Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

Formulation and Antibacterial Activity of *Bauhinia Variegate* Hydroalcoholic Extract Loaded Silver Nanoparticle

Amit Kumar Jain* and Kavita R. Loksh
Faculty of Pharmacy, Oriental University, Indore (M.P.)

ABSTRACT

Interest in green nanotechnology in nanoparticle biosynthesis is growing among researchers. Nanotechnologies due to their physicochemical and biological properties have applications in diverse fields, including drug delivery, sensors, optoelectronics and magnetic devices. Green synthesis of nanoparticles is an eco-friendly approach, which should be further explored for the potential of different plants to synthesize nanoparticles. The sizes of AgNPs are in the range of 1 to 100nm. Multi drug resistance has become an emerging and challenging issue for pharmaceutical industry as more and more bacteria are developing drug resistance towards many antibiotics, also over use of these synthetic drugs causes toxicity leading to further detrimental effects on human body. This study focused on the synthesis of silver nanoparticles, which is necessary for safe and effective exploitation of silver nanoparticles in collaboration with plant derived metabolites as a substitute for harmful synthetic drugs. Silver nanoparticles were synthesized using *Bauhinia variegata;* synthesized silver nanoparticles were then subjected to check antibacterial activity against multidrug resistant bacteria like *Staphylococcus aureus* and *Klebsiella pneumonia* by well diffusion method.AgNPs were synthesized by using 1mM $AgNO_3$ solution mixed with hydroalcoholic extract of *Bauhinia variegata*. The characterization of the prepared AgNPs was done by UV-Vis spectrometry and FTIR spectroscopy. The characterization results of the prepared AgNPs displayed that the silver nanoparticles are formed and stabilized by plant phyto-constituents and also exhibited virtuous antimicrobial property. Green synthesis process is a pivotal area in nanotechnology and usage of natural resources is the best choice for the making of NPs as a sustainable, eco-friendly, inexpensive and free of chemical contaminant method. These AgNPs have several potential biological and medical applications.

Keywords:*Bauhinia variegata*, Silver nanoparticles, FTIR spectroscopy, Antibacterial activity.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

Introduction

The utilization of nanotechnology for constructing nanoscale products in research and development divisions is growing [1]. Nanotechnology can be used to produce a broad range of products applicable to an equally broad array of scientific sectors. Creation, exploitation, and synthesis are terms associated with nanotechnology, which generally considers materials that measure less than 1 mm. Nano is derived from the Greek word nanos, meaning dwarf, tiny, or very small [2]. Nanotechnologies are generally classified as wet, dry and computational. Wet nanotechnology is associated with living organisms such as enzymes, tissues, membranes and other cellular components. Dry nanotechnology is associated with physical chemistry and the production of inorganic items, such as silicon and carbon. Computational nanotechnology is associated with simulations of nanometer-sized structures [3]. These three dimensions (wet, dry and computational) depend on each other for optimal functionality. Nanotechnology supports diverse unique industries, such as electronics, pesticides, medicine and parasitology and thus provides a platform for collaboration [4]. Nanobiotechnologies provide one such example, wherein the study and development combine multiple scientific sectors, including nanotechnology, biotechnology, material science, physics and chemistry [2, 5]. Biologically synthesized nanoparticles with antimicrobial, antioxidant and anticancer properties are possible through the collaboration of different natural science sectors. These nanotechnologies may provide novel resources for the evaluation and development of newer, safer and effective drug formulations [6]. Thus in recent time, usage of plant extract in the synthesis of nanoparticles is a quite novel practice [7]. So far various parts of the plants such as leaf, fruit, bark, peels, root and callus have been widely used in the synthesis of metal and metal oxide (silver, gold, platinum, titanium, iron and nickel) nanoparticles of varied sizes and shapes [8-15]. Currently, chemical, electrochemical, radiation, photochemical, Langmuir-Blodgett and biological methods have been extensively used in the synthesis of nanoparticles [16].Out of the said methods, plant-based biomimetic synthesis of silver nanoparticles judged to be the better method as it meets the demands of human health with the least toxicity. Plant-mediated nanoparticles are not only cost-effective and environmentally friendly but they can be synthesized in a single-step method as well [17]. Studies by various research groups demonstrate that Alfalfa roots could absorb silver nanoparticle from agar medium and at the oxidized state it transport to the plant shoot [18] Furthermore, silver nanoparticles synthesized using *Jatrophacurcas* latex, *Aloe vera*, *Acalyphaindica* and *Garciniamangostana*leaf extracts found to show enormous therapeutic applications such as antioxidant, antimicrobial, antifungal and anticancer [19,20].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

