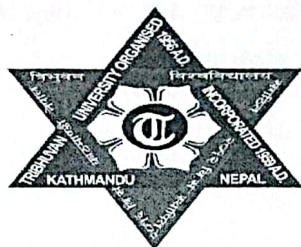


GREEN SYNTHESIS OF SILVER NANOPARTICLES USING RUDRAKSHA EXTRACTS: CHARACTERIZATION AND ITS APPLICATIONS FOR ANTIMICROBIAL ACTIVITIES



A RESEARCH FINAL REPORT SUBMITTED TO THE
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DECLARATION

This project work entitled “**GREEN SYNTHESIS OF SILVER NANOPARTICLES USING RUDRAKSHA EXTRACTS: CHARACTERIZATION ITS APPLICATIONS FOR ANTIMICROBIALACTIVITIES**” is being submitted to Institute of Science and technology (IoST) Tribhuvan University, Nepal for mini research project work under supervision of Dr. Bhoj Raj Poudel Department of Chemistry Tri Chandra Multiple Campus, Tribhuvan University This project work report has not been submitted in other university or institution.

..........

Gautam Kumar Jha

July, 2024

LETTER OF FOREWORD

Mr. Gautam Jha has carried out the entire research works presented entitled **GREEN SYNTHESIS OF SILVER NANOPARTICLES USING RUDRAKSHA EXTRACTS: CHARACTERIZATION AND ITS APPLICATIONS FOR ANTIMICROBIAL ACTIVITIES** under our supervision in year 2023. During the research period, he has performed the work sincerely and satisfactorily. No part of this project work has been submitted by any others.



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Lastly, I am very thankful to my Family, friends and my student Dhan Raj Gurung and well wishers for believing me and supporting me unconditionally in whatever I do.



Gautam Jha

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ABSTRACT

Different methods are reported for synthesis of AgNPs. In this research green synthesis of AgNPs is used as it is simple, economical, environmentally friendly and nontoxic process. Leaves extract of Rudraksha plant has been used for synthesis of AgNPs. Rudraksha and AgNPs both have antimicrobial activity. Green Reduction Ag^+ was carried out. Phytochemicals present in leaves extract was studied at room temperature. The Synthesized AgNPs was characterized using techniques UV -visible Spectral analysis, XRD, and FTIR followed by assessment of antimicrobial property against some bacteria and fungus of extract, AgNPs and their composite 1:1 mass ratio. The size of synthesized AgNPs was about 11.93nm. The antimicrobial activity of AgNPs was enhanced by leaf extract of Rudraksha.

Key words: Nanoparticles, Rudraksha extract, Green synthesis, Phytochemicals, Antimicrobial activities

LIST OF ABBREVIATIONS

°C:	Degree centigrade
FTIR:	Fourier transform infrared
nm:	Nanometer
SEM:	Scanning electron microscopy
NPs:	Nanoparticles
AgNPs:	Silver Nanoparticles
XRD:	X-Ray Diffraction
DLS:	Dynamic Light Scattering
TEM:	Transmission Electron Microscopy
SPR:	Surface Plasmon Resonance
DMSO:	Dimethyl Sulphoxide
LB:	Liquid Broth

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1. TITLE

The title of the research work is **“Green Synthesis of Silver Nanoparticles using Rudraksha Extracts: Characterization and its Applications for Antimicrobial Activities.”**

2. INTRODUCTION

2.1 Background

The word 'nano' means a dwarf substance that represents a billionth (10^{-9}) of a unit (Murty et al., 2012). Nanoparticles (NPs) are ultrafine microscopic particles that range between 1 to 100 nanometers in size. Materials having at least one of their dimension in this nanoscale range are nanomaterials (Giri et al., 2022; Murty et al., 2012). Nanomaterials can be zero, one, two or three dimensional based on their dimensions (Tiwari et al., 2012). Metal NPs are more important in the field of nanoscience and nanotechnology because of their potential applications (Li et al., 2001). The properties of nanomaterials are influenced by their shape, size and surface morphology (Raveendran et al., 2003). In addition to having large surface areas, NPs are mechanically strong, optically active and chemically reactive, all of which make them unique to various applications including optical, thermal and electrical conductivity; catalytic activity; antimicrobial, antioxidant and anticancer activity; and many more (Khan et al., 2019; Mali et al., 2020).

Silver nanoparticles (AgNPs) manufacturing methods that are eco-friendly that don't use or release toxic substances are becoming more and more in demand (Kalimuthu et al., 2008). Even though the synthesis of AgNPs by microorganism like algae, bacteria, fungi, and yeast is beneficial over chemical method, the requirements, such as culturing of biomass, safety precautions, low reaction kinetics, etc., had forced the researchers to search for other bio-resources with the necessary characteristics (Moradi et al., 2021). The quick, eco-friendly, aseptic, affordable, single-step procedures of plant-mediated AgNPs production have attracted interest (Abdelghany et al., 2018). Silver nanoparticles provide a potential solution to the problems posed by many illnesses, particularly those brought on by the world's rapidly proliferating multidrug-resistant bacteria (MDR) (Tang & Zhang, 2018).

Different approaches are available for the synthesis of NPs. These are broadly classified as “bottom-up” and “top-down” approaches that differ from each other in the size of starting materials (Murty et al., 2012). In biological or green synthesis methods, microorganisms such as bacteria, fungi, yeasts, algae and plant extract have been used (Alishah et al., 2017).

In comparison with chemical and physical methods of synthesis, green synthesis of metal/metal oxide NPs is an innovative and expanding research area. It is relatively simple, low cost, eco-friendly, energy efficient and easy to synthesize large-scale metal/metal oxide NPs using plant extract (Mohammadi et al., 2016). The biological component like enzymes, proteins, aldehydes, ketones, flavonoid, amino acids, polyphenols, polysaccharides, terpenoids and many more other phytochemicals present in plant extracts themselves acts as reducer and capping agents (Makarov et al., 2014).

