Available online on 15.05.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Study of Antibacterial activity of *Phyllanthus emblica* and its role in Green Synthesis of Silver Nanoparticles

Shah Pratibha J*, Malik Ruchi

Department of Microbiology K.C. College, Churchgate, Mumbai, India

ABSTRACT

Objective: *Phyllanthus emblica L.* or amla is known for its therapeutic properties. The aim of the present study was to evaluate the antibacterial activity of aqueous *Phyllanthus emblica* fruit extract (APE) against eight pathogenic cultures and its application in green synthesis of silver nanoparticles.

Methods: APE was screened for the presence of phytochemicals and its antibacterial activity was evaluated by agar well diffusion assay. The minimum inhibitory concentration (MIC) was quantified by broth macrodilution technique, and minimum bactericidal concentration (MBC) was determined. Further, APE was used in the biological synthesis of silver nanoparticles (AgNPs), which were characterized by an Ultraviolet–visible (UV-VIS) spectroscopy and Field emission gun-scanning electron microscopy (FEG-SEM) techniques. The antibacterial activity of the AgNPs was screened by agar well diffusion assay.

Results: The zone of inhibition (ZOI) for APE was found to be in the range of 10.7–21.3 mm, for varying concentrations. The MIC values were in the range of 12.5% - 50% (v/v) and the MBC values indicated that a concentration of 50% (v/v) APE could kill 75% (6/8) test cultures. The presence of AgNPs was confirmed by UV-VIS spectroscopy and the surface-plasmon resonance peak was observed at 420 nm. The FEG-SEM analysis revealed that the most of AgNPs were spherical in shape and had 30-40 nm size range. All the test cultures were inhibited by the AgNPs and the average ZOI measured 19.25±2.7 mm.

Conclusion: *Phyllanthus emblica* fruit extract might have therapeutic significance against pathogens and it can be used for green synthesis of silver nanoparticles.

Keywords: *Phyllanthus emblica*, MIC, MBC, silver nanoparticles, UV-VIS, FEG-SEM.

Article Info: Received 18 March 2019; Review Completed 20 April 2019; Accepted 25 April 2019; Available online 15 May 2019

Cite this article as:

Shah PJ, Malik R, Study of Antibacterial activity of *Phyllanthus emblica* and its role in Green Synthesis of Silver Nanoparticles, Journal of Drug Delivery and Therapeutics. 2019; 9(3):76-81

http://dx.doi.org/10.22270/jddt.v9i3.2753

***Address for Correspondence:**

Pratibha J Shah, Department of Microbiology K.C. College, Churchgate, Mumbai, India

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years in many parts of the world. Currently, there is a renewed interest in study of safer biologically active compounds isolated from natural products with acceptable therapeutic index for the development of novel drugs[1]. *Phyllanthus emblica* L. commonly known as Amla or Indian gooseberry, belongs to Euphorbiaceae family and has been frequently used in traditional medicine to treat diseases. The fruits of *P. emblica* mainly contain tannins, polyphenolics, and alkaloids along with other phytochemicals [2]. In *P. emblica* fruit, vitamin C is considered to be highly stable due to the presence of tannins and polyphenols. These phytochemicals contribute to the high antioxidant capacity and free radical scavenging, anti-cancer, antibacterial, antifungal, antiviral and anti-inflammatory activities [3].

The growing threat of microbial resistance against antibiotics has encouraged the development of antimicrobial nanoparticles (NPs), including silver NPs (AgNPs). Particles which have two or more dimensions in the size range as 1-100 nm or one billionth part of a meter are defined as one nanoparticle. NPs have a unique chemical and physical properties as compared to their solid bulk materials because of their high surface area and electronic properties. Nanotechnology can be implemented as preventives, diagnostics, drug carriers, antibacterial coatings for implantable devices, in development of antibacterial vaccines and synergetics in the antibacterial therapies [4, 5].

Various approaches are available for the synthesis of silver nanoparticles. Silver ions can be reduced by chemical, electrochemical, radiation, photochemical methods and biological techniques. The biological synthesis of nanoparticles can be done using microorganisms, enzymes,

and plant extracts. The synthesis of silver nanoparticles using plant extracts offer an economical, energy efficient and protective method for environment, leading to lesser hazardous waste [5]. The mechanism of formation of AgNPs using plant phytochemicals can proceed through three steps: first, charge transfer from reducing agents to Ag^+ results in the formation of Ag atoms, which subsequently nucleate to form small AgNPs; second, a condensation step occurs in which small particles grow to form larger ones, followed by surface reduction of any Ag^+ present on the surface of the formed NPs; and third, adsorption of excess negatively charged reducing agents ions on the surface of the formed particles, achieving electrostatic stabilization and thus controlling their sizes. It has also been studied that the antimicrobial activity of AgNPs depends on their size and shape [6].