Bauhinia variegata Linn (Caesalpiniaceae) grows as a medium-sized, deciduous tree found throughout India and is commonly called Shemmandarai in Tamil [21]. It is traditionally used in bronchitis, leprosy, tumours and ulcer [22] and its extracts have been found to have antibacterial and antifungal activity [23]. Phytochemical studies revealed the presence of 5,7-dimethoxy and β-dihydroxy flavonone-4-O-α-l-rhamnopyronosyl-β-d-glucopyranosides, 5-hydroxyl7,3,4,5-tetramethoxyflavone-5-O-β-d-xylopyronosyl-(1–>2)-α-l-hamnopyroanoside, lupeol, β-sitosterol and quercertin [24-27].The present study aimed to assess the green synthesis of *Bauhinia variegata*mediated silvernanoparticles; its characterization and antimicrobial activity were attempted as there is no earlier report on the green synthesis of *Bauhinia variegata*AgNPs.

Materials and methods

Plant material

The barks of *Bauhinia variegata* were collected from local area of GulabganjVidisha (M.P.) in the month of March, 2019. The sample was identified by senior Botanist Dr. PradeepTiwari, Doctor Hari Singh Gour Vishwavidyalaya (M.P.) by comparing with the voucher specimen.

Chemical reagents

Silver nitrate ($AgNO_3$) is purchased from Sigma-Aldrich Chemicals for this study. Dimethyl sulphoxide (DMSO) was purchased from Merck, India. The pH buffer tablets were purchased from Himedia. Nutrient Agar, Nutrient Broth, Agar Agar, Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar Media were purchased from Himedia Laboratories, Mumbai, India. The pathogenic bacteria used in the current study obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India.The aqueous solutions were prepared with triple distilled water. All the chemicals used in this study were of analytical grade.

Extraction of plant material

Powdered plant material (bark) of *Bauhinia variegata*was shade dried at room temperature. The shade dried bark was coarsely powdered and subjected to extraction with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

Green synthesis of silver nanoparticles

$AgNO_3$ powder wasdissolved in distilled water to prepare 10 mM $AgNO_3$ stocksolution from which a series of 1, 2 and 3mM $AgNO_3$ solutions were prepared [28]. The AgNO3solutions were mixed with the hydroalcoholic extract of *Bauhinia variegata*at a ratio of 1:1 and 1:2 v/v to a volume of 50 mlin a flask. The flask was wrapped with an aluminum foil andwas then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use Table 1.

Table 1: Different formulation of silver nanoparticles

Formulation Code	Extract (mg)	AgNO3 (mM)	Ratio
F1	250	1	1:1
F2	250	2	1:1
F3	250	3	1:1
F4	250	1	1:2
F5	250	2	1:2
F6	250	3	1:2

Characterization of synthesized silver nanoparticles formulations

Microscopic observation of prepared silver nanoparticles

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared silver nanoparticle formulation [29].

Percentage Yield

The prepared silver nanoparticle with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres [30].

$$\%\ \text{Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Silver nanoparticle entrapped extract were isolated from the free drug using dialysis

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free flavonoids could be obtained from the absorbance difference based on standard curve [31].

Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the silver nanoparticles was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm [32-34].

Antibacterial activity of silver nanoparticles

The well diffusion method was used to determine the antibacterial activity of the extract prepared from the *Bauhinia variegata* using standard procedure of Bauer *et al* [35].The drug used in standard preparation was ciprofloxacin of IP grade. The antibacterial activity was performed by using 24hr culture of *Staphylococcus aureus*and*Klebsiellapneumonia.* There were 3 concentration used which are 25, 50 and 100mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particularconcentration of drug. The diameter of zone of inhibition of each wall was recorded.

