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Synthesis of Silver Nanoparticles using *Azadirachta Indica* Leaves Extract and its Antimicrobial Study

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Abstract

In this work, we used a green synthesis approach to create plant extract-based silver nanoparticles [Ag NPs], which we then analyzed using FTIR and UV. The unique absorption spectra of silver nanoparticles were discovered using ultraviolet scanning spectroscopy. The maximum amount measured at 435 nm. These nanoparticles were added to the PBS solution to test both the electrocatalytic and antibacterial activities. Because of their special antibacterial qualities, silver nanoparticles have attracted a lot of attention among the many nanoparticles. The production of herbal-mediated silver nanoparticles [SNP] with antibacterial properties is the major topic of this study. The extract served as a reducing agent in the silver nanoparticle production. Finally, we conclude by discussing the future perspective of AgNPs.

Keywords: Neem extract, AgNPs, FTIR, UV-VISIBLE, Antimicrobial activity, electrical conductivity.

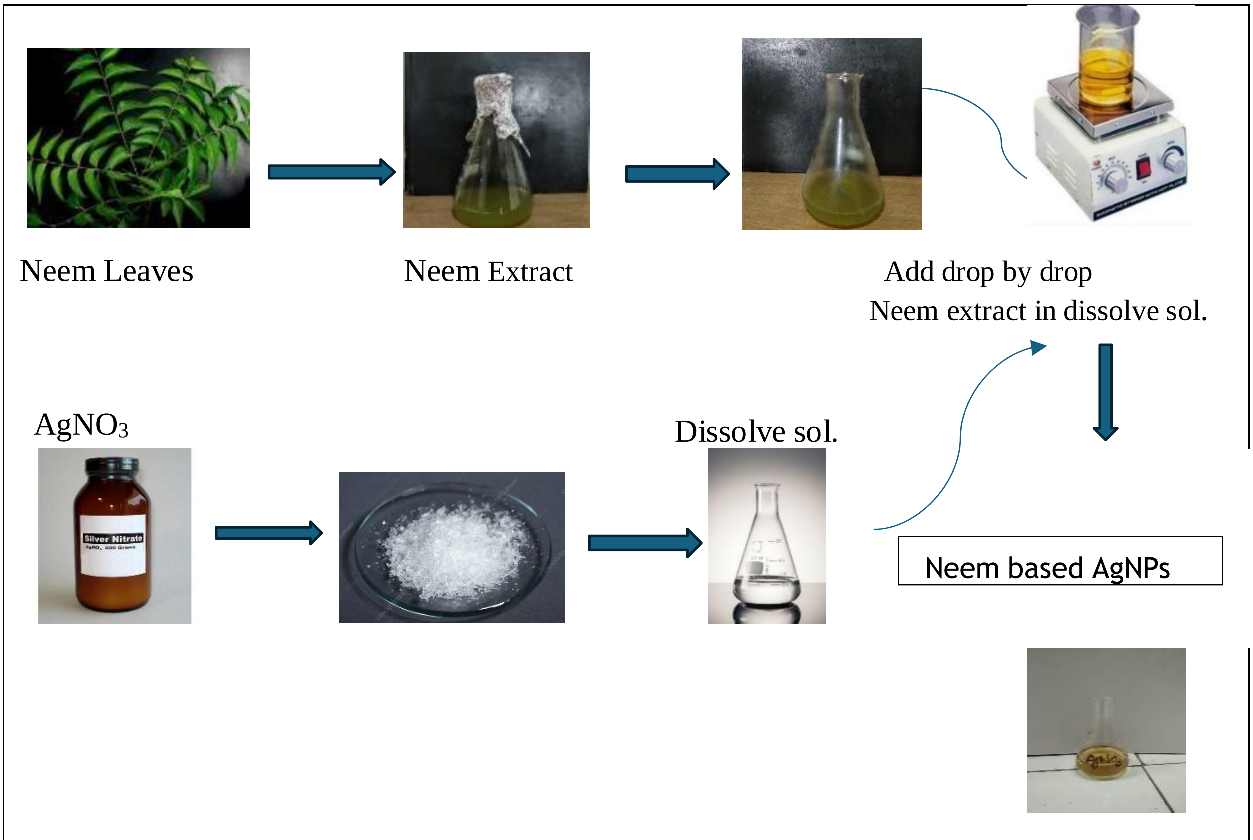


Fig.1 [Graphical representation making AgNPs]

