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ORIGINAL RESEARCH

Antibacterial Activity of Bio Synthetic Silver Nanoparticles Against Escherichia Coli And Salmonella Typhimurium Using Moringa Oleifera Leaves Extract

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ABSTRACT

Objectives: Antibacterial activity of bio synthetic silver nanoparticles against Escherichia coli (E. coli) ATCC 8739 and Salmonella typhimurium (S.typhi) ATCC 14028 using Moringa oleifera leaves extract was investigated.

Materials and Methods: Prepared silver nanoparticles were characterized by using different techniques included: Ultraviolet Visible Spectroscopy (UV-VIS spectra analysis), Fourier transform infrared spectroscopy (FTIR) and Transmission electron microscope (TEM). The comparison between the antibacterial activities of the concentration 100 µg/ml of bio synthetic silver nanoparticles using Moringa oleifera leaves extract and 100 µg/ml of silver nitrate solution tested against E. coli reference strain ATCC 8739 and S. typhi ATCC 14028 was evaluated by using agar diffusion assay method.

Results: FTIR analysis was showed the reduction action of Moringa oleifera leaves extract to form Silver Nanoparticles. TEM shows a dispersion of nanoparticles in a range (15.22 nm – 29.45) by using Moringa oleifera leaves extract. The antimicrobial activity of the concentration 100 µg/ml of synthetized silver nanoparticles was approved against E. coli reference strain ATCC 8739 by showing zone of inhibition against E. coli equal to 18 mm, while 100 µg/ml of the silver nitrate solution showed zone of inhibition against E. coli equal to 17 mm.

While The antimicrobial activity of the concentration 100 µg/ml of synthetized silver nanoparticles which tested against S.typhi reference strain ATCC 14028 by showing showed zone of inhibition against equal to 21 mm, while 100 µg/ml of the silver nitrate solution showed zone of inhibition against S.typhi equal to 15mm

KEY WORDS: *Silver nanoparticles, Antibacterial, Moringa oleifera leaves extract, green synthesis.*

INTRODUCTION

Nanotechnology is the study and use of small entities which used in various fields such as chemistry, biology, physics and engineering. Nanoparticles received massive attention from chemists, physicists, biologists, and engineers who demand to use them for the progress of a new Nano devices (Das, Parida, & Bindhani, 2013).Nano means a billionth or 10-9 unit and its size ranges from 1nm to100nm (Brian, Hemachitra, Deepa, & Selvi, 2016). Recently, the nanoparticles have been the most interesting research topic due to their unique optical, electronic, mechanical and

chemical properties that are considerably altered from those of bulk materials. For these causes, nanoparticles used in various applications in altered fields, such as catalysis, photonics, and electronics (Suresh, Annapurna, Bhikshamaiah, & Singh, 2014).

Researchers used physical and chemical devices to synthesize nanoparticles like gas phase deposition, lithography via use of electron, laser ablation, electrochemical deposition, and heat decomposition method. Although, both of physical and chemical techniques produce nanoparticles with different size and morphology but these techniques are quite costly, have a potentially dangerous effect to the environment and leads to various biological risks. So, it is essential to improve such methods which are eco-friendly, cheapest with extreme production, having no side effect on human health and environment. These biological methods have many benefits more than chemical and physical methods is as it eco-friendly, prevent pollution and wastes manufacture, decrease time and energy and the most importantly its economical synthesis of Nanoparticles.(Hasan et al., 2018,Mokgweetsi, 2018).

Biological synthesis of nanoparticles may be achieved by using plants (Lee et al., 2011) , bacteria (Li, Xu, Chen, & Chen, 2011) , fungi (Zhao et al., 2018) and human cells (El-Said, et al.,2014). The nanoparticles like silver, gold and many other metals synthesized by the biological method (Li, et al., 2011)

Many plants used for the synthesis of Nanoparticles. Moringa oleifera is one of this plants(Mokgweetsi, 2018). Moringa oleifera (Moringingaceae, English: drumstick tree) used as part of Indian diet since periods. It cultivated at all over the country and its leaves and fruits are used as vegetables. Moringa oleifera parts used in traditional medicine properties. The Moringa oleifera leaves have antitumor properties, hypotensive properties, cardio protective, wound curing activities and use for eye diseases. Moringa oleifera extract contains metabolites which help in the ions reduction and synthesize of nanoparticles faster than microbes (Behravan et al., 2019).There were found a highly antimicrobial activity against different pathogenic tested microorganisms (Tnvkv & Ek, 2014). The main advantage of using Moringa oleifera plant extract was the synthesized of silver nanoparticles.