The current study explores the antibacterial activity of a crude aqueous extract of *Phyllanthus emblica* L. (Amla) and its application to synthesize benign nanostructure materials as it is less toxic and eco-friendly and can serve as both reducing and capping agents, due to its phytochemicals.

MATERIALS AND METHODS

Plant material and extraction

Fresh *Phyllanthus emblica L.* fruit were purchased from a local market in Mumbai, India. The collected fruit was identified and authenticated by Blatter herbarium at St. Xavier's College, Mumbai. Washed 100 gm *P. emblica* fruit were deseed and crushed. The crushed material was filtered using a muslin cloth and the fresh extract obtained was collected into a sterile container. This extract was regarded as 100% extract and was stored at 4°C for further use in the study, after sterility testing of the extract.

Qualitative phytochemical studies

Phytochemical analysis of the *P. emblica* fruit extracts for the presence of flavonoids (Shinoda's test), triterpenoids (Libermann Burchard test), glycosides (Borntrager's test), anthraquinones, and tannins (Ferric chloride test) was conducted by previously described standard protocols [7,8].

Bacterial cultures

The crude extract of *P. emblica* fruit was tested for antibacterial activity against eight pure cultures known to be pathogenic to humans. The cultures selected were *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi A, Salmonella paratyphi B, Shigella spp.,* and *Bacillus cereus.* The cultures were obtained from Microbiology Department, K.C. college, Churchgate, Mumbai.

Evaluation of antibacterial activity by agar well diffusion

The primary antibacterial activity of aqueous *Phyllanthus emblica* fruit extract (APE) was screened by agar well diffusion method using Mueller-Hinton (MH) agar (HiMedia, Mumbai). The turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of 0.5 McFarland standards, resulting in suspension containing approximately 1.5×10^8 cfu/ml [9]. Further, 20 ml of molten media (MH agar) containing 100 µl of test inoculum (1.5×10^8 cfu/ml) was poured into a sterile petri-dish and allowed to solidify. Wells of 8 mm diameter were bored and various concentrations of APE (25%, 50%, 75%, and 100%, v/v); were prepared using sterile distilled water and then subsequently added to the wells. The broad spectrum standard antibiotic Amikacin (30 µg/ml), was used as positive control and sterile distilled water was used as the negative control. The plates were refrigerated for 1 h to diffuse the samples and incubated at 37°C for 24 h for detection of antibacterial activity. The inhibition zone diameter of the test samples were measured in mm. The experiment was repeated in triplicates and the average values were recorded [10,11].

Determination of MIC (minimum inhibitory concentration) and MBC (minimal bactericidal concentration) of APE

Minimum inhibitory concentration (MIC) of APE was determined by broth macrodilution method and Mueller Hinton broth (HiMedia, Mumbai) was used for the MIC assay. APE was first diluted to the highest concentration (50%) using MH broth and then serial two-fold dilution was done to obtain concentration range from 50% to 1.56% (v/v) of APE. 0.1 ml of standardized suspension of the eight bacterial cultures (10^6 cfu/ml) was added to each tube containing APE at the concentrations of 50.0, 25.0, 12.50, 6.25, 3.12, 1.56 (v/v) and 0% (control), in broth medium. The tubes were incubated at 37°C for 24 h and observed for visible growth after mixing the tubes gently. The lowest concentration of the extract in a tube that failed to show any visible growth (at the binocular microscope) was considered as the MIC of APE against the particular bacterial strain. A tube containing broth and inoculum but lacking APE was considered as control [12,13].

The minimum bactericidal concentration (MBC) is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 h under a standardized set of conditions. MBC can thus be determined after broth macrodilution, by sub-culturing a dilution yielding a negative microbial growth on the surface of MH agar plates. The lowest concentration that yielded no single bacterial colony after 24 h of incubation on the MH agar plates was reported as MBC [14].

