Green synthesis of Ag/Cu bimetallic nanoparticles and determination of it's antimicrobial and antioxidant activities

[1]Prashast Kumar Tripathi and [1]S C Sati
[1]Research Scholar, [1]Assistant Professor
Department of Chemistry

HNB Garhwal University (A Central University)
Srinagar, Garhwal Uttarakhand
India, 246174

Abstract: In the present study we have synthesized Ag/Cu bimetallic nanoparticle with average size of 18 -26 nm, which is confirmed by UV-Vis spectroscopy, Powder-XRD, SEM-EDX and HR-TEM. The plant, *Artemisia roxburghiana* root extract was used for the reduction purpose of metal ion formed during dissolution. The synthesized nanoparticles was screened for anti-oxidant and anti-microbial activities against many common pathogens.

Keywords : Green chemistry , NPs, Bimetallic, Artemisia roxburghiana, anti-microbial, antioxidant

Introduction

The alloy or core shell structure of bimetallic nanoparticles offers a great area of technological and scientific interest owing to its excellent application in the field of magnetic[1], optic[2], electronic[3] and heterogenous catalysis[4]. The properties of bimetallic nanoparticles are somewhere more advanced and diverse in comparison to their monometallic counterparts[5]. Several bimetallic nanoparticles have find its application as drug delivery agents[6] and biosensor[7]. The medicinal properties of silver can be tailored during the synthesis of mono or bimetallic properties and the engineered bimetallic nanoparticles can give desired specific application. In this way the synthesis of bimetallic nanoparticles by green route has broaden the horizon of Nano-chemistry. The Ag-Cu bimetallic nanoparticles[8] along with other bimetallic nanoparticles such Ag-Au[9] has been synthesized by using plants or its parts. The antioxidant[10] and antimicrobial activity[11] of reported bimetallic nanoparticles has shown to have enhanced activity. The bimetallic nanoparticles can be made by one of the several available methods such as seed mediated growth, laser ablation and chemical reduction. Despite the fact that the methods frequently used for the synthesis of bimetallic nanoparticles are associated with use of hazardous chemical and solvent and once the chemical waste generated come in contact with vital part of our environment, it effects are adverse. The use of chemicals for the synthesis of bimetallic nanoparticles is the not only environmental concern, the use of auxiliary stabilisers with high cost instruments which essential to provide required conditions for the synthesis, are also a matter of concern. The advantage of green chemistry[12] methods over conventional methods is not only its cost effectiveness but also its environmental compatibility which make it a better method for the synthesis of not only bimetallic nanoparticles but also any kind of nanomaterials. The synthesis of bimetallic nanoparticles by green chemistry route has been reported earlier in some details however the number of papers available on bimetallic nanoparticles are limited in numbers.

In this research paper we have synthesized Ag-Cu bimetallic nanoparticles for the first time by using *Artemisia roxburghiana* leaf extract as reducing and stabilising materials. The synthesized nanoparticles were characterised by FT-IR, Powder XRD, HR-SEM and HR-TEM which confirms the average size of bimetallic nanoparticles are in range of 18-26 nm. After the characterisation the bimetallic nanoparticles were screened against many common pathogens. The antioxidant activity of synthesized bimetallic nanoparticles were performed by DPPH method.

Materials and methods

Preparation of plant extract

5 grams of washed and dried roots of *Artemisia roxburghiana* was dipped into 100 ml of distilled water in a round bottom flask which was boiled for 30 min. at 80 ^{0}C . Then the mixture was allowed to cool at room temperature, filtered two times with Wattaman filter paper no. 4. The filtrate was placed in refrigerator for further experimentation.

Salt solution

4 mM solution of $AgNO_3$ (Silver nitrate) and $CuSO_4$ (Copper sulphate) was prepared by dissolving calculated amount of $AgNO_3$ and $CuSO_4$ in 100 ml of distilled water separately. The solutions of silver nitrate and that of copper sulphate were kept for 1 hr to obtain true homogenous solution.

All chemicals used for preparation of solution of $AgNO_3$ and $CuSO_4$ was purchased from Sigma Aldrich with maximum assay 99.999 %.

