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PHYTO-ASSISTED SYNTHESIS OF GOLD NANOPARTICLES BY AQUEOUS EXTRACT OF *Curcuma longa* and THE EVALUATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Growing low cost and eco-friendly methods for metallic nanoparticles is a growing need. Using plants towards synthesis of nanoparticles are beneficial with the presence of bio-molecules in plants, which can act as capping/stabilizing and reducing agents. In the present study we describe rapid biosynthesis of gold nanoparticles by *Curcuma longa* aqueous extract. Synthesized nanoparticles were characterized by UV–Visible spectroscopy, transmission electron microscopy (TEM) and the chemical groups in plant extract were detected by Fourier Transform Infra-Red (FT-IR) spectroscopy. TEM study showed that the mean diameter and standard deviation for the silver nanoparticles were 14.12 ± 8.26 nm. Total phenolic and flavonoid contents and radical scavenging activity of the aqueous extract and GNPs/extract mixture, were also evaluated in this study. It can be concluded that the rhizome of *C. longa* is a good source of phenolic compounds, a potent antioxidant and a valuable choice for bio-reduction and biosynthesis of gold.

Keywords: *Curcuma longa*; *nano* particles; TPC; DPPH assay.

1. INTRODUCTION

Metal nanoparticles (Me NPs) have been successfully developed for years with fascinating applications as catalysts, imaging reagents and magnetic materials in bio nanotechnology and biomedicine [1]. Owing to the interest and importance of nanoparticles several researchers have entered on the synthesis of nanoparticles using the various chemical and physical methods. These methods available for the synthesis of gold nanoparticles like particle sputtering, reverse micelle, chemical reduction, hydrothermal, sol gel,

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etc. but unfortunately, are expensive and potentially hazardous to the environment which involves use of toxic and perilous chemicals responsible for various biological risks. The techniques used naturally occurring reagents such as plant extracts, fungi, sugars, and bacteria, biodegradable polymers as reluctant and stabilizing agents can be thought of various synthesizes of inorganic nanoparticles. The synthesis of nanoparticles using plant extract provides advancement over different strategies other methods because it is simple, one step, cost-effective, environment friendly and comparatively reproducible [2].

Among the metallic nanoparticles, gold has been enormously utilized for its high antimicrobial, antioxidant, cytotoxic, and catalytic properties [3]. A variety of Gold nanoparticles synthesis has been previously reported such as *Mangifera indica* leaf [4]. *Hibiscus rosasinensis* [5], *Rosa indica* [6] have been reported.

In our approach to green synthesis, we selected turmeric rhizomes to produce gold nanoparticles. The plant *Curcuma longa* L. belongs to *Zingiberaceae* family. This rhizome has been used for many medical applications such as treatment of burns, hot swellings, small pox, ulcers of the mouth and stomach [7]. It also used as an anti-irritant and anti-microbial. The antioxidant property of curcumin can prevent rancidity of food and thus provides foodstuff with less oxidized fat and free radicals. Keeping the biological perspective in mind, in the present study, we demonstrate the use of turmeric rhizome extract for the synthesis of gold nanoparticles.

This study investigates a simple, speedy, green and sustainable route of Au NPs preparation by aqueous extract of *C.longa* and evaluates total phenolic and flavonoid contents of the extract as the mighty compounds which may be liable for the reduction of gold ion to Au NPs. Radical scavenging activity of the extract and synthesized Au NPs were also evaluated in this project.

2. MATERIALS AND METHODS

2.1 Reagents and Apparatus

Different chemicals and reagents used in this experiment include: Choloro auric acid ($HAuCl_4$, $3H_2O$ (G 4022-5G, 99.9%). Whatman no.1 filter paper, measuring cylinders, standard flasks, mortar and pestle, beakers, distilled water. The chemicals used were of analytical grade and were purchased from Sigma–Aldrich Chemical Limited Bangalore.

