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Original Research

Nanopackaging of Silver using Spice Extract and their Characterization

Atiya Sameen[1], Syeda Juveriya Fathima[1], Shylaja Ramlal[2], Sajeev Kumar[3] and Farhath Khanum[1]*

[1]Biochemistry and Nanosciences Discipline, Defence Food Research Laboratory (DFRL), Siddharthanagar, Mysore-570011, India

[2]Department of Microbiology, Defence Food Research Laboratory (DFRL), Siddharthanagar, Mysore-570011, India

[3]Department of Engineering and Packaging, Defence Food Research Laboratory (DFRL), Siddharthanagar, Mysore-570011, India

Abstract

The aim of the present study was to synthesize silver nanoparticles using spice extracts as reducing agents and further evaluate their anti-microbial activities. Silver has been shown to possess antimicrobial activity. The silver nanoparticles were prepared by solvent evaporation method. The silver nanoparticles were characterized by UV-Vis spectroscopy. The functional groups present in the phyto-constituents on the plant extract were determined by FT-IR studies. The particle size of the silver nanoparticles was determined by Dynamic Light Scattering and was found to be 143, 50 and 56 nm for cloves, cinnamon and neem silver nanoparticles respectively. They exhibited antibacterial property against *Escherichia coli, Staphylococcus aureus and Salmonella typhi* strains, tested using Well Diffusion method. Plant extracts however reduce the antimicrobial activity of the nanoparticles. In conclusion, antimicrobial activities of silver nanoparticles were reduced by plant extracts certifies vital potential in biomedical application.

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***Corresponding Author:**
Farhath Khanum
E-mail:
farhathkhanum@gmail.com

INTRODUCTION

Packaging of food and drugs has been a topic of research from time immemorial probably the history of packaging is as ancient as the history of mankind. Bactericidal activity of silver has also been known since a long time and silver compounds have been used in external preparations such as antiseptics in wound dressings. Some reports show a reduction in urinary tract infections when silver alloy catheters were used (Beattie and Taylor, 2011) and reductions in ventilator associated pneumonia. Nanoencapsulation is the latest trend in packaging in order to protect food supplement and drugs, so that they are not degraded before reaching the target organs of the body for which they are intended to. Degraded supplements can be less effective, not sufficient and may not be even bioavailable.

Metal nanoparticles have received considerable attention in recent years because of their unique properties and potential applications in catalyst, plasmonics, optoelectrics, biological sensors and pharmaceutical applications. Among various metal nanoparticles, silver nanoparticles and gold nanoparticles have several effective applications as bactericidal, sensors and detectors besides their biomedical application (Ojha *et al*., 2012 and Vivekanandhan *et al.,* 2012). Various plant extracts have been used as potential reductants in silver nano synthesis (Ojha *et al*., 2012), in place of toxic chemicals such as chemical reduction and photoreduction in reverse micelles (Pileni *et al*., 2000 and Sun *et al*., 2001), and radiation chemical reduction (Henglein *et al.,* 2001). These methods are expensive and involve toxic, hazardous chemicals which may pose serious biological and environmental risks.

Spices have been the spice of life of man since time immemorial and therefore there is no doubt on their toxicity. The captivating aroma and pungency are very appetizing and have become indispensable in the preparation of palatable dishes. In addition they possess antibacterial and medical/health benefits (Iyer *et al*., 2009, Nillius and Appendino., 2013). We therefore used the extracts of some of the spices like cloves; cinnamon and neem leaves to green synthesize the silver nanoparticles and evaluated the antimicrobial properties.

MATERIALS AND METHODS

Plant extract of *Azadirachta indica* (Neem), *Cinnamomum verum* (Cinnamon) and *Syzygium aromaticum* (Cloves). Butylated hydroxyanisole (BHA) was purchased from sigma. All organic solvents were of HPLC grade, and other chemicals were of analytical grade.

Preparation of Extracts

Azadirachta indica (Neem), *Cinnamomum verum* (Cinnamon) and *Syzygium aromaticum* (Cloves) were washed and cleaned with distilled water and dried with water absorbent paper, crushed with mortar and pestle, 100g of plant/spice powder were dispensed in 1000 ml of distilled water and heated for 30 minutes at 70-80ºC. The extract was then filtered using Whatman No. 1 filter paper. The filtrated was collected and was stored at 4ºC (Ojha *et al*., 2012).

