Evaluation of the antioxidant activity of *Annona muricata* (Guyabano) mediated silver nanoparticles and *Annona muricata* ethanolic leaf extract

ANN THERESE S. SOBRETODO, MICA ANTONIA D. JAEN, RICHLYN JOY S. VILLALOBOS, and RAMON ANGELO N. SINCO
Philippine Science High School - Western Visayas Campus, Brgy. Bito-on, Jaro, Iloilo City 5000, Department of Science and Technology – Science Education Institute, Philippines

Abstract

Silver nanoparticles (AgNPs) biosynthesized from various plant sources is an emerging material used for several applications. These may be used as antioxidants as several studies have focused on its ability to eliminate free radicals. *Annona muricata* (Guyabano) leaves are commonly known to possess such antioxidant properties due to its present bioactive compounds—thus becoming a suitable capping agent for AgNPs. The present study compared the antioxidant activity of *A. muricata* leaf extract, *A. muricata* mediated AgNPs, and commercially available AgNPs against ascorbic acid (positive control) and methanol (negative control) via DPPH Assay. Results show the leaf extract having the highest antioxidant activity after the positive control. This suggests that the plant extract can primarily be used as an antioxidant while the synthesized AgNPs do not contribute to its antioxidant property.

Keywords: *Annona muricata, silver nanoparticles, antioxidant, DPPH Assay, biosynthesis*

Introduction. Plant mediated silver nanoparticles (AgNPs) is a material known for inhibiting oxidation due to its infinitesimal size, larger surface area to volume ratio, and present medicinal properties [1,2]. The capability of plant mediated silver nanoparticles to inhibit oxidation is attributed to its present antioxidant properties, which may vary on how it is synthesized [3]. However, the factor which majorly contributes to the antioxidant activity of synthesized AgNPs is unknown [4,5].

Studies have speculated the presence of reducing and capping agents and functional groups in the extract as major contributors of the antioxidant activity of the system after AgNP synthesis [6,7]. Such factors are known to contribute to the antioxidant activity by reducing silver ions, stabilizing AgNPs, and possessing phytochemicals which form by-products after AgNPs synthesis [8]. These have been tested through the use of various experiments which are based on the presence of such factors. Phull et al. [9] tested the antioxidant activity of one experiment consisting of synthesized AgNPs mixed with its extract, resulting to increased antioxidant properties in the experiment containing both the capping and reducing agents and functional groups. This was shown to be more effective than the extract tested alone in the study. Mahmoud et al. [10], on the other hand, tested pure AgNPs centrifuged and washed twice with water, resulting to AgNPs with weakly-bonded capping agents. Consequently, the antioxidant activity of the AgNPs was also shown to be increased.

Annona muricata is known for its several bioactive compounds found within its leaves, making it suitable as a reducing and capping agent for the synthesis of silver nanoparticles [7]. These prevent AgNPs from further agglomeration. Its potency as a capping and reducing agent has been tested by Florence et al. [11], Muthu and Durairaj [12], Okalune, Onyechi and James [13], and Shaniba et al. [14]; establishing the plant as a producer of AgNPs. Due to the capability of plant extracts such as *A. muricata* to act as reducing and capping agents, this may also be a source for the antioxidant properties of synthesized AgNPs. Its leaves are also reported to possess enzymatic and non-enzymatic antioxidants moreso than *A. muricata* seeds, roots, and fruits [15]. However, compounds other than the reducing and capping agents may exhibit antioxidant activity.

Though there are studies which test the antioxidant activity of different kinds of AgNP experiments [9,16,17], it is unclear as to what majorly contributes to the antioxidant properties of synthesized AgNPs. However, this can be demonstrated by comparing the antioxidant activity of the leaf extract and the resultant nanoparticles biosynthesized from its extract. Thus, this study aims to evaluate the antioxidant activity using three experiments, namely *A. muricata* extract, *A. muricata* mediated AgNPs unfiltered from its originating extract, and commercially available AgNPs against ascorbic acid (positive control) and methanol (negative control). Studying the origin of the antioxidant activity of biosynthesized silver nanoparticles through the use of four experiments may lead to an increase in researches about these mechanisms. Moreover, this study would be able to contribute to understanding how silver nanoparticles may obtain its antioxidant properties and how other synthesis methods may relate to the source.

