©Biotechnology Society www.bti.org.in
ISSN 0974-1453
Research Article

FACILE GREEN SYNTHESIS AND NANOSTABILIZATION OF GOLD NANOPARTICLES USING COCONUT SHELL EXTRACT AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

Blessy Joseph[2, 3,] M. Vibin[1, 3*] ,

[1] Biochemistry Division, Post Graduate Department of Fisheries and Aquaculture, St. Albert's College, Affiliated to Mahatma Gandhi University, Banerji Road, Ernakulam-682018, Kerala, India.

[2]International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India

[3]Sree Buddha College of Engineering, Affiliated to University of Kerala, Pattoor, P.O, Pandalam, Alappuzha, Dist. Kerala

***Corresponding author: vbnano@gmail.com**

ABSTRACT

A simple one-pot approach for the green synthesis of gold nanoparticles (AuNPs) using coconut shell extract is reported. This method is rapid, facile, cost effective, non-toxic and eco-friendly and these features make it more attractive for biomedical applications. The reduction of auric chloride led to the formation of AuNPs within 7 min at room temperature (27 ^{0}C), suggesting a higher reaction rate than chemical methods reported for the synthesis. The structural characteristics of the AuNPs were analyzed by UV-visible spectroscopy, High Resolution Transmission Electron Microscopy (HR-TEM), X-ray diffraction (XRD), FT-IR, SEM and EDAX techniques. Characterization results showed that, AuNPs obtained were spherical and monodispersed with overall size 10-15 nm. Synthesis can be completed in a very short period (several min) and exhibits higher reproducibility, which will be more advantageous for their applications in biomedicine. The antibacterial activity of the synthesized gold nanoparticles was confirmed against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.*

Keywords: Gold nanoparticles, Coconut shell, Eco-friendly, Green synthesis, Nanostabilizers

INTRODUCTION

Gold nanoparticles have become the focus of intensive research owing to their wide range of applications (Alric *et al*., 2008;Cabuzu *et al*., 2015; Hainfeld *et al*., 2006; Das *et al*., 2012; Jongjinakool *et al*., 2014; Curry *et al*., 2014; Jiao *et al*., 2011). Since the gold nanoparticles (AuNPs) have to be employed for biomedical applications, nanoparticles synthesized using physical or chemical methods can have adverse effects. Hence alternate methods; preferably synthesis using plant extracts is greatly explored nowadays. The use of plants in gold nanoparticles synthesis is cost effective and eco-friendly. Plant based synthesis is catalyzed by the phytochemicals which act as reducing as well as stabilizing agents. Moreover nanoparticles prepared by biologically active polysaccharides and phytochemicals might exert synergistic effects by combining their biological activities with those of nanoparticles (Bhattacharya and Mukherjee, 2008). Researchers have introduced the concept of green chemistry combining the principles of nanotechnology. For the development of green chemistry, Raveendran *et al* (2003) suggested that three main factors in nanoparticle preparation should be considered i.e. solvent choice, environmentally benign reducing agent and the use of non-toxic material for nanoparticle stabilization.

Plants have been observed as good candidates for the synthesis of gold nanoparticles with well defined dimensions such as *Cinnamomum zeylanicum* (Smitha *et al*., 2009), *Mangifera indica* (Philip *et al*., 2010), *Achillea wilhelmsii* (Andeani *et al*., 2011), olive (Khalil *et al*., 2012) and *Piper pedicellatum* (Tamuly *et al*., 2013). *Cassia auriculata* leaf extract has been identified very recently for the production of gold nanoparticles (Kumar *et al*., 2011). In this study gold nanoparticles of size 10-15 nm were synthesized within 7min at room temperature. AuNPs are an obvious choice due to their amenability of synthesis and functionalization, less toxicity and ease of detection (Tiwari *et al*., 2011). AuNPs synthesized by *Bacillus* species showed antibacterial activity against various organisms, in combination with or without antibiotics (Singh *et al*., 2013). There are several reports on the antibacterial activity of gold nanoparticles against various pathogens (Bindhu *et al*., 2014; Cui *et al*., 2012). AuNPs are being used to deliver protein based drugs like ampicillin for antimicrobial activities (Chen *et al*., 2006; Chamundeeshwari *et al*., 2010). Suganya *et al* (2015) synthesized AuNPs from blue green alga *Spirulina platensis* and its antibacterial activity against gram positive organisms *B. subtilis* and *S. aureus* were studied. Here the nanoparticles had an average size of ~5nm. Burygin *et al* (2009) showed that the biocidal properties of AuNPs can be increased by adding antibiotics and also that for maximum bacterial susceptibility the antibiotic-nanoparticle conjugates should be stabilized.

