Title : Study of Synthesis ,Characterisation and antibacterial activity of Silver nanoparticles derived from roots of *Phyllanthus emblica*.

Main / Corresponding Author : *Rashmi V. Bordiwala(PhD Student)

Co-Author : Prof. Dr. Ramesh S. Yamgar (PhD guide)

Affiliations : C.S.'s S.S. & L.S. Patkar – Varde College

Abstract : In this study, synthesis of silver nanoparticles(AgNP's) is discussed using aqueous extract of roots of *Phyllanthus emblica*. Synthesised AgNP's showed maximum wavelength at 267 nm after 30 minutes of synthesis. Fourier Tranform Infrared (FTIR) Spectroscopy results are obtained giving functional groups responsible in formation of metal nanoparticles. Field Emission Gun -Scanning Electron Microscopy (FEG-SEM) showed particle size in the range of 30.0 nm, 37.5 nm and 35.6 nm for AgNP's .Whereas, Energy dispersive X-Ray spectroscopy (EDS) technique also determined composition of metal nanoparticles formed. Futher , antibacterial activity is highlighted using synthesised AgNP .

Keywords : Silver nanoparticles , *Phyllanthus emblica*, , antibacterial activity etc.

1.Introduction :

Nanotechnology is the branch of science which deals with study comprising at nanolevels. Over the years , it has gained lot of importance due to its applications in fields like pharmaceuticals, agriculture , industries etc[1-6]. Some of the examples like ceramics, carbon nanotubes metal nanoparticles are widely been researched in this branch[7]. Out of which Metal nanoparticles has been area of great interest to explore due its unique properties. To highlight , Silver metal nanoparticle has been one of the most advantageous noble metal nanoparticle. It has proven its efficacy in theraupeutic uses and catalyst as well[8-9]. There are many methods discovered for the synthesis of metal nanoparticle like laser ablation, ball milling, sol-gel , co-precipitation, green synthesis etc . Amongst all, green synthesis has been considerable due to several advantages like cost- effective, time saving, environmentally benign etc. as compared to other methods[10-11]. Green synthesis can be done using microbes or plant extracts . In this study, synthesis of silver nanoparticle has been discussed using roots of *Phyllanthus emblica* also called as Indian gooseberry. It is also called as Amla. This plant is listed in tropical trees category and found in most of the parts of Asia. Also ,the location of this plant collection is not much explored in ethanobotanical survey. Amla is itself known for its beneficial properties like it is high in Vitamin –C .It helps in healing scurvy . It also has many other medicinal properties such as antibacterial, antifungal, antioxidant properties [12-16]. Amla fruit is consumed in Indian diet in forms of pickles, juices , candies etc. it is also used in cosmetics such as hair oil, shampoos etc.

