Available online on 25.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Green Synthesis of Silver Nanoparticles using Aqueous Cranberry Fruit Extract and its Antibacterial Activity

Shah Pratibha J* and Malik Ruchi

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

ABSTRACT

Objective: Cranberry (*Vaccinium macrocarpon*) is popularly used in traditional folk medicine for treatment of microbial infections. The aim of the present study was to evaluate the antibacterial activity of aqueous cranberry fruit extract (ACE) against pathogenic cultures and its application in green synthesis of silver nanoparticles.

Methods: ACE was screened for its antibacterial activity by agar well diffusion assay. The minimum inhibitory concentration (MIC) was determined by broth macrodilution technique, and minimum bactericidal concentration (MBC) was quantified. ACE was used in the green 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. Agar well diffusion assay was used to evaluate the antibacterial activity of the AgNPs formed.

Results: The zone of inhibition (ZOI) for ACE was found to be in the range of 19 - 30.3 mm, for the concentration of 100% (v/v). The MIC values were in the range of 12.5% - 50% (v/v) and the values indicated that a concentration of 50% (v/v) ACE could inhibit 87.5% (7/8) test cultures. The formation of AgNPs was confirmed by UV-VIS spectroscopy and the surface-plasmon resonance peak was observed at 430 nm. The FEG-SEM analysis revealed that the most of AgNPs were spherical in shape and had 15-25 nm size range. All the test cultures were inhibited by the AgNPs and the average ZOI measured 25.7±5.6 mm.

Conclusion: Cranberry fruit extract has a potent antibacterial activity against pathogens and it can be applied in green synthesis of silver nanoparticles.

Keywords: Cranberry, MIC, MBC, silver nanoparticles, FEG-SEM.

Article Info: Received 21 June 2019; Review Completed 07 Aug 2019; Accepted 14 Aug 2019; Available online 25 August 2019

Cite this article as:

Shah PJ, Malik R, Green Synthesis of Silver Nanoparticles using Aqueous Cranberry Fruit Extract and its Antibacterial Activity, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):991-995 http://dx.doi.org/10.22270/jddt.v9i4-s.3705

***Address for Correspondence:**

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

INTRODUCTION

The increasing failure rate of conventional antibiotics and the continuous emergence of resistance among the pathogens has become a common occurrence leading to the development of antimicrobial nanoparticles (NPs). The development of nanoparticles for the delivery of therapeutic agents has introduced new opportunities for improvement in the field of therapeutics. Nanoparticles bridge the gap between bulk materials and atomic or molecular structures [1]. Among the various types of metallic NPs, silver NPs (AgNPs) have proven to be the most effective against bacteria and viruses [2]. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. Plant materials, including leaf, bark, fruit, peel, seed, and root extracts can aid in the green synthesis of AgNPs and replace the hazardous chemicals [3]. The use of plants for AgNPs synthesis is preferred as it is less expensive, with ease of scaling up and ability of plant extracts to act as reducing as well as capping agents. Thus green synthesis of nanoparticles with controlled size and shape is an interesting field of research [2].

Cranberry (*Vaccinium macrocarpon*) is cultivated throughout the northern United States, Canada and Chile. It is a rich source of polyphenols, which have been associated with antibacterial, antiviral, anti-mutagenic, anti-carcinogenic, anti-tumorigenic, anti-angiogenic, anti-inflammatory, and antioxidant properties. The diverse phytochemical profile of Cranberry fruit includes three classes of flavonoids (flavonols, anthocyanins, and proanthocyanidins), catechins, hydroxycinnamic and other phenolic acids, and triterpenoids [4].

The current study demonstrates the green synthesis of AgNPs through the reduction of an aqueous $AgNO_3$ solution using fresh aqueous cranberry extract (ACE) as it possesses large amounts of natural antioxidants. The AgNPs developed were characterized and their antimicrobial activity was assessed.

MATERIALS AND METHODS

Plant material and extraction

Vaccinium macrocarpon L. or cranberry fruit were purchased from a local market in Mumbai, India. Washed 100 gm *V. macrocarpon* fruit was deseeded and crushed and then filtered using a muslin cloth and the fresh extract obtained was collected into a sterile container. The extract obtained was regarded as 100% extract and after sterility testing, was stored at 4°C for further study [5].