Silver nanoparticle is a chemically stable substance; it has a special antimicrobial activity and has a good conductivity property. Silver nanoparticle shows a good antimicrobial character compared to its salts because it has high surface area, which delivers strongly interaction with microorganisms (Saware et al., 2014). The antimicrobial activity mechanism of silver nanoparticles can be explained by directly damage bacteria cell membranes or by increasing of the membrane permeability or by disordering of DNA replication. Silver nanoparticles effective biocides against bacteria such as E. coli (Marambio-Jones & Hoek, 2010).

This study investigates the antibacterial activity of bio synthetic silver nanoparticles against gram negative bacteria included E. coli ATCC 8739 which indicate the presence of fecal contamination and against S.typhi ATCC 14028 which causes typhoid disease using Moringa oleifera leaves extract which was an example of biological synthesis of nanoparticles was investigated.

MATERIALS AND METHODS

Collection and identification of Moringa oleifera leaves

Fresh leaves of Moringa oleifera were collected in October 2018 from Hassan Land at Al-Sadat City, Egypt.

Preparation of Moringa oleifera leaves powder and aqueous extract

Fresh leaves of Moringa oleifera dried under shade at room temperature for 11 days. The dried leaves of Moringa oleifera ground to form powder using a mortar and pestle(Mitiku & Yilma, 2017).Plant leaf extract prepared by mixing 10 g of powdered leaf weighted balance (Vibra-AJ-320E-Jaban) with 100 mL Purified water in 500 mL of Erlenmeyer flask and boiled for 20 minutes(Tnvkv & Ek, 2015); the solution was then kept at room temperature to cool down. The plant extract was then filtered out by using Whatman filter paper no.1.The dried leaf powder

The standard extracts obtained were then stored in a refrigerator at 4°C further use as a reducing, capping and

stabilizing agent for the synthesis of silver nanoparticles without further treatment (Brian et al., 2016).

Preparation of 1 mM of silver nitrate solution.

Seventeen milligrams (17 mg) of Silver nitrate (AgNO3, 99.99%) - Sigma Aldrich chemicals. AgNO3, MW = 169.87 g/mol) weighted using balance (Vibra-AJ-320E-Jaban) and transferred into 100 ml volumetric flask. The silver nitrate was slowly dissolved by slightly swirling the flask containing deionized water. After Silver nitrate dissolved, more deionized water was slowly added to bring the level of solution exactly to a volume mark of 100 ml (Malathi & Rajkumar, 2015)

Preparation of Microbial Strains Suspension

The bacterial strains used comprise a discs of E. coli ATCC 8739 and S.typhi ATCC 14028 bacteria- Selectrol -United Kingdom. According to supplier instruction, Place a discs of E. coli ATCC 8739 and S.typhi ATCC 14028 on Nutrient agar solid media ,the allow disc to soften for 10-15 minutes then incubate it in incubator at 33◌ C for 24 hours, then collect them in sterile bottle using 10 ml of 0.9% sterile saline.

Synthesis of silver nanoparticles

A volume of 10 ml of aqueous extract of Moringa oleifera leaves was added to 90 ml of 1 mM of silver nitrate solution in 500 ml Erlenmeyer flask for reduction of Ag+ ions and stabilization of silver nanoparticles(Tnvkv & Ek, 2015) ,then heated at 60°C for 3 hours .A change from yellowish to brown color was observed.

Separation and purification of silver nanoparticles from crude matrix

The samples were then centrifuged at 6000 rpm for 15 min to get a clear supernatant at room temperature (Vaghela, et al., 2017).The process of centrifugation and re-dispersion in distilled deionized water was repeated three times to ensure better removal of free entities from the powdered nanoparticles.

