A Comparative Study of Green Synthesis, Characterization and Antibacterial Activity of ZnO, Cu and AgNO3, Nanoparticle of Indian Sunflower Honey

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Abstract

Honey is a functional food with antibacterial, anti-inflammatory, and antioxidant properties. The aim of this study is that Honey-mediated green synthesis of nanoparticles has been proven as a fast, safe, biocompatible, and cost-effective method. These three nanoparticles were prepared by the green synthesis method. Honey acts as both a stabilizing and reducing agent in the NP synthesis process and serves as a precursor in the green synthesis sol-gel method. Nanoparticles offer strength and medical imaging applications. In this study, we compare the three metal nanoparticles—green synthesized ZnO NPs, Cu-NPs, and AgNO3 NPs—with Indian sunflower honey on the basis of their particle sizes and antibacterial activity. The observed particle size of honey ZnO, Cu & AgNo3 NPs were 117nm,94 nm, and 64nm with great thermal stability. The products were characterized using UV–visible (UV–vis) spectroscopy, X-ray diffraction (XRD), Field-Emission Scanning Electron Microscopy (FESEM), and Fourier Transform Infrared spectroscopy (FTIR). The antibacterial activity results of Ag NPs seen against Gram-positive (S. aureus) and Gram-negative (E. coli) Bacteria were significantly more than Zn and Cu NPs.Our research leads us to conclude that AgNPs are superior compared to ZnO and CU nanoparticles synthesized from sunflower honey. The result obtained for this study is useful for researchers working on these three metal NPs syntheses from Sunflower honey for their formulation and various herbal synthesis products to select the suitable NPs in their work according to the size of NPS, characteristics fit, and antibacterial activity

Keywords- Antibacterial activity, Characterization, green synthesis, Honey, Silver, ZnO and copper nanoparticles.

Introduction

One of the greatest blessings given to humanity, according to the ancient Vedic civilization, is Honey. Only honey is an insect-produced natural substance with uses in food, cosmetics, medicine, and commerce.[1,2]Honey has a number of beneficial qualities, including anti-inflammatory, antioxidant, antibacterial, antiviral, and antiparasitic activity; antimutagenic and antitumor activity.[3,4] Additionally, Ayurvedic professionals constantly advise honey for all imbalances, heart illness, palpitations, and skin disorders including burns, wounds, and burns.[5] Sunflower honey is bright goldish-yellow in color. Sunflower honey, owing to the high presence of natural glucose, gives instant energy. Benefits OF Sunflower honey are Conditions of the stomach and intestines, Lung and kidney health, as well as blood circulation in the hands and feet, and antimicrobial qualities. Hair growth.[6,7]

Nanoparticles

Scientists are very interested in nanoparticles (NPs), which are tiny particles with sizes ranging from 1 nm to 100 nm. Consequently, there has been a lot of interest in the controlled synthesis of nanomaterials with unique morphologies Nanomaterials have been employed in a variety of industries during the past ten years, including the cosmetics, pharmaceutical, biomedical, food and feed, gene therapy, environment, health, machinery, optical, chemical industry, electronics, aviation sector, and energy.[8,9]

Green synthesis

Nowadays, more and more studies are focused on the chemical, physical, and environmentally friendly production of nanoscale metals. Due to concerns about high energy consumption, the emission of poisonous and dangerous substances, the usage of complicated equipment, and the conditions of synthesis, physical and chemical processes are gradually being replaced by green synthesis methods. Green synthesis, in contrast, uses organic and eco-friendly components.[10] Metal nanoparticles can be created when environmentally friendly components are present and the proper circumstances are fulfilled (temperature, concentration, ambient air, etc.) [11]. This field of nanoscience ought to provide environmentally acceptable, safe NPs that are well-accepted by the nanotechnology community. The morphology of integrated particles, such as their size, physicochemical characteristics, and shape, is greatly influenced by the solvents and reducing operators used for the reduction of the NPs, and this morphology affects the utilization of NPs.