2.2 Plant Materials

The plant selected for nanoparticle synthesis; Fresh and healthy C. *longa* Linn plant .Curcuma *longa* **(***Zingiberaceae* family) were collected from the Botanical garden of Fatima Mata National College (Latitude: 8.8932^0 North and Longitude: 76.6141^0 East), Kollam district of Kerala during December 2015. The Collected samples have been identified by Department of Botany Fatima Mata National College Kollam district, Kerala

2.3 Preparation of *Curcuma longa* Rhizome Extract

Fresh and healthy rhizomes of *Curcuma longa* Linn was washed several times in running tap water and then with the aid of distilled water to dispose of debris. Exactly One gram of *C. longa* was mixed with 10 ml of distilled water and crushed in a mortar pestle. The aqueous extract of *C .longa* was filtered with Whatman No. 4 filter paper. The filtered extract was centrifuged at 1000 r/min for 10 minutes. The filtrate was gathered and kept at fridge for nano gold synthesis

2.4 Determination of Total Phenolic Content (TPC)

Total Phenolic contents (TPC) determined by following the Folin- Ciocalteu method of slight modifications [8]. About One ml of plant extract and gold NP-s extracts added separately to a 25 ml volumetric flask filled with 9 ml distilled water. Folin Ciocalteu phenol reagent (0.5 mL) added to the mixture and shaken vigorously. After 5 min, 5 ml of Na CO solution was mixed up. The solution was instantly diluted to 4 ml with distilled water and mixed thoroughly. Thereafter it is then allowed to stand for 60 min before measurement .The absorbance measured at 750 nm versus the prepared blank. The amount of total phenolic compounds was expressed in terms of Gallic acid equivalent (mg/L of the extract) using a regression equation that was obtained from Gallic acid calibration curve ($Y = 0.0105 + 0.0138$, $R = 0.9955$). Experiment was performed in triplicate and expressed as mean± Standard Deviation (SD).

2.5 Total Flavonoid Content Assay

To 1 ml of extract and AuNPs solution, 4 ml of double distilled water added. 0.3 ml of sodium nitrite solution (5%) was then added to all test tubes. Then, it was incubated at room temperature for 5 min. 0.3 ml of aluminium chloride (10%) was then added to all the tubes followed by 2 ml of 1 M NaOH. Then, the

volume made up to 10 ml with double distilled water, and absorbance was measured at 510 nm spectrophotometrically. Double distilled water taken as blank, and the same procedure followed [9]. The total Flavonoids content (TFC) was expressed as GAE in mg/g sample. The calibration curve was plotted for quercetin and a regression equation was obtained (Y= 0.0673 + 0.0051, R = 0.9961). Flavonoids content of the extract was expressed in terms of quercetin equivalent (mg/L of the extract). Experiment was performed in triplicate and expressed as mean ±Standard Deviation (SD).

2.6 DPPH Radical Scavenging Assay

The 2, 2-diphenyl-1-picrylhydrazil (DPPH*) free radical scavenging activity of the GNPs was determined following the method of Ramadan *et al*., 2003 with some modifications [10]. The antioxidant activity of the plant extract was estimated using the DPPH radical scavenging protocol. In the DPPH radical scavenging method, 0.1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was mixed with 3 mL of sample solutions in water at different concentrations (20- 100 µg/mL). The mixture was allowed to react at room temperature in the dark for 30 minutes. Ascorbic acid was used as standard controls. Ethanol was served as the blank. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 517 nm and converted into the percentage .For calculating the percentage of radical scavenging activity, the following equation: % = 100[Ac-As/Ac] was used. In the equation, As is the absorbance of sample and Ac is the absorbance of control.

2.7 Synthesis of Green Gold Nanoparticles

Preparation of gold nanoparticle done according to the method described by Sree lekshmi *et al*; 2013 with slight modifications [11]. To synthesize nanoparticles from *C. longa*, 20µl of aqueous solution of 0.3M Chloroauricacid ($HAuCl_4.3H_2O$) solution carefully added to 10 mL of *C. longa* extract from a conical flask at room temperature under static conditions. The physical parameters were optimized using the obtained reaction mixture *C.longa*.Within 10 min of addition of turmeric extracts, the initial colour of the solution changed from pale yellow to pink colour, which indicates formation of biogenic (Au/*C. longa)* gold NPs.

2.8 Characterization of Au- NPs

The reduction of Au-NPs was confirmed by using UV-vis spectroscopy in the range of 500 to 600nm (Shimadzu, UV-1601 UV-VIS Spectrometer). Transmission electron microscopy (TECNAI, G2 F20) was used to investigate the size and morphology of the Au-NPs using SC1000 Orius CCD camera. The stability of gold nanoparticle(Au-NPs) was measured using Particulate Systems Nano- Plus Zeta/Nano Particle Analyser, Japan. The bioreduction compounds that are responsible for the reaction were determined using Fourier Transform Infrared spectroscopy. The spectrum was obtained by Thermo Scientific Nicolet 6700 system with 16 scans per sample at the range of 550– 4000 cm−1.