Cocos nucifera L. (Family Arecaceae) commonly known as coconut, is considered as an important fruit crop in the tropical countries. *Cocos nucifera* has been recognized as an entity with multiple uses with every component being biologically active in one way or other. Coconut shell is supposed to be the hardest part of the fruit but ironically richest source of phenolic and flavonoid content. Green coconut shell was utilized as a cheap source of vegetable dye in silk fabric (Akhter *et al*., 2014). Preparation of azo dye from the green coconut shell extract is also an indication of a source of phenolic compounds (Ali and Akhter, 1997). Heating the coconut shells gives oil that is used against ringworm infections in Indian popular medicine. The alcoholic extracts of ripe dried coconut shell possess antifungal activity attributed to the high content of phenolic compounds (Venkataraman *et al*., 1980). Antimicrobial effect of dry distilled extract of *Cocos nucifera* endocarp has also been reported (Singla and Jagani, 2012). *Cocos nucifera* endocarp was also found to have significant vasorelaxant and antihypertensive effect when evaluated using isolated rat thoracic aorta and DOCA salt induced hypertensive rats (Bankar *et al*., 2011).

Herein, we report the one pot green synthesis of AuNPs (Fig.1) using antihypertensive potent coconut shell extract for biomedical applications.

MATERIALS AND METHODS

Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) was obtained from Hi Media, India. All other reagents used in the reaction were of analytical grade with maximum purity. Coconut shells were collected from Alappuzha District, Kerala, India, and was cleaned with double distilled water and shade dried. Further coconut shells were ground to powder and stored at 4°C for further study.

Synthesis and characterization of gold nanoparticles

Coconut shell extract was prepared by taking 10g of shell powder with 100 ml double distilled water in a conical flask. This was boiled for 20 min at 70°C. The extract thus obtained was filtered was stored at 4°C. For the synthesis of gold nanoparticles 1mM Chloroauric acid was transferred to a 100ml Erlenmeyer's flask and incubated with coconut shell extract for few minutes. The reaction was rapid as the ruby red color appeared within 7 min. The appearance of ruby red color in the reaction confirmed the formation of gold nanoparticles. The gold nanoparticles initially identified by color changes were followed by characterization using UV-vis spectroscopy. The UV-vis spectrophotometer readings were recorded for the samples in the nanometer range of 450–750 nm using Systronics 2202 spectrophotometer. *Cocos nucifera* shell reduced AuNPs were centrifuged at 10,000 rpm for 15 min to prepare pellet, washed with deionized water to remove the remaining biomass. HR-TEM studies were prepared by drop coating nanoparticles onto carbon-coated TEM grids using FEI (TECNAI G² 30S-TWIN). The film on the TEM grids was allowed to dry. Extra solution was removed using a blotting paper. XRD measurement of the *Cocos nucifera* shell reduced AuNPs was carried out on films of the respective solutions drop coated onto glass substrate on a Bruker Advance D8 XRD instrument operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiations. FT-IR was used to identify the possible biomass at a resolution of 4 cm^{-1} in the range of 4000–450 cm^{-1} and the FT-IR spectrum was recorded by employing KBr pellet technique using Shimadzu IR Prestige - 21 spectrophotometer. The surface morphology of the formed AuNPs was studied using XL 30 FSEM, Philips. The presence of elemental gold was determined by using EDAX, Hitachi H 7650 (D-71). Coconut shell reduced AuNPs were stored at room temperature and were found to be stable for several days.