The objective of the study is to determine if the *A. muricata* mediated AgNPs have inherent antioxidant activity or if the synthesis process contributes to the antioxidant activity of the *A. muricata* leaf extract. It specifically aims to:

(i) Synthesize AgNPs from *A. muricata* ethanolic leaf extracts.

(ii) Confirm the presence of *A. muricata* mediated AgNPs using an Ultraviolet-Visible Spectroscopy (UV-Vis).

(iii) Evaluate and compare the antioxidant activity of (a) ascorbic acid (positive control); (b) *A. muricata* mediated AgNPs; (c) commercially available AgNPs; and (d) 2% leaf extract using DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay.

Methods. The present study includes the biosynthesis of silver nanoparticles using *Annona muricata* leaf ethanolic extract. The *A. muricata* leaves were first collected from Brgy. Cabilauan, New Lucena, Iloilo (10° 52′ 26.1546″ N 122° 35′ 34.602″ E) and washed in the laboratory of Philippine Science High School - Western Visayas Campus (PSHS-WVC). These were then oven-dried, pulverized using a blender, macerated in 95% ethanol, and stored in a dark area. After maceration, the solvent was separated using the rotary evaporator (Eyela Autojack NAJ-100) of the Department of Science and Technology - Region 6. The leaf extract was then diluted with distilled water to obtain a 2% concentration. Silver nanoparticles were synthesized after plant extraction. The synthesized AgNPs were then characterized using Ultraviolet-Visible Spectroscopy. Four experiments were prepared, namely the *A. muricata* mediated AgNPs, commercially available AgNPs obtained from Cytodiagnostics Inc., leaf extract, and ascorbic acid (positive control). Methanol was used as the negative control. The antioxidant activity of the setups was tested using DPPH Assay. The absorbance of each experiment was used to calculate the Radical Scavenging Activity of the setups.

Collection of Annona muricata Leaves. Fresh and mature *Annona muricata* leaves were hand-picked from an *A. muricata* tree in Brgy. Cabilauan, New Lucena, Iloilo. These were transported in a sterile paper bag to the Research laboratory of Philippine Science High School - Western Visayas Campus wherein the leaves were placed on clean sheets of aluminum foil.

Preparation of Annona muricata Leaves. The leaves were first washed with tap water, followed by distilled water [18]. These were then placed on a table covered with newspaper for air-drying. The midrib and degraded parts of the leaves were manually cut out using clean scissors by the researchers. After cutting, the leaves were placed in the oven and were oven-dried at 43° Celsius for 72 hours for the removal of moisture within the leaves. The leaves were then pulverized using an herb grinder (Mophorn) at 28000 rpm and were weighed using a toploading balance.

Extraction of Annona muricata. The pulverized *A. muricata* leaves were mixed with 95% ethanol at a ratio of 1:8 until the leaves were submerged for maceration. This is to obtain the remaining secondary metabolites which give the *A. muricata* its capping and reducing properties [18]. The leaf suspension was placed in a beaker with a sealed cover and was left for at least 72 hours within a dark area. The remaining extract was then filtered using Whatman no. 1 filter paper. The residual leaves were wrapped with foil and were properly disposed in a trash bin. The rotary evaporator in the Department of Science and Technology laboratory was used to separate the ethanol from the extract [18].

