JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EXTRACTION OF MELANIN AND SYNTHESIS OF SILVER NANOPARTICLES FROM *AZADIRACHTA INDICA*, AS WELL AS ITS PHYTOCHEMICAL AND VARIOUS ACITIVITIES

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Abstract : Nanotechnology is a growing field of study in which we work with materials at nanodimensions. Metal nanoparticles are traditionally synthesised using complex and expensive tools or expensive ingredients. Furthermore, the approaches may be harmful to the environment. As a result, "green" technologies for nanoparticle creation are always favoured. This dissertation describes a "green" approach for producing silver nanoparticles. The process is easy, convenient, and environmentally beneficial. Scholars have showed a strong interest in nanotechnology in recent years. This research focuses on nanoparticles, which typically have dimensions of 1-100 nm in at least one dimension. Because of their small size and unique physical features, nanoparticles are frequently used in a variety of sectors, including medical chemistry and atomic physics. They are thought to impact the behaviour of other substances with which they come into contact. Different chemical, physical, and biological methods can be used to conveniently create these particles. The antibacterial activity of the synthesised silver nanoparticles was tested against Bacillus subtilis, Escherichia coli, and Klebisella pneumonia bacteria. The nanoparticles were antimicrobial. The effective inhibitory zone is substantially larger in E. coli and K. pnuemonia than in B. subtilis. It can be determined that nanoparticles are more effective against tested bacteria. The DPPH technique was used to investigate the anti-oxidant activity of the synthesised silver nanoparticles. Silver nanoparticles had a higher percentage of anti-oxidants than extract. Two methods were employed to evaluate the anti-inflammatory activity of the synthesised silver nanoparticles. 1. Denaturation of proteins 2. Hemolysis caused by heat. The powerful percentange was demonstrated by both ways. Silver nanoparticles have a higher percentage value than extract.Alpha amylase was used to investigate the anti-diabetic activity of the synthesised silver nanoparticles. It demonstrates the greater impact of silver nanoparticles than the extract. The presence of melanin was determined by various test that has been extracted by neem leaves. It was investigated and confirmed the existence of melanin in Azadirachta indica leaves. To determine which phytochemicals are present in neem leaves, a phytochemicals test was performed using several reagents, and it was discovered that nearly all types of phytochemicals are found in neem leaves. To determine the greatest SPR peak, UV-VIS spectroscopy of silver nanoparticles was performed within 24 hours of their synthesis.

IndexTerms **- Green leaf, Nanoparticles, Melanin, silver nanoparticles, bacteria, spectroscopy, phytochemicals, anti-oxidant, anti- inflammatory, anti- diabetic**

I. INTRODUCTION

Neem leaves have a long history of use in diabetes management, and there is some clinical evidence supporting their potential in regulating blood sugar levels. It is crucial to remember, however, that neem oil, bark, and leaves can be hazardous to pregnant women, potentially resulting in miscarriage. Neem oil, extracted from the seeds, is used directly as an insect and mite repellant, pesticide, and fungicide. It is a crucial element in many commercial pesticide formulations, including dusts, granules, and concentrates. The principal active insecticidal ingredient in neem, azadirachtin, works by altering the hormones involved in insect moulting. This disturbance hinders larvae from maturing properly into adults and acts as a feeding inhibitor. When soft-bodied insects come into touch with neem oil, it effectively kills them and also decreases mating and reproductive behaviours, lowering pest fecundity. Neem oil is often used as a fungicide to treat a variety of plant diseases such as rust, black spot, mildew, scab, anthracnose, and blight. However, because of its rapid breakdown when exposed to ultraviolet radiation, neem oil must frequently be reapplied. Insecticides based on neem are generally deemed non-toxic to mammals and are commonly employed in organic farming practises. (Vineet kumar shukla, October 2010). Plant products are becoming increasingly popular due to their biodegradability, low persistence, and low toxicity non-target organisms, economic and convenient availability.Approximately 200 plants having insecticidal activity are known today. Azadirachtin, an active chemical derived from the Azadirachta indica with antiviral, antifungal, antibacterial, and insecticidal effects, is one of the