Antimicrobial activity

The antimicrobial properties of the synthesized nanoparticles were analyzed using the Kirby-Bauer disc diffusion method. Bacterial suspensions of *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2685) and *Salmonella typhi* (NCIM 2501) grown overnight were swabbed on separate nutrient agar plates using L-rods. Antibiotic discs were separately impregnated with 20 μl of gold nanoparticle solution. The discs were dried and then impregnated on plates. Norfloxacin (10mcg/disc) was used as positive control.

RESULTS AND DISCUSSION

Synthesis of gold nanoparticles using coconut water through microwave irradiation has been reported (Babu *et al*., 2011). In our study of AuNP synthesis using coconut shell extract, no additional heating was required. The synthesis was rapid, eco-friendly and the complete synthesis required only 7 min. The color change involved during the synthesis of gold nanoparticles is shown in Fig. 1.

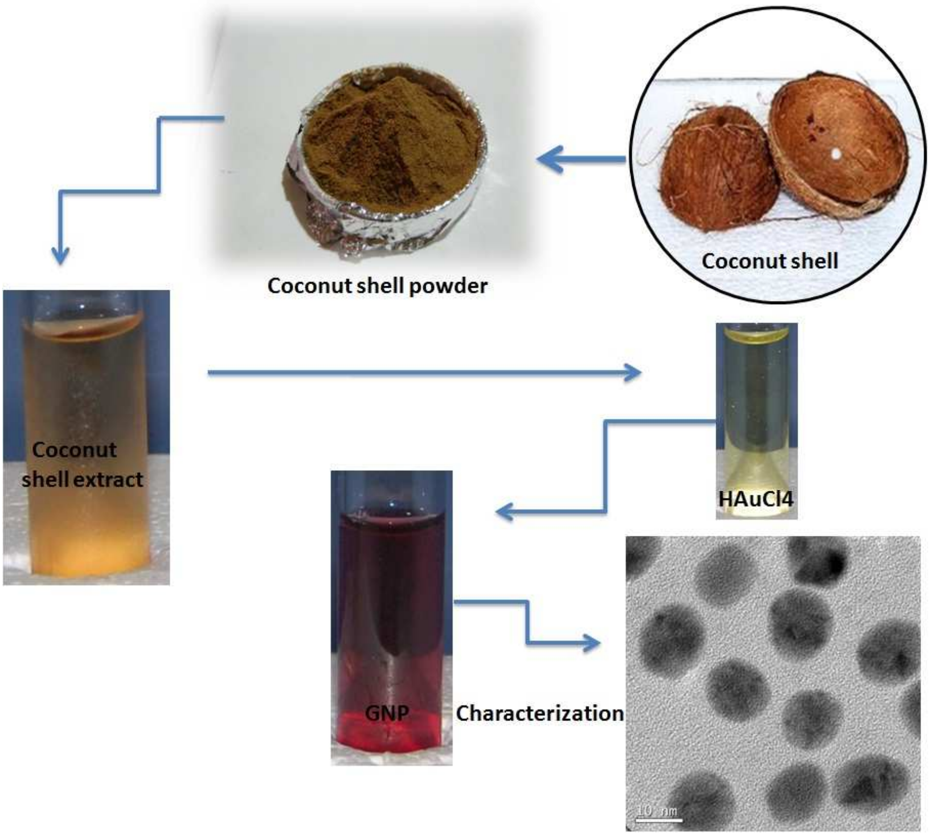


Figure 1: Graphical representation of GNP synthesis using coconut shell extract.

The appearance of the ruby red color and the maximum absorbance were indications of the formation of colloidal gold particles. It is well known that the optical properties of metal nanoparticles are dominated by Surface Plasmon Resonance (SPR), which shifts to longer wavelength with increasing particle size. The change in color was due to the collective coherent oscillation of conduction electrons at the surface of gold nanoparticles. The phytochemicals present in coconut shell extract may be responsible for the reduction of gold ions into metallic nanoparticles. This strategy reduces the cost of production and the environmental impact.

