*CIBTech Journal of Zoology ISSN: 2319–3883*
*Online, International Journal, Available at http://www.cibtech.org/cjz.htm*
*2024 Vol.13, pp.584-593/Abarna et al.*
***Research Article** (Open Access)*

# GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF *RAPHANUS SATIVUS* AND THEIR ANTIBACTERIAL ACTIVITIES

**Abarna V, Amtuz Zehra and *Farheen Khanam**
*Department of Zoology, Justice Basheer Ahmed Sayeed College for Women, Madras University, Tamil Nadu*
**Author for Correspondence: farheenkhader47@gmail.com*

## ABSTRACT
The radish (*Rhaphanus sativus*) is a medicinal plant that has long been used in traditional medicine for its antioxidant and anti-microbial properties. A preliminary approach was carried out to synthesize silver nanoparticles (AgNPs) using leaf extracts of R. sativus and evaluate it antibacterial potential. Silver nanoparticles were synthesized by electrochemical method and validated by UV-VIS spectroscopy with absorption maximum at 435 nm. The silver nanoparticles showed antibacterial activity against both Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Escherichia coli* and *Enterobacter soli*) microorganisms. The current study resulted in successful biosynthesis of green silver nanoparticles. The extract containing silver nitrate changed from colorless to brown and finally to dark brown at room temperature. This change of color is due to the presence of organic molecule present in the radish act as the capping agent and the reducing agent of silver ions. The antibacterial performance assessed by measuring the zone of inhibition (ZOI) showed high in silver nanoparticles (AgNps) in contrast to plant extract and silver nitrate (AgNO3). The results from the study shows that AgNps synthesized using *R. sativus* have antibacterial activity against bacterial pathogens.

***Keywords*:** *Silver Nanoparticles, Antibacterial activity, Staphylococcus aureus, Escherichia coli, Enterobacter soli*

## INTRODUCTION
The novel aspects of nanotechnology have been explored worldwide due to the technological innovations and progression in the field of science and technology. Nanotechnology is offering unique features and extensive application in various sectors and it is emerging as an attractive and popular area of research (*Erci et al.*, 2018). The term "nanotechnology" was defined correctly by Professor Norio Taniguchi of Tokyo Science University as "the processing of separation, consolidation, and deformation of materials by one atom or one molecule." In the twenty-first century, nanotechnology has emerged as a scientific breakthrough. It is a multidisciplinary field that deals with the creation, handling and application of materials with a scale of less than 100 nanometers.

Nanoscale structures (nanoparticles) in optics, electronics, biomedical science, mechanics, drug-gene delivery, chemical industry, optoelectronics devices, nonlinear optical devices, catalysis, space industries, energy science, and photo electrochemical applications are all important applications of nanotechnology (Singh *et al.*, 2019). Because of their enormous surface to volume ratio and extremely small size (in nm), nanoparticles attract a lot of attention because they induce both physical and chemical changes in their properties when compared to the majority of the same chemical composition (Ray, 2010; Bakand *et al*., 2012). Many researchers and scientists have shown significant interest in their unique characteristics and discovered that they have remarkable uses in a variety of sectors, however many nanoparticle materials have exhibited toxicity at the nanoscale scale. To address the issue of toxicity, nanotechnology and green

chemistry are combining to create environmentally benign nanoparticles using plants, microorganisms, and other natural resources (Lateef *et al.*, 2016). Many synthetic approaches for nanoparticle fabrication have been explored, revealing a significant advantage to nature and the environment via clean, nontoxic, and environmentally appropriate "green chemistry" processes that involve bacteria, fungus, and plants (Duan *et al.*, 2015). The theme of this research is to use plant extracts to synthesize a variety of nanoparticles, as this is the most widely used way of eco-friendly and green chemistry. Researchers and scientists were drawn to this path because of the easy availability and vast distribution of plants, as well as the fact that it is safe to use and a source of diverse metabolites. Nanoparticle synthesis by green methods is safe for the environment and should be explored and encouraged popularly since various plants have the high extent to form these nanoparticles (Emad *et al.*, 2024).