most promising natural compounds. During the isolation of nimbin, the first bitter chemical discovered from neem oil, more than 135 compounds were extracted from various portions of the neem tree (Bandyopadhyay, 2002). Neem produces a rich and complex variety of physiologically active chemicals. Several researchers looked at the medicinal benefits of the herb Azadirachta indica. They had antipyrectic, anti inflammatory (Okpanyi, 1981) (Khattak, 1985), anti-malarial and anti-tumor effects, as well as anti-ulcer, anti-diabetic (Patil, 2013), anti-fertility, central nervous system, and antioxidant activities. (Pillai, 1981) (Bandyopadhyay, 2002). Boiling neem leaf water is a great antiseptic for cleaning wounds, soothing, swelling, and alleviating skin problems (Bojar, 2004).

Plants frequently have black and brown seed colour. Melanin, a pigment with a high molecular weight formed by phenol oxidation and polymerization, could be the source of the colour. It is the most interesting pigment in plants, while being found in all kingdoms of living organisms. The paucity of scientific interest in this plant pigment is due to its lack of evident functions. For a long time, it was assumed that this plant pigment was not melanin since, according to the definition of the term "melanin," which was developed based on studies of melanin in animals, it must be a nitrogen-containing pigment, although melanin in plants does not include nitrogen. Except for the absence of nitrogen in plant and animal melanin, the physical and chemical properties of black pigments produced from microbes, plants, and animals were discovered to be the same. This resulted in a rethinking of the definition of "melanin" and the removal of the nitrogen requirement. At present, melanin is categorized into three different types: eumelanins, pheomelanins, and allomelanins.. Eumelanins are the most frequent type found in animals, microbes, and some fungi. Pheomelanins, on the other hand, are found only in higher creatures such as mammals and birds. Both eumelanins and pheomelanins are generated from tyrosine, however pheomelanins are made up of monomeric units containing sulphur, notably benzothiazine and benzothiazol, rather than the indole units found in eumelanins. Because of its extensive variety of antecedents, the group of allomelanins, which includes melanin found in plants and fungus but does not include nitrogen, is the most diversified type of melanin. Fungal melanin is produced from gamma-glutaminyl-3,4-dihydroxybenzene, catechol, and 1,8- dihydroxynaphthalene, whereas plant precursors include catechol, caffeic, chlorogenic, protocatechuic, and gallic acids. (Glagoleva AY, 2020 Jun 23)

Fig. 1. ***Azadirachta indica*** **(Neem Tree)**

II. MATERIALS AND METHODS

1. Extraction of Melanin

   Fresh Azadirachta indica (neem) leaves were purchased from a local market and washed multiple times with water to remove dirt particles or impurities before sundrying to eliminate the remaining moisture. After it has completely dried, it is ground to form a powder. In a conical flask, combine 5 grams of neem powder and 20 ml of distilled water. To extract the pigment, heat the conical flask on a heating mantle or in an electric water bath for 20 minutes at 70 to 80 degrees. After heating for 20 minutes, the extract absorbs the water and becomes dry; add 10 ml of distilled water and heat for another 5 minutes. Filter the solution through filter paper or cotton to remove any solid particles. Mix with 10 ml of ethanol to the filtered neem leaves extract solution. This will aid in the precipitation of melanin pigment. Cover with foil paper and set aside in the dark for 24 hours to allow the pigment to settle. After 24 hours, filter the solution with a funnel and filter paper to separate the precipitated melanin pigment. Wash the pigment with distilled water to eliminate any dirt or contaminants. In 5 mL of 1Molar NaOH solution, dissolve the pigment. Heat the solution for 2 hours in an electric water bath or hot plate at 70 to 80 degrees Celsius to completely dissolve the pigment. To modify the pH to roughly 2 to 3, add 0.5 ml of diluted HCL to the solution. This will also aid in the precipitation of the pigment. Allow the solution to

stand for 24 hours to allow the pigment to completely precipitate. After 24 hours, transfer the precipitate solution to an Eppendorf tube and centrifuge for 5 minutes at 8000 rpm. Eliminate the supernatant and wash the pellet with 100 microliters of acetone to eliminate contaminants before placing it in the incubator to dry.