3. RESULTS AND DISCUSSION

3.1 Phenol and Flavonoid Activity

Total phenol was higher in plant-AuNPs (84.07 ± 1.49 mg/g GAE) as compared to the aqueous extract alone (77.12± 1.29 mg/g GAE). The results presented in Table 1 also revealed that total flavonoid were higher in plant-AuNPs compared to those found in the plant extract alone and the recorded values were 17.6 ± 0.95 and 20.75 ± 0.531 mg/g respectively. The above phytoconstituents were tested as per the standard methods. Among the phytoconstituents, phenol was found to be present in the highest concentration.

3.2 Visual Observation

In the next part of our study, green synthesis of gold nanoparticles through *C. longa* aqueous extract was carried out. The appearance of yellow to pink coloration of the reaction mixture indicated the biosynthesis of gold nanoparticles (Fig. 1). It is well known that silver nanoparticles exhibit striking colors (yellow to pink) due to the excitation of surface Plasmon vibrations in the particles [12].

Table 1. Total phenolic and flavonoid contents of *Curcuma longa* aqueous extract and gold nanoparticles/extract mixture

Test	Plant extract	AuNPs
TPC (mg GAE/g extract)	77.12±1.29	84.07±1.49
TFC (mg GAE/g extract)	17.6±0.95	20.75±0.07

All values are expressed as Mean ±SD, *TFC=Total Flavonoid Content, TPC=Total Phenol Content GAE=Gallic acid equivalent AuNPs=gold nanoparticle*

Fig. 1. Colour dispersion before and after nanoparticles formation
(i) Final gold dispersion formed after reduction $HAuCl_4$ solution (ii) $HAuCl_4$ solution (iii) $HAuCl_4$ solution with extract before reduction

3.3 Ultraviolet-Visible Spectroscopy Analysis

The UV-Vis spectra illustrating optical properties of the biosynthesized gold nanoparticles (Au NPs), using the extract of *C. longa* is represented in Fig. 2. (a & b). The formation of Au-NPs was followed by measuring the Surface Plasmon resonance(SPR) of the *C. longa* and Au/*C. longa* emulsions over the wavelength range of 500–800 nm. The absorption spectrum of the C.L aqueous extract has shown in Fig. 2. (a) exhibits a characteristic band at 410nm. The disappearance of the UV-Vis absorption band at 410 nm and appearance of a new band at 540 nm could be attributed to the formation of AuNPs. The disappearance of the band at 410nm indicated that the precursor metal ions (chloroauric acid) are reduced to small metal ions in the presence of phenolic acids, flavonoids and other antioxidants present in *C.longa* extract, acting both as a reducing as well as stabilizing agents to form stable CL-AUNPs [13].

3.4 TEM Study

The morphology and size of the synthesized AuNPs had been investigated by using TEM analysis. Fig. 3 represents a magnified image of these gold nanoparticles at the scale bar of 100nm. Two different types of particles are clearly visible from these representative micrographs. One is nearly circular or hexagonal and other having some equilateral triangle shapes in small numbers is nearly negligible. The nano cluster shown in Figure ranged from 15 to 20nm in size, with a mean diameter of 14.12 ± 8.26 nm. This agrees with the histogram of the measured particles, displaying maximum frequency of particle distribution of the ranges of 15 – 20 nm.

3.5 FT-IR Spectrum of Green Gold Nanoparticles

FT-IR spectrum of green gold nanoparticles was carried out to identify the water soluble organic compounds from the extract which may take part in the reduction of gold ions (Fig. 4). A strong peak at 3313 cm–1 indicates the presence of O-H groups in phenolic compounds, and N-H groups in proteins.. The peaks at (1400 and 1600) cm– 1 indicates C=C stretching in alkenes and aromatic compounds and also C-H bending in aliphatic compounds. The peaks at (1000-1300) cm–1 indicate C-C and C-O stretching in the compounds like alcohols and phenols..FT-IR confirmed the presence of bio-reducing organic compounds on the surface of Au NPs which are likely to be responsible for nanoparticle synthesis and stability. This suggests that Phenolic compounds and proteins present in aqueous extract of the plant species could be responsible for the reduction of gold ions and for the stabilization of the phythosynthesized GNPs.