2. Materials and Methods :

2.1 Collection of Plant Material :

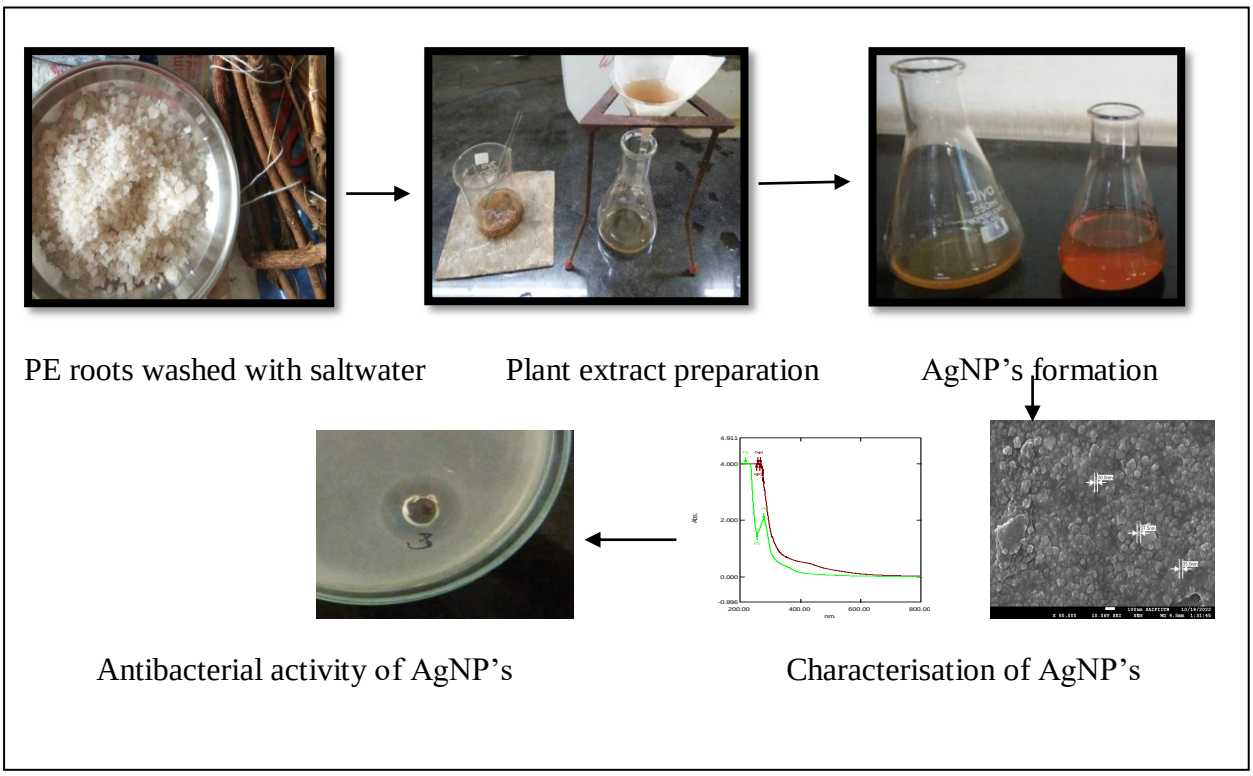
Roots of *Phyllanthus emblica* (PE) were collected from Bordi village located in Palghar district ,Maharashtra. Roots were first washed with normal water then with crystal salt water to remove any bacterial or fungal infestation. They were shadow dried for 20 days and stored in glass bottles.

2.2 Synthesis of aqueous plant extract :

Shadow dried roots were crushed in mortar and pestle manually. Approximately 2 gm of crushed roots were taken in 50 ml of distilled water and soaked overnight to follow cold maceration principle and allow percolation for better results. The soaked solution was gently boiled at mild temperature till its volume is reduced to half of its quantity. The solution was filtered through Filter paper. The Filtrate was used as Plant Extract.

2.3 Synthesis of Silver Nanoparticles :

1mM of Silver Nitrate($AgNO_3$) has been used as a precursor. $AgNO_3$ from Molychem manufacture has been used. 10 ml of prepared plant extract has been added dropwise to 90 ml of 1mM of Silver Nitrate solution while stirring. $AgNO_3$ solution with plant extract was placed in dark and monitored . Initially synthesised solution developed turbid creamish yellow color and gradually turned into reddish brown which indicated the formation of silver nanoparticles. Its maximum wavelength was measured using UV-visible spectrophotometer.



3. Characterisation:

3.1 UV – Visible Spectrophotometer analysis - Maximum wavelength was measured using UV-Visible spectrophotometer of Shimadzu -1800 model. Small aliquots of plant extract , and AgNP's were measured after 30 minutes to find its maximum wavelength in the range of 800-200 nm in spectrum mode. Maximum wavelength of plant extract was found to be at 278 nm, AgNP's at 267 nm . Whereas, 1mM of $AgNO_3$ maximum wavelength was obtained as 334.50 nm.

