Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 7
ISSN: 2321–3647(online)

Biosynthesis, antimicrobial effect and DNA damage study of gold nanoparticles with *Cinnamomum zeylanicum*

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ABSTRACT

Objective of the study was to synthesize gold nanoparticles using bark of *Cinnamomum zeylanicum* and study its antimicrobial property and DNA damage. Gold nanoparticles were synthesized using powdered aqueous extract of bark of *Cinnamomum zeylanicum*. Green synthesis method was used. It was done using two different concentrations of Chloroauric acid 2mM and 1mM. Characterization studies were done with the synthesized nanoparticles. They were also checked for its antibacterial and antifungal activity through microbiological techniques. Also the plant extract and solution containing were tested for DNA damage through Agarose gel electrophoresis. *Cinnamomum zeylanicum* supported the synthesis of gold nanoparticles. Characterization studies like FTIR, UV – VIS spectroscopy, SEM, and XRD were carried out. GNPs in the range of 35 – 80nm were obtained. The antibacterial and antifungal study showed clear zones of inhibition of microbial growth. The image projected through UV – Transilluminator proves that there is no DNA damage. The results indicate that *Cinnamomum zeylanicum* is a good support plant for synthesis of gold nanoparticles and also the biologically synthesized GNPs has antimicrobial activity and also it is safe since it does not damage DNA.

Keywords: *Cinnamomum zeylanicum*, gold nanoparticles, green synthesis, characterization, antimicrobial assay, DNA damage study

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Received 08 June 2016, Accepted 16 July 2016

Please cite this article as: Pulikkottil SS *et al*., Biosynthesis, antimicrobial effect and DNA damage study of gold nanoparticles with Cinnamomum zeylanicum. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Nano technology is an emerging field which offers a wide range of scope for research. Amongst variable nanomaterials and fields of their application, metal nanoparticles can be distinguished as the most popular in biology and medicine with gold (AuNPs) and silver (AgNPs) nano particles playing a prominent role.[1] Nowadays many methods of their synthesis have been elaborated in different studies. Generally, gold nanoparticles are produced with the help of liquid chemical methods. It involves reduction of chloroauric acid (H[AuCl4]). The common methods of preparation of gold nano particles are Turkevich method and Brust method. The method found by J. Turkevich et al. in 1951 and modified by G. Frens in 1970s, is the simplest. It is used to produce modestly monodisperse spherical gold nanoparticles suspended in water of around 10–20 nm in diameter. It involves the reaction of hot chlorauric acid and sodium citrate solution, result of which the colloidal gold will be formed. This is because the citrate ions act as reducing agent and a capping agent. Brust method was discovered by Brust and Schiffrin. It is used to produce gold nanoparticles in organic liquids. The reaction of a chloroauric acid solution with tetraoctylammonium bromide (TOAB) solution in toluene results in synthesis of nano particles. Sodium borohydride acts as an anti-coagulant and a reducing agent, respectively in this case.

Since the support of the new generation is for biological substances rather than chemical substances, this research work also attempts to fulfill the same. The material used for synthesis of gold nano particle is cinnamon, the common ingredient of our delicious Indian dishes.

Cinnamomum zeylanicum is an easily available variety, commonly called as Ceylon cinnamon. The bark extracts were easily obtained from the fields of spices board, Calicut. The ability of cinnamon to diminish the effect of gold nano particles has been proved.[2] These aspects led to the selection of this spice for the present research. Cinnamon has been used to synthesize gold nanoparticles and its antibacterial and antifungal activity has also been studied along with DNA damage studies.

MATERIALS AND METHOD

Chemical

Synthesis of gold nanoparticles was done with Chloroauric acid, purchased from Sigma Aldrich. Nutrient agar and potassium dextrose were purchased from Himedia which were used for plating. Agarose gel, TAE buffer, ethylene bromide from Himedia was used for Gel electrophoresis.

Preparation of plant extract

The barks of *Cinnamomum zeylanicum* was collected from the cinnamon fields of Spices board of India, Calicut, Kerala. It was dried and powdered. The powdered cinnamon was analyzed through gas chromatography to find out the components. Concentrated aqueous extract was obtained when the powder was mixed (in grams) with double distilled water (in ml) in the ratio 1:14. The mixed solution was kept in the water bath maintained at 90^0C for 1 hour. The filtrate was obtained by filtering with muslin cloth. The filtrate was centrifuged for 10 min at 4600 rpm for getting a clear supernatant. The clear supernatant was stored for further use.