Results and discussions

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of hydroalcoholicextraction was found to be 6. 34 % w/w.Practical yield of the prepared silver nanoparticles was in the range of 63.32±0.25 to76.65±0.16. The yield of nanoparticles decreased with increasing the concentration of

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

extract and silver nitrate, which might be due to generation of stickiness by extract. The maximum percentage yield was found in Formulation F4 (76.65±0.16).The EE was found to be in the range from 0.615±0.032 to 0.712±0.014%. It was observed that the encapsulation efficiency depends on the concentration of extract and silver nitrate ratio. On the basis of high yield, and encapsulation efficiency batch F4 was observed as optimized batch for the preparation of silver nanoparticles Table 2.Average particle size of nanoparticles (F4) was found to be 185.65 nm. The zeta potential is defined as the electrical potential between the medium and the layer of the fluid attached to the dispersed particles. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and is one of the fundamental parameters known to affect stability. Zeta potential of prepared nanoparticles (F4) was found - 34.5mV. It was found that higher the zeta potential less will be the particle aggregation, due to electric repulsion and hence more will be the stability of nanoparticles Figure 1 & 2.To study the antibacterial property of AgNPs, gram positive and gram negative bacteria were used and standard antibiotic Ciprofloxacin were used in this study. Zone of inhibition against bacterial growth produced by AgNPs was compared to standard antibiotic Ciprofloxacin. From the Table 3 & 4, it is concluded that synthesized AgNPs exhibit zone of inhibition nearly close to standard antibiotic values.

Table 2 Determination of % yield and entrapment efficiency of prepared formulations

Formulation	% Yield	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	63.32±0.25	0.623±0.025
F2	65.58±0.32	0.615±0.032
F3	67.74±0.14	0.627±0.021
F4	76.65±0.16	0.712±0.014
F5	71.12±0.52	0.658±0.025
F6	69.52±0.36	0.625±0.035

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

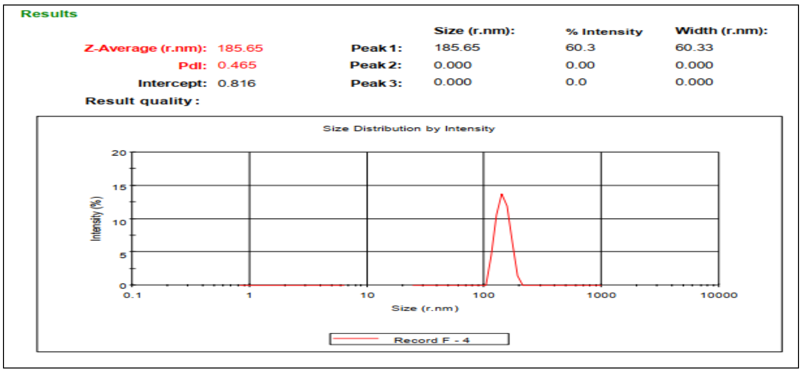

Figure 1: Graph of average particle size of formulation F4

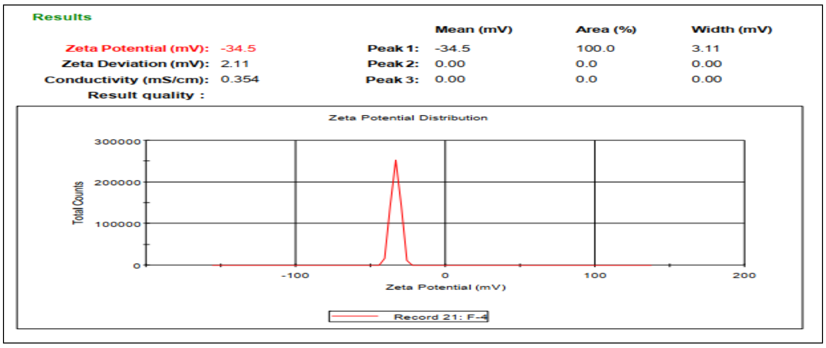

Figure 2: Graph of average vesicle size formulation F4

Table 3 Antibacterial activity of standard drugagainst selected microbes

S.N	Name of drug	Microbes	Zone of Inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Staphylococcus aureus*	17±1.69	18±2.62	22±2.16
		Klebsiellapneumoniae	19±4.71	28±1.24	36±1.699