UV–vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al*., 2006). It is well-known that AuNPs exhibit ruby red color in aqueous solution due to Surface Plasmon resonance (Guo *et al*., 2005). Therefore the reduction of $AuCl_4$ ions during exposure to the Coconut shell extract may be easily followed by UV–vis spectroscopy. The UV- visible spectra (Fig. 2) indicate a strong plasmon resonance that is located at 531 nm.

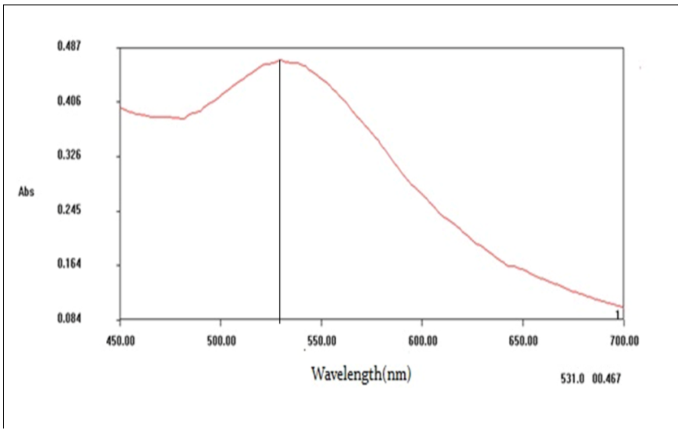


Figure 2: UV-vis spectrum of coconut shell extract encapsulated gold nanoparticles obtained at 531 nm range.

The size and shape of the bioreduced AuNPs were elucidated with the help of HR-TEM (Fig. 3).The results obtained from the HR-TEM analysis gives a clear indication regarding the shape and size of the nanoparticles. The synthesized particles were monodisperse, and the AuNPs formed were predominantly of diameter ranging from 10-15 nm. The results clearly illustrates that most of the particles were spherical and found to be effective for biomedical applications, similar to that reported by other workers (Philip, 2010).

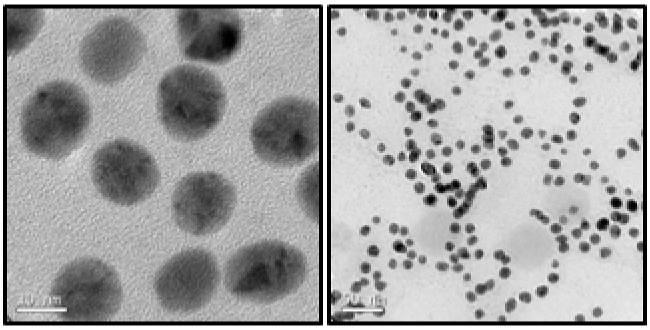


Figure 3: HR- TEM images indicating the presence of spherical nanoparticles recorded at various magnifications

The crystalline nature of the particles was analyzed using XRD technique. The XRD spectrum measured in this case resulted in three intense peaks observed in the spectrum which agrees to the Bragg's reflection of AuNPs. The XRD patterns (Fig. 4) of AuNPs obtained were similar to the results reported earlier (Dubey *et al*., 2010) where three diffraction peaks were observed in the 2θ range of 30-80°, which can be indexed as (1 1 1), (2 0 0), (2 2 0) reflections of fcc structure metallic gold respectively similar to Joint Committee on Powder Diffraction Standards (JCPDS, File no: 04-0784) revealing that synthesized AuNPs are of pure crystalline gold. The average crystal size was calculated by applying Scherrer equation and was found to be nearly 10 nm. This was in agreement with the particle size obtained from TEM image of the gold nanoparticles.