Synthesis of gold nano particles:

Synthesis was done using the green synthesis method. 2mM solution and 1mM solutions of HAuCl4 were prepared using distilled water and anhydrous Cloroauric acid (HAuCl4) was purchased from Sigma Aldrich. 5ml of plant extract were added to 10ml of 2mm and 1mm solutions.[6] The solutions were kept in darkness for 24 hours. Then the samples were studied for identification and characterization of nanoparticles using UV – Vis spectroscopy, FTIR, XRD and SEM.

Microbial plating:

Microbial plating was done for antibacterial and antifungal studies. Nutrient agar medium and Potato dextrose medium were used for bacteria and fungi respectively. The medium and the equipments were sterilized for 121^0c for 15 minutes and plating was done with well diffusion method. 100µl of cultures of bacteria were spread on petri plates containing nutrient agar and fungi were spread on potato dextrose medium. Wells were punched in the medium with sterile cork borer. 50µl of samples along with standard ofloxacin discs were inoculated into the wells and the plates were incubated at 37^0c overnight.[4] Petri plate inoculated with fungi was kept in room temperature. E.coli, Klebsiella and Staphylococcus are the bacteria used for studies and *Aspergillus niger* was the fungi used for study.

DNA damage study:

The study was carried out through agarose gel electrophoresis. The wells were filled with marker DNA, plant extract and Nano particles and were run at 50 v. After running the gel, the DNA damage was studied using UV Transilluminator. The purpose of this study was to check if the synthesized product can be used on animals.

RESULTS AND DISCUSSION

Gas chromatography results on analysis of powdered cinnamon bark.

Cinnamon bark powder was analyzed using gas chromatography to find out the components (table 1). Equipment used was THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II and the column was MS CAPILLARY STANDARD NON - POLAR COLUMN. Carrier gas was He with a flow rate of 1.0ml/min.

Table 1: Components present in the cinnamon bark powder analyzed through gas chromatography

No.	Name of the compound	Molecular Formula	MW	Compound Nature
1	ç-Terpinene	C10H16	136	Alkaloids
2	Cinnamaldehyde	C_9H_8O	132	Phenyl group
3	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (CAS)	C12H14O3	206	Alkaloids
4	cinnamaldehyde dimethyl acetal	C11H14O2	178	flavor and fragrance agents
5	*cis*-2-Methoxycinnamic acid	C10H10O3	178	Carbonyl Compounds; Carboxylic Acids
6	Caryophyllene	C15H24	204	essential oils
7	ortho methoxy cinnamic aldehyde	C10H10O2	162	Bio active compound
8	Tetradecanal	C14H28O	212	Myristic acid
9	9-Octadecenal	C18H34O	266	Aldehyde
10	trans-Z-à-Bisabolene epoxide	C15H24O	220	Sesquiterpene oxide
11	Campesterol	C28H48O	400	steroid,

Characterization of nano particles:

Color change

The first proof for synthesis of nano particles was the appearance of purple color. The change in colour was obtained within few minutes of addition of plant extract. After 24 hours, the color was intense with a powdered appearance of particles in solution (figure 1).

Figure 1: Appearance of purple color in solutions of 1mM and 2mM $HAuCl_4$ indicating the synthesis of gold nanoparticles

UV – Visible Spectroscopy:

The formation of gold nanoparticles was identified by scanning the solution containing gold nanoparticles at the wave length ranging from 400 – 700nm using Shimadzu UV – 1601 spectrophotometer. The maximum absorptions were obtained at a wave length of 460nm and 540nm in case of 1mm solution (figure 2) and 505nm and 420 nm in case of 2mm solution (figure 3). The SPR bands centered between 500 – 600nm, confirms the formation of GNPs in the solution. The appearance of the peak is due to the size dependent quantum mechanical phenomenon called Surface Plasmon Resonance (SPR). This effect become influential when the De – Brogile wavelength of the valence electrons becomes equal to or less than the size of the particle (less than 50nm).[3]