1. Introduction

Silver is one of the basic materials that comprise our environment. It is an extremely ductile and malleable element that occurs naturally. It is somewhat harder than gold. Infectious disorders including gonorrhoea and syphilis, as well as mental illness and epilepsy, have all been treated with soluble silver compounds like silver slats. Silver has been used in traditional medicine to treat disorders of the neurological and digestive systems. These days, numerous potent medications based on silver are being developed [1]. Silver-containing drugs have been found to be effective in treating several conditions, including cancer, inflammation of the tissues, eye, throat, and inflammation. Applications for bio diagnostic, imaging, therapeutic, and drug delivery make use of silver's special qualities [2]. The efficacy of antibiotics is compromised by microbial resistance, which is seen as a worldwide health concern and makes the treatment of common diseases impractical [3]. Cancer is the uncontrolled division of abnormal cells mainly caused due to external and genetic factors. Currently, silver nanoparticles are used as anti-cancerous substances [4]. The antiviral, antifungal, antibacterial, and anti-inflammatory properties of silver nanoparticles are well documented [5]. As a potent fungicide, silver nanoparticles can eradicate common fungal strains such as Aspergillus fumigatus, Mucor, Saccharomyces cerevisiae, and Candida tropical is [6]. Silver nanoparticles [Ag NPs] are typically the basis for the development of novel strategies that hinder the growth of microbes on building materials [7]. Taking into account the increasing need for novel antifungal medications as a result of microbial resistance to current therapies and the potential of silver nanoparticles as an antifungal substitute [8]. Because of their mechanical, electrical, and biological qualities as well as their thermal conductivity, biocompatible metallic nanoparticles [such as those made of gold, silver, titanium, iron, etc.] are utilized as biomaterials in items like shampoo, soap, hand lotion, toothpaste, textiles, and cosmetics [9]. They are also used in the pharmaceutical industry, catalysis, cancer diagnosis, and as antimicrobial agents [10]. Antibacterial compounds play a critical role in the food packaging, pharmaceutical, textile, and water disinfection industries [11]. Antibacterial substances eradicate germs on a localized level without doing any harm to the adjacent tissue [12]. The phenomenon known as "antibiotic resistance" refers to the ability of bacteria to resist antibiotics by a variety of strategies, such as neutralizing the drug, excreting it, altering structural elements of the cell to stop the drug from working, and DNA transfer between bacteria [13]. When antibiotics are used, they act on drug-sensitive bacteria, eradicating and leaving behind resistant bacteria which reproduce and proliferate [14]. Plants have bioactive substances alkaloids, phenols, saponins and quinines [15]. These chemicals have antimicrobial action as a result. In addition to being a significant source of chemical diversity, natural products have produced useful treatment medicines for a variety of bacterial illnesses [16]. It is explained by the ability to logically choose plants for antibacterial testing based on their ethno medicinal applications [17]. A class of heterocyclic chemical molecules found in plants and associated products like propolis, and honey are called flavonoids [18]. Metal nanoparticles which have the antibacterial property, also important in synthesis of biomaterial because antibacterial coatings can help to reduce the risk of implant-associated infections by inhibiting the growth of bacteria on the implant surface [19]. Electrically conductive metal nanoparticles play a key role in a variety of potential applications, such as electrochemical biosensors and biomedical healthcare monitoring [20]. Due to their special qualities, silver nanoparticles [Ag NPs] have attracted a lot of attention for a variety of uses, including antibacterial agents, biolabeling, selective coatings for solar energy absorption, optics, and catalysts [21]. The presence of biomolecules found in plants as its phytochemicals, such as proteins, amino acids, vitamins, polysaccharides, polyphenols, terpenoids, and organic acids like citrates, leads to the formation of nanoparticles mediated by plants [22]. Medical fields make use of the properties of nanoparticles. "Green synthesis" refers to the production of silver nanoparticles by environmentally friendly methods [23]. Because it is more affordable, environmentally friendly, and efficient than conventional synthesis, etc., green synthesis is favoured [24]. green production of silver nanoparticles with the help of fungi, plants, and other microorganisms [25]. The optimum capping material for the manufacture of silver nanoparticles is produced by plant extract [26]. There are numerous techniques for producing silver nanoparticles, but natural substances also can act as capping and reducing agents [27].

