

Silver Nanoparticles Are Biofabricated From *Withania Somnifera* Root Extract.

1.Pradeep 2.Archanaa 3.Yokeshwaran

UG Student P.S.V College of pharmaceutical science and research, Krishnagiri, India.

Submitted: 05-01-2023

Accepted: 13-01-2023

ABSTRACT

This work shows that green synthesis is a secure, affordable, and environmentally friendly way to create nanoparticles. In both the present and the future, the green synthesis of metal and metal oxide nanoparticles is very alluring. Different kinds of natural extracts, including those from plants, fungus, bacteria, and yeast, have been employed successfully as raw materials for the creation of products. Among them, biologically produced plant extracts have shown to have mostly stable goods that don't have any negative effects, and waste disposal is easy and toxin-free. In addition to being extensively used in the field of environmental remediation, the greenly produced silver nanoparticles have a wide range of additional vital uses in the pharmaceutical, food, cosmetic, and other industries. Nanotechnology is rapidly gaining importance in several areas like health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single-electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications. Nanomaterials are seen as a solution to several technological and environmental challenges within the field of solar power conversion, catalysis, medicine, and water treatment. In the context of worldwide efforts to scale back hazardous waste, the continuously increasing demand for nanomaterials must be amid green synthesis methods.

KEYWORDS: Silver nanoparticles, Biofabrication, green synthesis, nature extract, rhizome.

I. INTRODUCTION :

The present study showed the potential of *Withania Somnifera* root extract to reduce the silver ions to synthesize silver nanoparticles that can be used for different applications like Anti-bacterial and Anti-inflammatory activity. In the future, it can

be further formulated for various clinical applications as well. Biofabrication is usually defined as the production of complex biologic products from raw materials such as living cells, matrices, biomaterials, and molecules. This rapidly evolving technology has been stimulated by the development of 3D fabrication technologies. Ashwagandha (*Withania somnifera*) Ashwagandha, a perennial shrub, is also known as Indian ginseng or winter cherry, and belongs to the nightshade family Solanaceae. It has been used for centuries in Indian, Chinese, and Arabic traditional medicines. The root extract is recommended for medication and supplement purposes. Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size.[1] While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used silver nanoparticles are spherical, but diamond, octagonal, and thin sheets are also common.

Their extremely large surface area permits the coordination of a vast number of ligands. The properties of silver nanoparticles applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, biosafety, and biodistribution.

ADVANTAGES:

- (i) Improved bioavailability,
- (ii) Reduced toxicity,
- (iii) Sustained and controlled release,
- (iv) Ability to target,
- (v) Provide effective delivery to the brain and intracellular compartment

DISADVANTAGES:

1. Despite all that advantages, there have been certain disadvantages in pharmaceutical applications of nanotechnology.

2. Nanoparticles have a very large surface area compared to their volume, so they are active to react quickly.

3.a result, there is a potential for serious cellular damage.

BIOPRODUCTION OF NANOPARTICLES:

Due to incredible properties nanoparticles became significant in many fields within recent years, like energy, health care, environment, agriculture, etc. The preparation of nanoparticles is carried out either by (i) Nanoparticle synthesis or by (ii) the Processing of nanomaterials into nanostructure particles. The silver nanoparticles are prepared by using physical, chemical, and biological methods. The physical and chemical methods are very expensive. Biological methods of nanoparticles synthesis would help to get rid of harsh processing conditions by enabling the synthesis at physiological pH, temperature, pressure, and at an equivalent time at a lower cost. A large number of microorganisms have been found capable of synthesizing inorganic nanoparticles composite either intra or extracellularly. There are numerous methods available using various approaches including chemical, physical, and biological protocols for the synthesis of nanoparticles.

1. Physical Method
2. Chemical Method
3. Biosynthesis of Nanoparticles

GREEN SYNTHESIS:

Science and technology are moving at a rapid pace today. Environmental engineering science is witnessing drastic changes. Similarly, nanotechnology and green nanotechnology are moving from one visionary paradigm towards another. Green synthesis is defined as the use of environmentally compatible materials such as bacteria, fungi, and plants in the synthesis of nanoparticles. These attractive green strategies are free of the shortfalls associated with conventional synthetic strategies, i.e., they are eco-friendly. Alternatively, synthesis from biologically derived extracts offers several advantages such as rapid synthesis, high yields, and importantly, the lack of costly downstream processing required to produce the particles. Hence, nanoparticle synthesis from plant extracts

tentatively offers a route for the large-scale production of commercially attractive nanoparticles.