Biological synthesis of AgNPs using *P. emblica* fruit extract

10 g of finely cut *P. emblica* fruit was weighed and boiled for 20 minutes with 100ml distilled water. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected in a flask. The filtrate was used as reducing and capping agent of silver into silver nitrate ions [15]. $AgNO_3$ (HiMedia, Mumbai) was used as a precursor for synthesis of AgNPs. 10 ml of *P. emblica* extract was added to the 90 ml of 1.0 mM aqueous solutions of $AgNO_3$. The extract was added drop-wise under continuous stirring at 50°C-60°C. In this process, the *P. emblica* extract acts as the reducing and stabilizing agent and change in its color to dark brown, indicated the synthesis of AgNPs [4].

Characterization of the silver nanoparticles

The reduction of Ag^+ was monitored by measuring the UV-VIS spectrum. The spectrum was recorded throughout a range of 300–700 nm, and the wavelength corresponding to maximum absorption was determined. The shape and particle size of AgNPs was studied by Field emission gun-scanning electron microscopy (FEG-SEM) [4]. The colloidal solution of AgNPs made from *P. emblica* extract was analyzed under UV-Vis spectroscopic technique and the plasmon peak was recorded. The absorbance spectrum of AgNPs was observed at 420 nm. The topography of the nanoparticles was studied using FEG-SEM. The samples were washed with Milli Q water and centrifuged. 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, extra solution was removed using a blotting paper and

then the film on the SEM grid was allowed to dry and was observed.

Checking for antimicrobial activity of the nanoparticles

The antibacterial activity of AgNPs prepared using *P. emblica* fruit extract were screened using eight cultures by agar well diffusion method described above. The nanoparticles prepared using *P. emblica* were tested along with $AgNO_3$ as a control. 50µl of AgNps formed and $AgNO_3$ (control) were added into the wells under conditions described earlier for agar well diffusion assay. The inhibition zone diameter of the test sample was measured in mm. The experiment was repeated in triplicates and the average values were recorded [11].

RESULTS

The *Phyllanthus emblica* or Amla or Indian gooseberry is known for its therapeutic properties and holds a reputed position in the traditional system of medicine worldwide. Bioactive molecules or phytochemicals are found to have therapeutic significance. The phytochemical tests with the *P. emblica* showed the presence of flavonoids, tannins, triterpenoids and anthraquinones (Table 1).

Table 1: Phytochemical analysis of *Phyllanthus emblica L*

Phytochemicals	Observations
Flavonoids	+
Tannins	+
Triterpenoids	+
Anthraquinones	+
Glycosides	-

Key –'+' – Present; '-' - Absent

The above table depicts the types of phytochemicals present in APE.

The antibacterial activity of APE was carried out by the agar well diffusion method and the average zone of inhibition for the concentration of 25% (v/v) ranged from 10.7–15 mm with a mean of 12.2±1.48 mm against the test cultures. However, *Salmonella typhi* and *Escherichia coli* were not inhibited at this concentration. The average zone of inhibition for the concentration of 50% ranged from 13.3–17.3 mm with a mean of 15.4±1.6 mm, and all test cultures were inhibited. For 75% APE, the range was 14.7 – 18.7 mm with a mean of 17±1.7 mm and for 100% it was in the range of 16- 21.3 mm with a mean of 19±2.0 mm (Table 2).

Table 2: ZOI of APE against 8 pathogenic cultures by agar cup method

	Mean Zone of inhibition ± SD in mm			
Concentration (%) of APE	**25%**	**50%**	**75%**	**100%**
Salmonella typhi	-	14±1.5	18.3±1.1	21±1
Escherichia coli	-	15±1	17±0	20.7±0.58
Staphylococcus aureus	15±0	17.3±0.58	19.3±1.5	21.3±1.1
Vibrio spp	11.3±0.58	17±0	18.3±1.1	19.3±1.1
Salmonella paratyphi A	12±0	13.3±1.1	14.7±0.58	17.7±0.58
Salmonella paratyphi B	10.7±0.58	13.3±1.5	15±0	17±0
Shigella spp	12±0.58	15±0	15.7±1.5	16±1
Bacillus cereus	10.9±1.5	15.7±1.5	18.7±0.58	19.3±1.1

The above table depicts mean ± SD of zone of inhibition in mm for varying concentrations of APE (v/v) against the test cultures (n=3)

The MIC value of APE was determined by the broth macrodilution method by using various concentrations of APE. It was found that APE could inhibit the growth of all test cultures indicating its effectiveness, except *Salmonella typhi*, which could not be inhibited at the concentration of 50% (v/v). The MIC values were found to be in the range of 12.5% - 50%. The results were further confirmed by determining MBC values. It was found that the concentration of 50% APE could kill 75% (6/8) of the test cultures.