***Nanoparticles***

The building blocks of nanotechnology are nanoparticles. If one of any dimensions of a matter falls under nanoscale (1-100nm) in diameter, while changing or manipulating the matter is called nanotechnology. . Nanoparticles have high surface energy, quantum confinement and large surface area (Hussain *et al* 2016*),* due to an increase in the ratio of the surface area per volume of the particles, nanoscale material has unique chemical and physical properties when compared to its bulk structure *(*Cushing *et al.*, 2004*). Cinnamomum camphora (*Huang *et al.*, 2008*), Oryza sativa (*Dar *et al.*, 2016*), Medicago sativa (*Lukman *et al.*, 2011*)* in the biological and pharmaceutical field. To yield metal nanoparticles from bio reduction of silver ions using plant extracts such *Allium cepa* (Benjamin *et al.*,2011), *Corian drum sativum* (Satyavathi., 2010) have been reported.

The environmentally approved "green chemistry" concept has been applied to the biosynthesis of nanoparticles for the production of clean and environmentally friendly nanoparticles, which involves bacteria, fungus, plants, actinomycetes, and other organisms, is referred to as "green synthesis" (Pal *et al.*, 2019). Plants are considered as nature's chemical factories since they are cost-effective and low-maintenance. One advantage of plant-assisted nanoparticle synthesis is that its kinetics is much faster than other biosynthetic techniques that are comparable to chemical nanoparticle creation. Antibiotic-resistant bacteria are causing a growing amount of sickness; hence the development of efficient antimicrobials is becoming more important. Phytoconstituents, which also serve as a capping agent, ensured the stability of silver nanoparticles. Green synthesis can easily produce various nanoparticles such as silver, gold, palladium, iron, and zinc oxide (Singh *et al.*, 2018).

Radish (*Raphnus sativus)* is one of the easily available vegetables in India. It is a member of the *Brassicaceae* family. The *brassicaceae* family has about 350 genera and 4000 species around the world in different temperature zones. It has many varieties such as niger, maori, oleifera and radicle (Gutirrez *et al.*, 2009). Radishes can be cylindrical or tapering. The colour of radish root changes from white, pink, red, yellow and green. But the flush is usually white in colour. The colour of the root is due to the pigment anthocyanins. The leaves of the radish are green in color and have a fuzzy texture. They are usually 13 cm long and roots are round upto 2-5 in diameter. Radishes have 18 chromosome, it is a diploid species and its genome contains between 526-574 mb. True wild forms of *Raphnus sativus* were discovered from southeast asia. In full sunlight, radishes grow well with a ph 6.5 to 7.0. The conditions which are unsuitable for germination is dry weather. It regulates gastrointestinal issues by promoting peristaltic movements and alleviating constipation symptoms. It promotes bile flow and has a laxative impact on the intestine. Some people are sensitive to it, despite the fact that it aids indigestion. Asthma is treated with leaves, seeds, and ancient roots. Radish seeds have expectorant, laxative, and stomachic properties (Yeung *et al.*, 1985*).* It is an effective home cure for scorbutic disease, stone, and gravel (Grieve *et al.*, 1984*).*

This work uses *Raphnus sativus* leaves as an agent to synthesize silver nanoparticles in a straightforward, environmentally friendly, and cost-effective manner. This approach produces silver nanoparticles that have antibacterial activity against some gram positive and gram negative microorganisms.

## MATERIALS AND METHODS

The leaves of radish are wide, rough textured, green in colour with stems. They are edible. It is a fast growing crop and matures in 21-30 days. In the month of october fresh leaves of *Raphanus sativus* free from disease were collected from local market in northeast of Chennai city which lies in between 13.0576°N, 80.1545°E with humidity 83% and pressure 1.0017 atm . It was brought to the laboratory in an airtight polythene bag for further use. The leaves are surface sterilized with normal tap water to remove dirt and then again rinsed with distilled water twice thoroughly .10gram clean leaves were weighed in a weighing machine with butter paper as a base to maintain sterilization. The leaves were dried in the mild shade of natural sunlight for 24hrs to remove the moisture content.

The sample is finely chopped into tiny pieces. 100ml of distilled water is added to the chopped leaves.Then the 250 ml beaker containing sample and distilled water was kept in a boiling water bath at 60°C for 15 minutes. It is stirred at regular intervals using a glass rod. The extract was filtered by using Whatman's no.1 filter paper. After allowing the extract to cool down at room temperature it was transferred into a fresh falcon tube.The filtered extract was stored in the refrigerator at 4°C for future use.