Synthesis of Silver Nanoparticles. The leaf extract produced from the use of the rotary evaporator was diluted with distilled water to obtain a 2% concentration. To synthesize AgNPs, a beaker of 100 mL 1mM $AgNO_3$ solution was placed on a hot plate at 30° Celsius, 400 rpm. Four hundred mL of the extract was poured gradually in the solution while this was being vortexed with a magnetic stirrer. The ratio of the extract to the $AgNO_3$ solution is 1:4 respectively [4]. AgNPs were produced by *A. muricata* mediated synthesis through the reduction of the Ag^+ ion in the silver nitrate ($AgNO_3$) by the plant extract.

Characterization of AgNPs. The Ultraviolet-Visible spectroscopy (UV-Vis) of PSHS - WVC (Shimadzu UV-1800) was used to confirm the presence of the synthesized silver nanoparticles [4]. The wavelength range was set to 500 nm - 200 nm [19].

Evaluation of Antioxidant Activity using DPPH Assay. DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay will be used to evaluate the antioxidant activity of AgNPs as this was found to be a simpler, less expensive, and rapid way to measure the overall antioxidant capacity and free scavenging activity of plant extracts in comparison to other assays [20]. Four experiments were prepared for antioxidant activity evaluation. The experiments include the 2% leaf extract, biosynthesized AgNPs, commercially available AgNPs, and ascorbic acid as the positive control [21]. Methanol was used as the negative control. First, 1.2 mL of 1 mM DPPH was dissolved in methanol and placed in 2.4 mL of each of the three experiments. As the solution is sensitive to light, this was placed in a dark cabinet for 30 minutes [7]. The absorbance peak of the stable DPPH was evaluated under the UV-Vis, which should result to approximately 517 nm [7]. The absorbance peaks of the four experiments were then compared and evaluated. With the resulting data, the Radical Scavenging Activity (%) was computed using the formula [22]:

$$RSA = \frac{control\ absorbance - sample\ absorbance}{sample\ absorbance}$$

Safety Procedure. Solvents used in the maceration process and in the DPPH assay were properly handled inside the laboratory. Toxic chemicals such as silver nitrate and DPPH (2,2-diphenyl-1-picrylhydrazyl) were handled according to information found in their Material Safety Data Sheets (MSDS). The chemicals were collected in a waste bottle and forwarded to the biosafety officers of the laboratory for proper disposal. Laboratory protocols were also observed during the conduct of the data gathering such as wearing of protective clothing such as gloves, lab gowns, masks, and goggles.

Results and Discussion. A light yellow color was produced from the biosynthesis of silver nanoparticles due to the size of the AgNPs. The absorbance peak of the AgNPs was found to be 1.788 at the wavelength 264.20 nm when tested under Ultraviolet-Visible Spectroscopy. The absorbance peaks at 517 nm of each experiment were found to be

indirectly proportional to its corresponding Radical Scavenging Activity (RSA %) values. DPPH Assay results have shown that the experiment, ascorbic acid (positive control), was found to have the highest RSA (%) calculated amongst the other experiments; followed by the 2% leaf extract, *A. muricata* mediated AgNPs, and commercialized AgNPs.

Synthesis of AgNPs. Minimal change in color was observed in the synthesis of AgNPs. The color changed from light green to light yellow after 90 minutes. This may be due to the size of the AgNPs and the diversity of phytochemicals existing within the plant extract [23].

UV-Vis Data Analysis. The graph (Fig. 1) shows the UV-Visible spectra of the *A. muricata* extract-reduced AgNPs. The absorbance peak of the AgNPs was found to be 1.788 at 264.20 nm. As seen in Fig. 1, the wavelength of the absorbance peak is below 400-450 nm, which is the required wavelength range for optimum AgNP size [24]. This indicates that the reduction of $AgNO_3$ produced synthesized AgNPs which are smaller than the conventional AgNPs used as antioxidants. Since smaller AgNPs absorb less light, the color of the mixture would appear lighter in color, as opposed to the usual dark brown of large synthesized AgNPs. Additionally, aggregated AgNPs exhibit darker colors during synthesis, which indicates that the AgNPs synthesized by the authors of the present study have not agglomerated [25].