Table 3: MIC and MBC of APE by broth macrodilution method

Concentration of APE (%, v/v)		50	25	12.5	6.25	3.12	1.56
Salmonella typhi	MIC	+	+	+	+	+	+
	MBC	+	+	+	+	+	+
Escherichia coli	MIC	-	+	+	+	+	+
	MBC	-	+	+	+	+	+
Staphylococcus aureus	MIC	-	-	-	+	+	+
	MBC	-	-	+	+	+	+
Vibrio spp	MIC	-	-	+	+	+	+
	MBC	-	-	+	+	+	+
Salmonella paratyphi A	MIC	-	-	-	+	+	+
	MBC	-	-	+	+	+	+
Salmonella paratyphi B	MIC	-	-	-	+	+	+
	MBC	-	-	+	+	+	+
Shigella spp	MIC	-	-	-	+	+	+
	MBC	-	-	-	+	+	+
Bacillus cereus	MIC	-	+	+	+	+	+
	MBC	+	+	+	+	+	+
Percentage of cultures inhibited	**MIC**	**87.5**	**62.5**	**50**	**0**	**0**	**0**
Percentage of cultures killed	**MBC**	**75**	**62.5**	**12.5**	**0**	**0**	**0**

Key –'+' – Growth present; '-' – Growth absent

The above table depicts MIC and MBC values of varying concentrations of APE against the test cultures.

Further, in the study nanoparticles were formed using *P. emblica* fruit extract and the characterization of the nanoparticles was carried out by visual detection, UV-VIS spectroscopy and FEG-SEM analysis. The change of color of the solution in the flask to dark brown solution marked the formation of silver nanoparticles. A surface-plasmon resonance peak was obtained at 420 nm which confirmed the formation of silver nanoparticles (Fig 1).

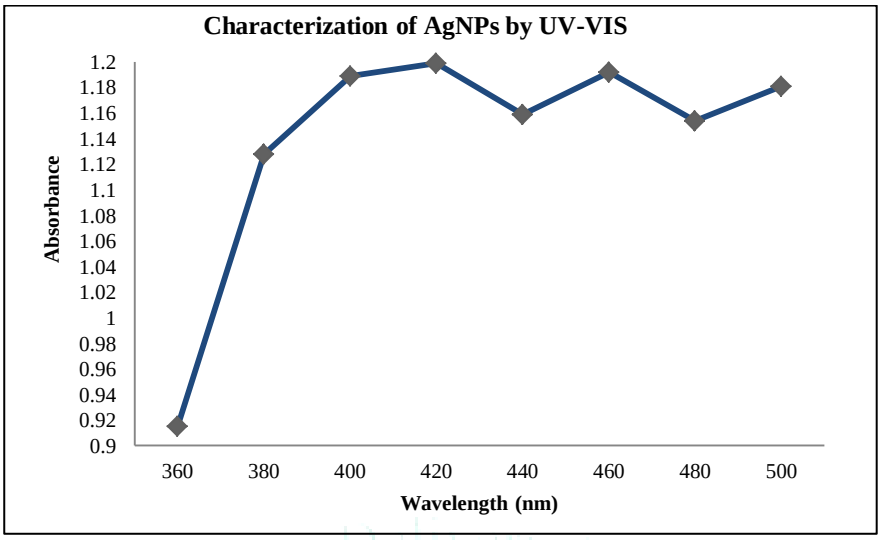

Figure 1: Line graph showing absorbance by AgNPs by UV-VIS spectrophotometer

The Field emission gun-scanning electron microscopy (FEG-SEM) technique was used to visualize the particles and to study the surface morphology, size and shape. From the FEG-SEM images (Fig. 2), it is evident that AgNPs formed using *P. emblica* fruit extract were mostly spherical in shape. The measured average size of AgNPs was 30-40nm. Occasional agglomeration of the AgNPs was observed.