Bacterial cultures

The crude extract of cranberry 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 antibacterial activity of aqueous cranberry fruit extract (ACE) was evaluated by agar well diffusion method using Mueller-Hinton (MH) agar (HiMedia, Mumbai). 20 ml of molten media (MH agar) containing 100 µl of test inoculum (0.5 McFarland standards, 1.5×10^8 cfu/ml) was poured into a sterile petri-dish and allowed to solidify. Various concentrations of ACE (25%, 50%, 75%, and 100%, v/v) were prepared using sterile distilled water and then subsequently added to the wells (8 mm). Amikacin (30 µg/ml) was used as a 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 was measured in mm. The experiment was repeated in triplicates and the average values were recorded [6].

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

The minimum inhibitory concentration (MIC) of ACE was determined by a broth macrodilution method using Mueller Hinton broth (HiMedia, Mumbai). Two-fold dilution was performed using ACE along with MH broth to obtain various concentrations ranging from 50% to 1.56% (v/v) of ACE. The density of the eight bacterial cultures was adjusted to 10^6 cfu/ml and 0.1 ml of each was added to the tubes containing ACE 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. Absence of any visible growth (at the binocular microscope) at the lowest concentration of the extract was recorded as the MIC of ACE against the particular bacterial strain. A tube containing broth and inoculum but lacking ACE was considered as control [7, 8].

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. Post broth macrodilution, MBC was determined 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 [8, 9].

Biological synthesis of AgNPs using aqueous Cranberry fruit extract

The aqueous Cranberry fruit extract was prepared by cutting 10 g of the fruit and boiling it for 20 minutes with 100 ml distilled water. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected and stored. The filtrate was used as reducing and capping agent of silver particles. Silver nitrate (HiMedia, Mumbai) was used as a precursor for synthesis of AgNPs. For preparation of AgNPs, 10 ml of ACE was added to the 90 ml of 1.0 mM aqueous solutions of $AgNO_3$. The extract was added drop-wise under continuous stirring and kept overnight in dark at room temperature. The nanoparticles showed a colour change from pink to yellowish brown after 24 h of incubation [2, 10]. The change in colour of ACE marked the synthesis of AgNPs, which was further confirmed by UV-VIS Spectrophotometry and FEG-SEM.

Characterization of the silver nanoparticles

The formation of AgNPs was monitored by UV-VIS spectrophotometry and the plasmon peak was recorded. The shape and particle size of AgNPs was studied by Field emission gun-scanning electron microscopy (FEG-SEM) [10]. The topography of the nanoparticles was studied using FEG-SEM [8].

Checking for antimicrobial activity of the nanoparticles

The antibacterial activity of AgNPs prepared using *V. macrocarpon* extract was evaluated by using eight cultures by agar well diffusion method described above. The nanoparticles prepared using ACE 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 recorded in mm, in triplicates, and the average values were determined [11].

RESULTS

The antibacterial activity of ACE was carried out by the agar well diffusion method and the average zone of inhibition for the concentration of 100% (v/v) ranged from 19–30.3 mm with a mean of 22.3±4.6 mm against the test cultures. All the cultures were inhibited at 75% and 100% concentration of ACE. The concentration of 25% (v/v) was unable to inhibit *Salmonella paratyphi B, Salmonella paratyphi A,* and *Shigella spp.* (Table 1).

Table 1: ZOI of ACE against 8 pathogenic cultures by agar cup method

	Mean Zone of inhibition ± SD in mm			
Concentration (%) of ACE	**25%**	**50%**	**75%**	**100%**
Bacillus cereus	15.3±0.58	17±1.0	20±0	20.7±1.15
Escherichia coli	15±1	17.7±0.58	21.3±0.58	24±1.0
Salmonella paratyphi A	-	13.7±1.1	15.7±0.58	19±1.0
Salmonella paratyphi B	-	-	13.7±0.58	16.7±0.58
Salmonella typhi	10.7±0.58	16.7±0.58	25±0	30.3±1.52
Shigella spp.	-	14.7±0.58	18.3±0.58	19.6±0.57
Staphylococcus aureus	14±0	20.7±1.15	24±1.0	26.3±1.15
Vibrio spp	16.7±1.15	18±0	21±0	26±0

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

The MIC value of ACE was determined by the broth macrodilution method by using various concentrations of ACE. It was studied that ACE could inhibit the growth of all test cultures except *Salmonella paratyphi B,* 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%. It was found that the concentration of 50% ACE could inhibit 87.5% (7/8) of the test cultures. The results were further confirmed by determining MBC values. It was found that the concentration of 50% ACE could kill 75% (6/8) of the test cultures (Table 2).