Synthesis of Silver Nanoparticles

Silver Nanoparticles were prepared using Solvent Evaporationn method. During the synthesis of silver nanoparticles both the precursor and the reducing agent were mixed in a clean sterilized flask. For the reduction of Ag+ ions, 0.2 µl of filtered plant extract was mixed with 5ml of $AgNO_3$ solution with constant magnetic stirring for 45 to 60 min. The silver nanoparticles thus prepared were stabilized by adding 1% of BHA (Ojha *et al*., 2012).

UV-Vis Spectra Analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles. The reduction of pure Ag+ to Ag^o was monitored by adjusting the pH from 4-9 and measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of Ag nanoparticles solution diluting the sample in 3 ml distilled water. UV-Vis spectra analysis was carried out using UV-Vis spectrophotometer. Sehimadzu observed the peak (Ojha *et al*., 2012).

FT-IR Studies

Infrared Spectra of all the lyophilized samples were measured using Thermo Nicolet FTIR Spectrometer, Madison USA, Model No. 5700. All spectra were recorded using ATR accessory with ZnSe as Internal Reflection Element (IRE). The spectra were recorded using lyophilized samples in transmission mode with 4 cm-1 resolution and averaging 32 scans.

Dynamic Light Scattering (DLS) Analysis

Particle size and size distribution of AgNPs was measured by dynamic laser scattering technique (DLS) using Malvern Zetasizer (Model no: nano-ZS90, Malvern Instruments, UK). Each sample was measured in triplicate. The particle size distribution of the nanoparticles is given as polydis-persity index (PDI). Around 1 ml of sample was taken in DLS cuvette and analyzed at 25°C with an angle of 90°.

Antimicrobial Activity

The antibacterial activities of the plant extracts reduced AgNPs were studied by well diffusion method. Luria bertoni or Nutrient Agar media was used, sterilized and solidified. Three bacterial strains (*E. coli, S. aureus, S.typhi*) were swabbed onto the plates. Wells were made and AgNPs solutions with different concentrations (50, 100 and 150 µl) were dipped and kept for incubation at 37ºC for 24hrs. Zone of inhibition for Control, AgNPs and $AgNO_3$ were measured (Ojha *et al*., 2012).

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

Clove, Cinnamon and Neem extracts were used to produce silver nanoparticles. Ag ions are reduced into Ag^o nanoparticles when plant extract is mixed with $AgNO_3$ solution. Reduction is followed by an immediate change in colour. It is well known that silver nitrate exhibit colorless appearance in distilled water. On mixing the plant extract with aqueous $AgNO_3$ solution it changed the colour of the solution immediately. Table 1 show the synthesized nanoparticles change in color before and after reduction of Ag ions.

Table 1: Synthesis of Silver Nanoparticles

No	Solution	Color change		Color Intensity	
		Before Reduction	After Reduction		
1	Clove extract	Dark reddish brown	Dark grey	Dark	
	0.1 M $AgNO_3$	Colorless			
2	Cinnamon extract	Light yellow	Faint yellow	Faint	
	0.1 M $AgNO_3$	Colorless			
3	Neem extract	Light Green	Light brown	Light	
	0.1 M $AgNO_3$	Colorless			

UV- Visible Spectroscopy

Silver nanoparticles absorb and scatter light with extraordinary efficiency. Their strong interaction with light occurs because the conduction electrons on the metal surface undergo a collective oscillation when they are excited by light at specific wavelengths. This oscillation is known as a surface plasmon resonance (SPR), and it causes the absorption and scattering intensities of silver nanoparticles to be much higher than identically sized non-plasmonic nanoparticles. It is well known that silver nanoparticles exhibit a yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Jae and Beom., 2009 and Forough and Farhadi., 2010). The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample with distilled water. The absorption spectrum of silver nanoparticles solution showed a surface Plasmon absorption band with a maximum of 413, 446, 515 nm for Cloves, Cinnamon and Neem respectively indicating the presence of silver nanoparticles. The UV spectra of Cloves, Cinnamon and Neem AgNPs are shown in Figure 1, 2, 3 respectively.