Synthesis of nanoparticle[13-16]

The synthesis of nanoparticles was done by using concept of green chemistry. Water was used as solvent in the synthesis. Plant extract was mixed with $AgNO_3$ solution at 100 ^{0}C for 30 min at magnetic stirrer. $CuSO_4$ solution was mixed with slight elevation in temperature upto 80^{0}C. The formation of nanoparticles was confirmed by colour changes and finally by recording UV-Visible spectra of the sample. On the addition of silver nitrate solution to the plant extract the colour of resulting solution was light green and further when copper sulphate solution was added to the silver colloid of silver the colour of the solution become pale yellow.

Characterisation

The characterisation of synthesized bimetallic nanoparticles was done by the instrumental techniques such UV-Vis spectroscopy, FT-IR, HR-SEM and HR-TEM. UV-Vis spectroscopy was used during the synthesis for the determination of formation of bimetallic nanoparticles. There are a range of biomolecules present in the various part of the plant *Artemisia roxburghiana.* As we have mentioned earlier that leaf of plant has been use for the reduction which contain many biologically active molecules. The functional group present in these biologically active molecules was studied with the help of FT-IR which in turn compared with FT-IR data of synthesized bimetallic nanoparticles. The crystallographic properties of nanoparticles was done examining powder XRD data. The surface and shape of nanoparticles were determined by HR-TEM and HR-TEM.

UV-Visible Spectroscopy : The UV spectrophotometer was used to monitor the synthesis of nanoparticles as it gives us information about the path of synthesis. When plant extract was mixed with silver salt solution then the reduction of Ag^+ ion get reduced due presence of active molecules present in the plant extract which gives light green colour to the solution with absorption in UV-Vis spectroscopy near 435 nm but when copper salt solution was mixed the spectrum become different due formation of composite structure which incorporates Cu^+ as second centre in the nanostructures. The UV-Visible spectrum was recorded at 0 min, 30 min ,1 hour and 1.5 hour which illustrate the corresponding changes occur during synthesis. The UV-Visible spectral analysis also confirms the formation of bimetallic nanoparticles by giving different absorption at 435 nm when silver nitrate was added to the plant material and it become changed when copper acetate was added to it.

IR-Spectroscopy

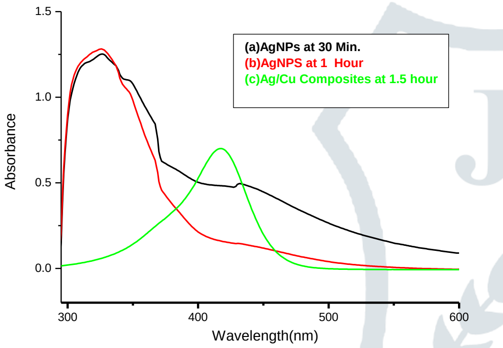
IR spectroscopy was done using plant material as well as synthesised nanoparticles which reveals that about 90% of the functional groups present in the plant material has been transferred to nanoparticles. Many functional groups such carbonyl group, hydroxyl group, amino group and multiple bonds are present in the nanoparticles.

In synthesised NPs. a small peak appeared at 1710 cm^{-1}which confirm the presence of carbonyl group in the NPs while it is very strongly present in the plant material. A band at 1635 cm^{-1} in FT-IR spectrum was due to carbon carbon double bond. The absorption peak at 3500 cm^{-1} was assigned for the hydroxyl group.

XRD Spectroscopy

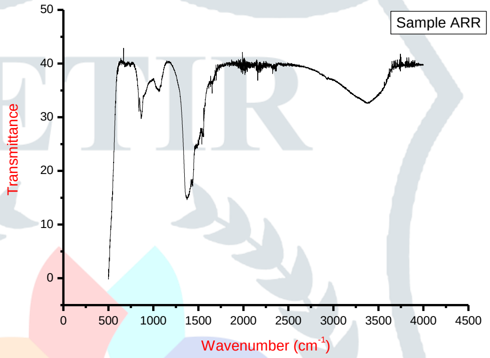
The XRD of synthesised nanoparticle was done by using PANalytical XPERT-PRO D3663 diffractometer. The average crystallite size of synthesized nanoparticles was varying from 16.0 nm to 36.5 nm with cubic structure. The peak position with 2Θ values are 38.32^0, 44.47^0, 64.64^0, 77.56^0 and 81.61^0 are indexed as (111), (200), (220), (311) and (222) planes, which is found to be in good agreement with those of silver copper composite data obtained from International Center Diffraction Data Card with reference code 01-077-6540.