METHOD OF PREPARATION

Solvent System-Based “Green” Synthesis Solvent systems are a fundamental component in the synthesis process, whether it is a “green” synthesis or not. Water is always considered an ideal and suitable solvent system for synthesis processes. According to Sheldon, “the best solvent is no solvent, and if a solvent is desirable then water is ideal”. Water is the cheapest and most commonly accessible solvent on earth. Since the advent of nanoscience and nanotechnology, the use of water as a solvent for the synthesis of various nanoparticles has been carried out. For instance, synthesized Au and Ag nanoparticles at room temperature using gallic acid, a bifunctional molecule, in an aqueous medium. Gold nanoparticles were produced via a laser ablation technique in an aqueous solution. The oxygen present in the water leads to partial oxidation of the synthesized gold nanoparticles, which finally enhanced its chemical reactivity and had a great impact on its growth. In the literature, “green” synthesis consists of two major routes:

- Wherein water is used as a solvent system.
- Wherein a natural source/extract is utilized as the main component.

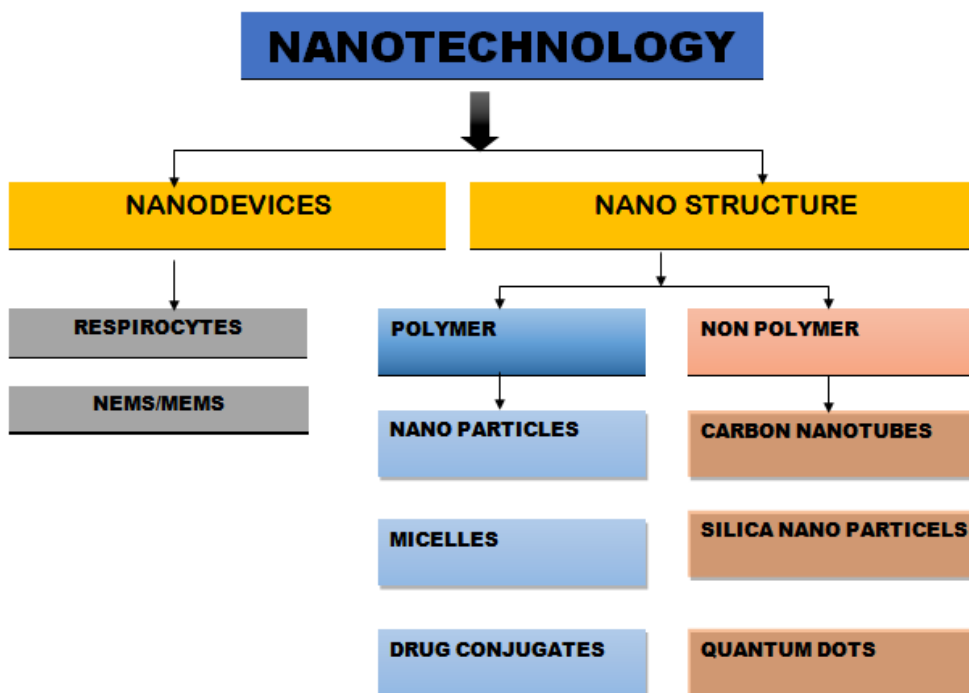
(a) Many metal catalysts, polar organic compounds, and gases are easily dissolved in ILs to support biocatalysts.

(b) ILs have constructive thermal stabilities to operate in a broad temperature range. Most of these melts below room temperature and begin to decompose above 300 or 400 °C. As such, they allow a broader synthesis temperature range (e.g., three to four times) than that of water.

(c) The solubility properties of IL can be modulated by modifying the cations and anions associated with them.

(d) Unlike other polar solvents or alcohols, ILs are non-coordinating. However, they have polarities comparable to alcohol.

(e) I do not evaporate into the environment like volatile solvents because they have no vapor pressure.



PART OF THE PLANT	USES
Leaf	Reduce stress, Fertility treatment Anti – inflammatory
Seeds	Antianxiety, Depression , Joint pain
Oil	Insomnia, Anti fungal ,swelling
Flowers	Tumor, inflammation, cytotoxic
Roots	Diabetes, Hypertension, stress, cancer
Barks	Anti inflammatory agent, liver tonic Stomach pain, ulcer ,anemia
Leaves	diabetes , Hypertension ,external Pain , painfull swelling , cuts Contraceptive remediesulcer.