1Mm of Agno3 was prepared by dissolving 0.1699 Agno3 in 1000 ml of distilled water and stored in a conical flask which is tightly wrapped by foil sheet. This was done in dark condition to prevent self-oxidation of silver nitrate solution

Test tubes were wrapped with aluminum foil and labeled as sample, positive control and negative control.

- 1 ml of plant extract is added to 9 ml of 1mM Agno3 solution in a test tube as control
- 1ml of sodium citrate is added to 9 ml of 1mM agno3 solution in a test tube as positive
- 1 ml of distilled water is added to 9 ml of 1mM agno3 in a test tube as negative control 1
- 1 ml of plant extract is added to 9 ml of distilled water in a test tube as negative control 2

These test tubes were incubated for 3 days in dark conditions. The characterization was visualized through a colour change exhibited by nanoparticles. When the particle size increases it shows colour change. The colour changed from colourless to dark brown colour, this indicates the presence of agno3.To confirm silver nanoparticles formation, a UV-VIS spectrometer was used ,it is one of the most commonly used techniques for structural characterization. The UV-vis spectrometer is in the range of 200-700 nm was used. At room temperature equal amounts of the suspension were taken and analyzed. The progress of the reaction between metal ions and the leaf extract was monitored by UV-visible spectra of silver nanoparticles. The in vitro antibacterial activity of synthesized nanoparticles was determined using agar well diffusion method. For inoculum preparation, in mueller -hinton broth gram positive and gram negative bacteria were pre cultured overnight in a rotary shaker at 37 degree celsius. Using 0.5 Mefarland standard, each strain was adjusted at a concentration of 10 cells|ml.

***Methods***

The agar medium was prepared by adding mueller hinton agar with 100ml of distilled water along with 1.2 grams of agar agar for proper solidification. It is autoclaved at 121°C for 20 minutes in 15lbs to obtain the texture. The agar mixture was dispensed into sterile petri plates. After letting it settle for some time the plates were incubated overnight. 5 ml of silver nitrate is added with 5 ml of sodium hydroxide (NaOH). It remained undisturbed for 30 mins. The precipitate formed at the bottom of the test tube is silver oxide. It is stored for further usage. Gram positive bacteria (*Staphylococcus aureus and Bacillus subtilis*)and gram negative organism (*Escherichia coli and Enterobacter soli)*were pre cultured .The organisms were spread evenly in each MHA plates (mueller hinton agar)by using sterile swabs. They were evenly spreaded in all directions. The wells were cut by sterile 6 mm cork borers in the inoculated plates and they were labeled properly. The wells were filled with 100 µl of the silver nanoparticles, 75µl of plant extract and 25µl of Ago**.** Then the plates were incubated at 37°C 24 hours. The presence of bacterial growth was indicated by a clear zone around the well, when the plates were examined. It was measured using a scale, the size of bacterial growth and zone of inhibition was expressed in terms of millimeter and the values were interpreted.

## RESULTS & DISCUSSION

After mixing the leaf extract with beaker containing aqueous solution of Agno3 nanoparticles starts to appear in about 3-4 days. It started to change colour from colourless to yellow brown solution and finally it turned to dark brown colour at room temperature. By Uv-Vis spectral analysis, formation of AgNps was again confirmed .The Uv-vis spectra recorded implied rapid bioreduction was achieved using Raphanus sativus leaf extract as reducing agent.. The maximum absorbance peak was seen at 435 nanometer