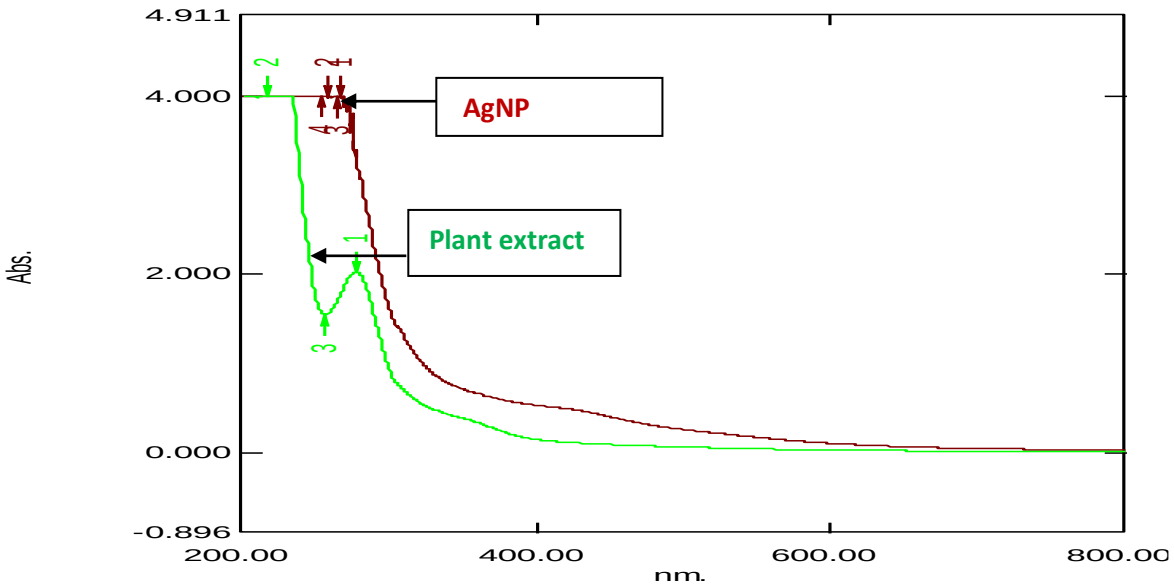


Fig.3.1 UV-Visible spectra of PE and AgNP's after 30 minutes of synthesis

3.2 FTIR analysis : Functional groups were detected using Bruker Vertex 80 FTIR model. 100 µl of sample was loaded on Potassium Bromide (KBr) pellet and compressed in hydraulic pellet press. Pellets were then dried under IR lamp for few minutes. Dried pellets were measured in the mid IR range of 400cm^{-1} to 4000cm^{-1} frequency.

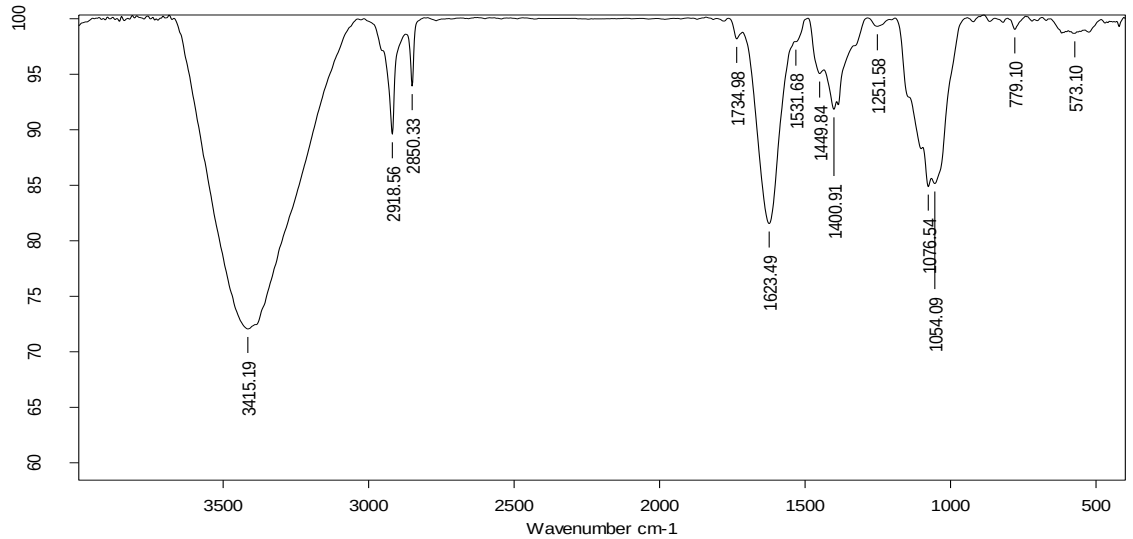


Fig . 3.2 a) FTIR spectra of aqueous extract of roots of *Phyllanthus emblica.*

In the Plant extract certain functional groups were seen such as 3415.19 cm^{-1} for Hydroxyl (OH) ,2918.56 cm^{-1} for (C-H) Aliphatic or alkanes , 2860.33 cm^{-1} for Aliphatic or alkanes (C-

H) ,1734.96 cm^{-1} for Aldehyde (C=O) ,1623.49 cm^{-1} ,1531.68 cm^{-1} for Aromatic (C=C) ,1449.84 cm^{-1} for Aromatic or Alkene (C=C),1400.91 cm^{-1} for Aromatic or alkene (C=C), 1251.58 cm^{-1} for Nitro group (NO_2),1076.54 cm^{-1} for C-O group ,1054.09 cm^{-1} for C-F Alkyl & Aryl halides,779.10 cm^{-1} for out of the plane C-H bending in trisubstituted alkene ,573.10 cm^{-1} halocompounds.