2. Preparation of Silver Nanoparticles
Fresh neem leaves were obtained from a nearby market and properly washed with distilled water. Using a blender, grind the leaves to a fine paste. Add 50 ml of distilled water to 5 grams of neem leaves powder and heat for 10 minutes in an electric water bath or heating mantle at 70 degrees Celsius. Filter the neem leaf extract with filter paper to eliminate any solid or dirt particles. To make a 10% neem leaves extract solution, combine 5 mL of neem leaf extract with 45 mL of distilled water. To make a 1% silver nitrate solution, dissolve 0.05 grams of silver nitrate in 5 mL of distilled water. To make a 1% melanin solution, dissolve 0.05 grams of melanin pigment isolated from neem leaves in 5 ml of distilled water. Combine 5 mL of AgNo3 solution and 5 mL of melanin solution. Add 50 mL of the 10% neem leaf extract solution to the silver nitrate melanin mixture. Using a NaOH solution, lower the pH of the mixture to 9 to 10. Incubate the mixture at room temperature for 24 to 48 hours with constant stirring. To obtain silver nanoparticles, centrifuge the mixture for 10 to 15 minutes at 10,000 to 15,000 rpm. Remove the supernatant, wash the nanoparticles with distilled water, and resuspend the silver nanoparticles with distilled water for future usage. During the analysis of silver nanoparticles, the Surface Plasmon Resonance (SPR) peak was identified in the 380 nm to 420 nm region.

3. Anti-microbial Activity
In a 45ml of Nutrient agar, fill the sterile petri plates with agar and allow to harden under laminar flow for 10-15 minutes. Cultures of E. coli, Klebsiella pneumoniae, and Bacillus subtilis were taken from Rapture Biotech, Mumbai . Inoculate 100 l of each, uniformly spread on agar, and let dry for 5-10 minutes. Using a 6-8 mm pipette, make 3 wells. Pour AgNP, neem extract, and ciprofloxacin into the wells. Rep with the other civilizations. Incubate for 24 to 48 hours at 37°C.

4. Anti-oxidant Activity
Stock solution can be prepared in a flask, dissolve 0.0072g DPPH in 30mL methanol. Mix 1.5 ml stock solution for the blank, then measure the optical density at 517 nm. Take 50 microlitre of leaf extract and AgNP in separate test tubes. In each, add 1.5 mL of stock solution and incubate in the dark for 30-35 minutes. Using a UV-VIS Spectrophotometer, determine the outside diameter at 517 nm. Antioxidant activity:% inhibition = (Ac - As / Ac) * 100, where Ac = Control/Blank, As = Sample (AgNP & leaf extract).

5. Anti- inflammatory Activity
a. Protein Denaturation: followed Gambhire et al.'s approach with Gunathilake et al.'s changes. 5ml - 0.2ml 1% bovine albumin, 4.78ml PBS, 0.02ml each sample (leaf extract and AgNP). Incubate at 37°C for 5 minutes, then at 70°C for 5 minutes. O.D. at 660 nm = 100 * (1 - As/Ac), Ac = PBS control, As = Sample.
b. Heat-Induced Hemolysis: Erythrocyte Suspension: Spin healthy blood, wash, and resuspend in 10% isotonic buffer (PBS). In test tubes, add 0.05mL blood cell suspension + 0.05mL sample (leaf extract and AgNP) + 2.95mL PBS. Incubate for 3 minutes, then centrifuge at 2500 rpm for 3 minutes and measure supernatant absorbance at 540 nm. % inhibition of hemolysis = 100 * (1 - As/Ac), Ac = PBS control, As = Sample.

6. Anti-dibaetic Activity
Make up to 25ml with distilled water by combining 5ml 3,5 DNSA with 12.5ml sodium potassium tartrate. Fill three test tubes with 200l each of AgNP, leaf extract, and PBS. To each, add 200l of alpha amylase + 1% starch solution. Incubate for 30 minutes at room temperature. Stop the process by applying DNSA reagent to all tubes. Heat in an electric water bath at 85°C for 15 minutes. Using a UV-VIS Spectrophotometer, take measurements at 540 nm. Determine the amount of maltose produced. The anti-diabetic activity is calculated using the formula: % inhibition = (Ac- As/Ac) * 100, where Ac- Control/ Blank and As- Sample (AgNP and leaf extract).