Already reported honey NPs

Only the ANTIOXIDANT ACTIVITY OF SUNFLOWER HONEY has been reported to date Concentration of polyphenols and flavonoids was confirmed with its antioxidant activity in the reported study.[12]As no other studies were stated for the activity of sunflower honey or any green synthesized nanoparticle from sunflower honey especially in the region of Maharashtra (INDIA), this study compares three green synthesized metal nanoparticles from Indian sunflower honey at various parameters like particle size, FTIR, XRD, SEM & it's Antibacterial Activity.

Due to the extreme abundance in their respective fields, many researchers manufacture nanoparticles using leaf extracts, which include ketone, phenol, flavonoids, and aldehydes that may operate as a capping and reducing agent. [13,14] Even if other studies employed fruit peels for biosynthesis, the authors have discovered a green synthesis using components that are widely accessible and biodegradable. [15,16] We attempted to manufacture three metal nanoparticles based on literature studies, using Sunflower honey in especially Maharashtra region in India as natural components because of their widespread availability. The choice of these materials was based on the fact that Sunflower honey is used as a capping agent and reducing agent in combustion reactions. Additionally, these processes need the use of synthetic precursors, expensive machinery, and high temperatures, which is particularly time-consuming. Many researchers are using eco-friendly, economical, simple, and non-toxic green synthesis methods to create NPs in order to solve the limitations. So, we synthesized the ZnO, Cu & AgNo3 NPs by using the green synthesis method with Sunflower honey (Indian) and compared the NPs on the basis of their physical and chemical characterization and their antibacterial activity

Materials and Methods

Chemicals and reagents

Sunflower Honey was purchased from the local market Madhuban Honey of Govt Khadi Gramodyog GVV2+CP6, Sagar Society, Wakadewadi, Shivajinagar, Pune, Maharashtra 411003. All glassware 135 used in the laboratory experiments were cleaned with doubly distilled water, and dried before use. Double distilled water was used in all experiments.

Copper II nitrate (Cu $(NO_3)_2.3H_2O$, ~99%), ascorbic acid ($C_6H_8O_6$, ~99%), sodium hydroxide (NaOH), Zn $(NO_3)_2·6H_2O$ (Merck 99%) and AgNo3, E. coli (ATCC 25922), B. subtilis (ATCC 6633). agar and nutrient broth were used to culture and maintain the bacterial strains from the Modern College of Pharmacy Nigdi Pune laboratory. Zn $(NO_3)_2·6H_2O$ (Merck 99%) and sunflower honey are used as raw materials.

AgNO3, Deionized water, Sunflower honey, UV spectrophotometer FTIR, SEM, XRD

Green synthesis of Zn, copper& AgNo3 nanoparticle

Synthesis of ZnO–NPs

ZnO NPs were produced using the combustion method related to sunflower honey. Here, honey has been used as a fuel to burn and produce nanostructures. ZnO NPs are primarily made with honey and analytical grade Zn (NO3)26H2O (Merck 99%). To keep the proper concentration of ZnO NPs, 0.5 mol of zinc nitrate is dissolved in 50 ml of distilled water. Before combining the precursor solutions, 50 ml of pure honey (natural fuel), which acts as a chelating agent, is added. The solution was thoroughly stirred for an hour. The mixed solution was then heated to 100 °C and stirred with a magnetic stirrer until the desired final powder was obtained. The material was finely ground.

Synthesis of Cu-NPs

To create Cu-NPs, a method involving chemical reduction and ultrasonic irradiation was used. Firstly, 0.025 mol of copper nitrate trihydrate was dissolved in 100 ml of double-distilled water. Then, honey (10%w/v) was mixed with copper nitrate while being stirred. The pH was increased to around 7.5 by slowly adding 0.6 M sodium hydroxide to the solution and stirring it continuously for 10 minutes. To the combined solution, 15 ml of 1 M ascorbic acid was added and subjected to ultrasonic irradiation for 10 minutes.