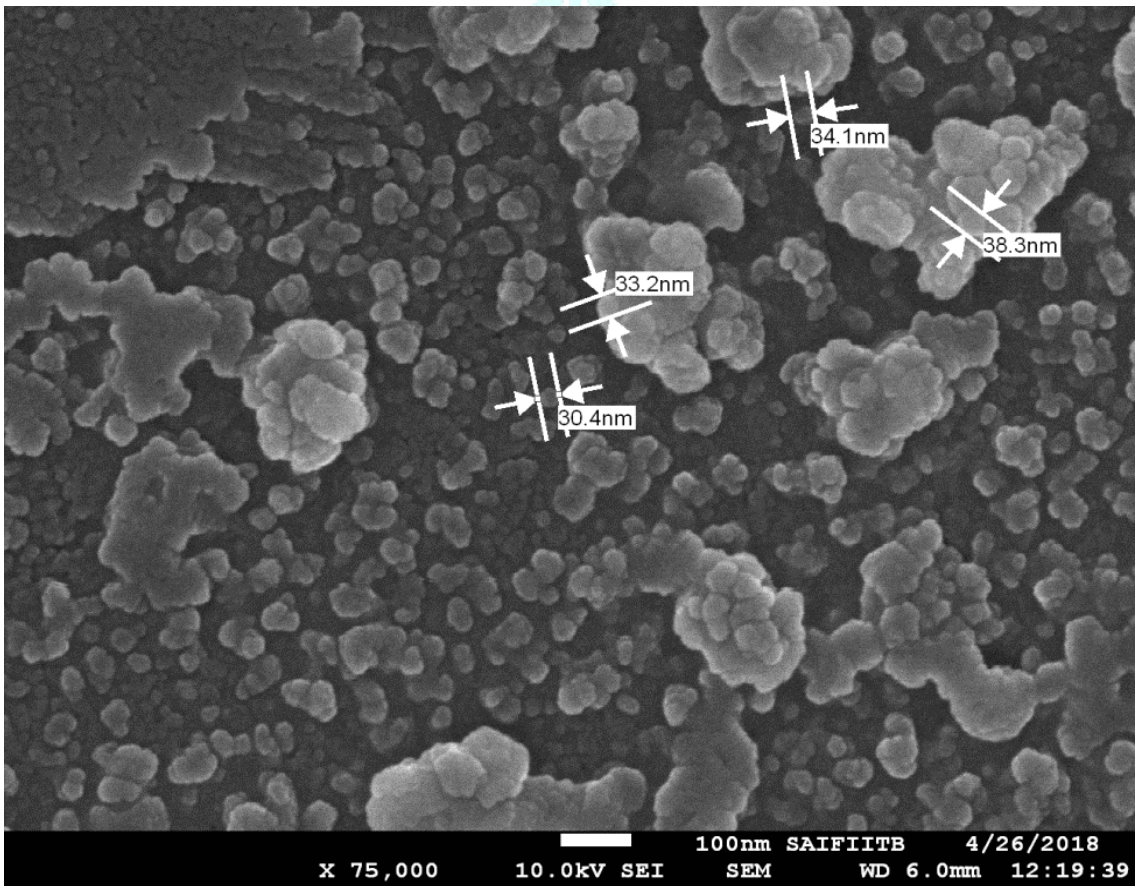

Figure 2: Characterization of AgNPs by Field emission gun-scanning electron microscopy

Antimicrobial activities of the synthesized AgNPs of *P. emblica* fruit extract were determined by using the agar well diffusion method. The size of ZOI for AgNPs formed was found to be ranging from 21.3 to 29.3 mm, against the test cultures used in the study. The maximum size of ZOI was found to be against *Salmonella paratyphi A* at 100% concentration, whereas minimum size of inhibition zone was observed against spore producing, *Bacillus cereus.* However, all the test cultures were inhibited by the AgNPs formed, indicating potent antibacterial activity. The average zone of inhibition measured 19.25±2.7 mm against the test cultures. A control containing $AgNO_3$ solution also inhibited the test cultures, but the difference in ZOI between $AgNO_3$ solution and AgNPs formed using APE was statistically significant (*P<0.05), indicating the efficacy of the AgNPs formed by green synthesis in killing pathogenic bacteria.

Table 4: ZOI of AgNPs against 8 pathogenic cultures by agar cup method

	Mean Zone of inhibition ± SD in mm	
	Control ($AgNO_3$)	AgNp-PE (100%)
Salmonella typhi	20.7±0.6	26±1.0
Escherichia coli	19±0	21.3±0.6
Staphylococcus aureus	18.3±0.6	22.7±1.1
Vibrio spp.	15.7±0.6	21.3±1.5
Salmonella paratyphi A	24.7±0.6	29.3±0.6
Salmonella paratyphi B	19±0	24±0.0
Shigella spp.	19.7±0.6	25±1.0
Bacillus cereus	17±0	20.7±0.6

The above table depicts mean ± SD of zone of inhibition in mm of AgNPs and $AgNO_3$ solution against the test cultures (n=3).

