showing the change in color from light yellow to dark green which confirmed the presence of polyphenolics in AEC

Folin Ciocalteu's Method

Total phenolics content was estimated by Folin Ciocalteu's method. For this, 1 mL of plant extracts or standard solution of prepared gallic acid (GE) (100, 200, 400, 600, 800, 1000 µg/mL) was added in test tube. Thereafter, 1 mL of Folin Ciocalteu's reagent (1N) was added to the above reaction mixture and shaken. After 5 minutes, 1.0 mL of 7 % Na_2CO_3 was added to the mixture, shaken and the total volume was made up to 10 mL with distilled water. After 90 min, the absorbance was determined against reagent blank at 650 nm using UV visible spectrophotometer. The experiments were performed in triplicates. The total phenolics content was expressed as mg Gallic acid Equivalents (GAE). The result showed that AEC was the rich source of phenolics (2.89 mg GAE/g).



$$TP = \frac{\text{Value of 'x'} \times \text{Volume used}}{\text{Amount of leaf added} \times \text{Amount of AEX}}$$

$$TP = \frac{722\ \mu g \times 100\ mL}{25\ g \times 1\ mL}$$

$$TP = 2891\ \mu g\ GAE/g$$

$$TP = 2.891\ mg\ GAE/g$$

Figure Supporting Information 1. Change in color of the reaction mixtures including Control, different standard solution of GA, and AEC **(A),** Calibration curve of standard solutions of GA **(B)**, and calculation of TP (**C**).

Biosynthesis

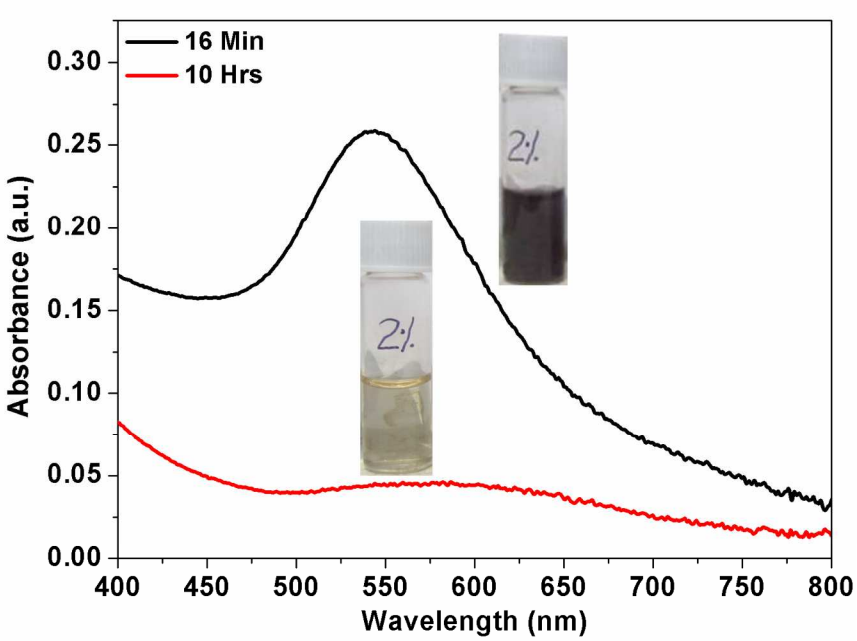


Figure Supporting Information 2. UV–vis absorption spectra of AuNPs in sunlight and in dark after 16 min and 10 hrs respectively with respective color change (conditions; $HAuCl_4.xH_2O$ conc. 0.8 mM, AEC inoculum dose 2% (v/v).

Characterization

XRD

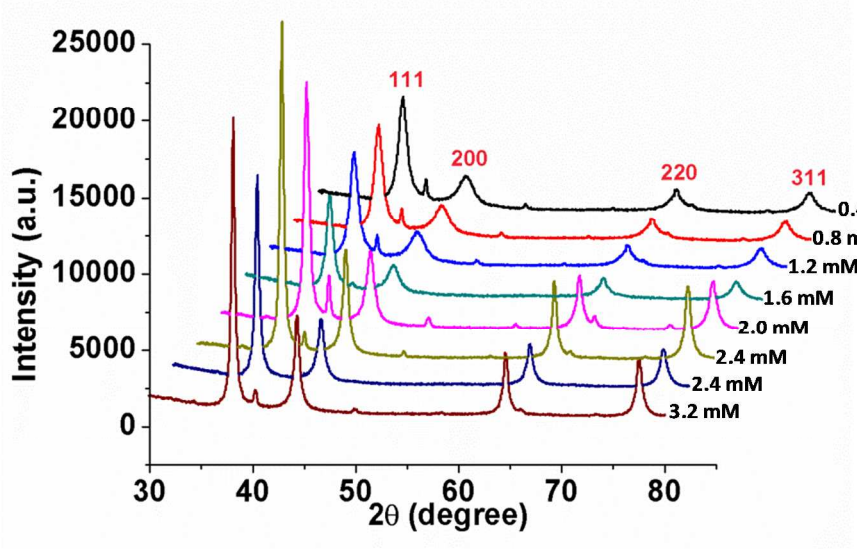


Figure Supporting Information 3. X-ray diffraction pattern of AuNPs obtained from different $HAuCl_4.xH_2O$ concentration (0.4 mM to 3.2 mM).