Synthesis of AgNo3-NPs

A precise mixture of 15 mL of 2% sunflower honey and 20 mL of AgNO3 (0.1 M) was used to create the silver nanoparticles. The reaction mixture was held at room temperature, The reaction's progress was noticed by a color change, and the surface plasmon resonance absorption was monitored using a UV-vis spectrophotometer in the scan range of 200-800 nm.

Characterization method and instrumentations

UV-vis spectroscopy (UV-2600, JASCO 360); ultraviolet-visible. Cells for the pure quartz solution were employed. For this experiment, a sample solution with the necessary concentration and a control sample (water) were both employed. In the quartz solution cells, the samples were homogenized before being put into the UV-vis chamber. The UV-vis spectra were captured between 220 and 1000 nm. To record XRD patterns, Ultima IV X-ray powder diffractometer radiation was used Mo Ka: Fine focus; Cu Ka: Microfocus goniometer head xyz and kappa (4 circles) Low-temperature device Oxford Cryostream 700 plus, Kryoflex II temperature range: 90 K to 400 K Bruker Smart Apex2 software. Fourier Transform Infrared Spectrometer (FTIR) Bruker - A Fourier Transform InfraRed (FT-IR) Spectrometer is an instrument that acquires broadband Near InfraRed (NIR) to Far InfraRed (FIR) spectra. That's Bruker FM. Cover the entire MIR and FIR spectral range from 80 cm^{-1} to 6000 cm^{-1} in one experiment, Carlzeiss, Gemini 300, with acceleration voltage 30 kV Field Emission Scanning Electron Microscope equipped with to take FESEM images and l Nanoparticle stability was analysed using Horiba SZ100, Zeta Potential Analyzer

Antimicrobial activity of ZnO, Cu&AgNo3 NPs

Nanoparticles of ZnO, Cu, and silver have antimicrobial properties. The test microorganisms were sourced from the microbiology lab at the Modern College of Pharmacy in Nigdi, Pune, in the form of pure stock cultures. S. aureus (ATCC 25932) and E. coli (ATCC 25922) are the organisms that were utilized. To create the inoculum, the sterile loop was used to transfer pure colonies of bacteria from the mother stock plates into the broth. The cultured inoculum was then incubated for 48 hours at 37°C in an incubator shaker. To conduct the antimicrobial investigation, we utilized the agar well diffusion technique. We drilled four 3mm-diameter wells into each nutritional agar plate using a sterile cork borer. For each triplicate, 100 µL of nanoparticles made from sunflower honey containing ZnO, Cu, and silver were added to the wells of a single bacterium. Before creating the wells, sterile nutrient agar plates were inoculated with 100 µL of the correct overnight bacterial broth. For the positive control, streptomycin was used, while ZnO, Cu, and silver were used for the negative control. After 24 hours of incubation at 37°C, the zone of inhibition was measured in millimetres (diameter) on all plates.

Result and discussion

UV–visible (UV–vis) absorption

The effective synthesis of ZnO, Cu, and AgNo3-NPs with honey was validated by UV-vis absorption, where peaks were seen at 372, 613, and 450 nm, respectively. The surface plasmon resonance of ZnO, Cu, and AgNo3-NPs allowed for the observation of these peaks. Cu-NPs are present in the honey, indicating that the size of the nanoparticles has reduced. This may be because honey contains protein and monosaccharides, which serve as stabilizing agents and inhibit the Cu-NPs from agglomerating.

The generation of silver nanoparticles by reduction of the aqueous silver ions during exposure to the various concentrations of sunflower honey was monitored using UV-visible spectroscopy in the scan range of 200-800. After a few minutes, the colorless aqueous silver nitrate solution became brownish brown with the addition of Honey samples. At 450 nm, the ideal absorption peak was seen.

UV–vis absorption spectrum of the ZnO–NPs. The ZnO–NPs were dispersed in water with a concentration of 0.1 wt.% and then the solution was used to perform the UV–vis measurement. The spectrum reveals a characteristic absorption peak of ZnO at a wavelength of 372 nm which can be assigned to the intrinsic band-gap absorption of ZnO due to the electron transitions from the valence band to the conduction band. In addition, this sharp peak shows that the particles are in nano-size and the particle size distribution is narrow.