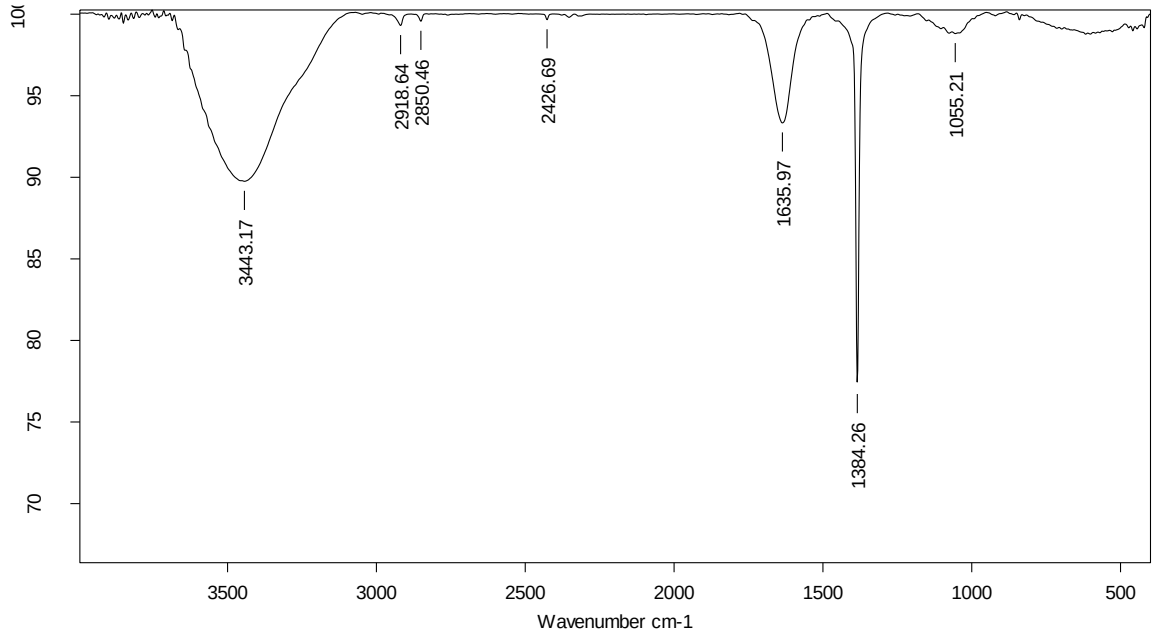


Fig.3.2.b) FTIR spectra of synthesised AgNP's

From the above obtained results it is found that functional groups present at 3443.17 cm^{-1} for Hydroxyl (OH) ,2918.64 cm^{-1},2850.46 cm^{-1} for Aliphatic or alkane C-H ,2426.69 cm^{-1} for nitrile (C≡N), 1636.97 cm^{-1},1384.26 cm^{-1} for Alkene or aromatic (C=C), 1066.21 cm^{-1} for C-O are responsible for the formation of AgNP's .

3.3.FEG-SEM analysis : Size determination was done using FEG-SEM instrument of JEOL JSM-7600F FEG-SEM model after 24 hours of Synthesis. Synthesised AgNP's were loaded on carbon tape placed on labelled stub. Loaded samples were dried in IR lamps for 15 minutes. It was placed in sputterer for platinum coating in Leica EM ACE600 model to enable charge effect for 20 minutes. Samples were placed in sample holder and images were captured at different magnifications. Size were determined using IMAGE-j software. From below images, It was found that AgNP's of sizes 30.0 nm, 37.5 nm and 35.6 nm were obtained.