Green synthesis methods are being advanced by changing plants or parts of the plant to prepare the extract and properties like temperature, pH, and concentration of precursors. Shapes, sizes, and structures of nanoparticles vary with these parameters, therefore, selecting a plant is crucial (Makarov et al., 2014). In this proposed study, the leaves and fruit of plant "rudraksha" are selected as they are easily available and have many medicinal values. The "King of Herbal Medicine," rudraksha, is well known for its potent antibacterial, antifungal, and cancer-preventing properties. *Elaeocarpus ganitrus*, also known as "rudraksha" in Sanskrit, is cultivated in Nepal, India, Indonesia, and the Himalayan foothills. For its gorgeous fruit endocarps and therapeutic benefits, rudraksha is primarily grown in Nepal in Sankhuwasabha-Khandbari, Dingla-Bhojpur, Kavre, Sindhupalchowk, Kathmandu, etc. Folk medicine employs it to treat conditions including stress, depression, palpitations, migraines, asthma, hypertension, arthritis, and liver illnesses as well as conditions like migraines and epilepsy.

In the proposed study, silver nanoparticles were created using Rudraksha leaf extract, and they were then characterized using UV-visible spectra, XRD, FTIR, and SEM. The study also focused on evaluating the antibacterial, antifungal, antiproliferative, and anticancer properties of these silver nanoparticles.

2.2 Statement of Problem

Physical and chemical processes for preparation of nanoparticles are costly, time- and energy-consuming, and require extreme conditions of temperature, pressure, etc. Therefore, a cost-effective, eco-friendly, nontoxic method is required to develop the preparation of nanoparticles. The green synthesis approach of nanoparticles will be the best alternative.

2.3 Objectives of the study

The main objective is to investigate the green synthesis and characterization of AgNPs using the leaves extract of rudraksha as a structure directing and stabilizer. The application of AgNPs will be investigated for its antimicrobial activities.

The specific objectives of the study are

- 1 To synthesize AgNPs by a green synthetic approach.
- 2 To carry out phytochemical screening of extract of rudraksha.
- 3 To characterize the AgNPs by using different characterization techniques.
- 4 To investigate the antimicrobial activities of synthesized AgNPs.

2.4. Limitations of the study:

- For the characterization (SEM-EDX) of the AgNPs will be sent to the laboratories abroad and due to unavailability of characterization instrument in the department, characterization will be done with the help of other research institutions in Nepal.
- The different environments and agro-climatic conditions affect the phytochemical contents of rudraksha and their potentiality. This might affect the properties of synthesized NPs.

3. LITERATURE REVIEW AND RESEARCH GAPS

Ahmed et al.(2016) investigated *Azadirachta indica* aqueous leaf extract to create silver nanoparticles quickly and easily. The plant extract serves as both a capping agent and a reducer. By using FTIR, the functional groups found in plant extract were examined in order to determine the substances which is responsible for the reduction of silver ions. Dynamic Light Scattering(DLS), photoluminescence, Transmission Electron Microscopy(TEM), and UV-Visible spectrophotometer are some of the methods used to characterize produced nanoparticles. A peak in absorbance was visible using a UV-Visible spectrophotometer between 436-446 nm. With regard to gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) pathogens, the silver nanoparticles demonstrated antibacterial properties. Additionally assessed were photoluminescence investigations of synthesized silver nanoparticles. Results supported the protocol's claims of being quick, easy, one step, safe for the environment, toxic-free, and an alternative to traditional physical/chemical approaches. Without the use of any dangerous chemicals, the conversion of silver ions into silver nanoparticles at room temperature only took 15 minutes.

Elaeocarpus ganitrus's (Roxb.) endocarp, known as rudraksha, is prized for its ethnopharmacological properties and spiritual significance in Hinduism. Mental disorders, convulsions, allergies, neurological diseases, asthma, diabetes, and many other problems with blood circulation are all treated with it traditionally. More than 50 components belonging to the genus *Elaeocarpus* are what give this plant its pharmacological and biological properties. Alkaloids, flavonoids, and phenols are thought to be among these elements that contribute to its pharmacological value. The current review summarizes the current body of knowledge regarding the pharmacology, toxicity, chemical makeup, and ethnopharmacology of *E. ganitrus* (Roxb.), (Singh et al. 2015).

Bhagat et al. (2015) used aqueous rhizome extract from the plant *Rheum australe* to biochemically produce silver nanoparticles (AgNPs) at room temperature. Further research was done on the as-synthesised AgNPs to characterize their morphology, biology, and electrical properties. Their effective synthesis was validated by morphological analyses using X-ray diffraction, scanning electron microscopy, and UV-vis spectroscopy. Their antioxidant activity was discovered through biological study using the DPPH assay (2,2-diphenyl-1-

picrylhydrazyl). Electrical analysis revealed that the mixture of AgNPs and DPPH test has a higher conductivity than the AgNPs dissolved in distilled water. The results that were acquired might be used as sensors.

Textiles are now frequently employed in things including clothes, home goods, and medication because of their antibacterial properties. In this investigation, either micro- or nano-sized silver particles combined with polypropylene. These polypropylene/silver compounds were created utilizing a traditional twin-screw mixer and direct melt compounding (Jeong et al., 2005). They used wide-angle X-ray diffractometry (WAXS), differential scanning calorimetry (DSC), and scanning electron microscopy to examine the properties of the compounds. In comparison to the pure polymer, the crystallinity of the polypropylene component marginally decreased, according to the DSC and WAXS results. The silver particles in the matrix displayed good dispersibility, according to the SEM micrographs. They quantified the antibacterial activity of these compounds using the AATCC-100 test technique and examined the mechanical qualities of these materials using a universal tensile tester. They draw the conclusion that the compounds including the silver nanoparticles demonstrated superior antibacterial activity in comparison to the samples containing micron-sized particles from these analyses of antibacterial activity.

Ibrahim, (2015) used the banana peel extract (BPE) as a reducer and capping agent in the present work is an eco-friendly, economical, quick, and simple technique for the synthesis of silver nanoparticles. Investigations were conducted into the many factors impacting silver reduction. Silver nitrate (1.75 mM), BPE (20.4 mg dry weight), pH (4.5), and incubation duration were the ideal values (72 h). The reaction mixture must be heated to between 40 and 100 °C for BPE to reduce silver ions into silver nanoparticles within 5 minutes, as shown by the reddish-brown hue that develops. A distinctive surface plasmon resonance (SPR) peak at 433 nm was seen in the UV-Vis spectra of silver nanoparticles. Nanoparticles of silver were described. Their crystalline nature was discovered via X-ray diffraction. Spherical-shaped and monodispersed nanoparticles were visible using scanning electron microscopy and field emission microscopy. The spherical shape and crystallinity of nanoparticles were confirmed by transmission electron microscopy. Dynamic light scattering indicated that the average size of nanoparticles was 23.7 nm. The presence of silver was confirmed by an energy dispersive

X-ray spectroscopy analysis peak in the silver area. The effectiveness of BPE as a silver ion reducer and scavenger was confirmed by FTIR spectroscopy. In tests with realistic yeast and bacterial pathogens, silver nanoparticles effectively combatted microorganisms. they established the minimal bactericidal and inhibitory concentrations. The antibacterial activity of the produced nanoparticles enhanced by 1.16–1.32 fold when combined with the antibiotic levofloxacin.