DISCUSSION

The current study investigated the ability of fresh crude aqueous *P. emblica* fruit extract to inhibit the growth of eight bacterial pathogens. The beneficial medicinal effects of natural products are generally attributed to secondary metabolites present in them. However, the antimicrobial activity is not due to a single compound, but a combination of the metabolites. Similarly, the crude APE was found to contain a myriad of phytochemicals like flavonoids, tannins, triterpenoids and anthraquinones, which is in corroboration with the study by Hutchings, et al. [4]. The findings suggested a potent antimicrobial activity by APE. The antibacterial activity of APE was studied by using agar well diffusion test and 100% (v/v) APE could inhibit all the strains indicating a effective antibacterial activity against the strains. The extract exhibited a more enhanced antibacterial activity against Gram-positive *S. aureus* in comparison to Gram-negative *E. coli*. Our results are in agreement with the findings of previous studies of Dharajiya et al. and Singh et al. [12,16]. The MBC studies also confirmed the strong antibacterial activity of APE against Gram-positive *S. aureus* in comparison to Gram-negative bacteria. The difference in susceptibility between Gram-positive and Gram-negative bacteria could be due to the morphological differences. Gram-negative bacteria have a complex cell wall, comprising of an outer membrane making it more resilient to damage[17]. Thus, it could be said that APE was effective in killing *E. coli*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Vibrio spp, Shigella spp,* and *Bacillus* cereus; but was most effective against *Staphylococcus aureus.* APE showed a reduced antibacterial activity against *Salmonella typhi*, which was also observed in a previous study by Singh et al. [16].

Silver nanoparticles are increasingly being used in therapeutics and diagnosis due to their unique physical, chemical and antibacterial properties. Many theories of antibacterial activity of colloidal silver solution have been suggested, like it can cause alteration of the permeability of cell membrane, release of lipopolysaccharides and membrane proteins, generation of free radicals responsible for the damage of the membrane and dissipation of the proton motive force resulting in the collapse of the membrane potential, however; the exact mechanism has not been fully deciphered [18].

In the green synthesis of silver nanoparticles, capping agents which aid in the stabilization of nanoparticles are organic molecules. Numerous studies have been reported regarding green synthesis of silver nanoparticles. However, there is a paucity of documented studies on fresh aqueous *P. emblica* fruit extract being used for synthesis of silver nanoparticles and its antibacterial activity.

In the current study, formation of silver nanoparticles using a fresh aqueous extract of *Phyllanthus emblica* fruit was indicated by the colour change from colourless to dark brown colour. Biosynthesized nanoparticles were characterized by UV-VIS (surface plasmon resonance peak at 420 nm) and FEG-SEM analysis. The FEG-SEM images provide further insight into the morphology and particle size, shape, and distribution profile of the AgNPs. It was determined that the nanoparticles were spherical in shape and ranging from 30-40 nm in size. Antimicrobial activities of the synthesized AgNPs were analyzed and all the test cultures were inhibited by the AgNPs formed, with the average zone of inhibition measuring 19.25±2.7 mm. However, smaller ZOIs were reported by the study carried out by Nayagam et al. [18]. Variation may occur due to factors like the geographical location of the plant's collection, the age of the plant, nutrient concentrations due to soil and environmental growth conditions, the extraction method and the biosynthesis method of AgNPs. The study reported by Nayagam et al. synthesized nanoparticles from dried *P. emblica* fruit residue, whereas in the current study fresh *P. emblica* fruit extract was utilized for synthesis of AgNPs. The size of AgNPs formed also affects the antibacterial activity,

smaller AgNPs having a large surface area available for interaction, would have stronger anti-bacterial activity [18]. The size of AgNPs formed in the study by Nayagam et al. was more in comparison to the current study, which may be leading to the differences in the antibacterial activity.

CONCLUSION

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective AgNPs, in controlling the growth of microbes. The results obtained from the current study indicated that fresh aqueous *P. emblica* fruit extract has potent antibacterial activity and suggested its application in a safe and economic synthesis of silver nanoparticles, as it was found to be a good bioreductant and capping agent. The prepared AgNPs possessed significant in-vitro antibacterial activity against Gram-positive and Gram-negative bacteria. Remarkably, Gram-negative bacteria were more susceptible to the effect of AgNPs than Gram-positive bacteria.