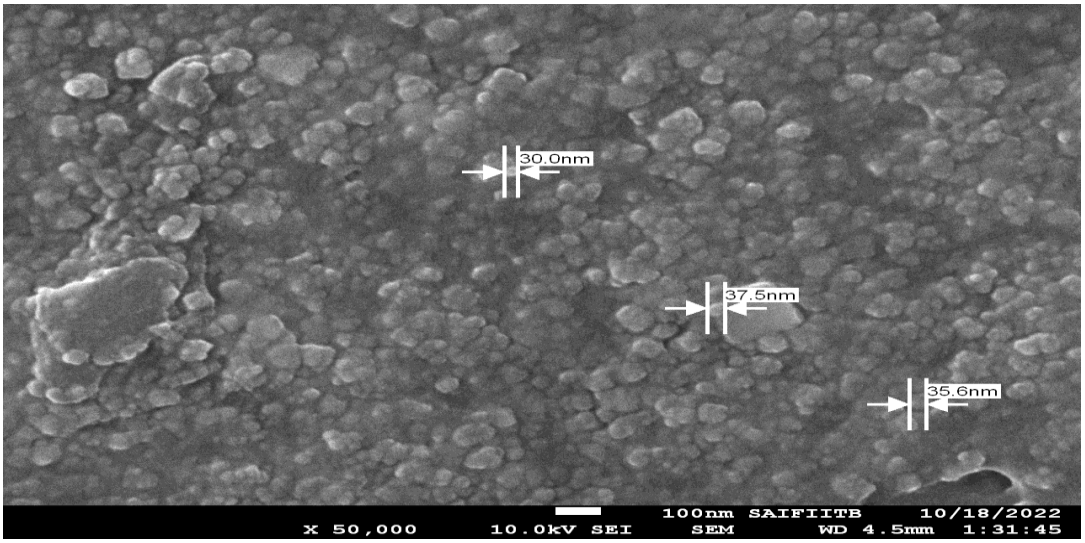
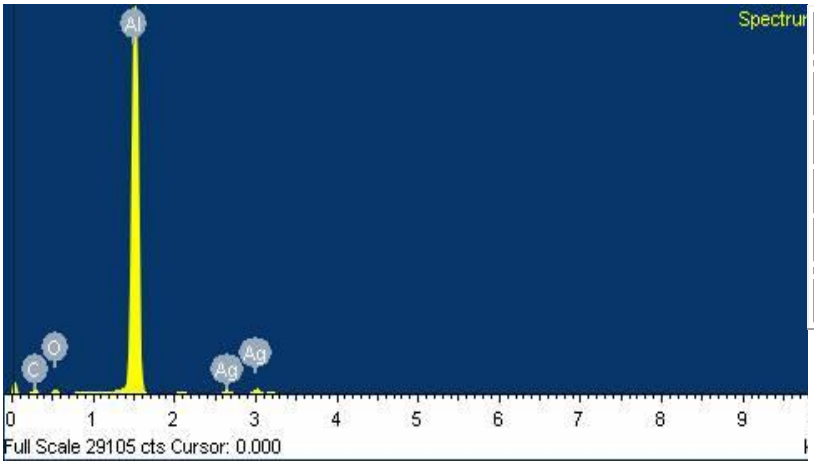


Fig. 3.3 FEG-SEM image of synthesised AgNP's

3.4 EDS analysis : 100 µl Sample was loaded on aluminium strip placed on labelled stub and dried in IR lamp for few minutes. It was coated with platinum to enable charge effect in platinum sputterer for 10 minutes. Samples were placed in sample holder and scanned for better image location and element composition detection was done using JEOL JSM-7600F FEG-SEM model. Below information confirms the presence of AgNP's in the samples.



Element	Weight%	Atomic%
C K	13.33	26.12
O K	4.22	6.21
Al K	75.98	66.26
Ag L	6.47	1.41
Totals	100.00	

Fig.3.4 a) EDS Of AgNP's

4. Antibacterial activity : Antibacterial activity was done using agar well diffusion method using Hi-media nutrient broth against *Staphylococcus aureus* strain no.(American Type Culture Collection) ATCC 6538 and *Salmonella abony* strain no.(National Collection Type Culture) NCTC 6017. *Staphylococcus aureus* is a gram- positive bacteria and *Salmonella abony* is a gram- negative bacteria. The mother culture was spread on autoclaved petridish and 16 mm well was made using sterile screw.100 µl of sample solution was added in agar well using micro pipette. These Petri dishes were kept in incubation for 24 hours to observe zone of inhibition. Zone of inhibition was measured using varnier calliper. Zone of Inhibition

(ZOI) of AgNP's against *Salmonella abony* was found to be 14.80 mm and ZOI of AgNP's against *Staphylococcus aureus* was found to be at 14.01 mm.