III. RESULTS AND DISCUSSIONS

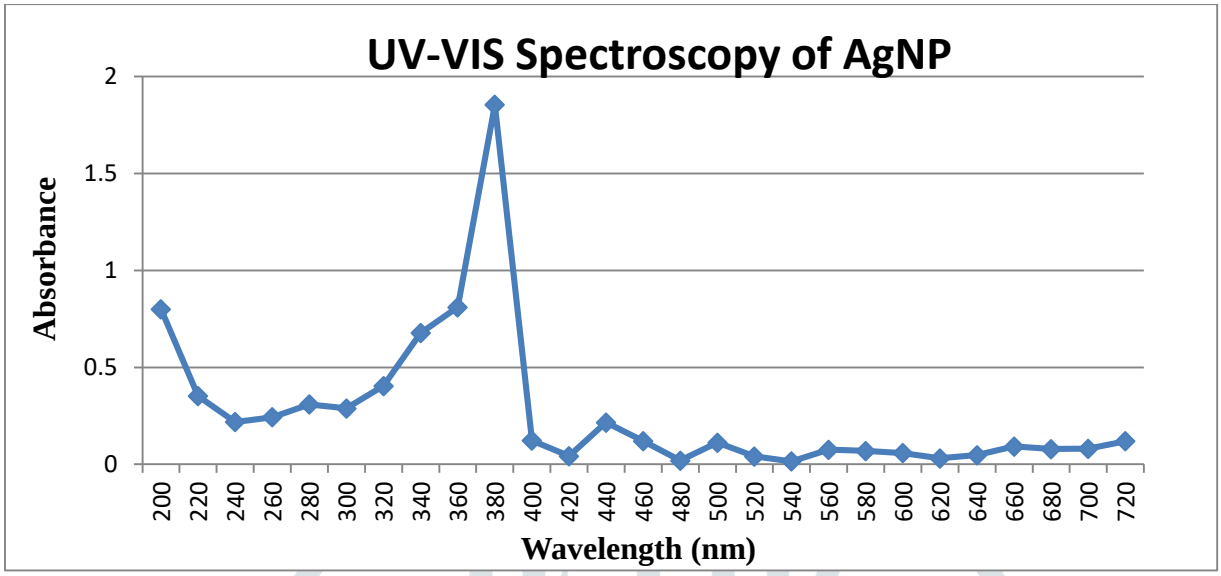
2.1 PRESENCE OF MELANIN

Sr. No	Test Performed	Observations
1	Sodium Hydroxide	++
2	Hydrogen peroxide	++
3	Copper Sulphate	++
4	Iron (III) Chloride	+++
5	Acid test	++
6	Sodium Bicarbonate	+

(+ Average, ++ highly, +++ very highly, - absent)

Table 1

2.2 CHARACTERIZATION OF SILVER NANOPARTICLES BY UV- VIS SPECTROPHOTOMETER



UV- VIS Spectra of AgNP of *Azadirachta indica* at 200 nm to 720 nm

Table 2

3.1 OBSERVATIONS OF ZONE OF INHIBITION OF ANTI- MICROBIAL ACTIVITY

These leaf extracts are often obtained through techniques like maceration or solvent extraction. The extracts are then tested against different microorganisms to determine their inhibitory or killing effects. Microbiological tests were performed against E. coli, K. Pneumonia, B. Subtilis and it was discovered that the antibiotic ciprofloxacin had a higher zone of inhibition than AgNP and leaf extract. The below figures shown below.