Table 2: MIC and MBC of ACE by broth macrodilution method

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

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

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

The characterization of the nanoparticles formed was carried out by visual detection, UV-VIS spectroscopy and FEG-SEM analysis. The change of colour of the solution in the flask marked the formation of silver nanoparticles. A surface-plasmon resonance peak was obtained at 430 nm which confirmed the formation of silver nanoparticles. The Field emission gun-scanning electron microscopy (FEG-SEM) technique was used to characterize the nanoparticles formed and to study the surface morphology, size and shape. From the FEG-SEM images (Fig. 1), it is evident that AgNPs formed using cranberry fruit extract were spherical in shape. The measured average size of AgNPs was ranging from 15-25nm.

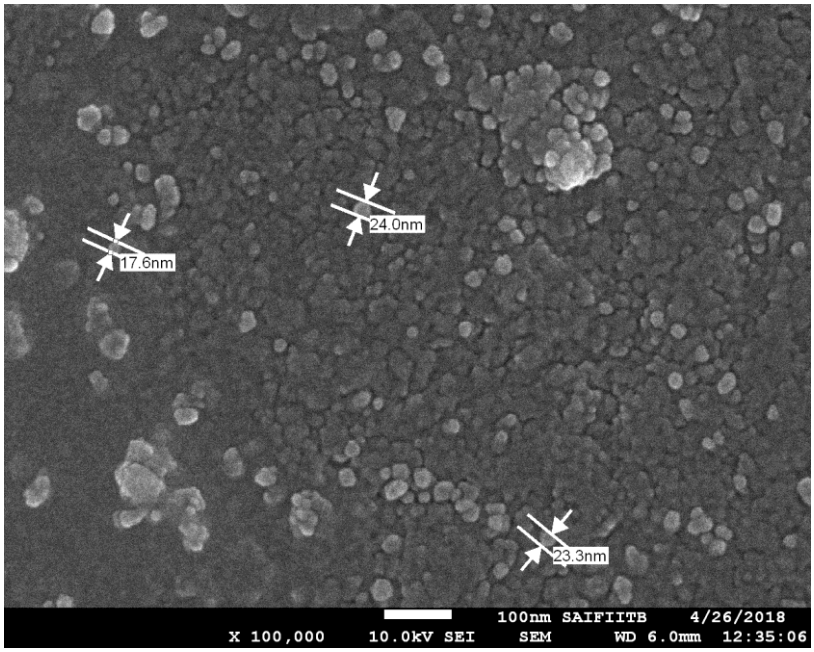

Fig. 1: Characterization of AgNPs by Field emission gun-scanning electron microscopy

Antimicrobial activities of the synthesized AgNPs using cranberry fruit extract were determined by using the agar well diffusion method. The size of ZOI for AgNPs formed was found to be ranging from 16.3 to 32.3 mm, against the test cultures used in the study. The maximum size of ZOI was found to be against *Salmonella typhi* at 100% concentration, whereas the minimum size of inhibition zone was observed against *Salmonella paratyphi B.* However, all the test cultures were inhibited by the AgNPs formed, indicating potent antibacterial activity (Table 3). The average zone of inhibition measured 25.7±5.6 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 ACE was statistically significant (*P<0.05), indicating effective antibacterial activity of the AgNPs formed by green synthesis.

Table 3: ZOI of AgNPs against 8 pathogenic cultures by agar cup method

Microorganism	Mean Zone of inhibition ± SD in mm	
	Control ($AgNO_3$)	AgNP-ACE (100%)
Bacillus cereus	16.7±0.58	30.0±1.0
Escherichia coli	20.0±1.0	27.3±0.58
Salmonella paratyphi A	17±0	18.7±0.58
Salmonella paratyphi B	13.3±0.58	16.3±0.58
Salmonella typhi	21.3±0.58	32.3±1.52
Shigella spp.	20.7±1.15	25.7±0.58
Staphylococcus aureus	21.3±0.58	29.7±0.58
Vibrio spp.	16.8±0.58	25.3±1.15

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

DISCUSSION

The present study analysed the antibacterial activity of fresh aqueous Cranberry fruit extract against eight bacterial pathogens. The results indicated a potent antimicrobial activity by ACE against the test strains. The antibacterial activity of ACE was evaluated by using agar well diffusion test. 75 % and 100% (v/v), ACE could inhibit all the strains indicating an effective antibacterial activity against the test strains. The study reaffirmed the results of a previous study carried out by Stobnicka et al., from Poland wherein the ACE displayed a more enhanced antibacterial activity against Gram-positive organisms in comparison to Gram-negative organisms [5], which was also further confirmed by the MBC studies. The difference in antibacterial activity against Gram-positive and Gram-negative bacteria could be attributed to the morphological differences between the cell wall of both types [8]. A study carried out by Ibrahim et al., in Iraq, attributes the antibacterial activity of ACE to the presence of secondary metabolites which can inhibit the adherence of pathogenic fimbriated *Escherichia coli* [12].