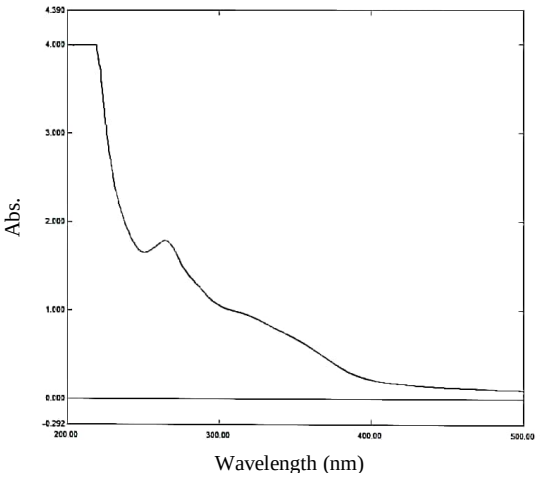

Figure 1. UV-Visible spectra of the *Annona muricata* extract-reduced AgNPs.

Quantification of Antioxidant Activity. For the conduct of DPPH Assay, visible observations of the discoloration were made in each samples during the preparations of the DPPH and the experiments. The positive control showed rapid change in color, closely followed by the sample of the leaf experiment and the mixture of the AgNPs with its extract. The absorbance levels at 517 nm were obtained using UV-Vis (Shimadzu UV - 1800) (Table 1). These values were used to calculate the RSA percentage of each experiment, which showed that the ascorbic acid had the highest RSA (44.8%), followed by the leaf extract (43.5%), *A. muricata* mediated AgNPs (29.6%), and the commercialized AgNPs (11.9%). Methanol was used as the negative control in the set up.

Table 1. Absorbance Peaks and RSA (%) of each of the four experiments.

Experiment	Absorbance at 517 nm	Radical Scavenging Activity (%)
Positive Control (Ascorbic Acid)	1.914	45
2% Leaf extract	1.961	43
A. muricata mediated AgNPs	2.441	30
Commercialized AgNPs	3.057	12

The speed of the discoloration of the experiments during the preparations could be attributed to the amount of antioxidants present within the samples. Higher amounts of antioxidants would result to quicker discoloration within the sample when mixed with DPPH. Based on the change in color, it could be seen that the positive control has the highest amount of antioxidants, followed by the 2% leaf extract, synthesized AgNPs mixed with the extract, and commercial AgNPs. The validity of this was then verified numerically using a UV-Vis.

For the antioxidant assay, it could be seen that the radical scavenging activity (%) of a experiment is dependent on the absorbance of each sample at 517 nm as this is the maximum absorption wavelength of DPPH. Trends in the results have proven that absorbance is indirectly proportional to the radical scavenging activity of each experiment. In effect, the experiment with the highest absorbance, the commercialized AgNPs, exhibited the lowest radical scavenging activity while the experiment with the lowest absorbance, the ascorbic acid, exhibited the highest radical scavenging activity.

Ascorbic acid was shown to have a higher antioxidant activity than that of the extracts (Fig. 2). Excluding the positive control, the 2% leaf extract proved to have the highest radical scavenging activity in comparison to the *A. muricata* mediated and commercialized AgNPs. This shows that good antioxidant compounds are present within the leaf extract. The high RSA of the experiment could furthermore be attributed to the stronger presence of phenols within the *A. muricata* extract as this is known to be a natural antioxidant [26]. The *A. muricata* mediated AgNPs, however, showed a lower RSA percentage than the leaf extract. This demonstrates that the *A. muricata* mediated AgNPs do not have inherent antioxidizing properties nor does synthesis yield by-products that have antioxidant properties. Hence, the presence of the unreacted leaf extract in the solution used to synthesize AgNPs is mainly if not

solely responsible for the RSA (%) data for the experiment. The commercialized AgNPs, on the other hand, is shown to have the lowest antioxidant activity, thus strengthening the claim that AgNPs do not have inherent antioxidant properties. This is because the commercialized AgNPs were not synthesized from plant extracts, thus lessening its chances of possessing naturally acquired antioxidant properties.