Optimization of synthesis parameters

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 30 minutes, 1 hour, 2 hours and 3 hours. At different temperature 22°C, 30°C, 60°C and 90°C, using to measure different pH condition 3,6.2 and 10 PH values

Characterization of silver nanoparticles Techniques

Visual observation

The color change in reaction mixture from yellowish to brown color (Gopalakrishnan & Gandhi Muniraj, 2007; Mitiku & Yilma, 2017) .

UV-VIS spectra analysis

UV-VIS spectroscopy or Ultraviolet-Visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region(Das et al., 2013). UV-VIS spectroscopy is used to determine the optical properties of nanoparticles. Light move through the sample solution and the amount of absorbed light wavelength is measured.

The solutions of silver nanoparticles with Moringa oleifera leaves extract were characterized using spectrophotometer UV–Vis (UV-1601PC -Shimadzu) at (400-800 nm)

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR measures interaction between silver salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of silver nanoparticles formed using Moringa oleifera leaves extract.

Analysis done by using for JASCO FT/IR-5300 model operated in the diffuse reflectance mode at a resolution of region 4000-500 cm^{-1}.

spectral analysis was carried out to detect biomolecules responsible for the reduction of Ag+ ions and stabilization of silver nanoparticles (Saminathan, 2015)

Dried powdered sample of tested silver nanoparticle showed peaks at 3436.8, 2067.7720, 1637.5216, 534.2410 cm^{1}.

Transmission Electron Microscopy (TEM)

The size of dried powdered nanoparticles was determined by TEM. JSM1400-PLUS-JEOL is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

Antimicrobial activity study

Antimicrobial activity of the synthesized silver nanoparticles was determined, using the agar well diffusion assay method.

Antibacterial activity of silver nanoparticles

Antimicrobial activity of the synthesized silver nanoparticles was determined against E. coli and using the agar well diffusion assay method. 25mL of molten and warmed media Nutrient agar (Hexa-Bioteck, Egypt) containing poured in sterile petri dishes and allowed to solidify (Gopalakrishnan & Gandhi Muniraj, 2007).

From the original Strain suspension bottles, the tested microbial organisms included E. coli and S.typhi was swabbed regularly on the different 2 plates using sterile cotton swab. The Powder of silver nanoparticle was prepared as a concentration of 100 µl / ml using deionized water of and tested against 100 µl/ml of silver nitrate using micropipette. 2 Pores were made in the 2 plate with sterilized stainless-steel cork poorer each plate filled with 100 µl / ml of silver nanoparticle and the other hole filled with 100 µl / ml of silver nitrate solution. The tested plates incubated at 37°C for 24 - 48 h. Zones of inhibition appear as a clear area around the Pores (Nilanjana etal., 2014, Perez etal., 1990.)

RESULTS AND DISCUSSION

Optimization of synthesis parameters

Effect of PH

pH value has importantly impact the size and morphology of the biologically synthesized nanoparticles, where pH strongly changed the electrical charges of biomolecules and capping agents and as a result changing their capacity to bind and reduce metal ions(Hamouda, Hussein, Abo-elmagd, & Bawazir, 2019).It is shown that maximum absorbance of the synthesized silver nanoparticles was in basic, then in 6.2 PH value and the acidic condition show the lowest absorbed level as shown in Figure 1.

PH measurement by using JENWAY3510 model

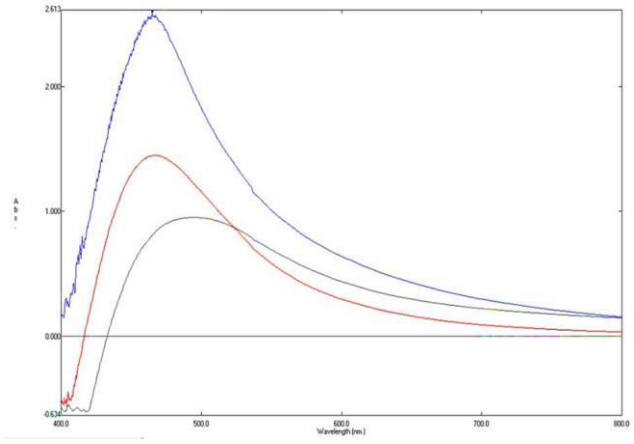

Figure 1. Effect of PH of bio synthesis of Silver nanoparticles using Moringa oleifera leaves extract