Khanal et al. (2020) investigated the antioxidant and antibacterial properties of extracts from the commonly used traditional medicinal plants *Rubus ellipticus*, *Ziziphus mauritiana*, *Pyrus pashia*, and *Drynaria coronans*. The secondary metabolites of the plant, including polyphenols, alkaloids, flavonoids, terpenoids, reducing sugar, glycosides, tannins, carotene, phytosterols, coumarins, saponins, and anthracenes, were examined by phytochemical analysis. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was used to test the in vitro antioxidant activity of the plants, and the agar well diffusion method was used to test the antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), and *Salmonella typhi* (ATCC 14028). Using the Resazurin Microtiter Assay (REMA) technique, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were calculated. In comparison to the standard ascorbic acid, which has an IC₅₀ of 28.44 0.97 g/ml, root extract from *R. ellipticus* was found to have the highest antioxidant activity, with a value of 42.40 1.5 g/ml. This was followed by root extract from *Z. mauritiana* (IC₅₀ 55.67 7.41 g/ml), leaf and bark extract from *P. pashia* (IC₅₀ 58.33 The plants had Zones of Inhibition (ZOI) for *R. ellipticus* (17 mm), *P. pashia* (12 mm), *Z. mauritiana* (9 mm), and *D. coronans* (8 mm) against Gram-positive *Staphylococcus aureus*. On all of the Gram-negative bacteria put to the test, the extracts had no impact. *R. ellipticus* and *P. pashia* have respective MIC and MBC values of 3.125 mg/ml, 12.5 mg/ml, and 12.5 mg/ml, 25 mg/ml.

Bhakya et al., (2016) were found that the stable AgNPs were produced as a result of the reduction in aqueous silver (Ag⁺) ions in solution, which were previously connected with the stem bark extract of *Helicteres isora*. Several methods were used to characterize these AgNPs. The nanoparticles have their highest absorbance in the ultraviolet-visible spectrum between 419 and 431 nm. Fourier transform-infrared (FTIR) spectroscopy was used to detect the

presence of the steroid sapogenin. Utilizing X-ray diffraction, the reduction of the Ag⁺ ions to elemental silver was studied (XRD). Energy dispersive spectroscopy (EDX) examination supported the existence of elemental silver, and transmission electron microscopy (TEM) demonstrated the development of monodisperse, with modest polydispersity, nanoparticles of 25.55 nm. In comparison to the conventional compounds, the AgNPs demonstrated antioxidant properties such as DPPH, hydrogen peroxide, and nitric oxide radical scavenging as well as a reducing power. Test strains were used to determine the antibacterial action, and substantial inhibition was found. Oral cancer (KB) cells were used to demonstrate the antiproliferative effect of the AgNPs using MTT, and this activity was then verified by AO/EtBr, comet assay, DCFH-DA, and Rhodamine 123 staining. With an IC₅₀ of 70 g mL⁻¹ and 108 h of exposure, a substantial mortality rate against *Artemia* was seen in the toxicity study. The NPs demonstrated cytotoxicity against *Artemia* at 108 hours, indicating that they can be hazardous at high doses and over an extended period.

Research gap

Different methods are reported for the synthesis of AgNPs. Based on literature, the green synthetic method of AgNPs provides a simple, economical, environmentally friendly, and non-toxic process, which can be carried out at ambient temperature and pressure. More attention needs to be paid to the applications of biologically synthesized AgNPs for antimicrobial effects. To the best of our knowledge, no study on a green synthesis of AgNPs using rudraksha extract and its antimicrobial applications. This study will explore the green synthesis of AgNPs, its characterization, and antimicrobial activities.

4. RESEARCH METHODOLOGY

4.1. Materials:

Methanol, silver nitrate, and dimethyl sulphoxide (DMSO) of high purity was used. Rudraksha leaves were collected from the areas of the Patan Multiple Campus for the preparation of extracts.

4.2 Preparation of rudraksha extracts:

The collected rudraksha leaves was washed and cleaned thoroughly with distilled water and Stored in dark for few days. Then, it was powdered in a blender. The powder was boiled in distilled water (5 g in 200 ml) in a conical flask until its volume becomes one-fourth. The boiled extract was filtered by using filter paper. The heavy and solid particles present in the extract was removed by centrifuging the filtrate. Then it was refiltered. Sterilized bottles were used to collect the plant extract and it was stored.

4.3 Preparation of AgNPs:

The green synthesis of AgNPs was carried out at the optimized condition. 60 mL of the rudraksha extract were added dropwise using a burette into 20 mL of AgNO_3 (0.1M) on a magnetic stirrer at neutral pH at 25°C (Adebayo-Tayo et al., 2019). The conical flask was air tightly covered to avoid light penetration. The conversion of color of mixture into reddish brown was a visual sign of synthesis of AgNPs . After 48 hours of reaction, the mixture was centrifuged for half an hour at room temperature and repeatedly washed. It was again centrifuged with distilled water for 30 minutes. Finally, the solid mass was dehydrated in ethyl alcohol, dried and collected for characterization and antimicrobial activity.

4.4 Characterization of NPs

The prepared AgNPs was characterized using the UV-visible analysis, FTIR, XRD,

4.5 Antimicrobial activity

The antimicrobial activity of synthesized NPs was studied by bioassay technique using bacteria and fungi (Murthy et al., 2020).