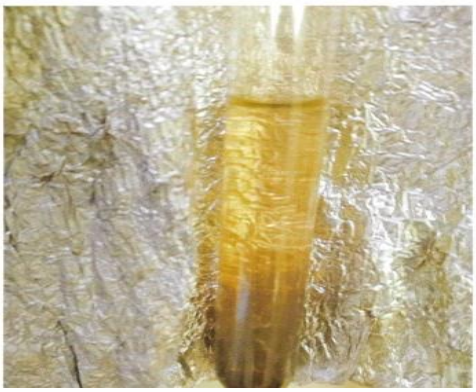

**Fig 1:** Macroscopic appearance of nanoparticles

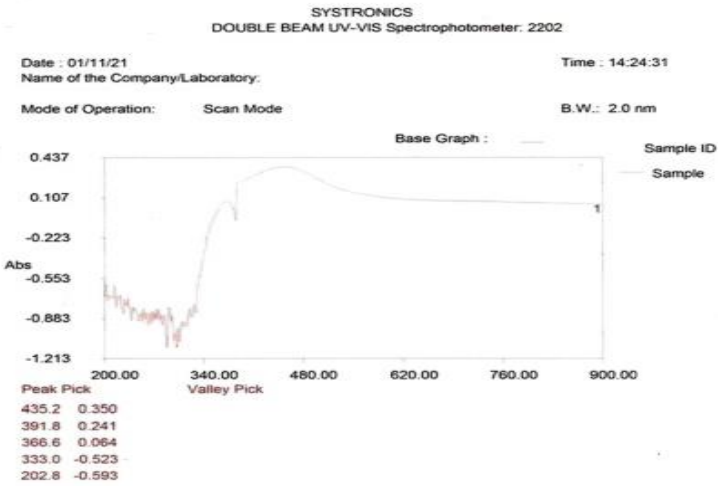


**Fig 2:** UV Vis spectrometer analysis

| ORGANISM | ZONE OF INHIBITION (mm) | |
|---|---|---|
| | Sample Mean | S.D. |
| *Staphylococcus aureus* | 8.2 | 0.836 |
| *Bacillus subtilis* | 12.6 | 0.547 |
| *Escherichia coli* | 10.4 | 0.894 |
| *Enterobacter soli* | 0.8 | 0.447 |

**Table 1: Zone of Inhibition**

PLATE A

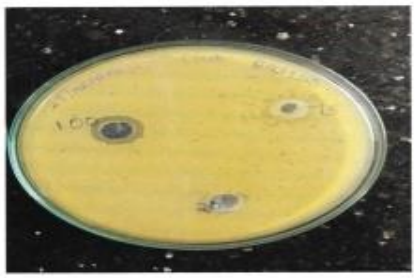

PLATE B

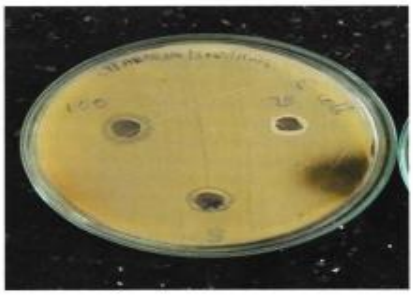

**Fig 3: Plate A: Growth of *Bacillus Subtilis* and Plate B: Growth of *Escherichia Coli***

respectively. This work investigated silver nanoparticles mediated by Raphanus sativus extract as potential agents. Plant extract and mediated silver nanoparticles were used to analyze the antimicrobial properties against both gram positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram negative (*Escherichia coli* and *Enterobacter soli)* bacteria was revealed and zone of inhibition was measured.The bacterial growth of inhibition is high in AgNps when compared with the plant extract (PE) and Ag0. *Escherichia coli* showed 11mm, *staphylococcus aureus* showed 9mm and *Enterobacter soli* exibited no zone of inhibition. Maximum growth was observed against *Bacillus subtilis*.

TABLE 2:ANOVA OF ANTIBACTERIAL ACTIVITY OF *RAPHNUS SATIVUS* AGAINST 4 DIFFERENT BACTERIAL SPECIES.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 311.25 | 3 | 103.75 | 191.5384615 | 0.0000000002 | 3.490294821 |
| Within Groups | 6.5 | 12 | 0.5416666667 | | | |
| Total | 317.75 | 15 | | | | |