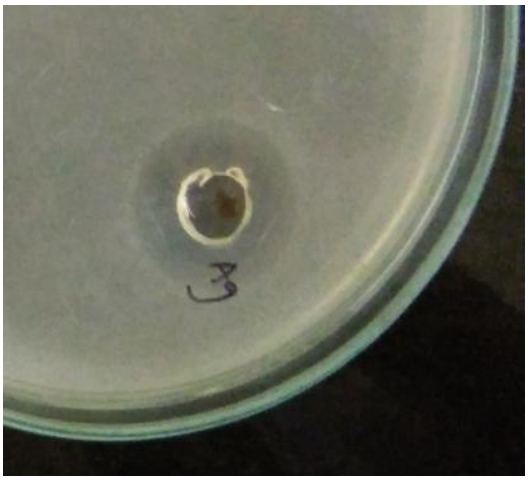


Fig.4.1 ZOI for *Salmonella abony*

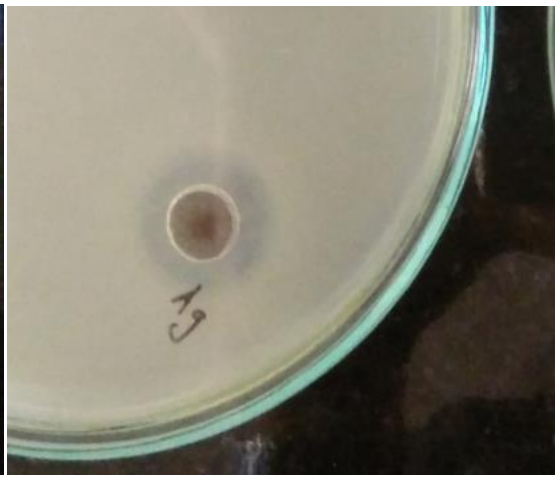


Fig.4.2 ZOI for *Staphylococcus aureus*

5.Results and discussion :

Hence , with the above results as stated in characteristic techniques AgNP's has been synthesised effectively using aqueous extract of roots of *Phyllanthus emblica*. Recorded λ max after 30 minutes proves that formation of AgNP's were in progress. Also, Functional groups were responsible for formation of AgNP's. FEG-SEM analysis was done after 24 hours of synthesis and hence particle sizes were obtained in desired range. For further studies synthesised AgNP's were stored at minus 4° C. Synthesised AgNP's were used to study antibacterial activity against Staphylococcus aureus and Salmonella abony which obtained effective results. Thereafter, through EDS analysis performance after few months elemental composition was determined and particle size aggregations were observed in scanned images.

6. Conclusion :

From the above results, it can be concluded that green AgNP's prepared from roots of the *phyllanthus emblica* using water is one pot synthesis environmental benign method. All the Instrumental Characterisation analysis results were performed in time bound span to overcome nanoparticle formation limitations such as agglomeration of size particles. Further, synthesised AgNP's effectively showed antibacterial activity against both the gram + bacteria and gram – negative bacterial strains effectively. I have also completed photocatalytic dye degradation studies of the synthesised metal nanoparticles and the same will be published soon. In future , my studies will be more focused on synthesis, characterisation and applications of nanoparticles derived from green waste eg. Vegetable , fruit peels etc.

7. Acknowledgments :

I would like to acknowledge Sophisticated Advanced Instrumentation Facility i.e.SAIF-IITB, Powai, Mumbai for allowing me to visit their laboratory analysis during my sample analysis. I would also like to acknowledge M.K.R. laboratories, Bombay College of Pharmacy for carrying out antimicrobial analysis related to my PhD research studies. Further, I would like to thank Head of the Chemistry department, Prof. Dr.Ramesh S. Yamgar for providing all the basic facilities and allowing me to perform PhD research analysis on UV-Visible Spectrophotometer in Patkar – Varde College, Chemistry laboratory(Aided Section).

8. Conflicts of Interest

I declare no conflicts of Interest.

References :

[1]I. Vasconcelos and T. Santos, "Nanotechnology Applications in Sepsis: Essential Knowledge for Clinicians," Pharmaceutics, vol. 15, no. 6, p. 1682, Jun. 2023, doi: 10.3390/pharmaceutics15061682.