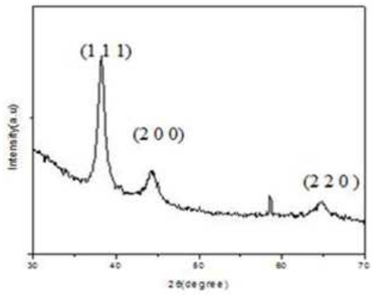


Figure 4: XRD pattern of gold nanoparticles obtained using coconut shell extract.

FT-IR spectra were analyzed to identify the possible surface interaction of biomolecules onto gold nanoparticles. The interaction of nanoparticles with biomolecules of coconut shell showed intense peaks at 3352.74, 2935.76, 1619.47, 1516 and 1063.23 $cm^{-1}$and relative shift in position and intensity distribution were confirmed with FT-IR (Fig. 5) recorded for dry powder of coconut shell, where the strong bands were observed at 3331.74, 2934.98, 1593.02, 1334.5 and 1059.34 cm^{-1}. The bands indicated the presence of alcohols and phenolic compounds. The presence of these bands confirms the biomolecular interaction at the gold nanoparticle surface. Comparison between spectra of untreated sample to the treated samples AuNPs reveal only minor changes in the positions as well as on the magnitude of the absorption bands. The stabilization of nanoparticles and the functional groups of the compounds responsible for synthesis were identified using FT-IR studies and were compared with previous works carried out to obtain AuNPs (Philip *et al*., 2011).

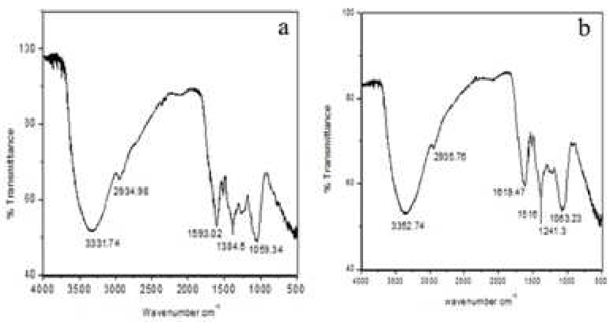
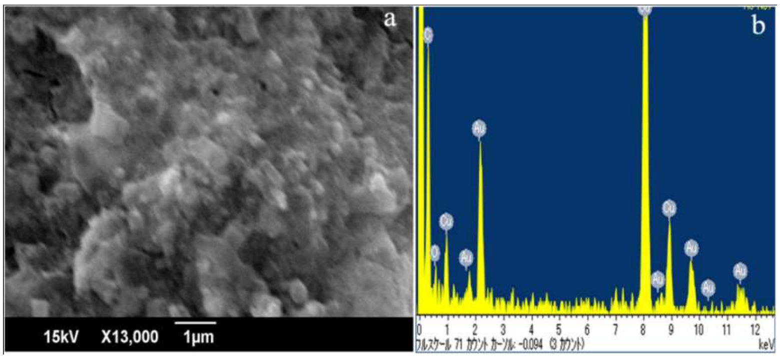


Figure 5: FT-IR spectra for a coconut shell extract and b gold nanoparticles.

The formation of gold nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the particles were not in direct contact with each other. This indicates the stabilization of nanoparticles by a capping agent. The capping agents are various phytochemicals based nanostabilizers present in the coconut shell. These phytochemicals are responsible for the creation of a robust coating on the gold nanoparticles and thus rendering the nanoparticles highly stable against agglomeration. This infers the presence of stabilizing molecules on nanoparticles (Fig. 6a). EDAX results gives a clear indication regarding the other elements involved in the synthesis of nanoparticles. The presence of some carbon and oxygen along with numerous gold signals suggests the presence of biomolecules in the gold nanoparticles, which were not observed in many biosynthesis of nanoparticles (Fig. 6b). These results are in agreement with previous report (Babu *et al*., 2011).



(a) **(b)**

Figure 6: **(a)** SEM and (**b)** EDAX images showing the presence of gold nanoparticles and bio-organic nanostabilizers of coconut shell.