2. Materials and Preparation of Silver Nanoparticles

a) To prepare the extract, the neem leaves were collected, washed under running tap water, and then rinsed with distilled water and ethanol to remove any remaining contaminants [28].

b) Using a motor and pestle, 100 grams of leaves were ground, and Whatman's filter paper was used for filtering [29].

c) For later usage, the filtered material was collected in a dry, clean 100 ml conical flask and refrigerated [30].

d) When 15 millilitres of neem leaf extract and 35 millilitres of 0.5 millilitre distilled water containing silver nitrate [$AgNO_3$] were combined, a colour shift that indicated the creation was seen [31].

2.1. Method and Materials

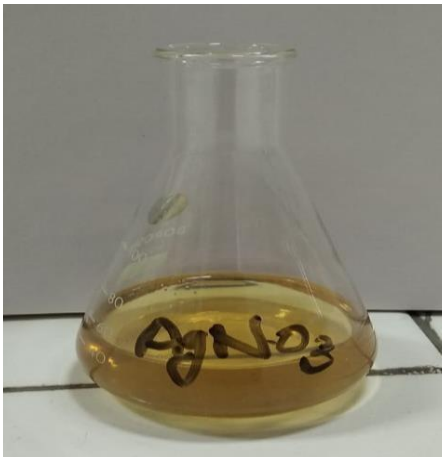
Utilities

Vertex mixer, heating element, laminar air flow, ph meter, incubator, weighing balance machine, and autoclave.

Chemicals

Agar powder, distilled water, and Mueller-Hinton broth [HIMEDIA] are used as solid media [32].

Distilled water is used for liquid media Nutrient broth [HIMEDIA]. 0.5 grams 100 cc of distilled water was added along with silver nitrate [$AgNO_3$]. Stirring, 15 millilitres of the leaf extracts were added to 35 millilitres of the 0.5 M $AgNO_3$ solution that had been produced at room temperature [33]. The solution's colour transitioned from brown to reddish brown. The mixture was used to create a character [34].



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[Neem Extract] *[Neem extract based AgNPs]*

Fig.2

2.2. Characterization

2.2.1. UV-VIS

The UV-Visible spectrophotometer [SYSTRONICS] of Shobhit University was used to record the UV-vis spectra of Ag NPS. The recorded spectra fell between 300 and 700 nm. Silver nanoparticles [Ag NPs] have an absorption peak in their UV-visible spectrum at approximately λ_{max}. 430 nm. Ag's surface plasmon resonance reflected the creation of Ag NPs, which is a hallmark of Ag. This was evidently demonstrated. The form and location of the surface Plasmon resonance [SPR] band frequently indicate the quality of the resulting Ag nanoparticles [NPs]; a sharp band at a lower wavelength indicates a smaller size of NPs.