**Table 2: Anova of four different bacterial species**

PLATE C

PLATE D

**Fig 4: Petriplate with bacteria**

The synthesis of nanoparticles was confirmed by the colour change observed in sample solution. Similarly, Rama koyyati *et al.,* (2013*)* reported that the silver nanoparticles exhibited striking colour from light yellow to brown which denoted the emergence of nanoparticles. Fadel *et al.,* (2017) reported that the colour was changed from crimson to dark brown after 30 mins in radish leaf extract. This colour change indicates the formation of AgNps. Bio-molecules such as protein, carbohydrates, flavonoids and phenols have been found to play a function not only in capping nanoparticles but also in decreasing ions to nanosize. The silver nanoparticles in solution change colour due to surface Plasmon vibration (Rai *et al.,* 2006). Because it has free electrons, silver metallic nanoparticles produce the SPR absorption band. The reduction rate and formation of nanoparticles can be increased further by increasing the incubation time. Uv-Vis spectroscopy was used to characterize the nanoparticles, which has proven to be a very helpful approach for nanoparticle characterization. The reduction of Ag+ ions in the aqueous solution of silver complex during the reaction with the components contained in plant leaf extracts measured by Uv-Vis spectroscopy revealed that silver nanoparticles in the solution may be associated with the Uv-Vis spectra. Using a quartz cuvette with silver nitrate as the reference, a Uv- vis spectrograph of the colloid of silver nanoparticles was recorded as a function of time.

Throughout the reaction period, the surface plasmon band in the silver nanoparticles solution remains close to 380 nm, showing that the particles are dispersed in the aqueous solution with no indication of aggregation. The nanoparticle solution was shown to be stable for more than six months with no evidence of aggregation (Saifuddin, 2009; Ankanna *et al*., 2010). The AgNps SPR peak is found in the spectra at 435 nanometers with a high absorption, which is extremely specific for silver nanoparticles. The greatest smooth and broad dispersion peak was observed at 470 nm, according to Rama koyyati *et al*., (2013). Similarly, the greatest peak for radish greens was discovered at 411 nm in a study conducted by Tamileswari *et al.*, (2015). Prasad *et al.*, (2011) reported that absorption spectra of silver nanoparticles formed in the reaction media have absorbance peaks at 430-440 nm. (Veeraswamy *et al*., 2011) reported that absorption spectra of silver nanoparticles formed in the reaction media have absorbance peaks at 438 nm. Radish is a medicinal plant that has long been used in traditional medicine for its antioxidant and anti-microbial properties. I chose to bring this ancient process back to life by employing *Raphanus sativus* leaves for green synthesis. The silver nanoparticles obtained have a high bacterial potential. Silver nanoparticles have been employed in the health industry, medicine, textile coating, dye reduction, wound dressing, food storage, antiseptic creams, and a variety of environmental applications due to their antimicrobial qualities. Since elemental silver and its compounds have been utilised as antibacterial agents, silver coins and silver containers have been used to conserve water. I investigated silver nanoparticles mediated by *R. sativus* extract as potential antibacterial agents. The antibacterial activity of manufactured silver nanoparticles against *Bacillus subtilis* and *Escherichia coli* shown to be good based on zone inhibition. It has been proposed that the perforation of the microbial cell membrane causes Antibacterial activity (Khan *et al*., 2017). Undoubtedly, The antibacterial activity is attributed to silver cations produced from AgNps in response to changes in the membrane structure of microorganisms, resulting in increased bacterial membrane permeability and finally cell death (Dibrov, 2002; Sondi, 2004; Lin *et al*., 2000).

This was correlated with the results obtained by (Tamileswari *et al.*, (2015) where they had a maximum zone of inhibition (14 mm) against *Bacillus subtilis*. Whereas a study done by (Rama koyyati *et al*., 2013) indicated that When compared to *ampicillin*, the results showed that silver nanoparticles generated from *Raphnus sativus* leaf extract had good antibacterial action in both gram positive and gram negative microorganisms. The results of this study were also supported by previous studies of antibacterial activity of plant based AgNps was reported by (Khan, 2017; Niraimathi *et al*., 2013). Similarly, (Jain *et al.*, 2009) stated that an antibacterial assay was performed on human pathogens utilizing papaya fruit extract mediated silver nanoparticles, which showed great toxicity against multidrug-resistant bacteria. Kumar *et al*., (2012) found that silver nanoparticles were moderately harmful to *Pseudomonas aeruginosa, Pseudomonas vulgaris, and E. coli, B. subtilis, and Pseudomonas putida.* Nanoparticles, on the other hand, had a low toxicity against *S. typhi.*

*Raphanus sativus* leaf extract at room temperature is a simple one pot green synthesis was successfully used for the biosynthesis of silver nanoparticles. Uv-Vis spectrum analysis confirmed the formation of silver nanoparticles .potential free radical scavenging activity and bacterial growth inhibition was shown by biosynthesized silver nanoparticles. For this purpose, in in-vivo conditions there is a special need of extensive research to find out the perfect dose and evaluation of toxicity before the application of nanoparticles.