To the best of our knowledge, this is the first report on synthesis of mono disperse and spherical gold nanoparticles using coconut shell extract for biomedical applications. The synthesized gold nano-particles also exhibited excellent activity against different bacteria, namely *S. aureus, E. coli and S. typhi* (Fig. 7).

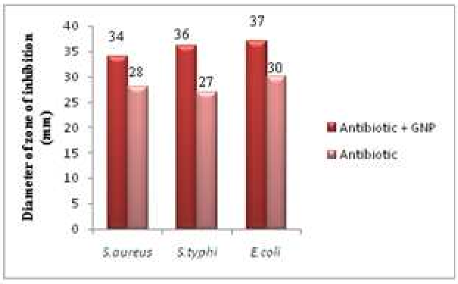


Figure 7: Antibacterial activity of Gold nanoparticles against various pathogens

The antibacterial study indicates that antibiotic with gold nanoparticles extracted from coconut shell extract exhibits more zone of inhibition when compared with standard antibiotic Norfloxacin. Gold nanoparticles are harmful to bacteria and fungi. They bind closely to the surface of microorganisms, causing visible damage to cells, with complete destruction of flagella, stimulated production of biofilm, and aggregation within the biofilm (Ghosh *et al*., 2008). Bindhu and Umadevi, 2014 recently synthesized gold nanoparticles that revealed optimum bacterial activity against gram positive and negative pathogens, which are found in water. Rajeev *et al*. (2011) studied the antimicrobial activity of *Cocos nucifera* endocarp extracts (Rajeev *et al*., 2011). They showed that the shell extracts exhibits strong activity against *B. subtilis, P. aeruginosa, S. aureus and M. luteus*. The activity was mainly attributed to the high content of phenolic compounds. Gu *et al*. synthesized stable gold nanoparticles covered with vancomycin and showed significant enhancement of antibacterial activity for this conjugate, in comparison with the activity of the free antibiotic (Gu *et al*., 2003). Our study indicates that antibiotic with gold nanoparticles extracted from coconut shell extract exhibit more zone of inhibition compared to standard antibiotics used. Moreover, our antimicrobial study results suggest that coconut shell mediated GNPs could act as an effective antimicrobial agent and prove as an alternative for the development of new antimicrobial agents to combat resistance problem, as well as aid to engineering of drugs for anticancer applications

CONCLUSION

Biocompatible **s**ynthesis of AuNPs using coconut shell extract resulted in spherical, monodisperse gold nanoparticles of average size 10–15 nm, which may have the potential to be used in anti-hypertensive applications. Overall, this novel one-pot method is rapid, cost effective, non-toxic, eco-friendly, stable and these features make it more attractive for biomedical applications.

Furthermore the low cost of the method as well as its simplicity and efficiency offers an alternative to other methods used for gold nanoparticle synthesis.

REFERENCES

Akhter, A., Hossain, M. K., Khatun, M. S., Rabbi, M. A., and Khatun, M. H.(2014). Dyeing effect on silk-fabric with vegetable dye using green-coconut (cocos nucifera) shell. IOSR Journal of Applied Chemistry.7:23-26.

Ali, M. A. and Akhter, A.(1997).Yellow-orange azo dye : Bangladesh Patent serial no.1002786

Alric, C., Taleb, J., Duc, G. L., Mandon, C., Billotey, C., Meur-Herland, A. L., and Tillement, O. (2008). Gadolinium chelate coated gold nanoparticles as contrast agents for both X-ray computed tomography and magnetic resonance imaging. Journal of the American Chemical Society. 130: 5908-5915.

Andeani, J. K., Kazemi, H., Mohsenzadeh, S., and Safavi, A. (2011). Biosynthesis of gold nanoparticles using dried flowers extract of Achillea wilhelmsii plant. Dig J Nanomater Bios. 6: 1011-1017.

Babu, P. J., Das, R. K., Kumar, A., and Bora, U. (2011). Microwave-mediated synthesis of gold nanoparticles using coconut water. International Journal of Green Nanotechnology. 3: 13-21.