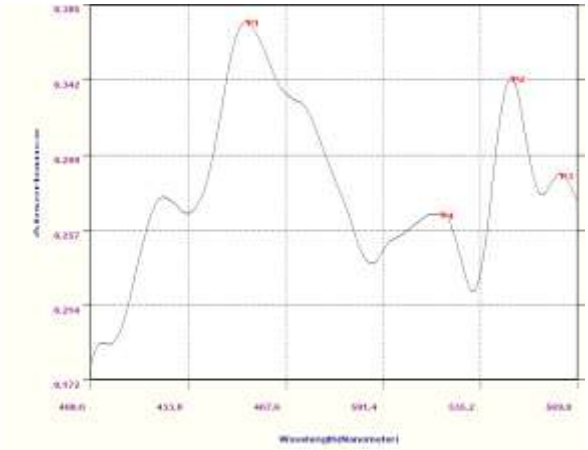

Figure 2: UV - Vis spectrum of 1mm gold nanoparticles

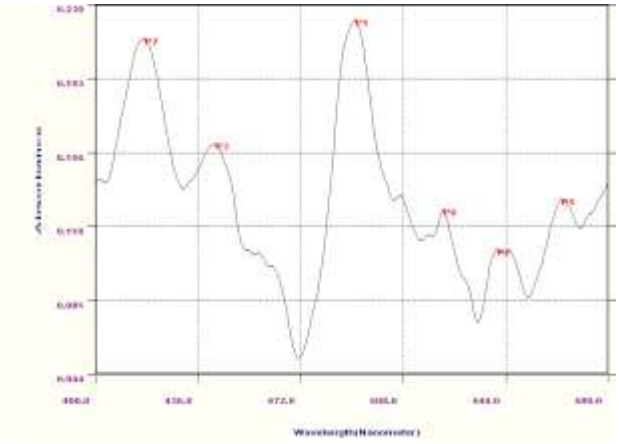

Figure 3: UV – Vis spectrum of 2mm gold nanoparticles.

EDX and Scanning Electron Microscopy Analysis:

Energy-dispersive X-ray (EDX) analysis was carried out using JEOL JEM 2100 high resolution transmission electron microscope to confirm the presence of gold in the particles as well as to detect other elementary compositions of the particles. Scanning Electron Microscopic (SEM)

analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, the extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The synthesis of gold nanoparticles using cinnamon extract was confirmed by the characteristic peak obtained in the EDX image and the structural view under scanning electron microscope.