5. RESULTS AND DISCUSSION

5.1 Phytochemical Screening

Phytochemical chemicals are compound present in plant which help them to fight against bacterial, fungal and plant viral infection. They have generally biological activity in plant and help in plant growth. Phytochemical analysis revealed the presence of alkaloids, phenol, glycosides, Carbohydrates, tannin and flavonoids. Experimental analysis of few phytochemicals was performed. The following results were observed and are tabulated as follows

S.N.	Experiments	Observations	Results
1	2-3 drops of Wagner's reagent was added to 1ml of plant extract	Brown color precipitate was observed	Presence of alkaloids
2	2mL plant extract was taken and few ml of 5% FeCl ₃ solution was added.	Formation of blue solution was observed	Presence of phenol
3	2ml plant extract was taken and 2 ml CHCl ₃ was added and acetic acid was added	Formation of green coloration was observed	Presence of Glycoside
4	To 2mL plant extract, few ml of Molisch's reagent was added and sulphuric acid was added	Purple ring was formed	Presence of carbohydrate

5.2 UV-vis Characterization of AgNPs

A UV- visible spectroscopy technique is for demonstration the creation of metallic nanoparticles have Surface Plasmon Resonance (SPR). The AgNPs of leaf extract of *Eleocarpus ganitrus* showed UV peak at 357 nm is the UV-vis spectroscopy(figure.1. The occurrence of silver metal SPR band in 350-400 nm range shows the synthesis of AgNPs (Anvesh et al. 2018)

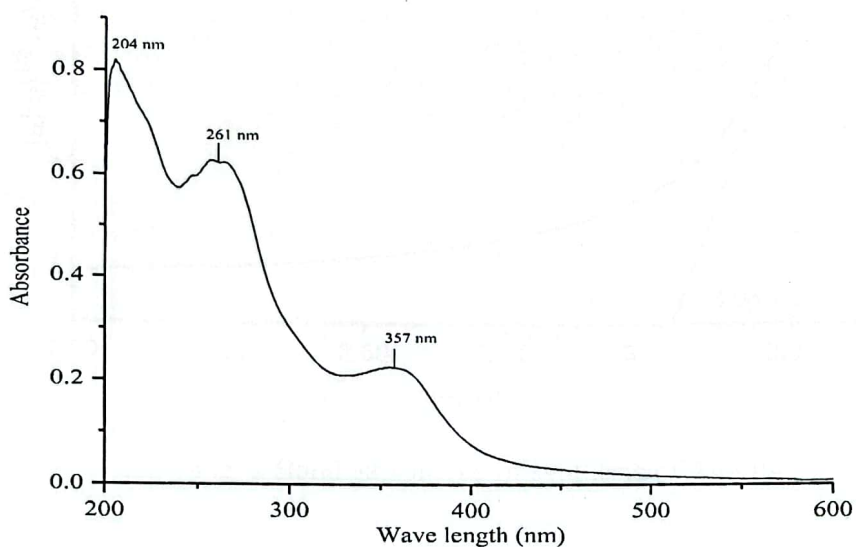


Figure. 1 UV -visible spectroscopy

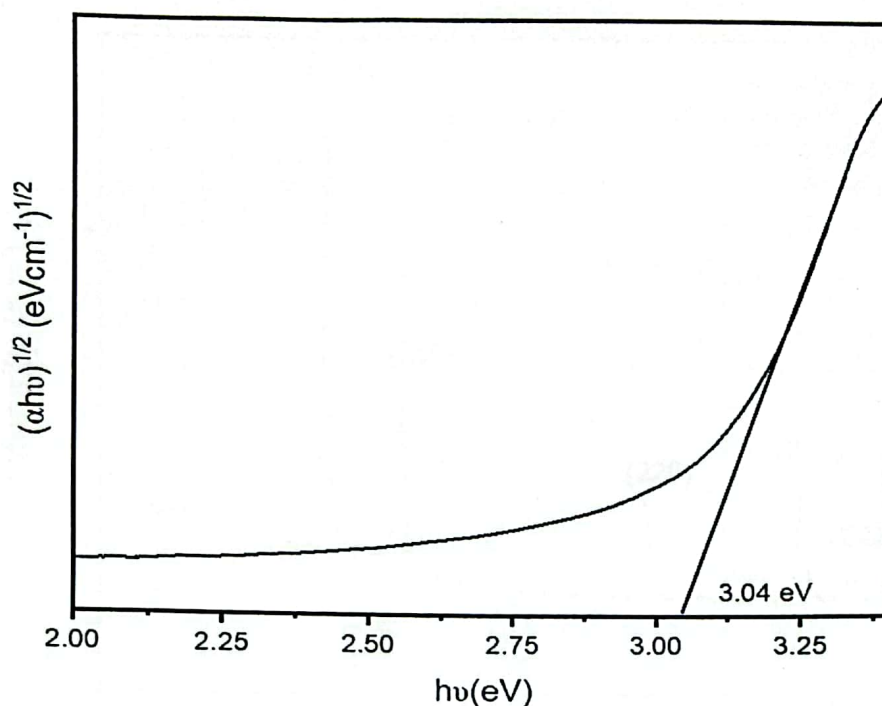


Fig. 2 Band gap energy of synthesis of AgNPs

The energy difference between the valance band and the conduction band is called band gap. The band gap is the minimum energy required to excite an electron up to a state in the conduction band where it can participate in conduction. The value of the band gap can be obtained by using the Tauc relation $(\alpha h \nu)^{1/n} = A (h \nu - E_g)$ where α is absorption coefficient being a function of wavelength $\alpha(\lambda)$, h is Planck. The band gap energy of green synthesized AgNPs is observed 3.04 eV

5.3 XRD Pattern of AgNPs

XRD characterization investigate the crystal structure of AgNPs . AgNPs of obtained from *Elaeocarpus ganitrus* extract observed diffraction peak in XRD at $2\theta = 38.3, 44.5, 64.5, 77.5$ and 82.1 . After comparison with the JCPDS, silver file no. 04-0783, these discovered XRD peaks revealed that biosynthesised AgNPs were crystalline in structure , with the peak matching the standard (Figure.2). The XRD peaks corresponds to the plane (111),(200), (220),(311) and (222) face of silver crystal respectively(Roy et.al. 2015)