FTIR Analysis

ZnO nanoparticle

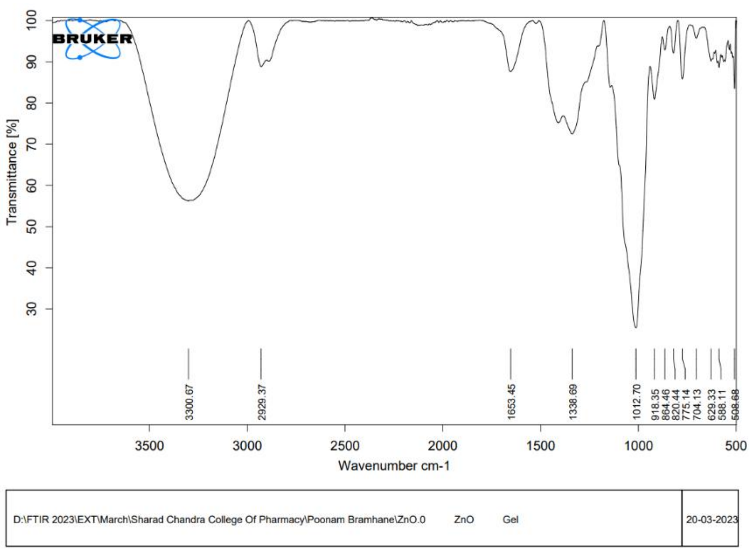


Figure 1. FT-IR spectra of ZnO NPs using Sunflower honey assisted combustion method.

In general, to determine the functional groups present in the sample, the FT-IR spectrum is used. the FTIR spectra of ZnO nanoparticles synthesized using honey. large range of 3437 cm-1 samples indicates the presence of O-H stretch and hydrogen bonded. Peaks are associated with alkyne-C-C stretching vibration at around 2920 cm-1. The tensile vibration of the C=O hydroxyl (or) carboxyl groups on the sample surface is indicated by high absorption peaks at 1630 cm to 1640 cm-1. The peak of about 1382 cm shows the nitrate ions' asymmetric -1 stretching vibration. The tensile vibration of the ZnO NP peak was observed at cm-1. Zn-O is assigned to the highest in the area between 400 cm-1 and 600 cm-1. The synthesized ZnO NPs' stabilization and capping agent may be due to the coordination of ZnO NPs with OH and C=O groups

Cu Nanoparticle

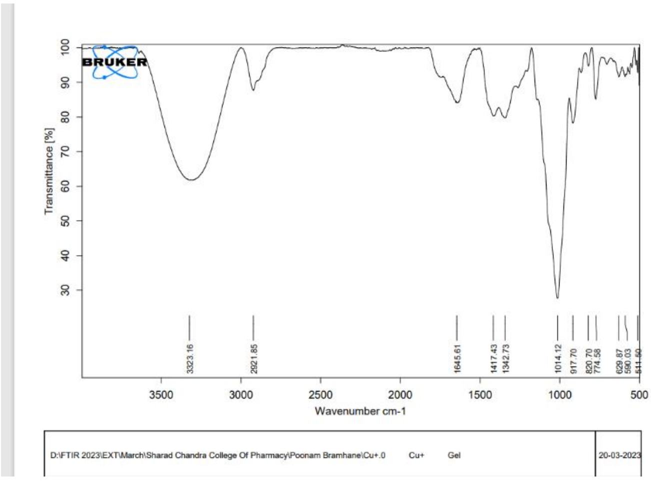


Figure 2. FTIR Analysis IR spectrum of CuNPs with Sunflower honey after 10 min ultrasonication respectively.