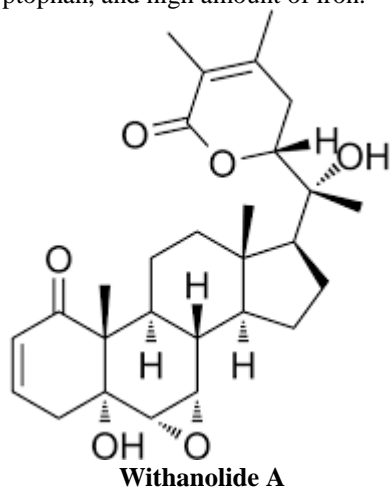
Traditional Uses

Traditionally, Ashwagandha is used for its Anxiolytic-Anti-depressive, Antifungal, Anti-malarial, Antibacterial, Hypoglycemic effect, Anticancer activity, Anti-inflammatory, Antitumor, Anti-stress, Antioxidant, Immunomodulatory, Antidiabetic.

- In Sanskrit, ashwagandha means “smell of the horse” due to its unique smell. (It doesn't smell like a horse per se, but it has a pungent barnlike, earthy fragrance. Gum is bland and mucilaginous. Seeds are acrid and stimulant.

Chemical Constituents Present in Roots

- Chemical constituents of *Withania Somnifera* are always of interest to researchers.
- The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine, etc.), steroidal compounds, including ergostane type steroidal lactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols AC, withanone, etc.
- Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with glucose at carbon 27 (sitoindoside IX and X).
- Apart from these contents plants also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron.



PHYSICAL APPEARANCE

Root extract is having:

Colour - pale yellowish

Odour - pungent (horses' urine)

pH - 8.5

PLAN OF WORK

The present works were carried out to bio fabricate and characterize the silver nanoparticle of *Withania Somnifera* root extract by using silver nitrate and investigating their anti-microbial and anti-inflammatory activity.

The following experimental protocol was therefore designed to allow a systemic approach to the study:

- Collection of literature related to green synthesis, silver nanoparticles, and the *Withania Somnifera* plant.
- Selection of plant part and its extraction method.
- Preparation of silver nanoparticles.
- Preliminary analysis (colour change, UV-analysis).
- Evaluation of silver nanoparticles for the following physicochemical parameters:

□UV Analysis.

□FTIR Studies.

□Particle Size Analysis.

□Zeta Potential Analysis.

□X-Ray Diffraction Studies.

□SEM (Scanning Electron Microscopy).

□In vitro Anti- microbial study,Agar Diffusion Method.

□In vitro Anti-inflammatory study,Denaturation of Protein Albumin.

MATERIALS

List of Materials Used:

The materials shown below were used for the biofabrication of silver nanoparticles.

The entire materials used were of best quality.

EXPERIMENTAL PROCEDURE

Plant Material Collection and Preparation of Extract

- ❖ The roots of the *Withania Somnifera* plant were collected from in and around areas of Palakkad District and good grade roots were selected from them.
- ❖ The roots selected were cut into thin slices.
- ❖ Freshly collected roots were washed thoroughly by using distilled water to remove the soil particles and other impurities present in the root part.
- ❖ The outer layer of the root was removed and allowed to dry in shade for 15 days.
- ❖ After the complete removal of water, the root was sliced into small pieces and grinded to a coarse powder using a mixer grinder.

- ❖ About 80gm of grinded powder was taken and boiled with 800 ml of doubledistilled water (DDW) for about 1 hour.
- ❖ After cooling to room temperature, the mixture was filtered using the Whatman filter paper no.1, and the root extract of Withania Somnifera was thus prepared.
- ❖ This was continued several times for obtaining the required quantity of the root extract.

The prepared root extract can be stored in the refrigerator (4°C) for future uses

Procurement of Ratio for Formulation of Silver Nanoparticle

To find out the optimum ratio of silver nanoparticle formulation. The different ratios of Withania Somnifera root extract:silver nitrate solution was F1 (1:1), F2 (1:3), F3 (1:6), and F4 (1:9). The selection of the appropriate ratio is done by taking the color of silver nitrate and Withania Somnifera root extract as control. Among the different ratios of the formulation showing an acceptable color change is selected and it is the first indication of the formation of the Withania Somnifera root extract Nanoparticle. UV-visible spectroscopy was conducted in a range of 200-800nm and the formulation showing absorbance within the limit of silver nanoparticles is selected for further study.