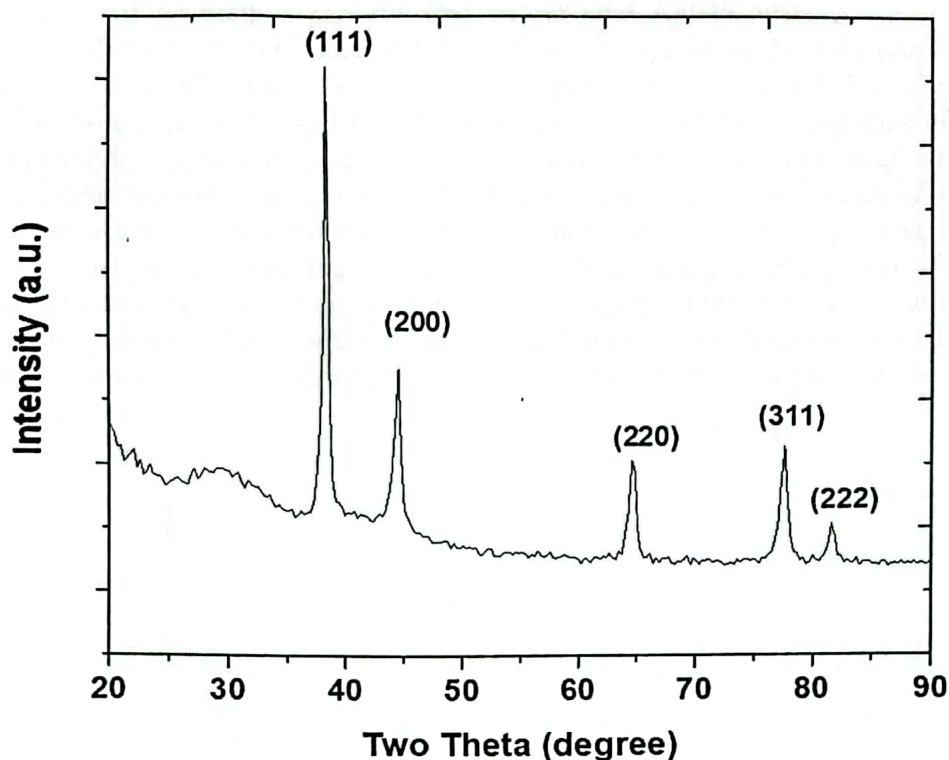


Figure. 3 XRD pattern of AgNPs synthesis from leaf extract of *Elaeocarpus ganitrus*

Size Calculation of AgNPs by using Scherrer equation

To calculate the crystalline size of the nanoparticles, Debye Scherrer equation, $D = K\lambda / \beta \cos\theta$, is used. Where D is the nanoparticles crystalline size, K represents the Scherrer constant (0.98), λ denotes the wavelength (1.54), β denotes the full width at half maximum (FWHM).

2theta (degree)	(FWHM)	crystallite size (D) nm	Average crystalline size(nm)
38.24	0.70277	11.96	11.93
44.36	0.82153	10.44	
64.56	0.80039	11.73	
77.52	0.85028	11.98	
81.6	0.77536	13.53	

Average particle size was 11.93 nm

5.4 FTIR Spectroscopic Analysis

FTIR spectra of *Elaeocarpus ganitrus* leaf extract and AgNPs were analysed to get the information about the biomolecules active functional groups in the bioreduction of silver ion and capping of AgNPs. The FTIR analysis was performed in 4000-500 cm^{-1} range. FTIR absorption bands were observed at 3400-3700 cm^{-1} range is due the presence of functional group hydroxyl(-OH) and the range of 1690-1630 cm^{-1} showed the presence of carbonyl group (-C=O). These functional groups were mainly responsible for bio-reduction of Ag^+ ion and synthesis of AgNPs. The absorption spectrum (Figure.3) recorded the peak at 3211 cm^{-1} is because of O-H bond stretching vibration in alcoholic and phenolic group of *Elaeocarpus ganitrus* synthesis AgNPs and the peak at 1696 cm^{-1} suggested the presence of carbonyl group. In the case of extract (Figure.4) the broad peak at 3469 cm^{-1} was because of O-H stretching vibration of alcoholic and phenolic group. The peak at 1648 cm^{-1} was due to carbonyl group.

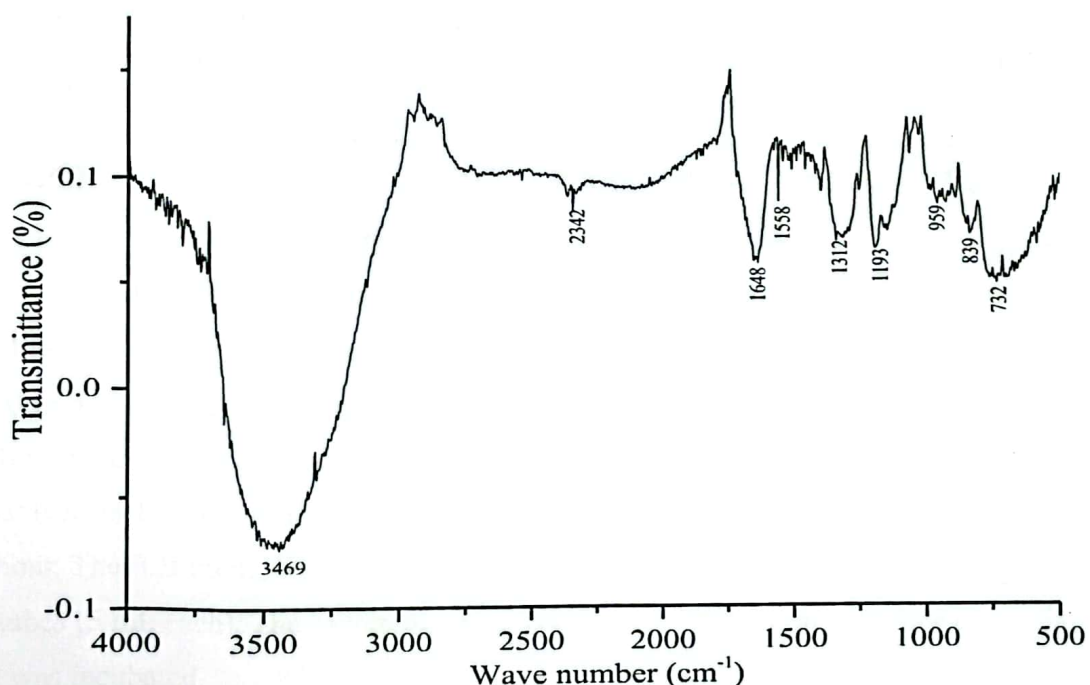


Figure. 4 FTIR spectrum of AgNPs synthesized from leaf extract of *Elaeocarpus ganitrus*