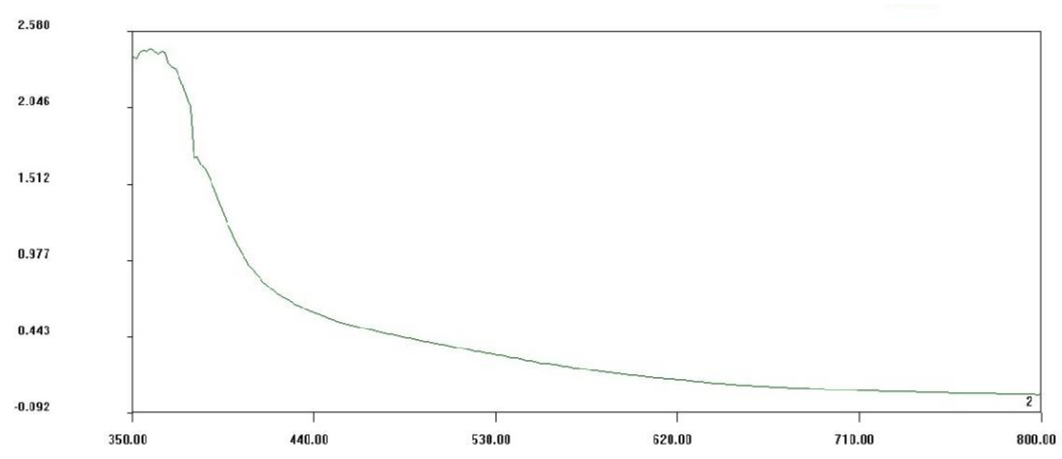


Fig. 3 [UV –VIS spectra showing the absorbance of neem extract in AgNPs]

2.2.2. Electrical Conductivity of Silver Nanoparticles

Silver nanoparticles [Ag NPs] are one of the many types of nanoparticle materials that have attracted a lot of attention because of their high electrical conductivity, inexpensive cost in comparison to gold, relative resistance to oxidation, and biocidal qualities [35]. Physical techniques need a lot of energy, but they yield nanoparticles with smoother surfaces. There are more benefits to biological synthesis than the others [36]. It seems to be a stable and economical method, but it has greater restrictions on the morphological control due to the chemicals' range. This is mostly because of its strong thermal and electrical conductivity, chemical stability, and relative affordability [in comparison to materials like graphene or gold, for example]. Its oxide form also has the capacity to conduct electricity. Because of their low melting point, silver nanoparticles may form conductive thin films at comparatively low temperatures, which is essential for applications involving flexible substrates like papers and polymers [37]. Comparing printed materials, sensors, and flexible circuitry to other conventional processes like drop casting or stamping, shows how far technology has come [38].

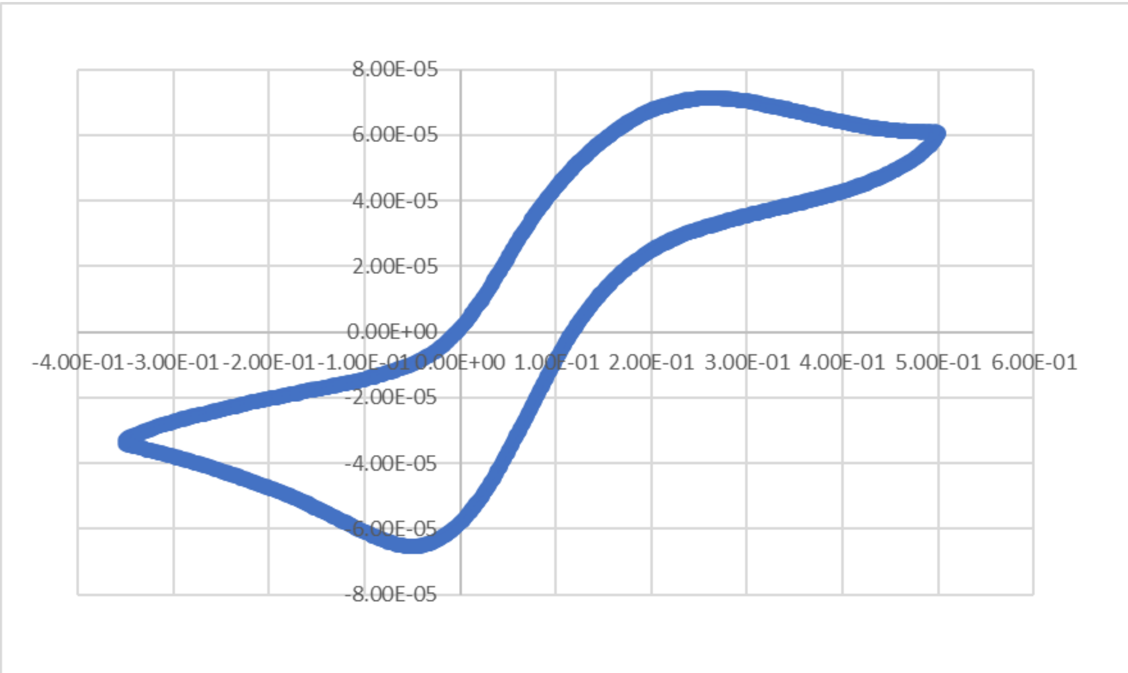


Fig. 4 [Showing electrical conductivity AgNPs]

3. Preparation of media for the test organism

Both liquid and solid media were ready for the test bacteria's single colony isolation. To disseminate germs on an agar plate and incubate them for the entire night at 37°C, solid media was utilized. The overnight incubation resulted in the appearance of distinct bacterial colonies.