Fig.1 Culture- Escherichia coli

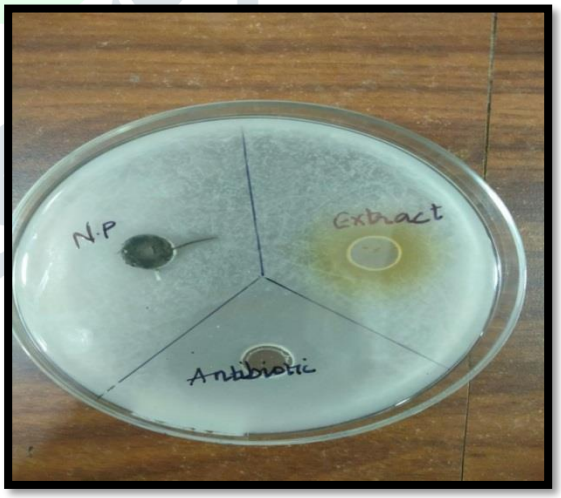
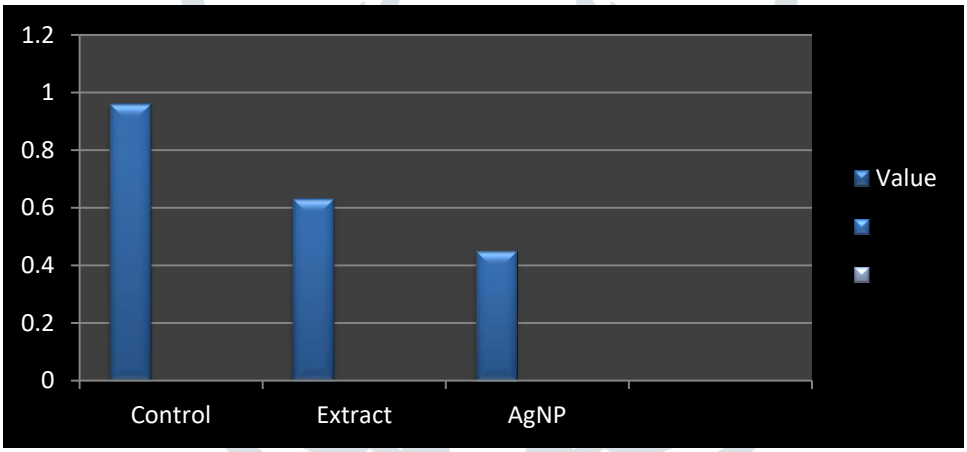


Fig. 2 Culture- Klebsiella pneumoniae

Fig. 3 Culture- Bacillus subtilis

4.1 OBSERVATIONS OF THE ANTI- OXIDANT ACTIVITY

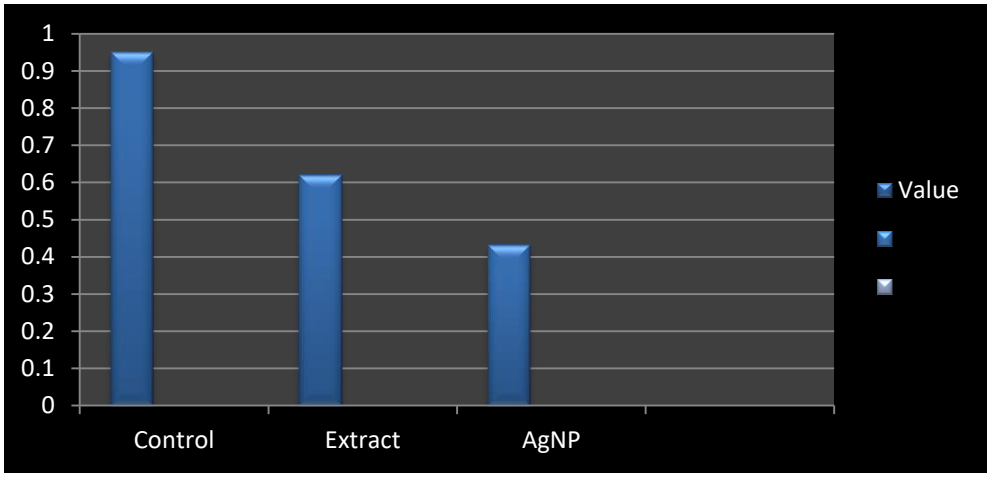
The anti-oxidants test was done and the absorbance of blank, neem leaf extract and AgNP was checked by the UV- VIS Spectrophotometer at 517 nm. It was observed that the anti-oxidants activity of silver nanoparticles is higher than the neem leaf extract. The percentage of leaf extract inhibiton was found to be 34.37% and the percentage of AgNp inhibition was found to be 53%. The below graph has shown.