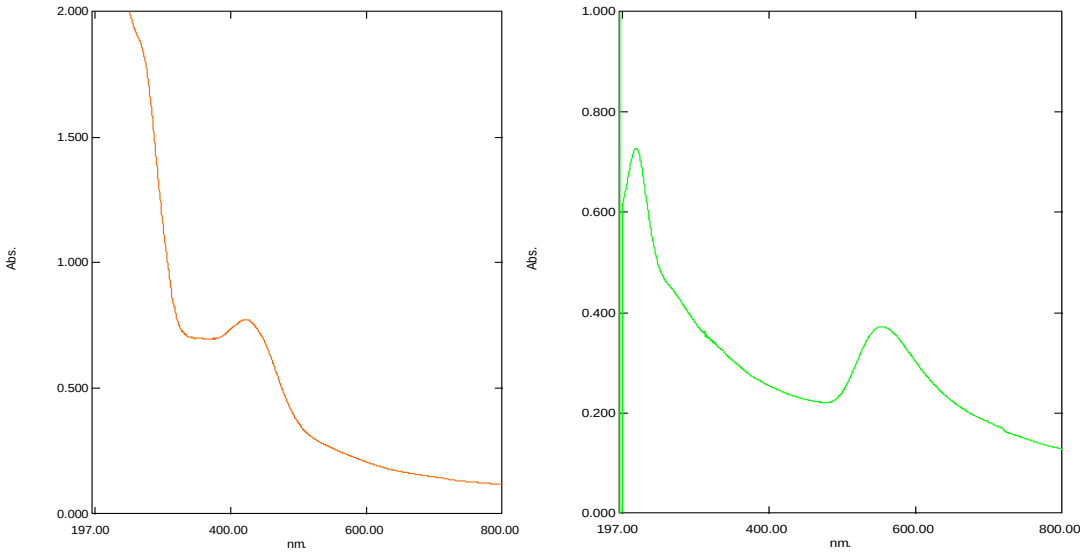

Fig. 2. UV-Vis spectrum of (a) *C. longa* aqueous extract and (b) UV-Vis absorption spectra of CL-AuNPs

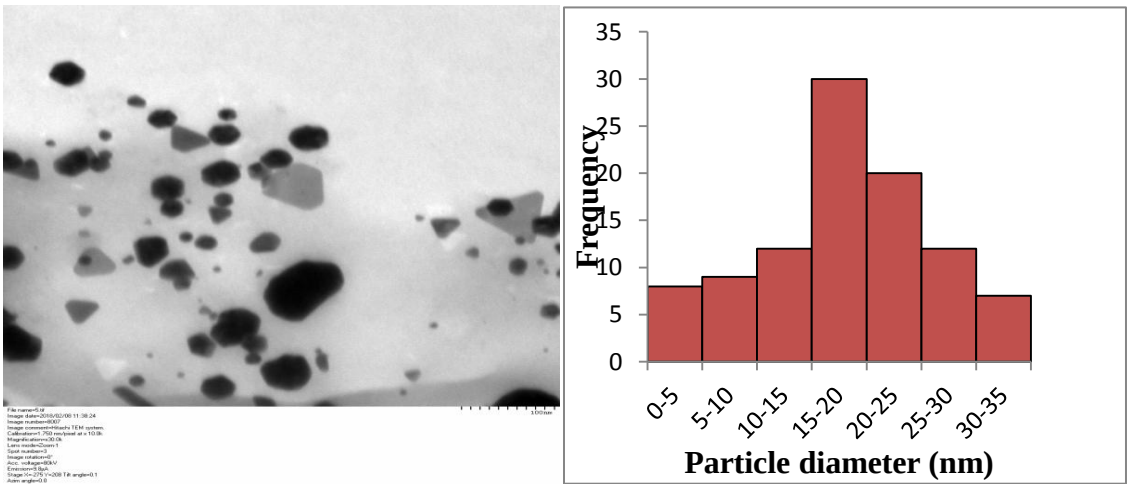

Fig. 3.TEM images and corresponding size of GNPs synthesized by *Curcuma longa* extract