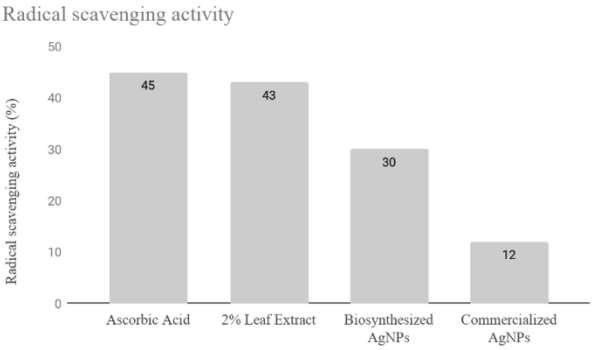

Figure 2. The column graph shows the radical scavenging activity of each of the four experiments.

Similar results were found in the study of Abdul-Aziz et al. [27] which compared the antioxidant and antimicrobial activity of *Chenopodium murale* mediated AgNPs and the plant extract. The study suggests that the plant extract majorly contributes to the antioxidant activity of AgNPs rather than the AgNPs in itself as this mainly serves as a carrier for biomedical properties. Additionally, higher antioxidant activity may also be obtained from the preferential adsorption of the antioxidant material of the plant extract to the surface of the silver nanoparticles [28]. These justify the lessened antioxidant activity of both the *A. muricata* mediated and commercialized AgNPs and the increased antioxidant activity of the 2% leaf extract experiment.

Results of the DPPH assay have also revealed that *A. muricata* extract is a good candidate for the synthesis of AgNPs. This is due to the high RSA value of the experiment, making this a suitable reducing agent as reducing agents scavenge oxygen from the medium to delay or prevent oxidation [29]. Additionally, antioxidants have been considered as reducing agents since they are able to perform antioxidant behavior by being oxidized [30]. Similar observations have been found in the study of Goodarzi et al. [31], which concluded that reducing substances in the extracts contribute significantly to the antioxidant potential of the tested plant species, and plants with a high reducing capacity are excellent sources for the green synthesis of metallic nanoparticles. Thus, it could also be concluded that the *A. muricata* extract is the strongest reducing agent after the positive control in comparison to the other experiments. This is then followed by the *A. muricata* mediated AgNPs and commercialized AgNPs.

Error Analysis. During the conduct of the study, some environmental factors were not taken into consideration, thus becoming possible factors for the error in the data obtained. Such factors are the pH and pressure during the synthesis of AgNPs and the particle size, temperature, and pH during the conduct of the DPPH Assay. The absence of replicates is also a factor that can produce an error in the study since there is no comparison to find the significance of the results.

Conclusion. *A. muricata* leaf extract successfully synthesized AgNPs with the help of its bioactive compounds as reducing and stabilizing agents. UV-Vis Spectroscopy showed the absorption peak of the AgNPs to be 1.788 at 264.20 nm, which alludes to the smaller size of the AgNPs in comparison to that of the usual AgNPs with an absorption peak at 390-420 nm. In the DPPH Assay, lower absorption values at 517 nm result to higher RSA percentages. In effect, the RSA percentage of the extract was proven to be higher than that of the *A. muricata* mediated AgNPs and commercialized AgNPs. This shows that the reducing agent contributes the most to the antioxidant activity of AgNPs.