Preformulation Study

Preformulation testing is an investigation of the physical and chemical properties of a drug substance alone and when combined with a polymer. The overall objective of Preformulation testing is to generate useful information for the investigator in developing a stable dosage form obviously, the type of information needed will be depended on the formation to be developed. The use of the preformulation parameter maximizes the chances of formulating an acceptable and stable product.

Colour, Odour, Appearance

The extract was evaluated for its color, odor, taste, and appearance and the results have been noted.

Melting Point Determination

The melting point is the main indication of the purity of the sample since the presence of a relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. Melting point determination of the

obtained Withania Somnifera sample was done by the open capillary method. Before performing the analysis, the equipment was calibrated and immediately after this the Withania Somnifera sample was compacted into capillary tubes each 6 mm long and 1mm diameter. The capillaries were introduced vertically into the equipment, with heating 10°C per minute. The reading was performed three times. The melting point of the drug was noted and compared with the melting point of the reference sample cited in Indian pharmacopeia.

Synthesis of Silver Nanoparticles of Withania Somnifera Root Extract

1mM of silver nitrate (AgNO₃) was prepared in a 1000ml beaker (0.1698 g AgNO₃ is added to 1000ml of distilled water). This beaker is placed on a magnetic stirrer for complete mixing of the silver nitrate solution. The 100 ml root extract of Withania Somnifera aqueous root extract was added drop by drop to different ratios of silver nitrate solution as 100ml (F1), 300ml (F2), and 600 ml (F3), 900 ml (F4) ratio by using a dropper. The reaction was carried out in a beaker kept on stirring at a temperature of 60°C for about 8 hours.

The change in the color of the solution of different ratios was noted because the color of the solution indicates the formation of Withania Somnifera root extract Nanoparticles. Then the formulation was kept under dark conditions for 24 hours and the solution was centrifuged at 4,000 rpm, 20°C for 15 minutes. Then the supernatant was removed from the tube and the product was taken which was placed for drying under dark conditions.

EVALUATION

To confirm the presence of silver nanoparticles in the formulation, UV-VISIBLE spectrophotometer analysis, and visual observations were performed along with the following.

UV- VISIBLE Spectrophotometer

To determine the time point of the maximum production of silver nanoparticles, the absorption spectra of the AgNO₃, Withania Somnifera root extract, and F1, F2, F3, and F4 formulations were taken in between 200 to 800nm using the UV-Visible spectrophotometer. The deionized water was used as the blank.

The best formulation can be selected from the absorption spectrum of the different formulations

by taking the characteristic peak of silver nanoparticles.

Fourier Transform Infra Red Spectroscopy (FTIR)

FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced silver nanoparticles synthesized. To determine the involvement of functional groups in the formation of Withania Somnifera root extract nanoparticles FTIR was conducted in the range of 4500 to 500 cm^{-1} . The FTIR of Silver nitrate, Withania Somnifera root extract, and the synthesized Withania Somnifera root extract nanoparticles were carried out. The synthesized nanoparticles of Withania Somnifera root extract also contains biomolecules that are not capped on nanoparticles; they are removed by dissolving in DDW and centrifuged at 500rpm for about 10 min, the procedure is repeated 3 times, and the final pellet obtained is dried at 60°C in a hot air oven and used for the characterization.

Particle Size Analysis

The particle size analysis of Withania Somnifera and different formulations of silver nanoparticles was carried out using a Microtrac Blue wave-particle size analyzer. Before measurement of the samples, they have to be diluted with de-ionized water to obtain a suitable concentration for measurement. The results obtained for particle size distributions were used to confirm the formation of nano-sized particles^[17].

Gram-Negative Bacteria

Escherichia coli

Klebsiella pneumonia

Standard: Amoxycillin

Solvent: Distilled

Anti-Bacterial Activity

It is evident from the literature that silver nanoparticles exhibit pronounced antibacterial activity. Anti-bacterial activity screening was performed for the synthesized compound (silver nanoparticle). Four bacterial strains were used, two were gram-positive and the other two were gram-negative. Various concentration of silver nanoparticle was used to test anti-bacterial activity. The standard used during the screening was Amoxycillin.