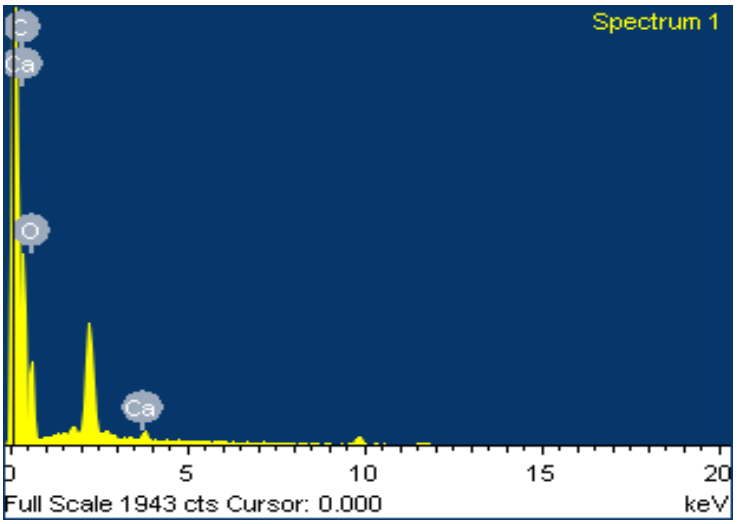

Figure 4: EDX image with four dominant peaks for C, Ca,O and gold

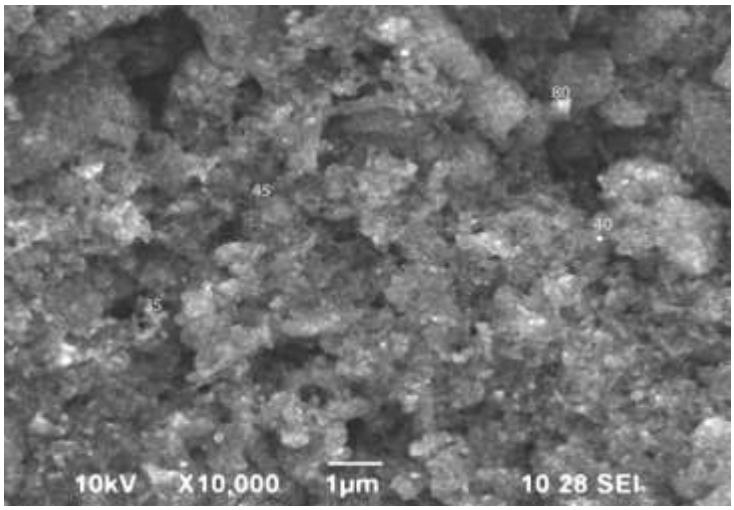

Figure 5: SEM showing NPs in the range of 35 –80 nm

FTIR Analysis

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm–1. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Shimadzu, Japan. The number of scans 16 and the resolution of 4 cm-1 characterized these measurements. The peaks show the presence of gold nanoparticles. Absorbance bands are observed in the region of 1200 -1800 cm^{-1}. The FTIR

spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins of plant extract has strong ability to bind metal, and most possibly might have formed a layer on the gold nanoparticles. Similar peaks were reported by other researchers' also.[6]

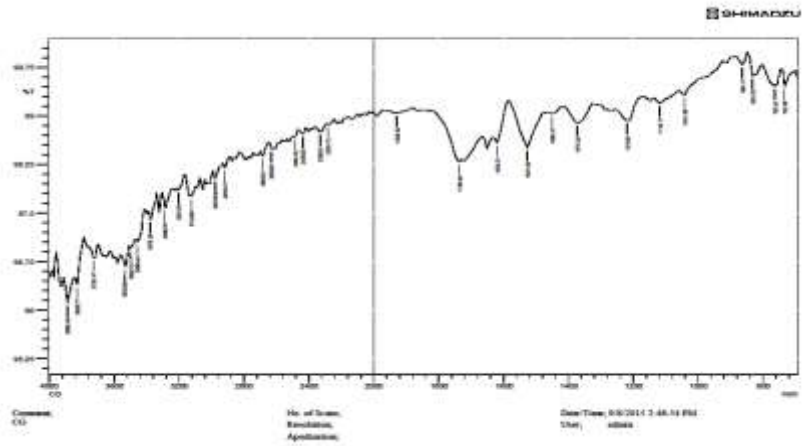

Figure 6: FTIR analysis of the solution containing gold nanoparticles

Anti Bacterial And Anti Fungal Activity

The microbial plating was done after all sterilizing techniques using well diffusion method. The bacteria used were *Klebsiella pneumoniae, E.coli, Staphylococcus aureus* and Fungi was *Aspergillus niger*. Bacterial plates were incubated and Fungi plate was maintained at room temperature. Efficient growth of bacteria with clear zones of inhibition of growth around the wells of nanoparticles was observed. The nanoparticles were efficient than the standard antimicrobial disc.

Table 2: Measurements of zone of inhibition of microbial growth in the petri plates (in mm)

Microorganism	1mM HAuCl4	2mM HAuCl4	1mM AUNP	2mMAUNP
S. aureus	0.5	1	2	5
K. pneumonia	1	1	1	2
E. coli	1	nil	4	3
A.niger	1	2	2	4
disc	nil			

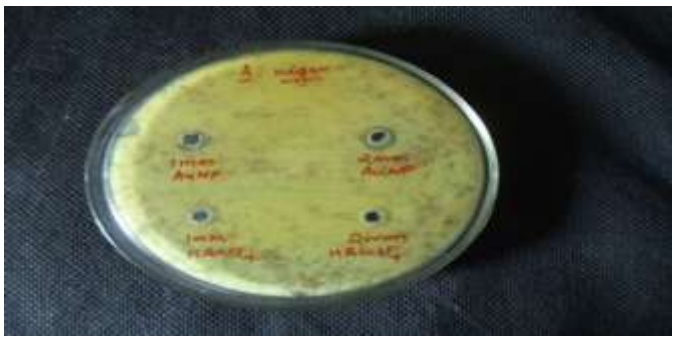

Figure 7: Antifungal activity of GNPs (A.niger)

Figure 8: antibacterial activity of GNPs (S.aureus)