The purpose of FTIR measurement is to identify the possible biomolecules that are responsible for capping and stabilizing the Cu-NPs synthesized using honey. the IR spectra of the honey, The peaks observed between 3100 and 3500 cm might be due to the hydroxyl group (-OH) and primary amine -NH bonded group of carboxylic acid -COOH. Next, the alkane C-H stretching peak was observed around 2928 cm for the Cu-NPs. A peak was seen for Cu-NPs around 2887-2906 cm^{1}, possibly due to the Cu-H bonding. Meanwhile, the peaks were observed between 1780 cm and 1607 cm-1 for the Cu-NPs with honey respectively which denote the C-C stretching aromatic rings, N-H vibration of amine,1417 cm-1 C-H bending in CuNPs with honey, and for 1047 cm-1 for C-O stretching for CuNPs with Honey

FTIR Analysis

Silver Nanoparticles

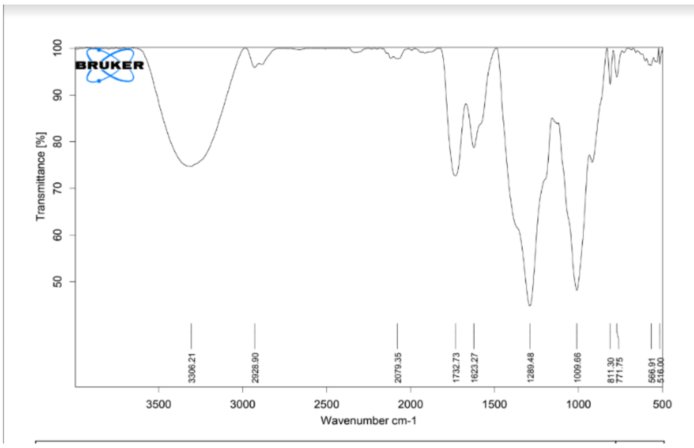


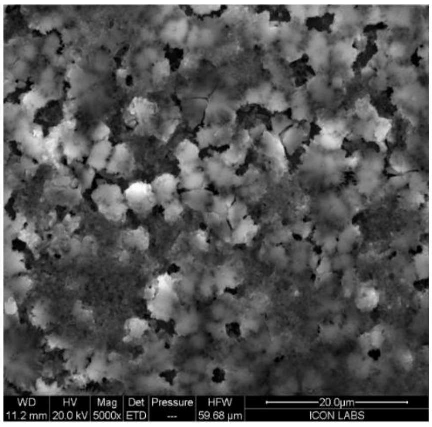
Figure 3. FTIR spectra of Sunflower honey-AgNPs

The peaks were observed at varying frequencies in the FTIR spectroscopy. The peaks corresponded to the presence of an amino group of proteins, aromatic compounds, aldehydes, unsaturated compounds like alkenes, primary amines, amides, and stretching of amines. The above spectrum depicts the presence of active functional groups in the infrared spectrum of different AgNPs obtained from sunflower honey

The presence of amine and amide group phenolic compounds, and carbonyl group(-coo-) in silver nanoparticles from the honey play an important role in reduction while. presence of 3282 cm-1 phenolic compounds O-H supports the antimicrobial activities in silver nanoparticles from sunflower honey. The results of FTIR indicated the importance of honey constituents in maintaining the stability of the produced nanoparticles within the matrix. The binding of honey proteins to AgNPs using both the free amine groups and carboxylate ions of the amino acid residue. The silver Nanoparticles are 2378 cm-1.

Field emission scanning electron microscopy [FESEM]

ZnO Nanoparticles



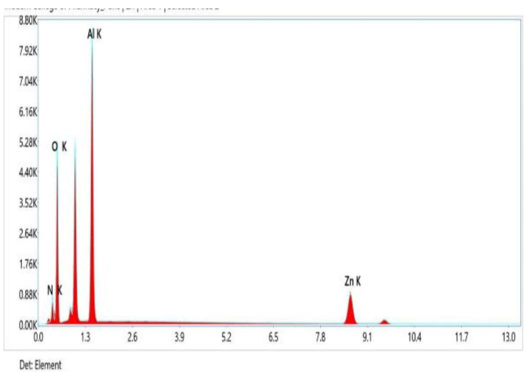
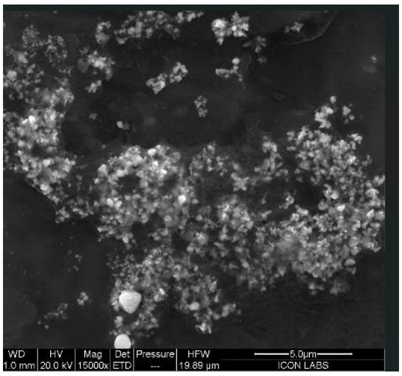


Figure 4. FE-SEM images of ZnO NPs using Sunflower honey-assisted combustion method.