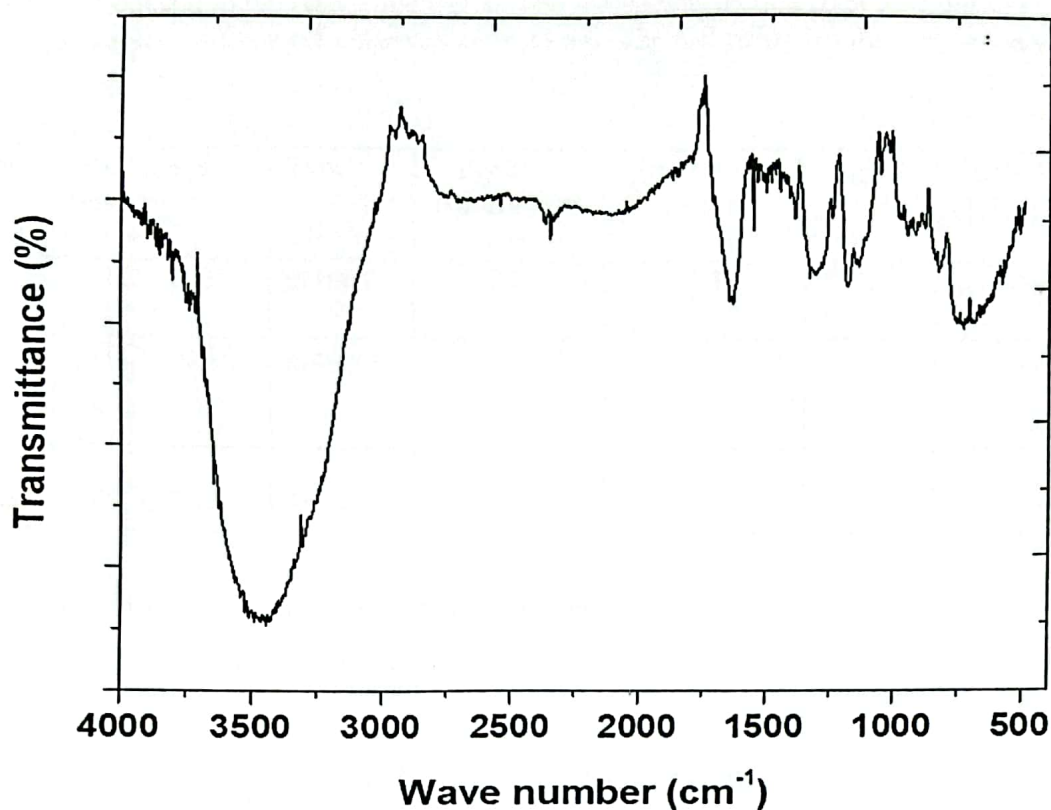


Figure. 5 FTIR spectrum of leaf extract of *Elaeocarpus ganitrus*

5.5 Antimicrobial properties of silver nanoparticles, rudraksha extract

5.5.1 Preparation of Liquid Broth(LB) media

To prepare culture media, 13 g of liquid broth LB powder (Sisco research Laboratories Pvt. Ltd, India) was dissolved in 1 L of water. It was autoclaved for 25 minute at 15psi and 121 °C for about half an hour. The LB media was cooled to 40-50 °C. Then it was transferred into sterilized 15 mL falcon tubes (5 mL each). The bacterial seed culture was co-cultured in each tube of media separately and was incubated.

5.5.2 Preparation of MHA media and antimicrobial study:

39 g of MH agar powder (Sisco research Laboratories Pvt. Ltd, India) was dissolved in 1 L of water to prepare MHA plate. The mixture was autoclaved at 15 psi and 121 °C for 25 minutes. The media was cooled to 40-50 °C, and was then transferred into Petri dishes (25 mL each). The media plates were labeled and 150 µL liquid bacterial seed were spread on the surface with the help of swab. The wells were made on the media and the sample powder (about 30 mg, of provided sample, in case of AgNP; 100 mg/mL dissolved in DMSO in case of rudhrakshya extract; and 15 mg : 15 mg of AgNP: Rudrakshhya sample (1:1 ratio) and kanamycin 5 mg/mL

(10 μ L) were introduced in the respective wells. The media was incubated at temperature of 37 $^{\circ}$ C. The antimicrobial results were observed after 24 hrs. The following results were observed

Bacterial strain	Reference culture	Type	Positive control (c+) cm	Rudraksha extract	AgNPs	Rudh:AgNPs (1:1)
<i>E.coli</i>	ATCC 8739	gram-ve	2.7	1.3	1.1	1.5
<i>Staphylococcus aureus</i>	ATCC 6538P	gram+ve	2.2	1.3	1.1	1.6
<i>C.albicans</i>	ATCC 2091	Fungi	2.3	1.4	1.1	1.4