Zeta potential

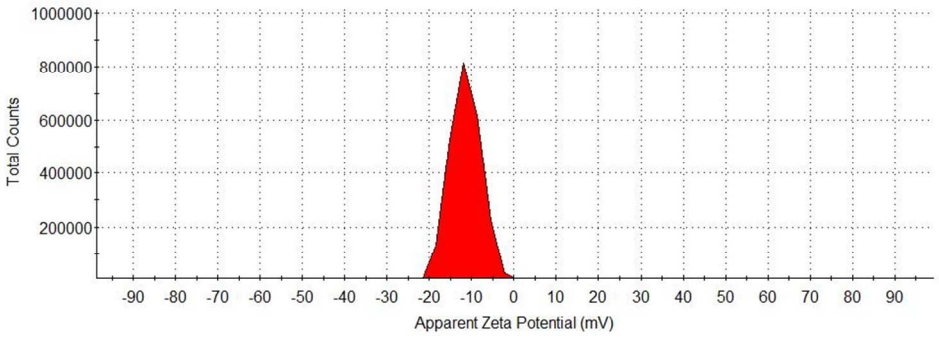


Figure Supporting Information 4. Zeta potential showing the negatively charged surface of AuNPs

Peroxidase-like activity

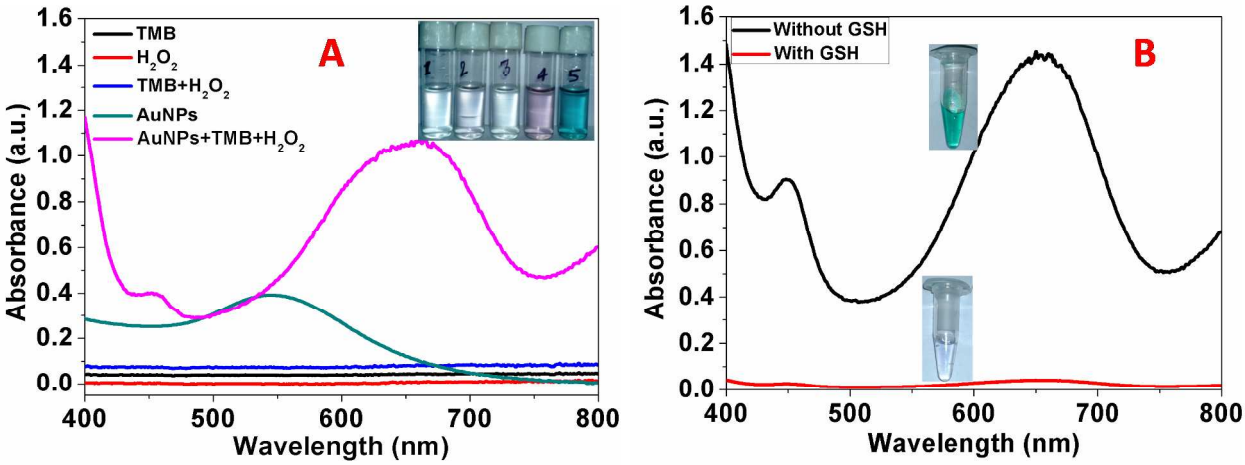


Figure Supporting Information 5. UV- visible absorbance spectra of (**A**) TMB, H_2O_2, TMB+H_2O_2, AuNPs, AuNPs+TMB+H_2O_2, and (**B**) AuNPs+TMB+H_2O_2 solution in the presence and absence of GSH and their corresponding digital images