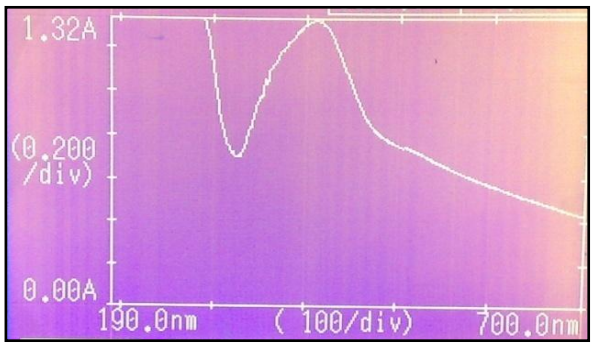


Figure 1: UV spectra of Cloves AgNPs

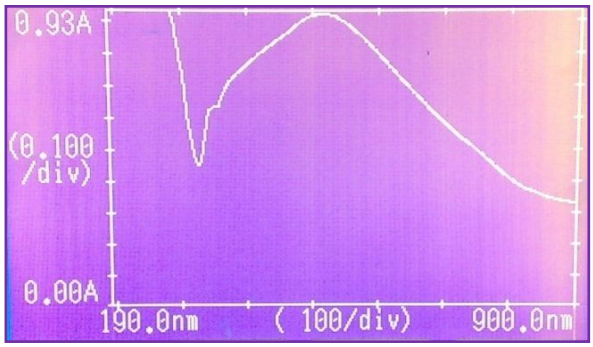


Figure 2: UV spectra of Cinnamon AgNPs

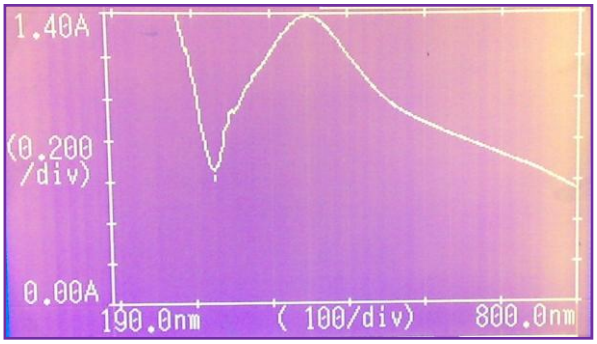


Figure 3: UV spectra of Neem AgNPs

DLS Analysis

The DLS size distribution of cloves AgNPs (pH 8) is shown in Figure 4. From the results, the calculated average particle size distribution of AgNPs was found to be 143nm. The size distribution of cinnamon AgNPs (pH 8) is shown in Figure 5. From the results, the calculated average particle size distribution of AgNPs was found to be 50nm. The DLS size distribution of neem AgNPs (pH 8) is shown in Figure 6. From the results, the average particle size distribution of AgNPs was found to be 56nm.