For starter culture, a single colony was inoculated in 10 millilitres of liquid media. A clean electronic weighing balance was used to weigh two grams of nutrient agar powder. A conical flask holding two grams of nutrient agar was then filled with one hundred millilitres of distilled water. Using a glass rod that had been sanitized, the mixture was heated and protected with a cotton plug. Next, aluminium foil was securely wrapped over the mixture and autoclaved for 15 minutes at 121°C.

Then, the media was given time to calm down. The media were kept in a molten state by placing them inside an autoclave after autoclaving. Then transfer into an inoculated petri dish. A test tube containing one millilitre of liquid medium.

Five different bacterial species were utilized to assess the antibacterial activity of neem extract

Staphylococcus aureus, Escherichia coli, Pseudomonas, Bacillus, and Clostridium Inoculation and culture preparation:

Before conducting the antimicrobial experiment, each bacterial strain was inoculated in 10 ml of LB broth and cultured separately for an entire night at 37°C. After each bacterial strain was cultured for overnight in 50 ul, it was individually transferred into 5 ml of LB broth, kept sterile, and in a shaking water bath at 37°C for 16 hours.

4. Antimicrobial Assay

Agar well diffusion method

To assess a plant's or microbial extract's antimicrobial activity, the Agar well diffusion method is frequently employed. The process of inoculating the agar plate surface involves covering the entire surface with a volume of the microbial inoculum, much like in the disk-diffusion approach. Subsequently, a 100 µL sterile tip is used to punch an aperture of 6 to 8 mm in diameter. 50 µL of the antimicrobial agent or extract solution at the intended concentration is then added to the well. After that, agar plates are incubated in the appropriate environment for the test microorganism. The studied microbial strain's growth is inhibited by the antimicrobial agent as it diffuses throughout the agar media.

5. Result and Discussion

5.1. UV-VISIBLE

The spectra that were recorded ranged from λmax. 300 to 700 nm. Silver nanoparticles [Ag NPs] have an absorption peak in their UV-visible spectrum at approximately λmax. 430 nm.

5.2. FTIR

FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. By using FTIR spectroscopy, it becomes possible to detect small absorbance changes on the order of 10^{-3}.