Babu, P. J., Sharma, P., Kalita, M. C., and Bora, U. (2011). Green synthesis of biocompatible gold nanoparticles using Fagopyrum esculentum leaf extract. Frontiers of Materials Science. 5: 379-387.

Bankar, G. R., Nayak, P. G., Bansal, P., Paul, P., Pai, K. S. R., Singla, R. K., and Bhat, V. G. (2011). Vasorelaxant and antihypertensive effect of Cocos nucifera Linn. endocarp on isolated rat thoracic aorta and DOCA salt-induced hypertensive rats. Journal of Ethnopharmacology. 134: 50-54.

Bhattacharya, R., and Mukherjee, P. (2008). Biological properties of “naked” metal nanoparticles. Advanced drug delivery reviews. 60: 1289-1306.

Bindhu, M. R., and Umadevi, M. (2014). Antibacterial activities of green synthesized gold nanoparticles. Materials Letters. 120: 122-125.

Bindhu, M. R., Rekha, P. V., Umamaheswari, T., and Umadevi, M. (2014). Antibacterial activities of Hibiscus cannabinus stem-assisted silver and gold nanoparticles. Materials Letters. 131: 194-197.

Burygin, G. L., Khlebtsov, B. N., Shantrokha, A. N., Dykman, L. A., Bogatyrev, V. A., and Khlebtsov, N. G. (2009). On the enhanced antibacterial activity of antibiotics mixed with gold nanoparticles. Nanoscale research letters. 4: 794-801.

Cabuzu, D., Cirja, A., Puiu, R., and Mihai Grumezescu, A. (2015). Biomedical Applications of Gold Nanoparticles. Current topics in medicinal chemistry. 15: 1605-1613..

Chamundeeswari, M., Sobhana, S. S., Jacob, J. P., Kumar, M. G., Devi, M. P., Sastry, T. P., and Mandal, A. B. (2010). Preparation, characterization and evaluation of a biopolymeric gold nanocomposite with antimicrobial activity.Biotechnology and applied biochemistry. 55:29-35.

Chen, M., Cai, Y., Yan, Z., and Goodman, D. W. (2006). On the origin of the unique properties of supported Au nanoparticles. Journal of the American Chemical Society. 128:6341-6346.

Cui, Y., Zhao, Y., Tian, Y., Zhang, W., Lü, X., and Jiang, X. (2012). The molecular mechanism of action of bactericidal gold nanoparticles on Escherichia coli. Biomaterials. 33: 2327-2333.

Curry, T., Kopelman, R., Shilo, M., and Popovtzer, R. (2014). Multifunctional theranostic gold nanoparticles for targeted CT imaging and photothermal therapy. Contrast media and molecular imaging, 9: 53-61.

Das, S. K., Dickinson, C., Lafir, F., Brougham, D. F., and Marsili, E. (2012). Synthesis, characterization and catalytic activity of gold nanoparticles biosynthesized with Rhizopus oryzae protein extract. Green Chemistry. 14:1322-1334.

Dubey, S. P., Lahtinen, M., and Sillanpää, M. (2010). Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of Rosa rugosa. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 364: 34-41

F Jiao, P., Y Zhou, H., X Chen, L., and Yan, B. (2011). Cancer-targeting multifunctionalized gold nanoparticles in imaging and therapy. Current medicinal chemistry. 18:2086-2102.

Ghosh, P., Han, G., De, M., Kim, C. K., and Rotello, V. M. (2008). Gold nanoparticles in delivery applications. Advanced drug delivery reviews. 60: 1307-1315.

Gu, H., Ho, P. L., Tong, E., Wang, L., and Xu, B. (2003). Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano letters. 3:1261-1263.

Guo, R., Song, Y., Wang, G., and Murray, R. W. (2005). Does core size matter in the kinetics of ligand exchanges of monolayer-protected Au clusters?. Journal of the American Chemical Society. 127: 2752-2757.