The formation of bimetallic nanoparticles was also confirmed by the comparison with the reported work on the bimetallic nanoparticles[13-15]. The similar lattice constant of Ag and Cu confirms that no mismatch observed for Ag/Cu bimetallic nanoparticles further it also resemble with monometallic counterparts

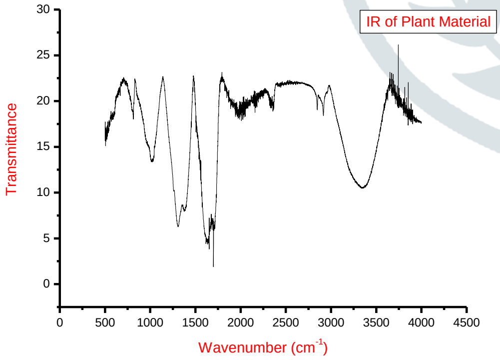


UV-Visible spectroscopy of
(a) AgNPs at 30 min. (b) AgNPs at 1 hour (c) Ag/Cu composite at 1.5 hour

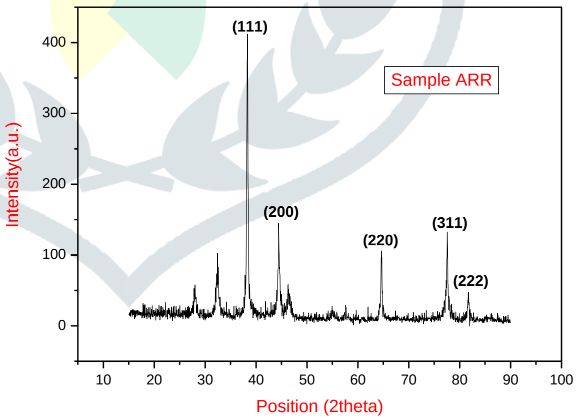
graph 1



graph 2



graph 3

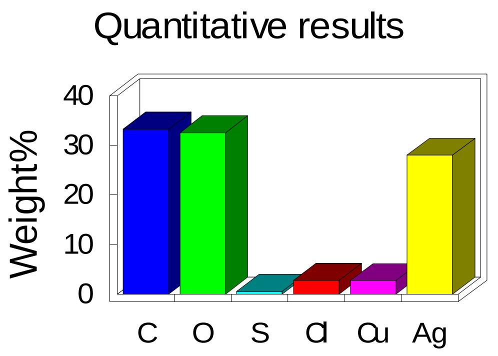


graph 4

The graphs (1) is UV-Vis monitoring of green synthesis of composit material at time intervals at 30 min, 1 hour and 1.5 hours. Graph (2) is for the is IR spectrum of plant material in the powder form. Graph (3) is IR spectra of synthesized nanoparticles for the comparative study. Graph (4) is XRD pattern for the value of 2theta.

S.No.	h	k	l	PP(2Θ)	Crystallite size
1	1	1	1	38.32	24.2 nm
2	2	0	0	44.47	21.1 nm
3	2	2	0	64.64	33.0 nm
4	3	1	1	77.56	36.5 nm
5	2	2	2	81.61	16.6 nm

Table.1 X-Ray Diffraction pattern Ag/Cu composite.



Elemental composition of NPs

SEM of Sample : The scanning electron micrography was done by using Carl Zeiss MA15EVO18 model of SEM instrument which gives the surface morphology and EDX data of the synthesised composite nanoparticles. The SEM data revealed that the synthesised nanoparticles has lower volume maximum surface area with good surface morphology. The Energy Dispersive X-Ray analysis (EDX) revealed that the percentage composition of Elements present in the synthesised NPs. The EDX analysis shows an intense signal at 5 KeV thus indicating the presence of elemental silver and copper with weak signals of oxygen, chlorine and sulphur.