Requirements:

Nutrient agar, nutrient broth, Petri dishes, spatula, test compounds, standard drug, solvent, Whatmann filter paper.

The following strains have been used for the study.

Gram-Positive Bacteria

Staphylococcus aureus - Bacillus subtilis - water

II. RESULT AND DISCUSSION : PREFORMULATION STUDIES CHARACTERIZATION OF ROOT:

The root of Withania Somnifera was evaluated by physical characters and determining the melting point.

PHYSICAL OBSERVATION STUDIES:

Colour - Pale yellowish

Odour - Characteristic

Taste - Bitter

SOLUBILITY STUDIES:

Withania Somnifera is soluble in hot water, quite soluble in alcohol and insoluble in cold water.

MELTING POINT DETERMINATION:

The melting point of Withania Somnifera was found to be 189°C.

III. CONCLUSION

The synthesized silver nanoparticles by green synthesis are having a wide range of applications that are applied extensively both in the field of environmental remediation and in other important areas like pharmaceutical, food, cosmetic industries, and many more. In the study, the biological synthesis of Withania Somnifera silver nanoparticles was successfully prepared. The method was found to be simple and does not need any specialized equipment or isolation techniques. Different characterization methods prove the formation of silver nanoparticles. The synthesized silver nanoparticles were of an estimated size, the first indication was visual observation and further characterization using the UV-Visible spectroscopy showed a peak at 431nm for F4 formulation, which confirmed the synthesis of silver nanoparticles. The FTIR result of formulated silver nanoparticles showed no interaction between silver nitrate and the extract. The particle size of the nanoparticles is homogeneous in size and the size distribution is compared with that of the extract. All the formulations showed lower particle sizes. Among these, the F4 formulation showed the lowest particle size range of (64.14 d.nm). Zeta potential is an indication of the stability of the formulations which should be around ± 20 mV. The Zeta

potential of F4 (14.9 mV) indicated good quality and showed the best formulation. The image of the SEM result showed a good surface morphology in small rectangular needle-shaped structure for F4 formulation when compared with that of *Withania Somnifera* root extract. The XRD technique for F4 formulation showed reduced diffraction peaks when compared with that of *Withania Somnifera* root extract and the XRD technique showed the face centered cubic in nature of the nanoparticles.

REFERENCE

- [1]. S. Thakur, Ram; Agrawal, Ruchi. Application of Nanotechnology in Pharmaceutical Formulation Design and Development. Bentham Science Publishers. Current Drug Therapy, Volume 10, Number 1, 2015, pp. 20-34(15).
- [2]. Parisa Ghsemiyeh and Soluman Mohammadi-Samani. Potential of Nanoparticles as Permeation Enhancers and Targeted Delivery Options for Skin: Advantages and Disadvantages. Drug Des Devel Ther. 2020;14 3271-3289.
- [3]. Hadel A, Abo Enin. Nanotechnology. International Journal of Pharmaceutical sciences Review and Research. 29(1), November-December 2014; Article No. 48, Pages: 247257.
- [4]. Haider A, Kang IK. Preparation of silver nanoparticles and their industrial and biomedical applications: a comprehensive review. Advances in materials science and engineering. 2015.
- [5]. Singh J, Dutta T, Kim KH, Rawat M, Samddar P, kumar P. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of nanobiotechnology, 2018 Dec;16(1):84.
- [6]. Meenal Kowshik, Shriwas Ashtaputre, Sharmin Kharrazi. Extracellular synthesis of silver nanoparticles by silver-tolerant yeast strain MKY3. Nanotechnology, Volume 14.
- [7]. Jagpreet Singh, Tanushree Dutta, Mohit Rawat, Pallabi Samddar & Pawan Kumar. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of Nanobiotechnology. 30 October 2018.
- [8]. Kushreed Ali, Tijo Cherian, Saher Fatima, Quaiser Saquib, Mohammad Faisal, Abdulrahman. Role of Solvent System in Green Synthesis of Nanoparticles. October 2020.
- [9]. Vicky V Mody, Rodney Siwale, Ajat Singh. Introduction to metallic Nanoparticles. Journal of Pharmacy and Bioallied Sciences. 2010 Oct-Dec;2(4): 282-289.
- [10]. Amit Kumar Mittal, Yusuf Chisti, Uttam Chand Banerjee. Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, Volume 31, Issue 2, March-April 2013, Pages 346-356.
- [11]. Jagpreet Singh, Tanushree Dutta, Mohit Rawat, Pallabi Samddar & Pawan Kumar. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. Journal of Nanobiotechnology. 30 October 2018.
- [12]. Mukti Sharma, Saurabh Yadav, Narayanan Ganesh, Man Mohan Srivastava & Shalini Srivastava. Bio fabrication and characterization of flavonoid-loaded Ag, Au, Au-Ag bimetallic nanoparticles using seed extract of plant *Madhuca longifolia* for the enhancement in wound healing bio-efficacy. Progress in Biomaterials volume 8, pages 51-63 (2019).
- [13]. R. Vijayaraj, G. Dinesh kumar, N. Sri Kumaran. In vitro anti-inflammatory activity of silver nanoparticle synthesized *avicennia marina* (Forssk.) Vierh.: a green synthetic approach. International Journal of Green Pharmacy. July-Sep 2018.
- [14]. Lakshmanan G, Sathyaseelan A, Kalaichelvan P.T, Murugesan K. Plant-mediated synthesis of silver nanoparticles using frit extract of *Cleome viscosa* L. Assessment of their antibacterial and anticancer activity. Karbala International Journal of Modern Science, Volume 4, Issue 1, March 2018, Pages 61-68.
- [15]. Mohana Sriramulu and Shanmugam Sumathi. Photocatalytic, antioxidant, antibacterial and anti-inflammatory activity of silver nanoparticles synthesised using forest and edible mushroom. Advances in natural sciences: Nanoscience and Nanotechnology, Volume 8.