Nowadays, the silver nanoparticles are of high interest due to their particular properties and wide applications. AgNPs are used to inhibit many pathogens, including bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa* and fungi like *Aspergillus* and *Candida* spp [13]. It has been found that silver nanoparticles can cause alteration of the permeability of cell membrane, release of lipopolysaccharides and membrane proteins, damage of cell membrane, leading to cell death [8].

The antimicrobial activity of AgNPs depends on their size and shape. It was found that the smaller particle size gave the highest antimicrobial activity. It is considered that smaller size AgNPs having the large surface area are more easily available for interaction with microorganism than the

larger size AgNPs, thus smaller AgNPs would have stronger anti-bacterial activity [13]. In the current study, the synthesized nanoparticles were characterized by UV-VIS (surface plasmon resonance peak at 430 nm) and FEG-SEM analysis. The FEG-SEM images provided an insight into the morphology and particle size, shape, and distribution profile of the AgNPs. It was observed that the nanoparticles were spherical in shape and ranging from 15-25 nm in size. The AgNPs synthesized using ACE were bigger than the study reported by Ashour et al., which reported a size of 8.6 ± 2.5 nm with 0.8% dried cranberry extract [2]. This variation in size may be due to the change in concentration of ACE used.

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 25.7±5.6 mm. The concentration of AgNPs was found to increase with increasing ACE concentration. Addition of increasing concentration of reducing agent – ACE caused an increase in zone of inhibition of the synthesized AgNPs. The results were in corroboration with the study reported by Ashour et al [2]. Numerous studies have been carried out to study green synthesis of silver nanoparticles. However, there is a paucity of documented studies on fresh aqueous cranberry fruit extract for synthesis of silver nanoparticles. Most of the studies have used dried powder of cranberry fruit for formation of AgNPs.

CONCLUSION

There is a continuous increase in the number of multi-drug resistant bacteria worldwide and to control them different approaches are being tested. Silver nanoparticles have exhibited excellent bactericidal properties against a wide range of microorganisms. The synthesis of AgNPs by chemical and physical methods poses a risk to the environment. Plants contain several types of secondary metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-products. The results obtained from the current study indicated that fresh aqueous cranberry fruit extract has potent in-vitro antibacterial activity against Gram-positive and Gram-negative bacteria and was also found to be a good reducing and capping agent. The prepared AgNPs possessed significant in-vitro antibacterial activity. Thus, it can be suggested that the aqueous cranberry extract can serve as a safe and economic tool for synthesis of silver nanoparticles.

ACKNOWLEDGEMENT:

The authors are grateful to the Head of the Microbiology Department, K.C. College.

CONFLICT OF INTEREST STATEMENT:

The authors declare that they have no competing interests with whomsoever.

REFERENCES

1. Banerjee J, Narendhirakanan RT. Biosynthesis of silver nanoparticles from Syzygium cumini (L.) seed extract and evaluation of their in vitro antioxidant activities. Digest Journal of Nanomaterials and Biostructures. 2011; 6:961-968.
2. 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.
3. Kumar B, Smita K, Cumbal L. Debut, A. Green synthesis of silver nanoparticles using Andean blackberry fruit extract. Saudi journal of biological sciences. 2017; 24(1), 45-50.
4. Blumberg JB, Camesano TA, Cassidy A, Kris-Etherton P, Howell A, Manach C et al. Cranberries and their bioactive constituents in human health. Advances in Nutrition. 2013; 4(6):618-632.
5. Stobnicka A, Jungfer E, Gniewosz, M. Composition and antibacterial properties of fresh cranberry (Vaccinium macrocarpon L.) juice. Postepy Fitoterapii. 2013; 2:85-89.
6. 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.
7. 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.
8. Shah, P., & 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.
9. 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.
10. 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.
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. Ibrahim OMS, Sarhan SR, Hameed AA. In Vivo and in vitro antibacterial activities of cranberry extract against E. coli O157:H7 in urinary tract infected rats. Advances in animal and veterinary Sciences. 2015; 3(4):233-244.
13. Suwan T, Wanachantararak P, Khongkhunthian S, Okonogi S. Antioxidant activity and potential of Caesalpinia sappan aqueous extract on synthesis of silver nanoparticles. Drug Discoveries and Therapeutics. 2018; 12(5):259-266.