## CONCLUSION

A low-cost, simple biological approach for the stable silver nanoparticles by reducing silver nitrate solution with the bio reduction method using *Raphanus sativus* leaf extract as the reducing agent. It was discovered that leaves can be a good source of silver nanoparticle production. Plants are being used to synthesis nanoparticles for the first time, resulting in a totally green chemistry pathway. This green chemistry approach to nanoparticle synthesis has a number of advantages, including process scaling,

economic viability, and a safe method of producing nanoparticles. Therefore, all the conditions of a 100% green chemical process were satisfied by this reaction pathway. This green synthesis could be a competitive alternative to the physical chemical methods used to synthesize Np, and so has a potential utility in biochemical applications. It will also play a significant role in optic electronics and medical devices in the near future. Because of these applications, prepared nanoparticles can be utilized as bactericidal agents, as well as in wound healing, water purification, and medicine. Still more clinical trials are needed to support its therapeutic uses.

**ACKNOWLEDGEMENT**
The authors are grateful to the management of JBAS College for women, Chennai, for providing facilities to carry out this work.

**REFERENCES**

**Akhilesh Rai, Amit Singh, Absar Ahmad, Murali Sastry, (2006)**. Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles, *Acs publication, Langmuir* **22**(2), 736-741.

**Ankanna S, Prasad TNVKV, Elumalai EK, Savithramma N, (2010)**. Production of biogenic silver nanoparticles using Boswellia ovalifoliolata stem bark. *Digital Journal of Nanomaterial biostructure* ;5(2):369-372.

**Armendariz V, Herreral, Jose-yacaman M, Troiani H. Santiago P, Gardea-Torresdey J, (2010)**. Aspergillus species. *Polish Journal of Microbiology* **61**, 61-3.

**Bakand S, Hayes A, Dechsakulthorn F, (2012).** Nanoparticles: a review of particle toxicology following inhalation exposure. *Inhalation Toxicology* **24** 125-135

**Benjamin. G, Bharathwaj, (2011)**. S. Biological Synthesis of Silver Nanoparticles from Allium Cepa (Onion) Estimating its Antibacterial Activity. International Conference on Bioscience, Biochemistry Bioinformatics, IPCBEE, 5 *Bioresource technology;* **101**(20) 7958-65.

**Cushing BL, Kolesnichenko VIL. O' Connor CJ, (2004)**. Recent advances in the liquid-phase synthesis of inorganic nanoparticles. *Chemical Review* **104**(9) 3893-946.

**Dar P, Waqas U, Hina A, (2016)**. Biogenic synthesis, characterization of silver nanoparticles using *Multani mitti* (Fullers Earth), Tomato (*Solanum lycopersicum*) seeds, Rice Husk (*Oryza sativa*) and evaluation of their potential antimicrobial activity. *Journal of Chemical Society of Pakistan* **38** 665

**Devi, L.S., N Joshi, S.R, (2015)**. Ultrastructures of silver nanoparticles biosynthesized using endophytic fungi. Journal of microscopy and ultrastructure, 3, 29-37.

**Dibrov, J. Dzioba, K.K. Gosink, C.C. Hase, (2002)**. Antimicrobial Agents. *Chemotherapy*. **46** 2670.

**Duan H. Wang D, Li Y, (2015)**. Green chemistry for nanoparticle synthesis of gold nanoparticles by the fungus Penicillium sp. *Journal of Nanoparticle Research* **13** 921- 930.

**El-Nour KM, Eftaiha AA, Al-Warthan A. Ammar RA, (2010)**. Synthesis and applications of silver nanoparticles. *Arabian Journal of Chemistry* **33** 135-40.

**Elumalai K. Velmurugan S, Ravi S, Kathiravan V, Ashokkumar S, (2015)**. Green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity. Spectrochim *Acta A Molecular Biomolecule Spectroscopy* **143** 158-64

**Emad Abada, Abdullah Mashraqi, Yosra Modafer, Mohamed A.Al Abboud, A El- Shabasy (2024)** Review green synthesis of silver nanoparticles by using plant extracts and their antimicrobial activity, Saudi journal of biological science, volume 31. 2024.