- [16]. Muhammad Rafique, Igra Sadaf, M. Shahid Rafique and M. Bilal Tahir. A review on green synthesis of silver nanoparticles and their applications. *Artificial cells, Nanomedicine and Biotechnology and international journal*, Vol 45, 2017. 1272-1291.
- [17]. S Anu Mary Ealia and M.P. Saravanakumar. A review on the classification, characterisation, synthesis of nanoparticles and their application. *IOP conference series: Materials science and Engineering*, Volume 236, Issue 3.
- [18]. K. Anandalakshmi. Green synthesis of silver nanoparticles using plant extracts- A review. *Plant archives* vol. 21, supplement 1, 2021, Pages 2091-2097.
- [19]. S. Saranya, A. Eswari, E. Gayathri, S. Eswari and K. Vijayarani. Green synthesis of metallic nanoparticles using aqueous plant extract and their antibacterial activity. *International journal of current microbiology and applied sciences* ISSN: 2319-7706 volume 6 (2017).
- [20]. Nivedita Patel, Pinal Patel, Dhara Patel, Sharav Desai & Dhananhay Meshram. Phytochemical analysis and antibacterial activity of moringa oleifera. *International Journal of medicine and pharmaceutical sciences (IJMPS)* ISSN(P): 2250-0049; ISSN(E): 2321-0095.
- [21]. Shivraj Hariram Nile, Arti Nile, Enkhataivan Gansukh. Subcritical water extraction of withanosides and withanolides from ashwagandha (*Withania Somnifera* L) and their biological activities. *Food and Chemical toxicology*. Volume 132, October 2019.
- [22]. Tushar Dhanani, Sonal Shah, N.A. Gajbhiye. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania Somnifera*. *Arabian journal of chemistry*, Volume 10, supplement 1, February 2017, Pages S1193-S1199.
- [23]. Dada Patil, Manish Gautam, Umesh Jadhav, Sanjay Mishra, Suresh Karpothula. Physicochemical stability and biological activity of *Withania Somnifera* extract under Real-time and accelerated storage conditions. *Journal of medical plant and natural product research*. 76(5): 481-488.
- [24]. Yue tying Loo, Buong Woei Chieng, Mitsuaki Nishibuchi. Synthesis of silver nanoparticles by using tea leaf extract from *Camellia sinensis*. *International journal of nanomedicine*. 2012; 7: 4263-4267.
- [25]. Jerushka S Moodley, Suresh babu, Naidu Krishna, Karen Pillay Sershen and Patrick Govender. Green synthesis of silver nanoparticles from moringa oleifera leaf extracts and its antimicrobial potential. *Advances in natural sciences and nanotechnology*. 2043-6254.