Graph 1

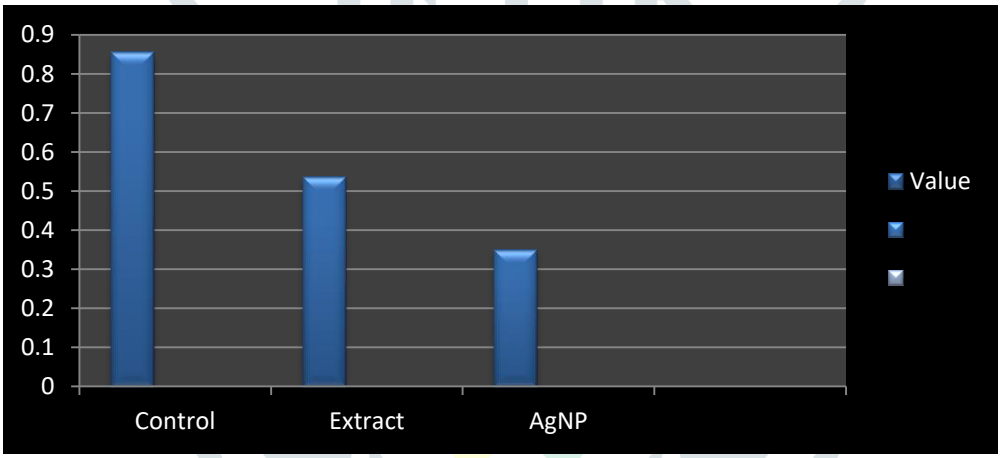
5.1 OBSERVATIONS OF ANTI- INFLAMMATORY ACTIVITY

The protein denaturation of anti-inflammatory activity has been observed. Protein denaturation is frequently employed as a model system in anti-inflammatory activity assays to assess a compound's or extract's capacity to inhibit or prevent protein denaturation, which can be produced by a variety of inflammatory triggers. Inflammation can alter the local environment of cells and tissues, resulting in protein denaturation and aggregation. Its shows the AgNP has highly effective to prevent protein from denatures itself than the leaf extract. The percentage of leaf extract inhibition was found to be 40% and the percentage of AgNP inhibiton was found to be 60%. The below graph has shown.



Graph 2

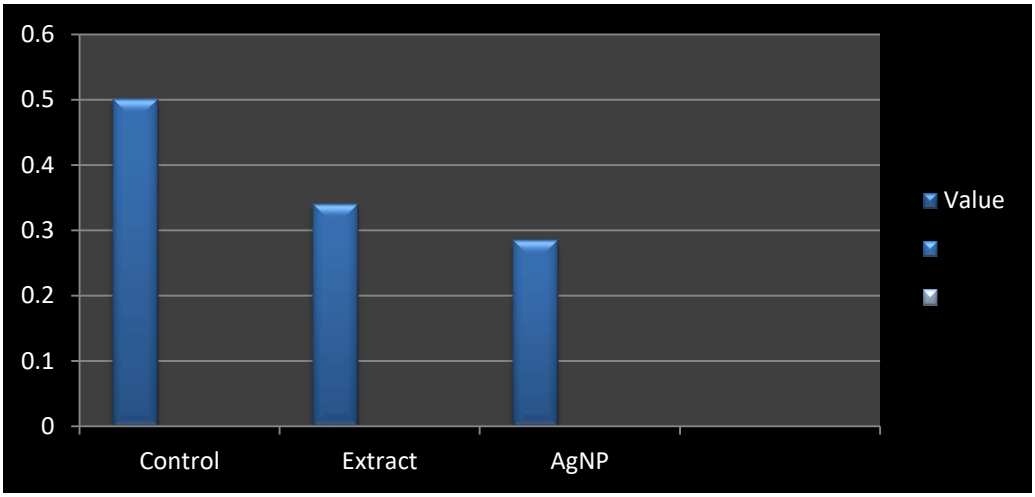
In the context of anti-inflammatory activity assays, heat-induced haemolysis is frequently employed as a model system to assess a compound's or extract's capacity to protect red blood cells from heat-induced damage. The test sample is commonly incubated with a suspension of red blood cells before being subjected to high temperatures. The amount of haemoglobin released from injured cells is used to assess the extent of haemolysis. Its shows the AgNP has highly effective to prevent red blood cells before it gets extreme heat than the leaf extract. The percentage of leaf extract inhibition was found to be 54% and the percentage of AgNP inhibiton was found to be 76%. The below graph has shown.