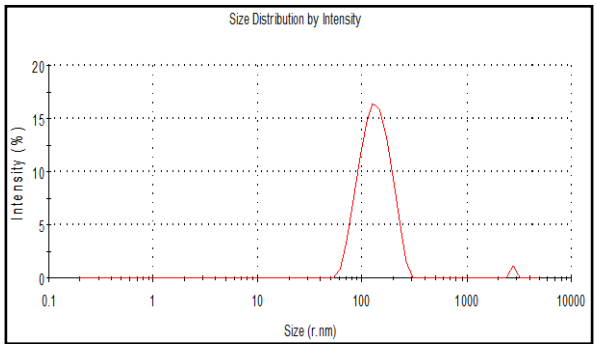


Figure 4: DLS of cloves AgNPs

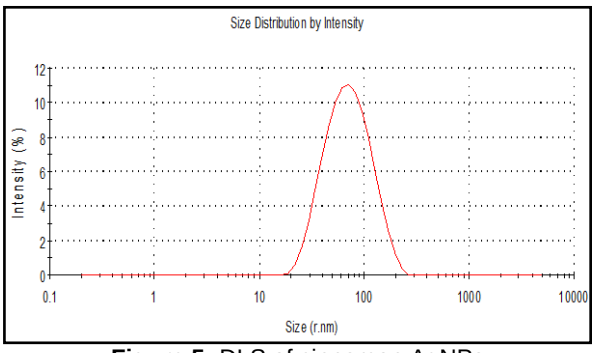


Figure 5: DLS of cinnamon AgNPs

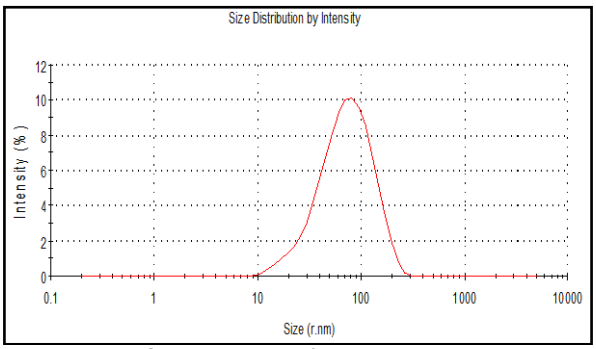


Figure 6: DLS of neem AgNPs

FTIR Analysis

Infrared Spectral analysis of the samples was carried out to identify the probable molecules responsible for capping the Ag nanoparticles formed. In Figure 7 is shown the FTIR spectra of silver nitrate, clove extract and the system after reduction of Ag NO_3. The absence of band around 1306 cm-1 (due to $AgNO_3$) in the clove plant extract after reduction of $AgNO_3$, indicates the absence of Ag^+ ions in the system. Since the UV Visible spectra indicated the presence of silver nanoparticles (Surface Plasmon Resonance at 413 nm), complete reduction of Ag+ ions could be expected in this system. The prominent IR bands of the clove plant extract system with silver nanoparticles are 1697 cm-1 and 1098 cm^{-1} which could be due to C=O stretching vibration and C-O stretching vibration. These bands have been found to be shifted towards a lower wave number from the corresponding bands of clove extract alone, indicating stronger interaction between the capping agent and the silver nanoparticles.

In Figure 8 and 9 are shown the FTIR spectra of Cinnamon plant extract system and Neem plant extract system after reduction of Ag NO_3. In both these cases, it can be seen that the band around 1306 cm-1 (due to $AgNO_3$) is very prominent in the plant extract nanoparticle system. This indicates the presence of Ag+ ions in the mixture along with the Ag nanoparticles. The presence of Ag nanoparticles have been confirmed by the UV spectral studies. In both these plant extract nanoparticle system a small band around 2255 cm-1 was appearing confirming the presence of an Ag+ ions. In these cases also a shift of C=O stretching vibration band to lower wave number is seen in their respective extract nano particle system indicating strong interaction between the capping agent and the silver nano particles. It has been reported that flavonoids and terpenoids in plant extracts are mainly responsible for capping the nanoparticles (Huang *et al*., 2007 and Priya *et al*., 2014). In our present studies also flavonoids and terpenoids could be mainly responsible for

efficient capping and there by stabilization of the silver nano particles generated. Thus the FTIR studies indicated that clove extract is a better system for complete reduction of silver nitrate than the other two plant extract. However, in all the cases stabilization of the nano particle is effected through flavonoids and terpenoids.