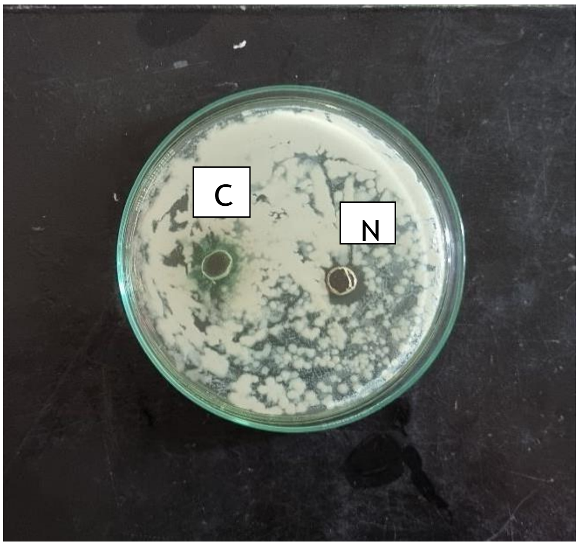
5.3. Antimicrobial Activity

Table 1. Zone of inhibition based on AgNPs and neem

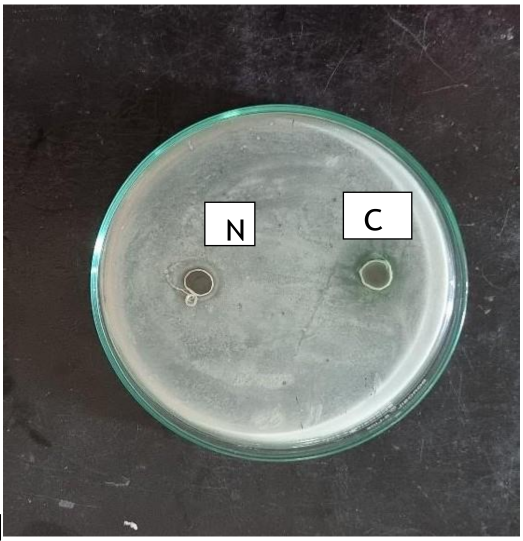
Zone of Inhibition		
Sample	**Neem Based [Neem]**	**Control Ag NPs**
A [E. Coli]	1.16mm	1.16mm
B [S. aureus]	0.5mm	1.3mm
C [Pseudomonas]	1.66mm	0.5mm
D [Bacillus]	2.5mm	4mm
E [Clostridium]	3.33mm	2.83mm

Depending on the bacterial type and extract concentration, the neem extract's antibacterial activity changed. The highest zone of inhibition appeared in 100µL of neem extract and neem-based Ag NPs, allowing researchers to examine the effects of these substances against the strains under investigation.

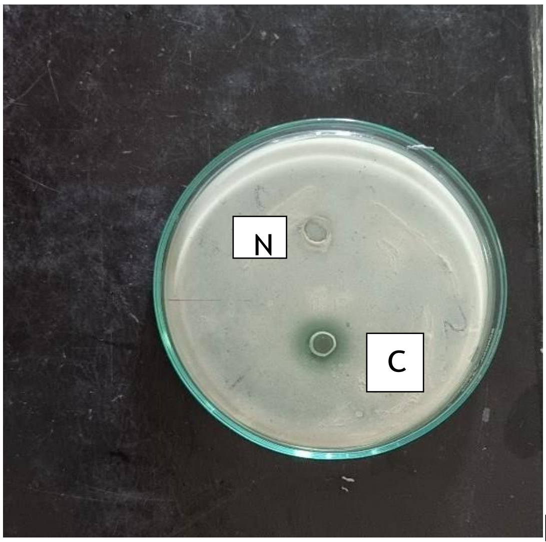
Staphylococcus aureus [0.5±1.3], Escherichia coli [1.16±1.16], Pseudomonas [1.66±0.5], Bacillus [2.5±4], and Clostridium [3.33±2.83] all have lowest zones of inhibition in 100µL of neem extract was evident. The 5µl of Ag NPs with neem extract.



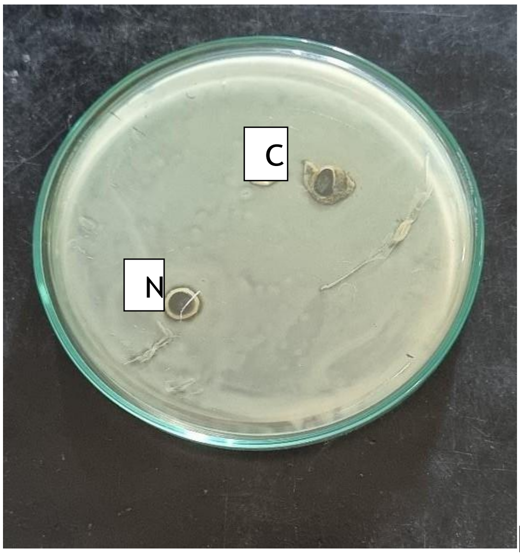
[a]



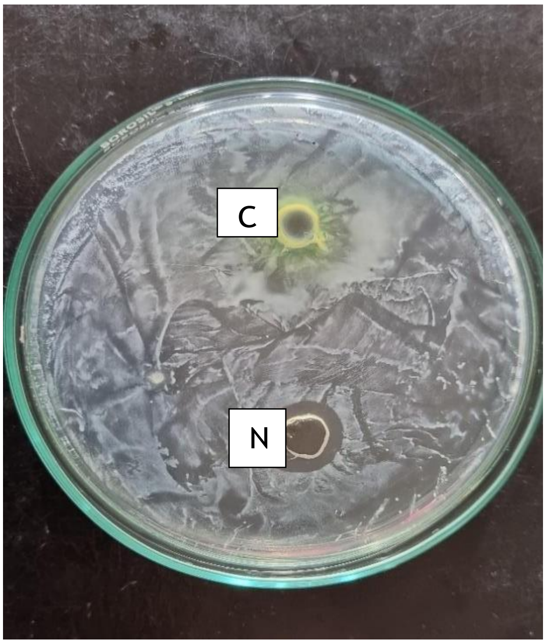
[b]