Graph 3

6.1 OBSERVATIONS OF ANTI- DIABETIC ACTIVITY

A substance's potential to reduce blood glucose levels and/or improve glucose metabolism in diabetics. Diabetes is a chronic disease typified by high blood glucose levels caused by the body's inability to produce or use insulin effectively. Diabetes, if left uncontrolled, can lead to a wide range of health issues, including cardiovascular disease, kidney failure, and blindness. It has been observed that the AgNP shows the effective anti- diabetic activity than leaf extract. The percentage of inhibition of leaf extract was found to be 32% and the inhibition of AgNP was found to be 43%. The below graph has shown.



Graph 4

7. PHYTOCHEMICALS TEST

Sr. No	Test Performed	Observations
1	Alkaloid	++
2	Tannins	+++
3	Flavanoids	++
4	Saponins	+++
5	Terpenoids	++
6	Steroids	+
7	Glycosides	++
8	Carbohydrates	++
9	Anthraquinones	-

(+ Average, ++ highly, +++ very highly, - absent)

Table 2

IV. CONCLUSION

The quick synthesis of silver nanoparticles by Azadirachta indica leaf extract provides a handy and effective method for manufacturing harmless nanoparticles in an environmentally responsible manner. We extracted the melanin pigment from the leaf, and this melanin pigment is used to synthesise silver nanoparticles. Silver nitrate serves as a reducing agent in this context. As a result of the presence of melanin, the colour obtained following the creation of silver nanoparticles is slightly dark greenish-blackish. Significant progress has been made in understanding the process of melanin synthesis in plants over the last ten years. One of the approaches used to analyse silver nanoparticles was the use of UV-VIS Spectroscopy, which identified a peak wavelength of 420 nm as the greatest absorption point. Recognising the presence of melanin throughout the plant world is a big accomplishment in the area. While the precise role of melanin in seed coatings is unknown, its widespread distribution suggests a variety of possible purposes, with disease prevention being the most likely. Another significant achievement in plant melanin research is the identification of its relationship with intracellular plastids, implying a link between melanin synthesis and these organelles. Only one plant species has shown melanin formation in plastids of grain envelopes; more research on other plant species is needed to corroborate this conclusion. Analysing the many qualities of silver nanoparticles from neem leaves extract, such as antimicrobial, antioxidants, anti-inflammatory, and anti-diabetic, revealed that neem leaves are extremely effective for human health. Dried neem leaves can be ingested and offer potential benefits. Neem contains compounds that have been associated with potential advantages such as lowering blood sugar levels, promoting the healing of stomach ulcers, acting as a contraceptive, combating bacteria, and preventing the formation of dental plaque.The synthesis process exhibited high efficiency in terms of reaction time and the stability of the produced nanoparticles, without the need for external stabilizers or reducing agents. This eco-friendly and rapid approach offers a cost-effective and efficient method for manufacturing silver nanoparticles, satisfying the requirements for a completely environmentally friendly chemical process. The synthesized silver nanoparticles displayed significant antibacterial activity against both E. coli and K. pneumonia. Utilizing plant extract for synthesis presents several advantages, including energy efficiency, cost-effectiveness, and the promotion of human health and environmental protection by reducing waste and ensuring safer products. Additionally, specific confirmatory tests revealed the presence of melanin in neem leaves. Neem leaves have a wide spectrum of phytochemical qualities, emphasising its important worth and advantages to both the environment and human well-being. This environmentally friendly technique offers a viable alternative to conventional physical and chemical processes used in silver nanoparticle production, making it a promising candidate for biomedical applications and potential contributions to opto-electronics and medical devices in the near future. Technologically, the generated silver nanoparticles have the potential for biomedical applications. This simple process has several advantages, including scalability for commercial production, affordability, suitability for medical and pharmaceutical uses, and the potential to be manufactured on a big scale to fulfil medical and pharmaceutical demands.

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