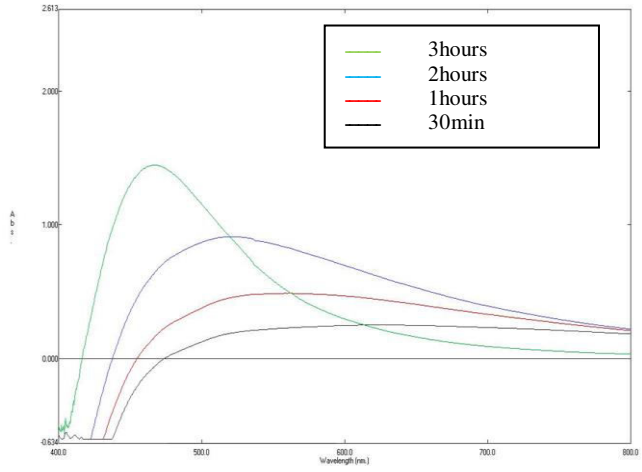

Figure 2. Effect of Time of bio synthesis of Silver nanoparticles using Moringa oleifera leaves extract.

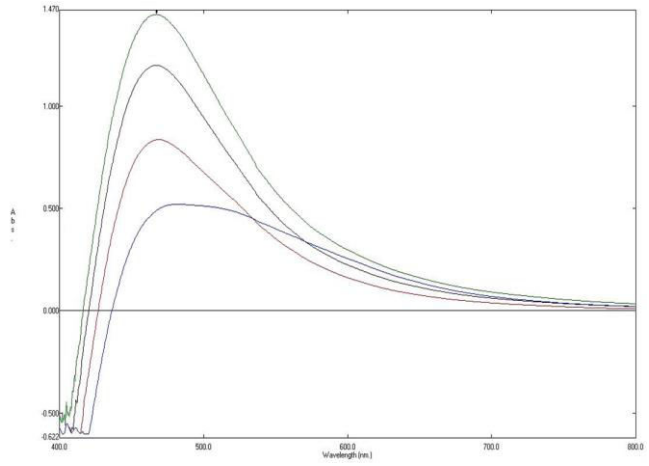

Figure 3. Effect of Temperature bio synthesis of Silver nanoparticles using Moringa oleifera leaves extract.

stage of reaction the nucleation starts by the reduction of metallic ions that starts forming the small aggregates which combine to each other to form nanoparticles at variable rates depending on the reaction conditions like reaction time as shown in Figure 2.

Heating temperature of extract

Temperature is one of the most essential factors affecting the synthesis and crystal structure of nanoparticles using plant extracts (Hasan et al., 2018) Maximum absorbance of the synthesized silver nanoparticles increased step by step with increase in heating temperature from 22°C to 60°C which led to Maximum absorbance value equal to 1.45 and began to decline until it reached 90°C due to aggregation of nanoparticles.(Figure 3).

Characterization of silver nanoparticles

Visual observation

The synthesis of silver nanoparticles was mainly observed by color change of the aqueous extract Moringa oleifera leaves from yellowish color; (Figure 4) after the reaction with silver nitrate which colorless solution to dark brown color for silver nanoparticles (Sultana et al., 2015)

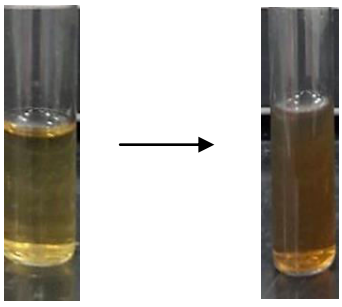

Figure 4. Color change from yellow color to brown due to formation of silver nanoparticles.

TEM analysis of silver nanoparticles

Transmission electron microscopy (TEM) used to characterize the size, shape and morphology of synthesized silver nanoparticles(Suresh et al., 2014)

Nanoparticles are the particles which have size between (1-100 nm). The silver nanoparticles synthesized with the help of Moringa oleifera leaf extract was observed by using TEM (JSM1400-PLUS-JEOL). TEM show silver nanoparticle size (29.45-24.97-15.22nm) and this is an indication of formation silver nanoparticles as shown in Figure 5.