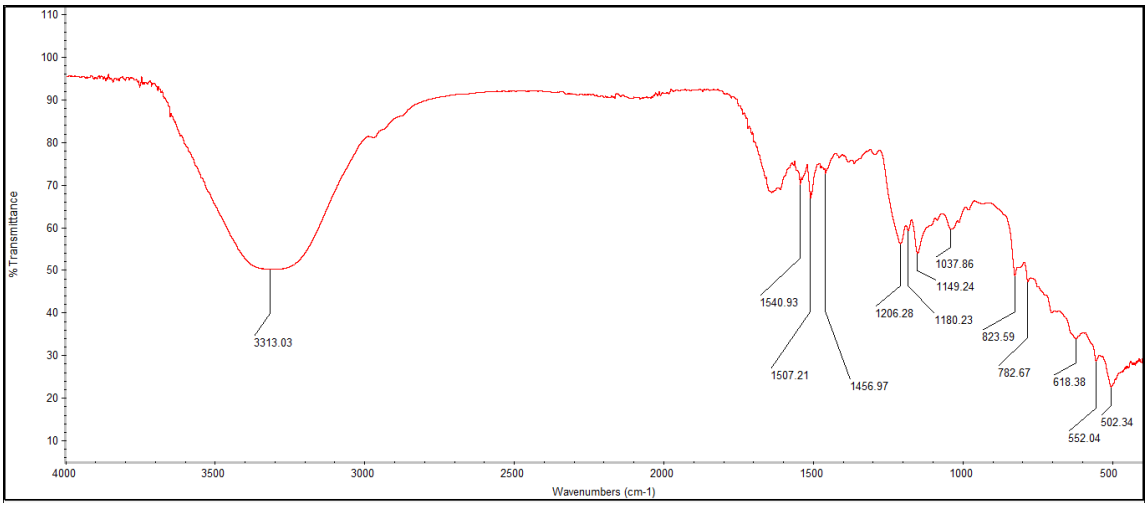

Fig. 4. FTIR spectrum analysis of gold nanoparticles from *C.Longa* extract

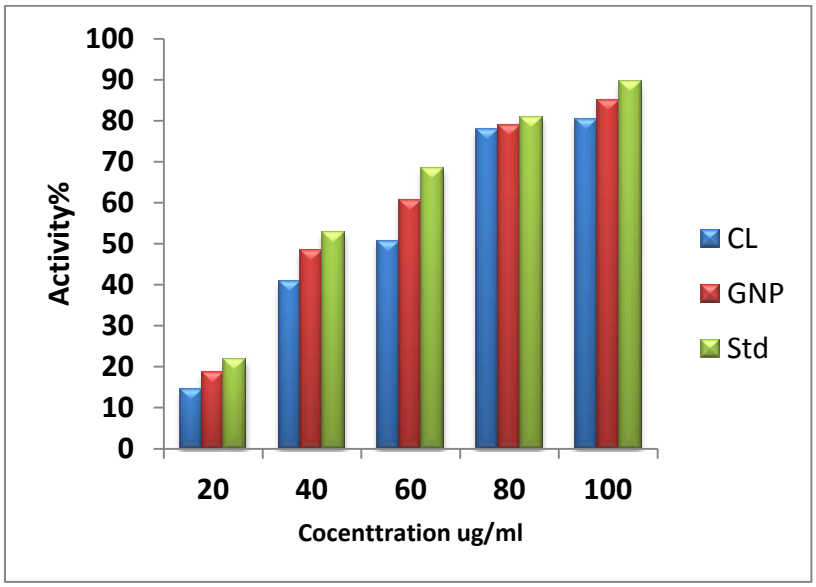

Fig. 5. DPPH radical scavenging effect of *C. longa* aqueous extract and Au NPs/extract suspension in comparison with ascorbic acid

3.6 Radical Scavenging Activity

Four different concentrations (20-100 µg/ml) of *C. longa* extracts and their nanoparticles were used. *C. longa* extract exhibited the higher reducing power at 100 µg/ml concentrations with 80.45% whereas low reducing power of 14.47% was observed in 20 g/ml. Consequently the CL AuNPs revealed the elevated 85% of reducing power at 100 g/ml. The lesser 18.4% of reducing power was found at 20 µg/ml. Fig. 5 explained the Radical scavenging activity of *C. longa* and their gold nanoparticles. Plant extracts and gold nanoparticles scavenging percentage were correlated with the standard control Ascorbic acid; it showed the greater scavenging percentage than the extracts. While increasing the concentration of test compound, scavenging ability also increased, it indicates the dose dependant activity.