Recommendations. In order to improve the results of the study, using different concentrations of extract for synthesis of AgNP is recommended to determine which concentration has improved antioxidant activity. Different concentrations of ascorbic acid in DPPH assay is suggested to correlate the RSA of these different concentrations to the RSA of the other set ups. The time of exposure of the experiments to DPPH can also be manipulated to have better results in the evaluation of the antioxidant activity. Determination of compounds present in the antioxidant activity which help in reducing and stabilizing the AgNPs is also recommended to further understand which compounds majorly contribute in synthesis and giving antioxidant activity.

Acknowledgement. We would like to extend our thanks to the staff of Department of Science and Technology - Region 6 for constantly granting us the permission to use their laboratory, materials, and equipment for free. Special thanks to Mr. Flouelin Malfarta for his guidance and for always accommodating us on short notice.

References

[1] Ramos AP, Cruz MAE, Tovani CB, Ciancaglini P. 2017. Biomedical applications of nanotechnology. Biophys Rev. 9(2):79–89.

[2] Zhang XF, Liu ZG, Shen W, Gurunathan S. 2016. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int. J. Mol. Sci. 17(9):1534. DOI: 10.3390/ijms17091534

[3] Iravani S, Korbekandi H, Mirmohammadi S, Zolfaghari B. 2014. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 9(6):385-406. PMID: 2633925

[4] Ghozali SZ, Vuanghao I, Ahmad NH. 2015. Biosynthesis and Characterization of Silver

Nanoparticles using *Catharanthus roseus* Leaf Extract and its Proliferative Effects on Cancer Cell Lines. J Nanomed Nanotechnol. 6:4. DOI: 10.4172/2157-7439.1000305

[5] Jeyaraj M, Sathiskumar G, Sivanandhan G, MubarakAli D, Rajesh M, Arun R, Kapildev G. 2013. Biogenic silver nanoparticles for cancer treatment: An experimental report. Colloids and Surfaces B: Biointerfaces. 106:86-92. DOI: 10.1016/j.colsurfb.2013.01.027

[6] Ahmed FA, Ali RFM. 2013. Bioactive Compounds and Antioxidant Activity of Fresh and Processed White Cauliflower. Biomed Research International. DOI: 10.1155/2013/367819

[7] Gavamukulya Y, Abou-Elella F, Wamunyokoli F, Ael-Shem H. 2014. Phytochemical screening, antioxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). Asian Pac J Trop Med. 7(Suppl 1):S355-S363. DOI: 10.1016/S1995-7645(14)60258-3

[8] Kulisic T, Radonic A, Katalinic V, Milosa M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry 85:633–640. DOI: 10.1016/j.foodchem.2003.07.024

[9] Phull AR, Abbas Q, Alib A, Razaa H, Kim SJ, Zia M. 2016. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliata.* Future Journal of Pharmaceutical Sciences. 2:31-36. DOI: 10.1016/j.fjps.2016.03.001

[10] Mahmoud W, Elazzazy AM, Danial EN. 2017. In vitro evaluation of antioxidant, biochemical and antimicrobial properties of biosynthesized silver nanoparticles against multidrug-resistant bacterial pathogens. Biotechnology & Biotechnological Equipment. 31(2):373-379. DOI: 10.1080/13102818.2017.1282836

[11] Florence NG, Benoit MZ, Jonas K, Alexandra T, Désiré DDP, Pierre K, Théophile K. 2014. Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 151:784–790. DOI: 10.1016/j.jep.2013.09.021

[12] Muthu S, Durairaj B. 2015. Evaluation of antioxidant and free radical scavenging activity of *Annona muricata.* European Journal of Experimental Biology. 5(3):39-45. ISSN: 2248 –9215

[13] Okalunle S, Onyechi O, James O. 2014. Toxicity, anti-lipid peroxidation, invitro and invivo evaluation of antioxidant activity of *Annona muricata* ethanol stem bark extract. American Journal of Life Sciences. 2(5):271-277. DOI: 10.11648/j.ajls.20140205.14

[14] Shaniba VS, Aziz AA, Kumar PRM. 2016. Phyto-mediated Synthesis of Silver Nanoparticles from *Annona muricata* Fruit Extract, Assessment of their Biomedical and Photocatalytic Potential. International Journal of Pharmaceutical Sciences and Research. 8(1):170-181. DOI: 10.13040/IJPSR.0975-8232.8(1).170-81.