[2] Srinivas, P. R., Philbert, M., Vu, T. Q., Huang, Q., Kokini, J. L., Saltos, E., Chen, H., Peterson, C. M., Friedl, K. E., McDade-Ngutter, C., Hubbard, V., Starke-Reed, P., Miller, N., Betz, J. M., Dwyer, J., Milner, J., & Ross, S. A. (2010). Nanotechnology research: applications in nutritional sciences. *The Journal of nutrition*, *140*(1), 119–124. https://doi.org/10.3945/jn.109.115048

[3] A. Haleem, M. Javaid, R. P. Singh, S. Rab, R. Suman, Applications of nanotechnology in medical field: a brief review,Global Health Journal,Volume 7, Issue 2,2023, 70-77,ISSN 2414-6447,https://doi.org/10.1016/j.glohj.2023.02.008

[4] D. Dodoo-Arhin, T. Asiedu, B. Agyei-Tuffour, E. Nyankson, D. Obada, J.M. Mwabora, Photocatalytic degradation of Rhodamine dyes using zinc oxide nanoparticles, Materials Today: Proceedings,Volume 38, Part 2,2021, 809-815,ISSN 2214-7853,https://doi.org/10.1016/j.matpr.2020.04.597

[5]J. Wang et al., "How can nanobiotechnology oversight advance science and industry: examples from environmental, health, and safety studies of nanoparticles (nano-EHS)," J Nanopart Res, vol. 13, no. 4, pp. 1373–1387, Apr. 2011, doi: 10.1007/s11051-011-0236-z.

[6]F. Martinez-Gutierrez et al., "Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and copper nanoparticles," Nanomedicine: Nanotechnology, Biology and Medicine, vol. 6, no. 5, pp. 681–688, Oct. 2010, doi: 10.1016/j.nano.2010.02.001

[7]R. A. Marcus, "Interaction of theory and experiment: examples from single molecule studies of nanoparticles," Phil. Trans. R. Soc. A., vol. 368, no. 1914, pp. 1109–1124, Mar. 2010, doi: 10.1098/rsta.2009.0261.

[8]J. R. Morones et al., "The bactericidal effect of silver nanoparticles," Nanotechnology, vol. 16, no. 10, pp. 2346–2353, Oct. 2005, doi: 10.1088/0957-4484/16/10/059.

[9]J. S. Kim et al., “Antimicrobial effects of silver nanoparticles,” Nanomedicine: Nanotechnology, Biology and Medicine, vol. 3, no. 1, pp. 95–101, Mar. 2007, doi: 10.1016/j.nano.2006.12.001.

[10]Lee SH, Jun B-H. Silver Nanoparticles: Synthesis and Application for Nanomedicine. *International Journal of Molecular Sciences*. 2019; 20(4):865. https://doi.org/10.3390/ijms20040865

[11]K. M. Metz et al., “Green Synthesis of Metal Nanoparticles via Natural Extracts: The Biogenic Nanoparticle Corona and Its Effects on Reactivity,” ACS Sustainable Chem. Eng., vol. 3, no. 7, pp. 1610–1617, Jul. 2015, doi: 10.1021/acssuschemeng.5b00304.

[12]D. A, M. A. S. S, and K. Shanmugam, “Green Synthesis of Silver Nanoparticles from Various Medicinal Plants,” *OJMR*, vol. 1, no. 1, pp. 25–32, Aug. 2022, doi: 10.31586/ojmr.2022.399.

[13]X. Liu et al., “Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities,” Food Chemistry, vol. 109, no. 4, pp. 909–915, Aug. 2008, doi: 10.1016/j.foodchem.2008.01.071.

[14] Saha, S., Verma, R.J. Antioxidant activity of polyphenolic extract of *Phyllanthus emblica* against lead acetate induced oxidative stress. *Toxicol. Environ. Health Sci.* **7**, 82–90 (2015). https://doi.org/10.1007/s13530-015-0224-2

[15] Prananda, A. T., Dalimunthe, A., Harahap, U., Simanjuntak, Y., Peronika, E., Karosekali, N. E., Hasibuan, P. A. Z., Syahputra, R. A., Situmorang, P. C., & Nurkolis, F. (2023). *Phyllanthus emblica*: a comprehensive review of its phytochemical composition and pharmacological properties. Frontiers in pharmacology, *14*, 1288618. https://doi.org/10.3389/fphar.2023.1288618

.

[16]Bruna T, Maldonado-Bravo F, Jara P, Caro N. Silver Nanoparticles and Their Antibacterial Applications. Int J Mol Sci. 2021 Jul 4;22(13):7202. doi: 10.3390/ijms22137202. PMID: 34281254; PMCID: PMC8268496.