ACKNOWLEDGEMENT

The authors are grateful to the Head of the Microbiology department, K.C.college and Dr. Rajendra Shinde, Director Blatter Herbarium, St. Xavier's college, Mumbai.

CONFLICT OF INTEREST STATEMENT: The authors declare that they have no competing interests with whomsoever.

REFERENCES

1. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma R S. Antibacterial activity of the plant used in Indian herbal medicine, International Journal of Green Pharmacy 2010; 10:22-28.
2. Srinivasan. Vitamin C in plants: Indian gooseberry *phyllanthus emblica.* Nature. 1994; 153:684-684.
3. Hutchings A, Cock IE. The Interactive Antimicrobial Activity of *Embelica officinalis* Gaertn. Fruit Extracts and Conventional Antibiotics against some Bacterial Triggers of Autoimmune Inflammatory Diseases. Pharmacognosy Journal. 2018; 10(4):654-662.
4. Tyagi S, Kumar A, Tyagi PK. Comparative analysis of metal nanoparticles synthesized from Hibiscus rosa sinesis and their antibacterial activity estimation against nine pathogenic bacteria. Asian Journal of Pharmaceutical and Clinical Research. 2017 10(5):323-329.
5. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. International Journal of Nanomedicine. 2017; 12:1227-1249.
6. Ashour AA, Raafat D, El-Gowelli HM, El-Kamel AH. Green synthesis of silver nanoparticles using cranberry powder aqueous extract: characterization and antimicrobial properties. International Journal of Nanomedicine. 2015; 10:7207–7221.
7. Khandelwal KR. Practical Pharmacognosy. Techniques and Experiments. 10th ed. Pune, India: Nirali Prakashan; 2006. p. 149-156
8. Senthilnath R, Balu PM, Murugesan K. Phyto-chemical Screening and Antibacterial Activity of Five Indian Medicinal Plants against Human Pathogens. International Journal of Current Microbiology and Applied Sciences. 2013; 2(3):75-84.
9. Madduluri S, Rao BK, Sitaram B. In vitro evaluation of antibacterial activity of five indigenous plants extract against five bacterial pathogens of human. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(4):679-684
10. Punjabi K, Chitalia VK, Mukadam T, Sharma J, Maniar J, Vasave S, Vaidya S, Dikho Mao L. Evaluation of the antimicrobial activity of Gooseberry and Wild Apple fruit juices of the northeast region of India. Journal of Medicinal Herbs and Ethnomedicine. 2017; 3:1-7.
11. Balouiri, M.; Sadiki, M.; Ibnsouda, S.A. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical and Biomedical Analysis. 2016; 6, 71–79.
12. Dharajiya D, Patel P, Moitra N. Antibacterial activity of Emblica officinalis (Gaertn.) Fruits and Vitex negundo (L.) Leaves. Current Trends in Biotechnology and Pharmacy. 2015: 9(4), 357-368.
13. Veljić M, Tarbuk M, Marin P, Ćirić A, Soković M, Marin M. Antimicrobial Activity of Methanol Extracts of Mosses from Serbia. Pharmaceutical Biology. 2008; 46(12):871-875.
14. CLSI, Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved Guideline, CLSI document M26-A. Clinical and Laboratory Standards Institute, 950 West Valley Roadn Suite 2500, Wayne, Pennsylvania 19087, USA, 1998.
15. Palanisamy K, Kalaiselvi PA, Gabriel M, Thangavel J, Sundaram L. Emblica officinalis leaf extract mediated green synthesis of antibacterial silver nanoparticles against human pathogens World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(3):2019-2032.
16. Singh SN, Moses A, David A. Antimicrobial Activity of *Emblica officinalis* extracts against selected bacterial pathogens. International journal of basic and applied research, 2019; 9(1):325-330.
17. Ramgopal M, Saisushma C, Attitalla HI, Abobaker M. A Facile Green Synthesis of Silver Nanoparticles using Soap Nuts. Research Journal of Microbiology, 2011; 6:432-438
18. Nayagam V, Gabriel M, Palanisamy K. Green synthesis of silver nanoparticles mediated by Coccinia grandis and Phyllanthus emblica: a comparative comprehension. Applied Nanoscience. 2018; 8(3):205-219.