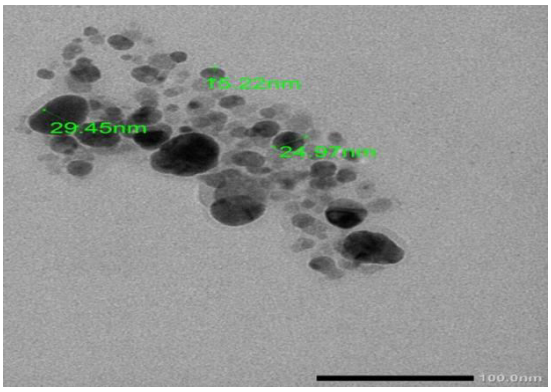

Figure. 5. TEM show silver nanoparticles size (29.45-24.97-15.22nm)

FTIR spectral analysis

FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic AgNO3 to silver nanoparticles by the action of the different Substances which would act simultaneously as reducing, stabilizing and capping agent.

It is recognizable that the biomolecules are the reasons for the reduction of Ag+ to Ag0(Dada et al., 2018)

The carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of plant extract shown in the FTIR spectrum are mostly elaborate in reduction of Ag+ to Ag0 nanoparticles. These bands refers to stretching vibrational bands responsible for compounds such as flavonoids and terpenoids and so may be held responsible for effective capping and stabilization of obtained silver nanoparticles.(Sultana et al., 2015). Figure 6 shows several absorption peaks observed at (3436.81- 2067.77 – 1637.52 - 534.24 cm−1)

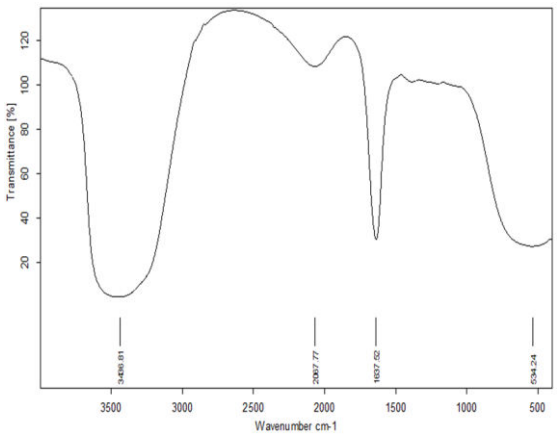

Figure 6. FTIR showing functional groups present in the synthesized silver nanoparticles using Moringa oleifera leaves extract

3436.81 refers to N-H & O-H Functional groups

2067.77 refers to C=C Aromatic Functional groups

1637.52 refers to N-H & C=O Functional groups

534.241 refers to C-Br Alkyl halide Functional groups

The peak at 3436.81 cm-1 in the FTIR spectrum refers to N–H stretching vibration of amino groups and OH stretching of hydroxyl group in phenols (Hassan et al., 2016). The peak at 2067.77 cm-1 refers to C=C Aromatic Functional groups 1637.52 cm-1 relates to amine groups of–N–H bending vibrations of proteins and specific of -C=O carbonyl groups (Selvam and Sivakumar, 2015). The peak at 534.24 cm-1 refers to C-Br Alkyl halide Functional groups.

Antibacterial activity of silver nanoparticles

The antibacterial activity of pure silver nanoparticles compared to silver nitrate against E. coli and S.typhi was reported. Silver nanoparticles show higher antibacterial activity than silver nitrate (Marslin et al., 2018). When 100 µg/ml of pure silver nanoparticles tested against E. coli it made 18 mm Zone of inhibition while 100 µg/ml of silver nitrate solution made 17 mm zone of inhibition as shown in Figure 7.

When 100 µg/ml concentration of pure silver nanoparticles tested against S.typhi, it made 21 mm Zone of inhibition while 100 µg/ml of silver nitrate solution made 15 mm zone of inhibition as shown in Figure 8.