In the current study, the mechanism by means of which the plant extract will be synthesise AuNPs can be explained with the aid of the higher total phenolics and flavonoid content in the plant. This plant phenolics/ flavonoid are strong antioxidants with high reducing capacity [14] which can be used for AuNPs synthesis [15]. The higher content of total phenol/ flavonoid contents in *C. longa* extract to facilitate the reduction of gold ions to nanoscale- sized gold particles due to the electron donating ability of these compounds. Furthermore, the quinoid compound produced due to the oxidation of the phenol group of phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension stabilization [16].

4. CONCLUSION

High amounts of flavonoids and phenolic compounds and a high potent of radical scavenging activity were evaluated for *C.longa* extract. A simple green synthesis of stable gold nanoparticles using *Curcuma longa* aqueous extract was also reported in this study. These nanoparticles were synthesized with an average size of 14.12 ± 8.26 nm and spherical in shape and were characterized by TEM, UV–Visible and FT-IR spectroscopy. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for synthesis of gold nanoparticles. Plants with high antioxidant and reducing capacities are not only useful for the green synthesis of metallic NPs, but also for the prevention or reduction of the harmful effects of reactive oxygen species (ROS), generated during normal cellular metabolism of plants and animals.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jose M, Palomo. Nanobiohybrids: A new concept for metal nanoparticles synthesis. Chem. Commun. 2019;55:9583.
2. Abhijith KS, Thakur MS. Application of green synthesis of gold nanoparticle for sensitive detection of aflaxiin B1 based on metal enhanced fluroscence. Analytical Metods. 2012;4(12):4250-4256.
3. Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, et al. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assay based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. Eur. Food Res. Technol. 2001;212:319–328.
4. Philip D. Rapid green synthesis of spherical gold nanoparticles using *Mangiferaindica* leaf. *Spectrochim* Acta Part A: Mol Biomol Spectrosc. 2010;77**:**807–103.
5. Philip D. Green synthesis of gold and silver nanoparticles using *Hibiscus rosaSinensis.* Physica E: Low-dimensional Syst Nanostruct. 2010;42:1417–1418.
6. Manikandan R, Manikandan B, Raman T, Arunagirinathan K, Prabhu NM. Biosynthesis of silver nanoparticles using ethanolic petals extract of *Rosa indica* and characterization of its antibacterial, anticancer and anti-inflammatory activities. Spectrochim Acta A Mol Biomol Spectrosc. 2014;138C:120-129.
7. Khanna NK. Cur. Sci. 1999;76:1351.
8. Singleton VL, Orthofer RM, Ramuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu Reagent. Methods Enzymol. 1999;299:152-178.
9. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002;10:178–182.
10. Ramadan MF, Kroh LW, Morsel JT. Radical scavenging activity of black cumin (*Nigella sativa L.),* coriander (*Coriandrum sativum L.),* and niger (*Guizotia abyssinica Cass.)* crude seed oils and oil fractions. Journal of Agricultural and Food Chemistry. 2003;51(24):6961–6969.
11. Sreelakshmi CH, Nidhi Goel, Datta KK. Anthony Addlagatta B, Ramesh Ummanni V. Green Synthesis of Curcumin Capped Gold Nanoparticles and Evaluation of Their Cytotoxicity. Nanoscience and Nanotechnology Letters. 2013;5:1–8.
12. Prabha S, Supraja N, Garud M Prasad. Synthesis, characterization and antimicrobial activity of Alstonia scholaris bark-extract-mediated silver nanoparticles. J. Nanostruc. Chem. 2014;4(4):161–170.
13. Mahitha BD, Raju T, Madhavi CH, Mahalakshmi NJ. Evaluation of antibacterial efficacy of phyto fabricated gold nanoparticles using *Bacopa monniera* plant extract. Indian Journal of Advances in Chemical Science. 2013;1(2):94-98.
14. Pietta PG. Flavonoid as antioxidants. J. Nat. Prod. 2000;63:1035–1042.
15. Martinez-Castanon GA, Nino-Martinez N, Martinez-Gutierrez F, Martinez-mondeza JR, Ruiz F. Synthesis and antibacterial activity of silver nanoparticles with different size. J. Nanopart Res. 2008;10:1343–1348.
16. Wang WQ, Chen C, Jiang D, Yang X, Liu S. One step synthesis of biocompatible gold nanopartcles using gallic acid in the presence of poly-(N-vinyl-2-pyrrolidone). Colloids Surf. A Physicochem. Eng. Asp. 2007;301:73–79.