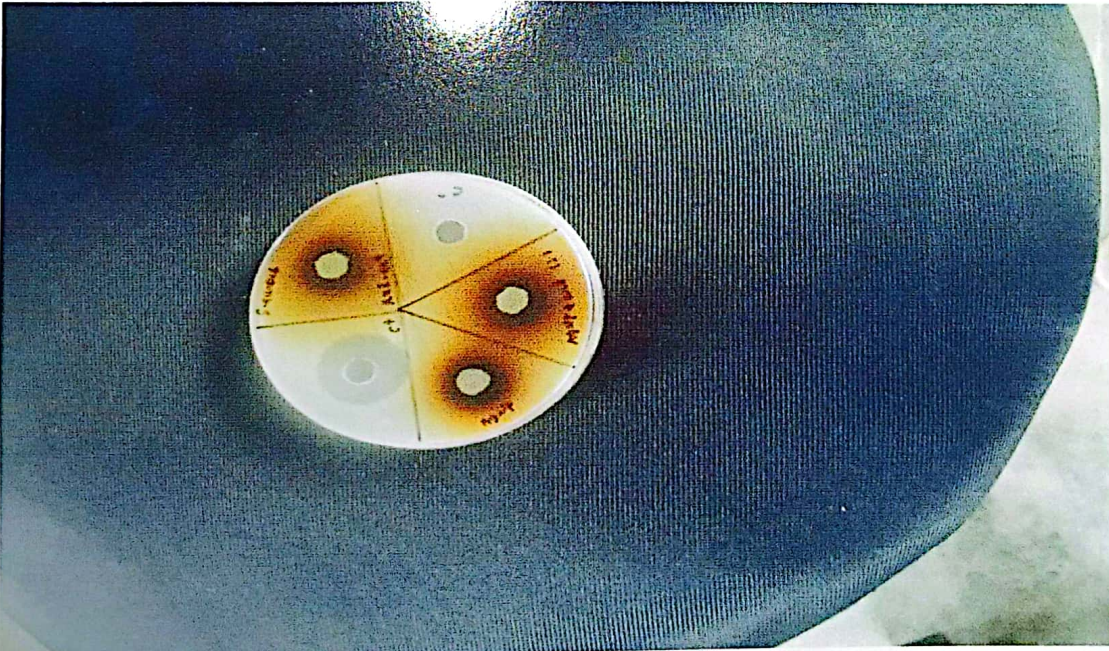
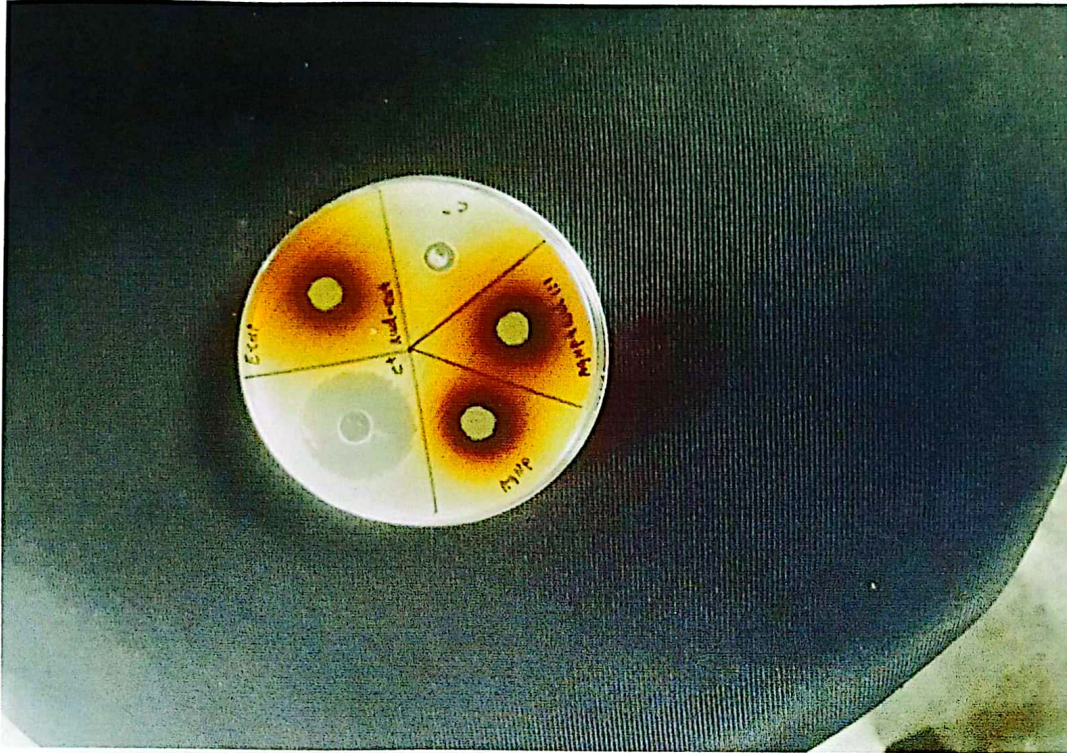
*The zone of inhibition (ZOI) has been tabulated in cm.

* Kanamycin antibiotic in the concentration of 5 mg/mL as positive control (c+) has been used

*DMSO has been used as a negative control.

The antimicrobial effect of AgNPs and Rudraksha extract on E.Coli , Staphylococcus aureus and C.albicans were analyzed. The zone of inhibition(ZOI) for Rudraksha and AgNPs composite is greater than that of extract and AgNPs individuals in bacteria. Rudraksha extract has greater antimicrobial activity than AgNPs. Composite shows most antimicrobial activity for gram + ve bacteria.

The result obtained shows that both plant extract and AgNPs have antimicrobial activity