The high surface area of silver nanoparticles increases their contact with micro-organisms. Free radicals induced by silver nanoparticles damage the cell membrane of bacteria lead to cell death. (Tashi, Vishal Gupta, & Mbuya, 2016)

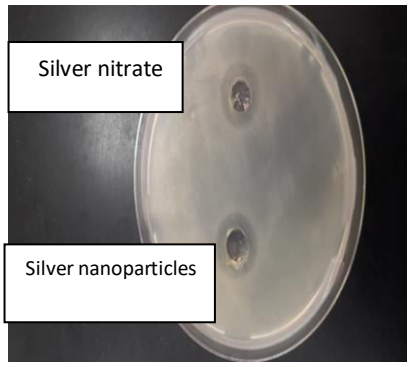

Figure.7. Effect of Silver nanoparticles and silver nitrate against E. coli.

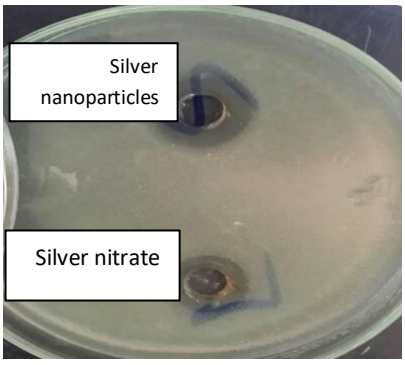

Figure.8. Effect of Silver nanoparticles and silver nitrate against S.typhi.

Conclusion

The biological synthesis of nanoparticles is considered simple, clean, non-toxic and eco-friendly than physical and chemical synthesis of nanoparticles. Moringa oleifera is an example of biological method of nanoparticles synthesis. Moringa oleifera leaves extract used in synthesis of various nanoparticles such as silver nanoparticles. Synthesized silver nanoparticles related to various factors like PH, mixture temperature and time. Silver nanoparticles which synthesized from Moringa oleifera leaves extract showed efficient antimicrobial activity against E. coli and S.typhi. , So silver nanoparticles are promising antibacterial agents for water and diseases cure applications.

REFERENCE

Behravan, M., Hossein Panahi, A., Naghizadeh, A., Ziaee, M., Mahdavi, R., & Mirzapour, A. (2019). Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. International Journal of Biological Macromolecules, 124, 148–154. https://doi.org/10.1016/j.ijbiomac.2018.11.101

Brian, M. O., Hemachitra, P., Deepa, R., & Selvi, V. S. (2016). Synthesis of silver nanoparticles and its antibacterial activity from Moringa oleifera , Murraya koingii and Ocimum sanctum against E . coli and S . aureus, 8(10), 150–160.

Dada, A. O., Inyinbor, A. A., Idu, E. I., Bello, O. M., Oluyori, A. P., Adelani-akande, T. A., & Okunola, A. A. (2018). Effect of operational parameters , characterization and antibacterial studies of green synthesis of silver nanoparticles using Tithonia diversifolia, 1–17. https://doi.org/10.7717/peerj.5865

Das, S., Parida, U. K., & Bindhani, B. K. (2013). Green Biosynthesis of silver nanoparticles using Moringa Oleiferal leaf, 3(2), 51–62.

El-Said, W. A., Cho, H. Y., Yea, C. H., & Choi, J. W. (2014). Synthesis of metal nanoparticles inside living human cells based on the intracellular formation process. Advanced Materials, 26(6), 910–918. https://doi.org/10.1002/adma.201303699

Gopalakrishnan, V., & Gandhi Muniraj, Nn. S. (2007). Biosynthesis of Silver Nanoparticles Using Aqueous AzadirachtaIndica (Neem) Flower Extract-Optimization, CharacterizationAnd Study of Antimicrobial and Anti-Oxidant Effects. International Journal of Innovative Research in Science, Engineering and Technology (An ISO Certified Organization), 3297(1), 11–21. Retrieved from www.ijirset.com

Hamouda, R. A., Hussein, M. H., Abo-elmagd, R. A., & Bawazir, S. S. (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium Oscillatoria limnetica, (August), 1–17. https://doi.org/10.1038/s41598-019-49444-y