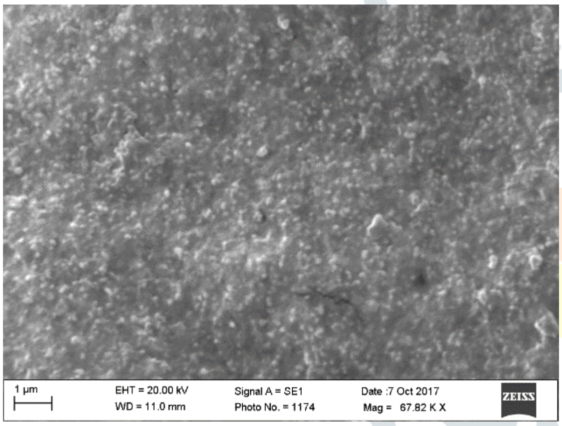


image (1)

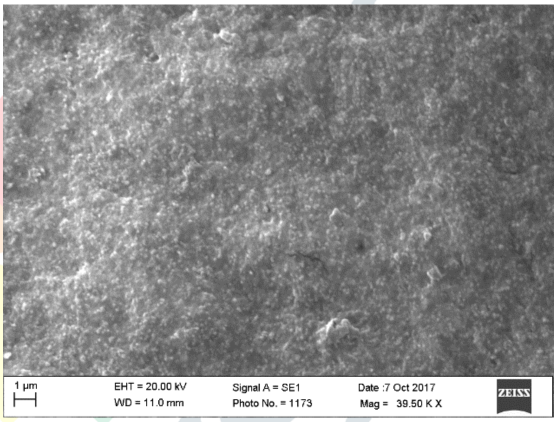


image (2)

Image (1) SEM image of sample at 67.82 KX magnification and image (2) is the SEM image of same sample at 35.50 KX magnification

The silver present 28.2% while Cu present in 2.76% by weight which indicate that the composite material of Ag and Cu has been formed.

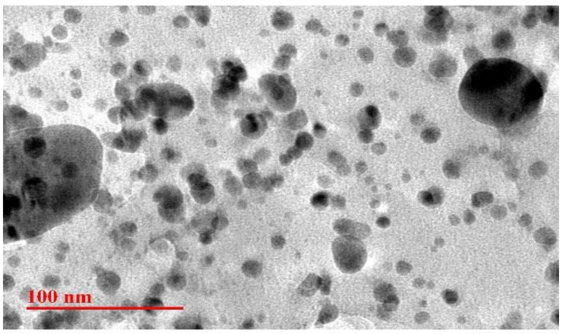


image (3)

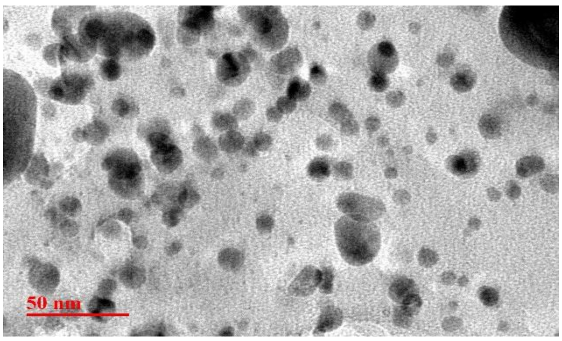


image (4)

HR-TEM images of synthesized nanoparticles (1) image at 100 nm magnification image (4) at 50 nm magnification

TEM Analysis The Transmission Electron Microscopic (TEM) analysis has performed Tecnai, u-Twin 50-300 KV instrument has shown That the particle size vary from 8 nm to 26 nm with spherical shape.

Antimicrobial activity of synthesised bimetallic NPs.[17-18]

The MIC based antibacterial activity of aqueous Ag/Cu bimetallic nanoparticles against ***E. coli, S. aureus, B. cereus*** and ***P. aeruginosa*** has been provided in the table 2. The bimetallic nanoparticles has shown interesting activity against both gram positive and gram negative bacteria in relatively low concentration. It can be concluded that the aqueous solution releases silver and copper ion due to its formation of stable dispersion at molecular level. The diffusion of silver and copper ions was slow which is one of the important factor in the antibacterial activity of the bimetallic nanoparticles. Therefore it can be deduced that the prolonged action of the materials can be obtained due its slow release of ions in stabilised medium. The mechanism by which bimetallic nanoparticles inhibit the growth of ***E. coli***[19]**, *S. aureus, B. cereus*** and ***P. aeruginosa***[20,21] has been reported earlier. The synthesized bimetallic nanoparticles ensure the continuous release of silver and copper along with certain biologically active metabolites that may attaches itself with the negatively charged cell wall of bacteria that get ruptured due to these oppositely charged ions.