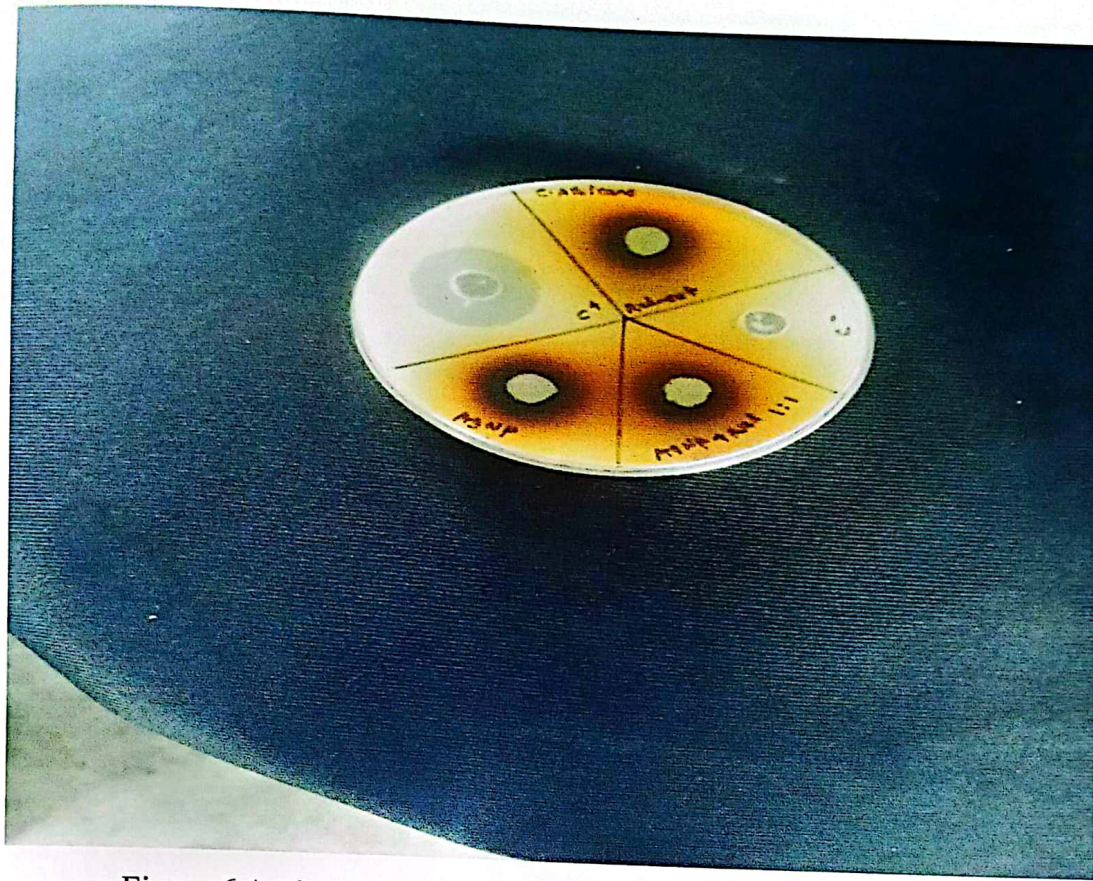


Figure.6 Antimicrobial test of leaf extract of rudraksha and AgNPs

6. REFERENCES

- Abdelghany, T. M., Al-Rajhi, A. M., Al Abboud, M. A., Alawlaqi, M. M., Ganash Magdah, A., Helmy, E. A., & Mabrouk, A. S. (2018). Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review. *BioNanoScience*, 8, 5-16.
- Adebayo-Tayo, B., Salaam, A., & Ajibade, A. (2019). Green synthesis of silver nanoparticle using *Oscillatoria* sp. extract, its antibacterial, antibiofilm potential and cytotoxicity activity. *Heliyon*, 5(10), e02502.
- Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *Journal of radiation research and applied sciences*, 9(1), 1-7.
- Alishah, H., Pourseyedi, S., Ebrahimipour, S. Y., Mahani, S. E., & Rafiei, N. (2017). Green synthesis of starch-mediated CuO nanoparticles: preparation, characterization, antimicrobial activities and in vitro MTT assay against MCF-7 cell line. *Rendiconti Lincei*, 28(1), 65–71.
- Bhagat, M., Rajput, S., Arya, S., Khan, S., & Lehana, P. (2015). Biological and electrical properties of biosynthesized silver nanoparticles. *Bulletin of Materials Science*, 38, 1253-1258.
- Bhakya, S., Muthukrishnan, S., Sukumaran, M., Grijalva, M., Cumbal, L., Franklin Benjamin, J. H., Senthil Kumar, T., & Rao, M. V. (2016). Antimicrobial, antioxidant and anticancer activity of biogenic silver nanoparticles – an experimental report. *RSC Advances*, 6(84), 81436–81446.
- Giri, A. K., Jena, B., Biswal, B., Pradhan, A. K., Arakha, M., Acharya, S., & Acharya, L. (2022). Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. *Scientific Reports*,

- Ibrahim, H. M. (2015). Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of radiation research and applied sciences*, 8(3), 265-275.
- Jeong, S. H., Yeo, S. Y., & Yi, S. C. (2005). The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. *Journal of Materials Science*, 40, 5407-5411.
- Kalimuthu, K., Babu, R. S., Venkataraman, D., Bilal, M., & Gurunathan, S. (2008). Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids and surfaces B: Biointerfaces*, 65(1), 150-153.
- Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, 12(7), 908–931.
- Khanal, L. N., Sharma, K. R., Pokharel, Y. R., & Kalauni, S. K. (2020). Assessment of phytochemical, antioxidant and antimicrobial activities of some medicinal plants from Kaski District of Nepal. *American Journal of Plant Sciences*, 11(09), 1383.
- Li, L. S., Hu, J., Yang, W., & Alivisatos, A. P. (2001). Band Gap Variation of Size- and Shape-Controlled Colloidal CdSe Quantum Rods. *Nano Letters*, 1(7), 349–351
- Makarov, V. V., Love, A. J., O.V., S., S.S., M., & Yaminsky, I. V. (2014). Green” Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants V. *ACTA NATURAE*, 6(20), 35–44.
- Mali, S. C., Dhaka, A., Githala, C. K., & Trivedi, R. (2020). Green synthesis of copper nanoparticles using *Celastrus paniculatus* Willd. leaf extract and their photocatalytic and antifungal properties. *Biotechnology Reports*, 27, e00518.
- Mohammadi, S., Pourseyedi, S., & Amini, A. (2016). Green synthesis of silver nanoparticles with a long lasting stability using colloidal solution of cowpea seeds (*Vigna sp. L*). *Journal of Environmental Chemical Engineering*, 4(2), 2023–2032

- Moradi, F., Sedaghat, S., Moradi, O., & Arab Salmanabadi, S. (2021). Review on green nanobiosynthesis of silver nanoparticles and their biological activities: With an emphasis on medicinal plants. *Inorganic and Nano-Metal Chemistry*, 51(1), 133-142.
- Murthy, H. C. A., Desalegn, T., Kassa, M., Abebe, B., & Assefa, T. (2020). Synthesis of Green Copper Nanoparticles Using Medicinal Plant *Hagenia abyssinica* (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties. *Journal of Nanomaterials*, 2020.
- Raveendran, P., Fu, J., & Wallen, S. L. (2003). Completely 'Green' Synthesis and Stabilization of Metal Nanoparticles. *Journal of the American Chemical Society*, 125(46), 13940–13941.
- Tang, S., & Zheng, J. (2018). Antibacterial activity of silver nanoparticles: structural effects. *Advanced healthcare materials*, 7(13), 1701503.
- Tiwari, J. N., Tiwari, R. N., & Kim, K. S. (2012). Zero-dimensional, one-dimensional, two-dimensional and three-dimensional nanostructured materials for advanced electrochemical energy devices. *Progress in Materials Science*, 57(4), 724–803.

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