Hasan, M., Ullah, I., Zulfiqar, H., Naeem, K., Iqbal, A., Gul, H., … Mahmood, N. (2018). Biological entities as chemical reactors for synthesis of nanomaterials: Progress, challenges and future perspective. Materials Today Chemistry, 8(March 2019), 13–28. https://doi.org/10.1016/j.mtchem.2018.02.003

Lee, H. J., Lee, G., Jang, N. R., Yun, J. H., Song, J. Y., & Kim, B. S. (2011). Biological synthesis of copper nanoparticles using plant extract. Technical Proceedings of the 2011 NSTI Nanotechnology Conference and Expo, NSTI-Nanotech 2011, 1(January 2011), 371–374.

Li, X., Xu, H., Chen, Z. S., & Chen, G. (2011). Biosynthesis of nanoparticles by microorganisms and their applications. Journal of Nanomaterials, 2011. https://doi.org/10.1155/2011/270974

Malathi, R., & Rajkumar, K. (2015). Synthesis of silver nanoparticles and its applications 4(09), 673–678.

Marambio-Jones, C., & Hoek, E. M. V. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research, 12(5), 1531–1551. https://doi.org/10.1007/s11051-010-9900-y

Marslin, G., Siram, K., Maqbool, Q., Selvakesavan, R. K., Kruszka, D., Kachlicki, P., & Franklin, G. (2018). Secondary metabolites in the green synthesis of metallic nanoparticles. Materials, 11(6), 1–25. https://doi.org/10.3390/ma11060940

Mitiku, A. A., & Yilma, B. (2017). Antibacterial and antioxidant activity of silver nanoparticles synthesized using aqueous extract of Moringa stenopetala leaves, 16(32), 1705–1716. https://doi.org/10.5897/AJB2017.16010

Mokgweetsi, P. (2018). Green Synthesis of Silver Nanoparticles Using Moringa Oleifera, Employing Multivariate Optimization Methodologies. International Journal of Advanced Research, 6(7), 953–962. https://doi.org/10.21474/ijar01/7445

Nilanjana, G., Samrat, P., & Piyali, B. (2014). Research article silver nanoparticles of moringa oleifera-green synthesis, characterization and its antimicrobial efficacy., 42–46.

Saware, K., Sawle, B., Salimath, B., Jayanthi, K., & Abbaraju, V. (2014). Biosynthesis and characterization of

silver nanoparticles using ficus beghalensis leaf extract, 3(12 M), 867–874.

Sultana, F., Barman, J., Banik, B., & Saikia, M. (2015). A biological approach to synthesis of silver nanoparticles using aqueous leaf extract of Houttuynia cordata Thunb and comparative antioxidant study of plant extract and synthesized ... ISSN 2249 – 9679 Original Article A biological approach to synthesis o, (May 2017).

Suresh, Y., Annapurna, S., Bhikshamaiah, G., & Singh, A. K. (2014). Copper Nanoparticles: Green Synthesis and Characterization, 5(3), 156–160.

Tashi, T., Vishal Gupta, N., & Mbuya, V. B. (2016). Silver nanoparticles: Synthesis, mechanism of antimicrobial action, characterization, medical applications, and toxicity effects. Journal of Chemical and Pharmaceutical Research, 8(2), 526–537.

Tnvkv, P., & Ek, E. (2014). Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity, 1691(December 2011), 1–5. https://doi.org/10.1016/S2221-1691(11)60096-8

Tnvkv, P., & Ek, E. (2015). Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity, (December 2011). https://doi.org/10.1016/S2221-1691(11)60096-8

Vaghela, H., Shah, R., & Parmar, K. (2017). Biogenic Synthesis of Silver Nanoparticles using Bauhinia Variegata Bark Extract and its Antibacterial Efficacy. International Journal of Nanomaterials and Chemistry, 3(2), 45–49. https://doi.org/10.18576/ijnc/030205

Zhao, X., Zhou, L., Riaz Rajoka, M. S., Yan, L., Jiang, C., Shao, D., … Jin, M. (2018). Fungal silver nanoparticles: synthesis, application and challenges. Critical Reviews in Biotechnology, 38(6), 817–835. https://doi.org/10.1080/07388551.2017.1414141