Petriplates containing 20ml Muller Hinton medium (for bacterial species) and 20ml Sabouraud Dextrose agar (for fungal isolates) were seeded with 24hr culture of bacterial and fungal strains respectively. Wells were cut and 20 µl of the sample diluted in distilled water for different concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibiotic activity was assayed by measuring the diameter of the inhibition zone formed around the well. **Oxytetracyclin (800ppm)** disc was used as a positive control for bacterial strains while **Fluconazole (1000ppm)** was used as positive control for fungal strains.

Image (5)

Image (6)

Image (7)

Image (8)

The image (5) is the for the antimicrobial activity against *E.coli* image (6) for the activity against *S. aureus*, image (7) activity against *B.serius* and image (8) for the activity against *P. aeruginosa*

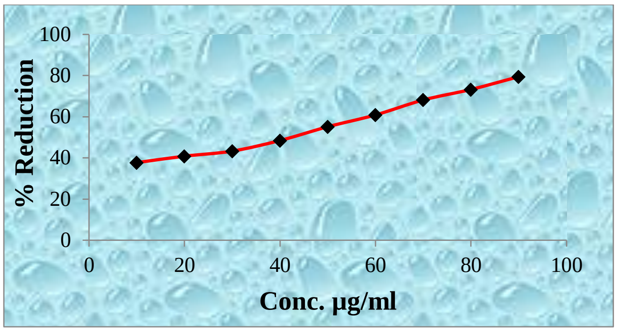
Zone of Inhibition (cm)				
Name of Pathogen	Nanoparticles			Oxytetracyclin (800ppm)
	25 mg/ml	50 mg/ml	100 mg/ml	+ve Control
E. coli	1.0	1.2	1.4	4.0
S. aureus	0.9	1.1	1.3	3.4
B. cereus	Nil	0.8	1.2	3.0
P. aeruginosa	1.1	1.4	1.7	2.3

Table 2 represents zone of inhibition in cm at various concentration of nanoparticles against positive control which is Oxytetracyclin (800ppm concentration).

Antioxidant oxidant activity[19-21] The antioxidant activity of the synthesized nanomaterials was performed through DPPH (2,2-diphenyl-1-picrylhydrazyl, λ_{max}=517 nm) methods. DPPH method is widely used to determine the free radical scavanging activity of natural compound or nanomaterials. This method is based on the detrmination of the scavanging ability of antioxidant materials towards stable radicals. The antioxidant activity through DPPH method which furnish a IC_{50} value 48 in comparison to the standard Ascorbic acid. It was found that the synthesised composite material is capable for the reduction of free radicals in value nearby Ascorbic acid.

S. No.	Conc. (µg/ml)	Absorbance	% Reduction	IC_{50} Value
1.	10	0.280	37.71	48
2.	20	0.270	41.86	
3.	30	0.250	43.31	
4.	40	0.230	50.45	
5.	50	0.210	55.13	
6.	60	0.180	60.85	
7.	70	0.149	68.11	

Table 3.**Antioxidant Activity of Sample in Ethanol**



Graph 5. DPPH Free Radical Scavenging Activity of Sample

Conclusion. It can be concluded that the synthesised nanoparticles has good biological and antioxidant activity due to presence of Ag and Cu on association with plant's active metabolites the activity has been enhanced. Further due to larger surface area and lower volume of the synthesised nanoparticles has potential to be used in heterogenous catalysis for many specific reactions.

Acknowledgments. Authors are thankful to Dept. of botany, HNB Garhwal University for the identification of plant material, USIC HNB Garhwal University for SEM and XRD characterisation, Centre for Nanoscience, Jamiya Millia Islamia for TEM analysis and Cytogene Lucknow for Antimicrobial assay.