Optimization of pH and temperature

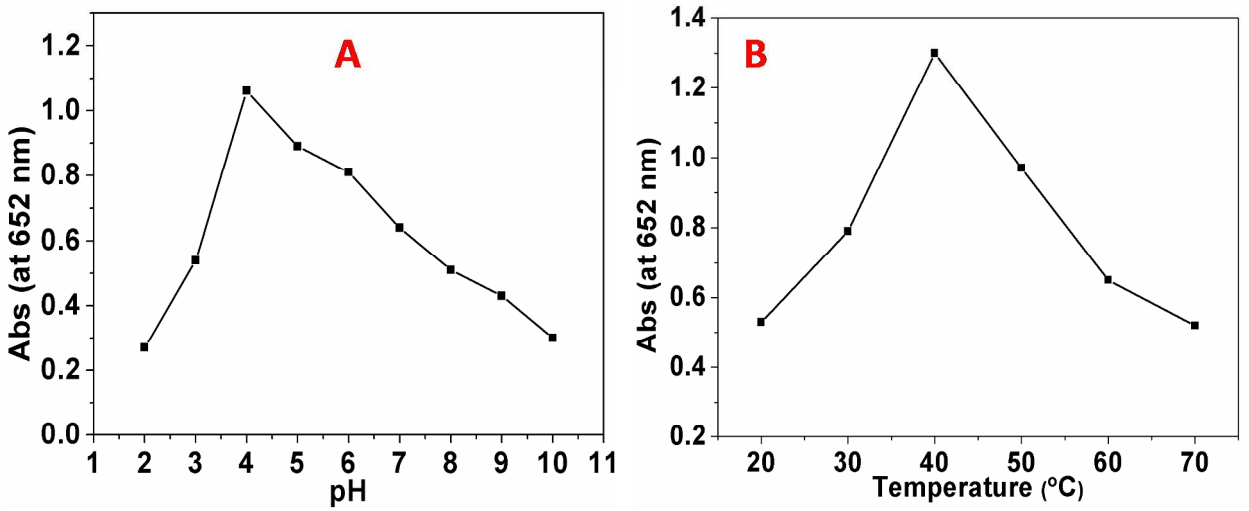


Figure Supporting Information 6. Peroxidase like activity of AuNPs at different **(A)** pHs (2 – 10) at 37 ^{o}C, and **(B)** temperatures, (20 – 70 ^{o}C) at pH 4 using AuNPs (50 µL) + TMB (50 µL, 1mM) + H_2O_2 (50 µL, 1 mM) in 0.2 M NaAc buffer solution

Detection of GSH

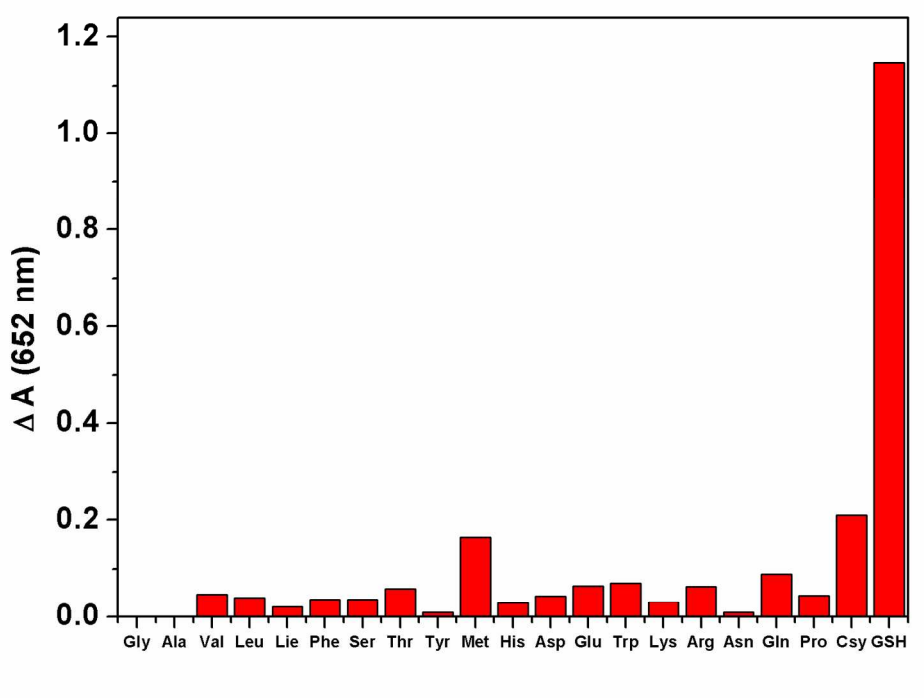


Figure Supporting Information 7. Selectivity of the TMB+H_2O_2+AuNPs system for the detection of GSH

Table Supporting Information 1. Comparison table of average size obtained from TEM and XRD analysis

S.N.		TEM	XRD
	Metal ion concentration (mM)	Average Size (nm)	Average size (nm)
1	0.4	5.6	7.1
2	0.8	5.7	7.4
3	1.2	6.1	9.3
4	1.6	8.6	10.4
5	2.0	13.2	13.4
6	2.4	14.5	16.4
7	2.8	17.7	20.3
8	3.2	19.4	22.2