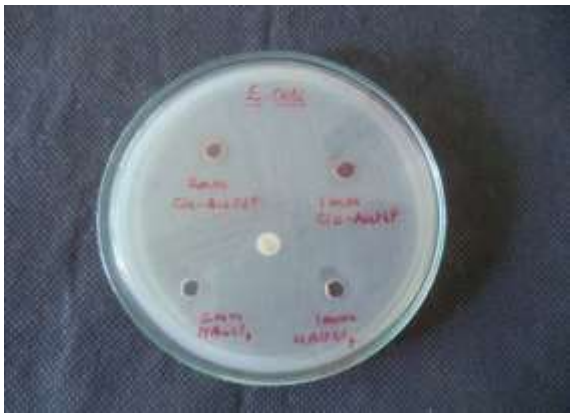

Figure 9: Antibacterial activity of GNPs (E.coli)

Figure 10: Antibacterial activity of GNPS (K.pneumoniae)

DNA damage study

DNA damage study is used to find out the toxicity of synthesized gold nanoparticles. All metals are known to have toxic effects on living cells. But in this research biologically synthesized gold nanoparticles do not show any damaging effect on the DNA. Therefore this can be used for various purposes on animals. The photograph (figure 11) shows clear orange color bands

representing the presence of DNA and the comparison was done between marker DNA, plant extract and biocompatible nanoparticles.

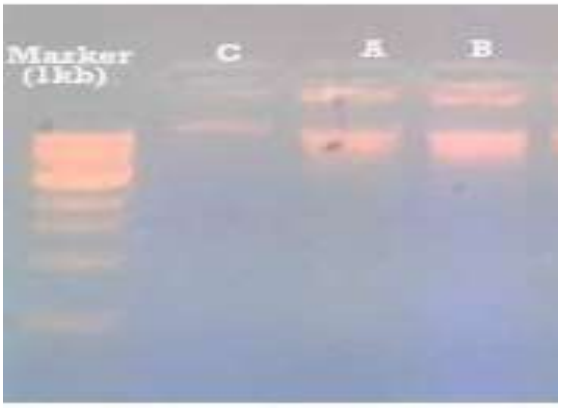

Figure 11: Photograph showing the illumination of the gel in UV –Transilluminator

C – Control, A- plant extract, B – GNPs

The synthesis of gold nano particles was effectively done using *Cinnamomum zeylanicum* following green synthesis method. The alakaloids, terpenes and phenyl groups were mainly responsible for the reduction of gold ions to Nano sized gold particles by capping around the gold particles.[6] Immediate color change was well observed in case of this extract. The color of the solution changed from yellow to purple color [5] which proves the effectiveness of the support provided by the plant extract in reduction and precipitation of gold ions. Kimling et al. [7] synthesized the gold nanoparticles using Turckevich method in the range of 9 – 120nm in the presence of sodium citrate and ascorbic acid whereas Bhargava et al. synthesized GNPs in the range of 5- 15 nm employing different amino acids.[8] The characterization using scanning electron microscopy shows that the nanoparticles formed in this research were in the size of 35 - 80nm.[5] The produced nanoparticles were stable for more than 2 months. The GNPs synthesized with cinnamon was efficient in proving its anti-microbial and antifungal activity. Three different bacteria were used for the study. The GNPs were more effective against *Staphylococcus aureus* than *Klebsiella* and *E.coli* (table 2). Also petri plates inoculated with bacteria were treated with 1mM solution GNPs and 2mM solution of GNPs. Nanoparticles synthesized in 2mM solution was more effective. Even in the studies for characterization and identification, 2mM solution of plant extract with $HAuCl_4$ showed effective synthesis of GNPs. Clear zones of inhibition of growth were obtained in case of 2mM solution of GNPs. DNA damage studies also shows that the biologically synthesized GNPs can be used effectively in the field of medicine, since there is no damage caused to the sample DNA.

CONCLUSION

The research work proves that the *Cinnamomum Zeylanicum* supports the synthesis of gold nanoparticles and also reduces the toxicity and makes it biocompatible. The gold nanoparticles synthesized with cinnamon possessed antimicrobial property. When compared the gold nanoparticles synthesized using 2mM solution were more effective. Also the DNA damage studies shows that the plants extract as well as the biocompatible gold nanoparticles do not cause damage to DNA, thus ensuring a scope to use the product in the field of medicine.

ACKNOWLEDGEMENT

Authors acknowledge Prof. Ragunathan, CNBR, Echanari, Coimbatore for all the support extended for carrying out the research work.

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