[c]



[d]



[e]

Fig. 5 [a, b, c, d, e the different zones formed of neem extract and AgNPs of samples on Gram positive bacteria]

6. Conclusion

The synthesis, characterisation, and bio applications of silver nanoparticles were thoroughly covered in this study, with a focus on the processes underlying the anticancer activity of AgNPs as well as their potential use in cancer therapy. Because of the common adverse effects of chemotherapy and radiation therapy, research from both academic and commercial sources has recently looked into the potential use of AgNPs as a next-generation anticancer therapeutic agent. Even though AgNPs are crucial for clinical research, there are a number of things to take into account: where the raw materials come from, how they are made, their stability, biodistribution, controlled release, accumulation, cell-specific targeting, and, lastly, any potential toxicity to humans. Although many approaches are accessible, the synergistic effects of AgNPs and antibiotics on antibacterial medicines or multiple therapeutic agents on anti-cancer activity/tumour reduction are still ambiguous. If we succeed in all of these investigations, it would allow researchers in the nanoscience and nanotechnology community to develop safer, biocompatible, efficient cancer or anti-angiogenic treatments containing AgNPs.

6.1. FTIR

The Fourier transfer infrared radiation [FTIR] approach is utilized to determine the distinctive functional groups from the spectral bands that provide information on the conjugation between the adsorbed biomolecules and the nanomaterial. FTIR has been applied to the investigation of Nano-scaled materials, such as confirming functional molecules covalently grafted onto silver, carbon nanotubes, graphene, and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process.

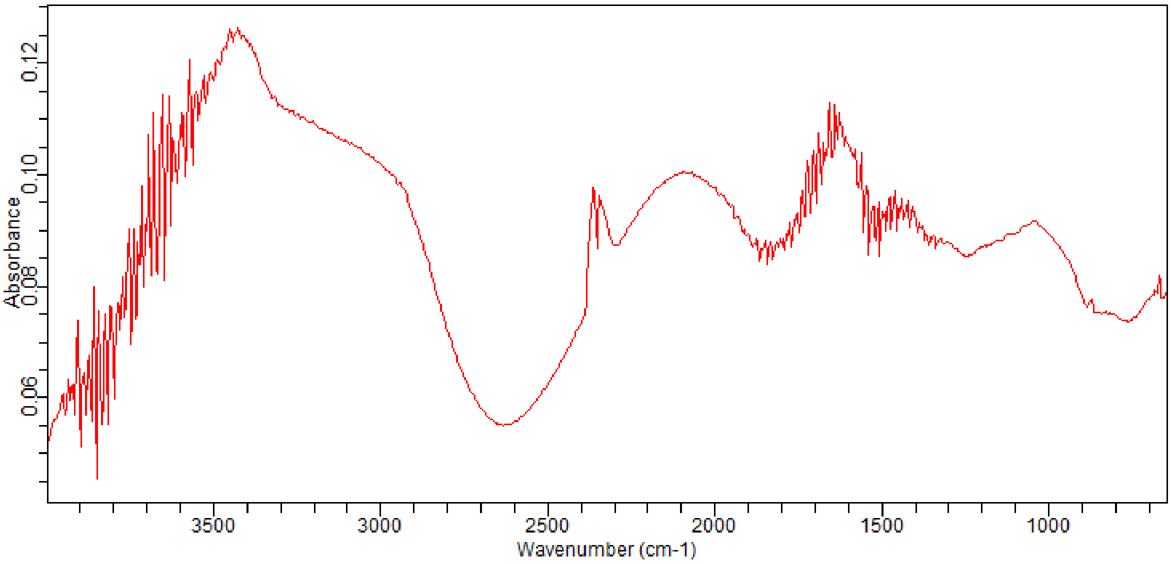


Fig. 6 FTIR Result "showing functional group absorbance"

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