References

1. Yurkov, G. Yu, 2007 "Electrical and magnetic properties of nanomaterials containing iron or cobalt nanoparticles." *Inorganic Materials* 43.8 : 834-844.
2. Ray, Paresh Chandra.2010 "Size and shape dependent second order nonlinear optical properties of nanomaterials and their application in biological and chemical sensing." *Chemical reviews* 110.9 : 5332-5365.
3. Iqbal, Muhammad Javed, and Mah Rukh Siddiquah.2008 "Electrical and magnetic properties of chromium-substituted cobalt ferrite nanomaterials." *Journal of Alloys and Compounds* 453.1-2 : 513-518.
4. Banerjee, Madhuchanda, et al 2011. "Enhanced antibacterial activity of bimetallic gold-silver core–shell nanoparticles at low silver concentration." *Nanoscale* 3.12 : 5120-5125.
5. Liu, Xiangwen, Dingsheng Wang, and Yadong Li.2012 "Synthesis and catalytic properties of bimetallic nanomaterials with various architectures." *Nano Today* 7.5: 448-466.
6. Sun, Conroy, Jerry SH Lee, and Miqin Zhang.2008 "Magnetic nanoparticles in MR imaging and drug delivery." *Advanced drug delivery reviews* 60.11 : 1252-1265.
7. Chen, Guanying, et al.2016 "Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy." *Chemical reviews* 116.5 : 2826-2885.
8. Valodkar, Mayur, et al.2011 "Synthesis and anti-bacterial activity of Cu, Ag and Cu–Ag alloy nanoparticles: a green approach." *Materials Research Bulletin* 46.3 : 384-389.
9. Raveendran, Poovathinthodiyil, Jie Fu, and Scott L. Wallen.2006 "A simple and "green" method for the synthesis of Au, Ag, and Au–Ag alloy nanoparticles." *Green Chemistry* 8.1 : 34-38.
10. Scampicchio, Matteo, et al.2006 "Nanoparticle-based assays of antioxidant activity." *Analytical chemistry* 78.6 : 2060-2063.
11. Astruc, Didier, Feng Lu, and Jaime Ruiz Aranzaes. 2005"Nanoparticles as recyclable catalysts: the frontier between homogeneous and heterogeneous catalysis." *Angewandte Chemie International Edition* 44.48.: 7852-7872.
12. Anastas, Panl T., and J. C. Warner. 1998 "Green chemistry." *Frontiers* (1998).
13. Smuleac, V., et al.2011 "Green synthesis of Fe and Fe/Pd bimetallic nanoparticles in membranes for reductive degradation of chlorinated organics." *Journal of membrane science* 379.1-2 : 131-137.
14. Zhan, Guowu, et al. 2011 "Green synthesis of Au–Pd bimetallic nanoparticles: single-step bioreduction method with plant extract." *Materials Letters* 65.19-20 : 2989-2991.
15. Kumari, M. Meena, John Jacob, and Daizy Philip.2015 "Green synthesis and applications of Au–Ag bimetallic nanoparticles." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 137 : 185-192.
16. Xia, Bihua, Fang He, and Lidong Li.2013 "Preparation of bimetallic nanoparticles using a facile green synthesis method and their application." *Langmuir* 29.15: 4901-4907.
17. Emami-Karvani, Zarrindokht, and Pegah Chehrazi. 2011 "Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria." *African Journal of Microbiology Research* 5.12 : 1368-1373.

18. Panáček, Aleš, et al.2006 "Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity." *The Journal of Physical Chemistry B* 110.33 : 16248-16253.
19. Shahverdi, Ahmad R., et al.2007 "Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli." *Nanomedicine: Nanotechnology, Biology and Medicine*3.2 : 168-171.
20. Valero, M., and M. C. Salmeron. 2003 "Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth." *International journal of food microbiology* 85.1-2 : 73-81.
21. Nascimento, Gislene GF, et al. 2000 "Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria." *Brazilian journal of microbiology* 31.4 : 247-256.
22. Re, Roberta, et al. 1999 "Antioxidant activity applying an improved ABTS radical cation decolorization assay." *Free radical biology and medicine* 26.9-10 : 1231-1237.
23. Brand-Williams, Wendy, Marie-Elisabeth Cuvelier, and C. L. W. T. Berset. 1995 "Use of a free radical method to evaluate antioxidant activity." *LWT-Food science and Technology* 28.1 : 25-30.
24. Gopinath, Kasi, et al. 2016 "Green synthesis of silver, gold and silver/gold bimetallic nanoparticles using the Gloriosa superba leaf extract and their antibacterial and antibiofilm activities." *Microbial pathogenesis* 101 : 1-11.