Table 4 Antibacterial activity ofprepared silver nanoparticlesagainstselected microbes

S. No.	Name of microbes	Zone of inhibition		
		Hydroalcoholic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	11.25±0.05	13.65±0.78	15.65±0.50
2.	*Klebsiellapneumoniae*	18.45±0.35	20.23±0.75	23.25±0.82

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

Conclusion

The present investigation involved the green synthesis of AgNPs using hydroalcoholic extract; characterization and antibacterial activity of synthesized nanoparticles. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the plants and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of green synthesis of silver nanoparticles for this plant. Being exhibiting greater antibacterial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

References

1. Albrecht MA, Evans CW, Raston CL (2006) Green chemistry and the health implications of nanoparticles. Green Chem 8:417-432
2. Rai M, Yadav A, Gade A (2008) Current trends in phytosynthesis of metal nanoparticles. Crit Rev Biotechnol 28(4):277–284
3. Sinha S, Pan I, Chanda P, Sen SK (2009) Nanoparticles fabrication using ambient biological resources. J ApplBiosci 19:1113–1130
4. Bhattacharyya A, Bhaumik A, Rani PU, Mandal S, Epidi TT (2010) Nanoparticles—a recent approach to insect pest control. Afr J Biotechnol 9:3489–3493
5. Huang J, Chen C, He N, Hong J, Lu Y, Qingbiao L et al (2007) Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomumcamphora leaf. Nanotechnology 18:105–106
6. Dipankar C, Murugan S (2012) The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresineherbstii leaf aqueous extracts. Colloids Surf B: Biointerfaces 98:112–119.
7. S. Ahmed,M. Ahmad, B.L. Swami, S. Ikram, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise, J. Adv. Res. 7 (1) (2016) 17–28.
8. A.D. Mubarak, N. Thajuddin, K. Jeganathan, M. Gunasegaram, Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens, Colloids Surf. B: Biointerfaces 85 (2011) 360-365.

9. F. Benakashani, A.R. Allafchian, S.A.H. Jalali, Biosynthesis of silver nanoparticles using Capparisspinosa L. leaf extract and their antibacterial activity, Karbala International Journal of Modern Science 2 (2016) 251–258.
10. T.C. Prathna, N. Chandrasekaran, A.M. Raichur, A. Mukherjee, Biomimetic synthesisof silver nanoparticles by Citrus Limon (lemon) aqueous extract and theoretical prediction of particle size, Colloids Surf. B: Biointerfaces 82 (2011) 152–159.
11. M. Satishkumar, K. Sneha, S.W. Won, C.W. Cho, S. Kim, Y.S. Yun, Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its antibacterial activity, Colloids Surf. B: Biointerfaces 73 (2009) 332-338.
12. A.S. Annu, G. Kaur, P. Sharma, S. Singh, S. Ikram, Fruit waste (peel) as bio-reductant to synthesize silver nanoparticles with antimicrobial, antioxidant and cytotoxic activities, J. Appl. Biomed. 16 (3) (2018) 221–231.
13. N. Ahmad, S. Sharma,M.K. Alam, V.N. Singh, S.F. Shamsi, B.R.Mehta, Rapid synthesis of silver nanoparticles using dried medicinal plant of basil, Colloids Surf. B: Biointerfaces 81 (2010) 81–86.
14. A. Nabikhan, K. Kandasamy, A. Raj, N. Alikunhi, Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuviumportulacastrum L, Colloids Surf. B: Biointerfaces 79 (2010) 488–493.
15. Ali A. Annu, S. Ahmed, Green synthesis of metal, metal oxide nanoparticles, and their various applications, in: L.M.T. Martínez, O.V. Kharissova, B.I. Kharisov (Eds.), Handbook of Ecomaterials, Springer International Publishing, Cham 2018, pp. 1–45.
16. R.V. Kalaydina, K. Bajwa, B. Qorri, A. DeCarlo, M.R. Szewczuk, Recent advances in “smart” delivery systems for extended drug release in cancer therapy, Int. J. Nanomedicine 13 (2018) 4727–4745.
17. V. Kumar, S.K. Yadav, Plant-mediated synthesis of silver and gold nanoparticles and their applications, J. Chem. Technol. Biotechnol. 84 (2009) 151–157.
18. J.L. Gardea-Torresdey, E. Gomez, J.R. Peralta-Videa, J.G. Parsons, H. Troiani, M. Jose-Yacaman, Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles, Langmuir 19 (2003) 1357–1361.
19. P. Tippayawat, N. Phromviyo, P. Boueroy, A. Chompoosor, Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. Corbo MR, ed, Peer J. 4 (2016) 2589.