[15] Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. 2015. Int J Mol Sci. 16(7):15625-15658. DOI: 10.3390/ijms160715625

[16] Banerjee J, Narendhirakannan RT. 2011. Biosynthesis of Silver Nanoparticles from *Synygium cumini* (L.) Seed Extract and Evaluation of their in vitro Antioxidant Activities. Digest Journal of Nanomaterials and Biostructures. 6(3):961-968.

[17] Ezealisiji KM, Noundou XS, Ukwueze SE. 2017. Green synthesis and characterization of monodispersed silver nanoparticles using root bark aqueous extract of *Annona muricata* Linn and their antimicrobial activity. Appl Nanosci. 7:905. DOI: 10.1007/s13204-017-0632-5

[18] Santhosh SB, Yuvarajan R, Natarajan D. 2015. *Annona muricata* leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector. Parasitol Res. DOI: 10.1007/s00436 015-4511-2

[19] Mirzajani F, Ghassempour A, Aliahmadi A, Esmaeili MA. 2011. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus.* Research in Microbiology. 162:542-549. DOI: 10.1016/j.resmic.2011.04.009

[20] Kedare SB, Singh RP. 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 48(4):412-22. DOI: 10.1007/s13197-011-0251-1

[21] Zadra M, Piana M, Brum TF, Boligon AA. 2012. Antioxidant Activity and Phytochemical Composition of the Leaves of *Solanum guaraniticum* A. St.-Hil. Molecules. 17:12560 - 12574. DOI: 10.3390/ molecules171112560.

[22] Nagaich U, Gulati N, Chauhan S. 2016. Antioxidant and Antibacterial Potential of Silver Nanoparticles: Biogenic Synthesis Utilizing Apple Extract. J Pharm (Cairo). DOI: 10.1155/2016/7141523

[23] Oldenburg SJ. 2016. Silver Nanoparticles: Properties and Applications. DOI: 10.3897/bdj.4.e7720.figure2f

[24] Khan NT, Jameel M. 2016. Optimization Studies of Silver Nanoparticle Synthesis by *Aspergillus terreus.* J Microb Biochem Technol. 8:6. DOI: 10.4172/1948-5948.1000330

[25] Nanocomposix. 2012. Uv/Vis/Ir Spectroscopy Analysis of Nanoparticles. 1.1:1-6

[26] Spiridon I, Bodirlau R, Teaca CA. 2011. Total phenolic content and antioxidant activity of plants used in traditional Romanian herbal medicine. Central European Journal of Biology. 6(3):388–396. DOI: 10.2478/s11535-011-0028-6

[27] Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA. 2014. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. Journal of Saudi Chemical Society. 18(4):356-363. DOI: 10.1016/j.jscs.2013.09.011

[28] Shirinwas P, Subhash K. 2013. Antioxidant, antibacterial and cytotoxic potential of silver nanoparticles synthesized using terpenes rich extract of *Lantana camara* L. leaves. Biochem Biophys Rep. 10:76–81.

[29] Loftsson T. 2014. Drug Stability for Pharma - ceutical Scientists. [Internet]. (n.p.): Academic Press; [cited 2019 February 18]. Available from: bit.ly/2UgXCpn

[30] Rao VR. 2016. Antioxidant Agents. In: Penta S, editors. Advances in Structure and Activity Relationship of Coumarin Derivatives. Available from: bit.ly/2ISEWLJ

[31] Goodarzi V, Zamani H, Bajuli L, Moradshahi A. 2014. Evaluation of antioxidant potential and reduction capacity of some plant extracts in silver nanoparticles' synthesis. Mol Biol Res Commun. 3(3):165–174. PMID: 27