**Erci, F., Cakir-Koc, R., & Isildak, I, (2018)**. Green synthesis of silver nanoparticles using *Thymbra spicata L. var. spicata* (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity.

**Evans, B, (2018)**. Nano-particle drag prediction at low Reynolds number using a direct Boltzmann-BGK solution approach" (PDF). *Journal of Computational Physics*. **352** 123-141. *Bibcode:2018JCoPh.352..123E.doi:10.1016/j.jcp.2017.09.038.*

**Gao, X., Yourick, J. J., Topping, V. D., Black, T., Olejnik, N., Keltner (2014)**. Toxicogenomic study in rat thymus of F1 generation offspring following maternal exposure to silver ion. *Toxicology Reports*, *http:/dx.doi.org/10.1016/j.toxrep.2014.12.008*.

**Gutierrez, Sophie Lefevre, Gilmer Govaert, Isabelle EJA François, Frank Madeo, Renata Santos, Bruno PA Cammue, Karin Thevissen, (2009)**. The antifungal plant defensin RSAFP2 from radish induces apoptosis in a metacaspase independent way in Candida albicans, *FEBS letters* **583** (15), 2513-2516, 2009

**Huang J, Lin L, Li Q, (2008)**. Continuous-flow biosynthesis of silver nanoparticles by lixivium of sundried *Cinnamomum camphora* leaf in tubular microreactors. *Indian English Chemical Research* **47** 6081-6090.

**Hussain I. Singh NB, Singh A. Singh H, Singh SC, (2016).** Green synthesis of nanoparticles and its potential applications, *Biotechnology Letters*, **38**(4) 545-60

**Jain D. Kumar Daima H, Kachhwaha S, Kothari SL (2009).** Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Digital Journal of Nanometer Bios* 4:557- 563

**Jayesh P. R, Arup Kumar C, Siddhartha P. D, Suparna M, (2008).** Strain specificity in antimicrobial activity of silver and copper nanoparticles, *Acta Biomaterialia*, **4**, 3, Pages 707-716.

**Joyita B, Narendhirakannan R, T, 2011()**. Biosynthesis of silver nanoparticles from Syzygium cumini (1) seed extract and evaluation of their in vitro antioxidant activities. *Digest Journal of Nanomaterials and Biostructures* **1**, 3,961-968

**Khan, T. Abbasi, B.H, Afridi, M.S, Tanveer, F.; Ullah; Bashir, S,; Hani, C, (2017).** Melatonin enhanced biosynthesis of Antibacterial AgNps by improving the phytochemical reducing potential of a. Callus culture of *ocimum basilicum* L. *var*. thyrsiflora. *Rsc Advances* **7**,38699-38713.

**Kumar, S. S. Selvi, A. L. Prabha, K. P. Kumar, R. S. Ganesh Kumar, and M. Govindaraju, (2012).** Synthesis of silver nanoparticles from Sargassum tenerrimum and screening phytochemicals for Pp. 12-16.

**Lateef A, Ojo SA, Elegbede JA, (2016)**. The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles. Nanotechnology Review **5**:601-622

**Lin. J. Wu, R. Xue, Y. Yong (2005).** Spectroscopic characterization of Au biomass of Saccharomyces waste biosorption by Spectrochim. *Acta Part A* **61** 761-765.

**Lipińska-Palka, Paulina; Józefiak, Damian; Kamińska, Agnieszka; Atanasov, Atanas, (2018).** The Effect of Different Levels of Cu, Zn and Mn Nanoparticles in Hen Turkey Diet on the Activity of Aminopeptidases. *Molecules*. **23**(5) 1150. *doi:10.3390/molecules 23051150.* PMC 6100587. PMID 29751626.

**Liu Y. Jin X. Chen Z (2018)**. The formation of iron nanoparticles by Eucalyptus leaf extract and used to remove Cr (V). *Science Total Environment* **627** 470-9.36

**Lukman AI, Gong B, Marjo CE *et al.*, (2011).** Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using Medicago sativa seed exudates. *Journal of Colloid Interface Science* **353** 433-444

**Niraimathi, K.L, V. Sudha, R. Lavanya, and Brindha, P, (2013)**. Biosynthesis of silver Alternanthera sessilis (Linn.) extract and their antimicrobial, antioxidant activities, *Bio-international* **102** 288-29

**Neha Joshi, Neha Jain, Abhishek Pathak, Jai Singh, Ram Prasad, Chandrama Prakash Upadhyaya, (2018)**. Biosynthesis of silver nanoparticles using Carissa carandas berries and its potential antibacterial activities. *Journal of Sol-Gel Science and Technology* **86**(3), 682-689, 2018.