20. Annu, S. Ahmed, G. Kaur, P. Sharma, S. Singh, S. Ikram, Evaluation of the antioxidant, antibacterial and anticancer (lung cancer cell line A549) activity of Punicagranatum mediated silver nanoparticles, Toxicol. Res. 7 (2018) 923–930.
21. Nadkarni, A.K., 1996. Indian MateriaMedica, vol. I. Popular Prakashan Pvt. Ltd., Bombay, pp. 184–185.
22. Kirtikar, K.R., Basu, B.D., 1993. Indian Medicinal Plants, vol. II. International Book Publisher, Dehradun, pp. 898–900.
23. Ali, M.S., Azhar, I., Amtul, Z., et al., 1999. Antimicrobial screening of Caesalpiniaceae. Fitoterapia 70, 299–304.
24. Gupta, A.K., Vidyapati, T.J., Chauhan, J.S., 1979. 5,7-Dihydroxy flavonone-4 -O--l-rhamnopyronosyl--d-glucopyranosides from the stem of Bauhinia variegata. Indian Journal of Chemistry: Section B 18, 85–86.
25. Gupta, A.K., Vidyapati, T.J., Chauhan, J.S., 1980. Chemical examination of stem of Bauhinia variegata. PlantaMedica 38, 174–176.
26. Yadava, R.N., Reddy, V.M.S., 2001. A new flavone glycoside 5-hydroxyl-7,3 4 5 -tetra-methoxyflavone-5-O-β-d-xylopyronosyl-(1–>2)-β-lrhamnopyroanoside from Bauhinia variegate Linn. Journal of Asian Natural Product Research 3, 341–346.
27. Duret, S., Paris, R.R., 1977. The flavonoid of several species of Bauhinia. Plant Medicinal Phytotherapy 11, 213–216.
28. Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K, Thangamani S. Synthesis of silver nanoparticles using leaves of *Catharanthusroseus* Linn. G. Don and their antiplasmodial activities. Asian Pac J Trop Biomed 2012; 2(7): 574-580.
29. Vanaja, M., Gnanajobitha, G., Paulkumar, K., Rajeshkumar, S., Malarkodi, C., Annadurai, G. Phytosynthesis of silver nanoparticles by Cissusquadrangularis: infuence of physicochemical factors. J. Nanostruct. Chem. 3, 1–8 (2013).
30. Umashankari, J., Inbakandan, D., Ajithkumar, T.T., Balasubramanian, T.: Mangrove plant, *Rhizophoramucronata* (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens. Aquat. Biosyst. 8, 1–8 (2012).
31. Banerjee, P., Satapathy, M., Mukhopahayay, A., Das, P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresour. Bioprocess. 1, 1–10 (2014)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 2050 - 2060
Received 08 November 2020; Accepted 15 December 2020.

32. Raut Rajesh, W., Lakkakula Jaya, R., KolekarNiranjan, S., Mendhulkar Vijay, D., KashidSahebrao, B.: Phytosynthesis of silver nanoparticle using Gliricidiasepium (Jacq.). Curr. Nanosci. 5, 117–122 (2009)
33. De Jesus Ruíz-Baltazar, Á., Reyes-Lopez, S.Y., Larrañaga, D., Estévez, M., Pérez, R.: Green synthesis of silver nanoparticles using a Melissa ofcinalis leaf extract with antibacterial properties. Results Phys. 7, 2639–2643 (2017).
34. B. Ajitha, Y. Ashok Kumar Reddy, and P. Sreedhara Reddy, "Green synthesis and characterization of silver nanoparticles using Lantana camara leaf extract," Materials Science and Engineering: C, vol. 49, pp. 373–381, 2015.
35. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 1966, 45:493–496.