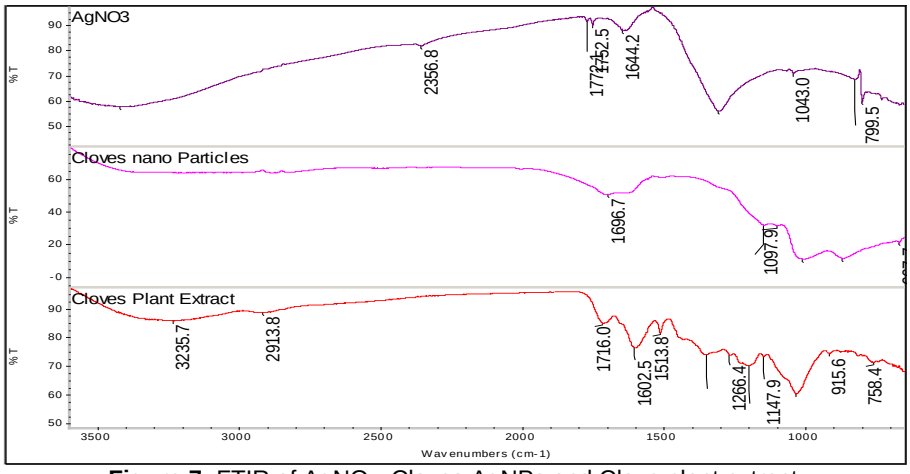


Figure 7: FTIR of $AgNO_3$, Cloves AgNPs and Clove plant extract

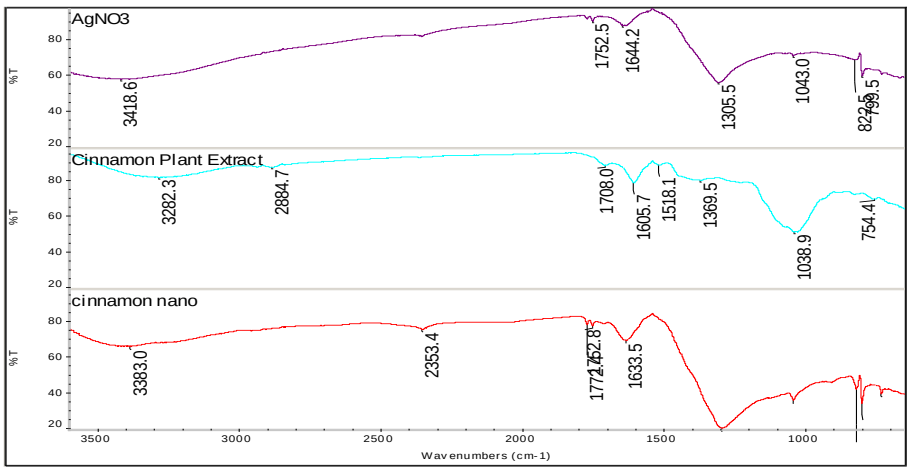


Figure 8: FTIR of $AgNO_3$, Cinnamon AgNPs and Cinnamon plant extract

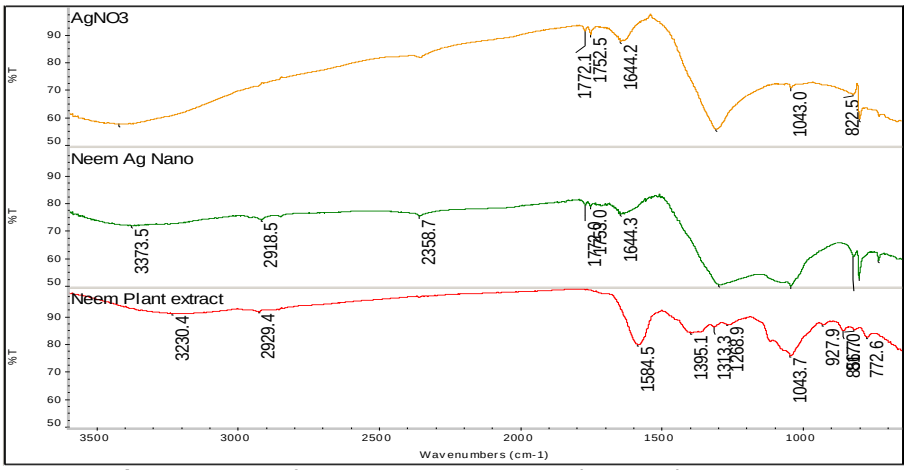


Figure 9: FTIR of $AgNO_3$, Neem AgNPs and Neem plant extract

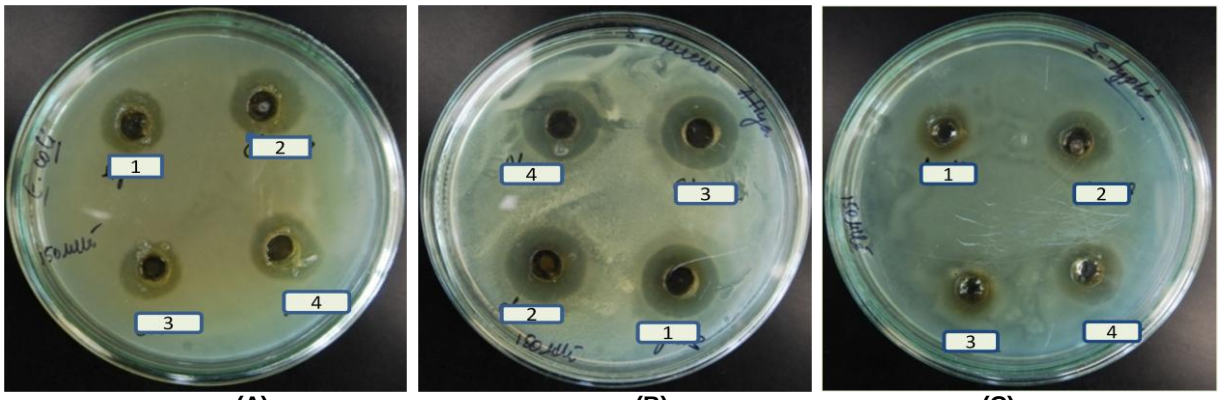
Antimicrobial Activity

The antimicrobial effect of silver nanoparticles reduced by cloves, cinnamon and neem extract has been studied against three bacterial strains by well diffusion method and the results are shown in Table 2. The silver nanoparticles has shown activity against the tested microorganism and the maximum zone of inhibition was found against in bacterial growth with maximum resistance against *S. aureus* suggesting that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane and ultimately cell death.