Hainfeld, J. F., Slatkin, D. N., Focella, T. M., and Smilowitz, H. M. (2014). Gold nanoparticles: a new X-ray contrast agent. The British journal of radiology. 79:248-253.

Jongjinakool, S., Palasak, K., Bousod, N., and Teepoo, S. (2014). Gold Nanoparticles-based Colorimetric Sensor for Cysteine Detection. Energy Procedia. 56: 10-18.

Khalil, M. M., Ismail, E. H., and El-Magdoub, F. (2012). Biosynthesis of Au nanoparticles using olive leaf extract: 1st nano updates. Arabian Journal of Chemistry. 5: 431-437.

Kumar, V. G., Gokavarapu, S. D., Rajeswari, A., Dhas, T. S., Karthick, V., Kapadia, Z., and Sinha, S. (2011). Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent Cassia auriculata. Colloids and Surfaces B: Biointerfaces. 87: 159-163.

Philip, D. (2010). Green synthesis of gold and silver nanoparticles using Hibiscus rosa sinensis. Physica E: Low-dimensional Systems and Nanostructures. 42: 1417-1424.

Philip, D. (2010). Rapid green synthesis of spherical gold nanoparticles using Mangifera indica leaf. Spectrochimica Acta Part A: Molecular and Biomolecular spectroscopy.77:807-810.

Philip, D., Unni, C., Aromal, S. A., and Vidhu, V. K. (2011). Murraya koenigii leaf-assisted rapid green synthesis of silver and gold nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 78: 899-904.

Rajeev, K. S., Nitesh, J., Varadaraj, B. G., and Hitesh, J. (2011). Antioxidant and Antimicrobial Activities of Cocos nucifera Linn.(Arecaceae) Endocarp extracts. India. Indo Global J. Pharmaceutical Sci. 1: 354-361.

Raveendran, P., Fu, J., and Wallen, S. L. (2003). Completely “green” synthesis and stabilization of metal nanoparticles. Journal of the American Chemical Society. 125:13940-13941.

Singh, S., Vidyarthi, A. S., Nigam, V. K., and Dev, A. (2014). Extracellular facile biosynthesis, characterization and stability of gold nanoparticles by Bacillus licheniformis. Artificial cells, nanomedicine, and biotechnology. 42: 6-12.

Singla, R. K, Jagani, H. (2012) Investigation of Antimicrobial Effect of Dry Distilled Extract of Cocos Nucifera Linn Endocarp. Webmed Central Pharmaceutical Sciences. 3(8):WMC003671

Smitha, S. L., Philip, D., and Gopchandran, K. G. (2009). Green synthesis of gold nanoparticles using Cinnamomum zeylanicum leaf broth. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 74:735-739.

Suganya, K. U., Govindaraju, K., Kumar, V. G., Dhas, T. S., Karthick, V., Singaravelu, G., and Elanchezhiyan, M. (2015). Blue green alga mediated synthesis of gold nanoparticles and its antibacterial efficacy against Gram positive organisms. Materials Science and Engineering. 47: 351-356.

Tamuly, C., Hazarika, M., Borah, S. C., Das, M. R., and Boruah, M. P. (2013). In situ biosynthesis of Ag, Au and bimetallic nanoparticles using Piper pedicellatum C. DC: Green chemistry approach. Colloids and Surfaces B: Biointerfaces. 102: 627-634.

Tiwari, P. M., Vig, K., Dennis, V. A., and Singh, S. R. (2011). Functionalized gold nanoparticles and their biomedical applications Nanomaterials. 1:31-63.

Venkataraman, S., Ramanujam, T. R., and Venkatasubbu, V. S. (1980). Antifungal activity of the alcoholic extract of coconut shell—Cocos nucifera Linn. Journal of Ethnopharmacology. 2: 291-293.

Wiley, B. J., Im, S. H., Li, Z. Y., McLellan, J., Siekkinen, A., and Xia, Y. (2006). Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. The Journal of Physical Chemistry B. 110: 15666-15675.