**Pall G, Rai P, Pandey A, (2019)**. Chapter 1 -green synthesis of nanoparticles: a greener approach for a cleaner future. In: Shukla AK,Iravani (eds) Characterization and applications of nanoparticles SBT-GS micro and nano technologies. Elsevier, Amsterdam, 1-26.

**Pavani KV. Kumar NS. Sangameswaran BB, (2012)**. Synthesis of lead nanoparticles by Aspergillus species. *Polish Journal of Microbiology* 6i:61-3.

**Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K, Thangamani S, (2012).** Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn G. Don used in their antiplasmodial activities. *Asian pacific Journal of Tropical Biomedicine* **2**(7) 574-580.

**Prasad TN V K and E. K. Elumalai, (2011)**. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity, *Asian Pacific Journal of Tropical Biomedicine*, **1**(6), 439-442.

**Rai A, Singh A, Ahmad A, Sastry M (2006)**. Role of Halide ions and temperature on the morphology of biologically synthesized gold nanoparticles. *Langmuir*, **22**, 736-741.

**Rama koyyati, Veerabahu nagari, Ramchander merugu & Prataprudra manthurpadigya (2013)**. Biological synthesis of silver nanoparticles using Raphnus sativus var.Longipinnatus leaf extract and evaluating their antioxidant and antibacterial activity. *International Journal of medicine and pharmaceutical science (IJMPS)* **3**(4), 89-100.

**Ray PC, (2010)**. Size and shape dependent second order nonlinear optical properties of nanomaterials and their application in biological and chemical sensing. *Chemical Review* 110:5332-5365

**Solomon SD, M. Bahadory, A. V. Jeyarajasingam,S. A. Rutkowsky, and C. Boritz (2007)**. "Synthesis and study of silver nanoparticles," Journal of Chemical Education,**84(**2), 322-325.39

**Saifuddin N, Wong CW, Nur Yasumira Aa, (2009)**. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation EJ chem **6**(1):61-70.

**Saraniya devi J, valentin bhimba B, krupa R (2003)**. In Vitro anticancer activity of silver nanoparticles synthesized using the extracl of Gelidiella sp. *International Journal of Pharmaceutical Sciences*, **4**, suppl 4, 710-715.

**Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R, and Narayana Rao D, (2010)**. Biosynthesis of Silver Nanoparticles Using 40 Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics. *Advanced Science Letters*. **3**, 138-143.

**Singh, T., Singh, A., Wang, W., Yadav, D., Kumar, A., & Singh, P. K, (2019).** Biosynthesized nanoparticles and its implications in agriculture. *In Biological Synthesis of Nanoparticles and Their Applications* (257-274). CRC Press.

**Sur UK. Ankamwar B. Karmakar S. Halder A, Das P, (2018)**. Green synthesis of Silver nanoparticles using the plant extract of Shikakai and Reetha. *Mater Today Proceedings* **5**(1) 2321-9.

**Tilahun Ayane Debele, Cheng-Fa Yeh, Wen-Pin Su, 2020()**. Cancer Cancers Immunotherapy and Application of Nanoparticles in Immunotherapy as the Delivery of Immunotherapeutic Agents and as the Immunomodulators, *Cancers* 12 (12), 3773.

**Veerasamy, T. 2. Xin, S. Gunasagaran *et al.*, (2011).** Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their xantimicrobial activities, *Journal of Saudi Chemical Society*, **15**, 2, pp. 113-120.

**Yasmin A. Ramesh K, Rajeshkumar S, (2014)**. Optimization and stabilization of gold nanoparticles by using herbal plant extract with microwave bearing. *Nano Convergence*, **1**(1) 12.

**Yeung, H.C, (1985)**. Handbook of Chinese Herbs and Formulas. *Institute of Chinese Medicine*, Los angeles.43