Silver nanoparticles activity was compared with the silver nitrate and plant extract. Silver nitrate solution has shown the maximum activity against *S. aureus* with inhibition zone of 6 and 5 mm was observed for silver nanoparticle against *S. aureus*.

Table 1: Synthesis of Silver Nanoparticles

No		Inhibition Zone (mm)								
		E.coli			*S. aureus*			*S.typhi*		
		50µl	100µl	150µl	50µl	100µl	150µl	50µl	100µl	150µl
1	Control	3	3	3	3	3	3	2	2	2
2	Cinnamon	3	4	3	6	4	6	3	2	2
3	Clove	2	4	3	5	5	6	2	3	4
4	Neem	3	3	4	6	5	6	3	2	5
5	$AgNO_3$	4	4	4	6	5	5	4	4	4



(A) **(B)** **(C)**

Figure 10: Antibacterial activity of Ag Nanoparticles (Nps) A) Against *E.coli* B) Against *S.aureus* C) Against *S.typhi* (1- $AgNO_3$; 2- Cloves Nps; 3- Cinnamon Nps; 4- Neem Nps)

CONCLUSIONS

The study has demonstrated that cloves, cinnamon and neem extract is capable of producing silver nanoparticles. $AgNO_3$ with reducing agent that is plant extract has shown a color change with concerned change in pH. The metal ions reduction occurs, and the reduction of Ag ions will be completed within 1 hour. DLS studies had shown that the synthesized Silver Nanoparticles are having the size around 143, 50 and 56 nm for cloves, cinnamon and neem silver nanoparticles respectively. Antimicrobial activities of silver nanoparticles reduced by plant extracts certify the vital potential in biomedical application. Silver nanoparticles have been used in numerous technologies and can be incorporated into a wide array of consumer products with a desirable advantage in enhancing optical spectroscopy including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS), in conductive inks, in composites to enhance thermal and electrical conductivity, in plastics for their antibacterial properties and also in biosensors and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection.

REFERENCES

Beattie, M. and Taylor, J. (2011). Silver alloy versus uncoated urinary catheters: a systematic review of the literature. *Journal of Clinical Nursing* 20(15-16): 2098-108.

Henglein, A. (2001). Reduction of Ag(CN)−2 on Silver and Platinum Colloidal Nanoparticles. *Langmuir* 17: 2329-33.

Huang, Q., Li, D., Sun, Y., Lu, Y., Su, X., Yang, H., Wang, Y., Wang, W., Shao, N., Hong, J. and Chen, C. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology* 18:105104.

Iyer, A., Panchal, O.S., Poudyal, H., Brown, L. (2009). Potential health benefits of Indian spices in the symptoms of the metabolic syndrome, a review. *Indian Journal of Biochemistry and Biophysics* 46(6): 467-481.

Jae, Y.S. and Beom, S.K. (2009). Rapid Biological Synthesis of Silver Nanoparticles Using Plant Leaf Extracts, *Bioprocess Biosystem Engineering* 32: 79-84.

Ojha, A.K., Behera, S., Rout, J., Dash, M.P. and Nayak, P.L. (2012). Green synthesis of silver nanoparticles from syzygium aromaticum and their antibacterial efficacy. *International Journal of Applied Pharmaceutical Sciences and Bio Medical Sciences*, 1(4), 335-341.

Nilius, B. and Appendino, G. (2013). Spices: The savory and beneficial science of pungency. *Reviews of Physiology Biochemistry and Pharmacology* 164:1-76.

Mehrdad Forough and Khalil Farhadi. (2010). Biological and green synthesis of silver nanoparticles. *Turkish Journal of Engineering and Environmental Science* 34: 281- 287.

Pileni, M.P. (2000). Fabrication and Physical Properties of Self-Organized Silver Nanocrystals, *Pure* and *Applied Chemistry* 72: 53-65.

Priya Banerjee., Mantosh Satapathy., Aniruddha Mukhopahayay. and Papita Das. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing* 1: 3.

Sun, Y.P., Atorngitjawat, P. and Meziani, M.J., (2001). Preparation of Silver Nanoparticles Via Rapid Expansion of Water in Carbon Dioxide Microemulsion into Reductant Solution. *Langmuir* 17: 5707-5710.

Vivekanandhan, S., Christensen, L., Misra, M. and Mohanty, A.K. (2012). Green process for impregnation of silver nanoparticles into microcrystalline cellulose and thin antimicrobial bionanocomposite films. *Journal of Biomaterials and Nanobiotechnology* 3: 371-376.