As it is confirmed by the FESEM images at different magnifications of ZnO, Cu&AgNo3–NPs, the crystalline size of the obtained samples is in nanoscale which was implied by the broadening of the peaks, and through achieving such fine and small sizes, a satisfying result was gained. The morphology of prepared ZnO–NPs by FESEM analysis is shown in Fig. The Nano powders possess a spherical and uniform shape in size about 20nm. The as-prepared ZnO–NPs were calcined at different temperatures for 2 h, PXRD peaks became sharper with increasing calcination temperatures, and full width at half maximum (FWHM) decreased, indicating that the crystallinity of ZnO–NPs is accelerated by the calcination process

Silver Nanoparticle



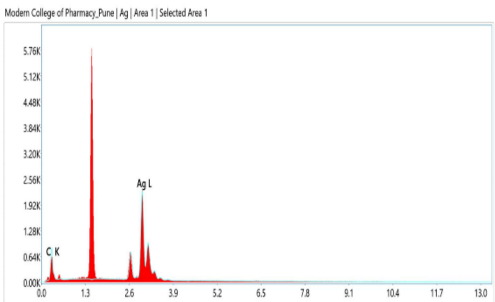


Figure 5. SEM image of Sunflower honey-AgNPs

The surface morphology of Sunflower honey is shown in Fig, with large size and irregular shape of silver nanoparticles, Similar results were also reported for Phyto-synthesized silver nanoparticles. Amounts of silver nanoparticles with spherical size were very few. The aggregation of particles due to the presence of cell components on the surface of silver nanoparticles as a capping agent and the spherical size of AgNPs 19nm provide the ability to penetrate into microbial cells and carry out their bactericidal properties. This result strongly confirms that Honey might act as a reducing and capping agent in the production of silver nanoparticles

Copper Nanoparticle

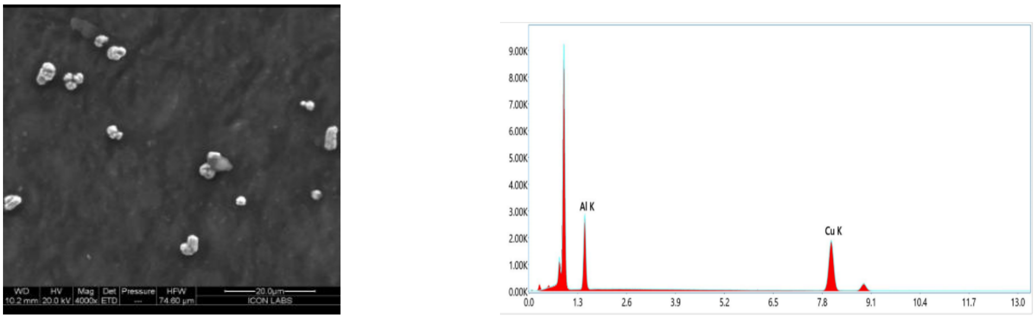


Figure 6**.** FESEM micrographs of Cu-NPs with honey respectively

Figure 6 shows the difference between the morphology and shape of the Cu-NPs synthesized with honey. The shape of CuNPs with honey was consistent This may suggest that the agglomeration of Cu-NPs with honey might occur slower. The size of the nanoparticle of cooper nanoparticle is 20nm. The size of the nanoparticles with honey was smaller. This could support that honey can act as a capping agent to prevent oxidation. The carbon element might come from either ascorbic acid or compounds in honey as it was detected in both Cu-NPs

XRD Analysis

Silver Nanoparticles

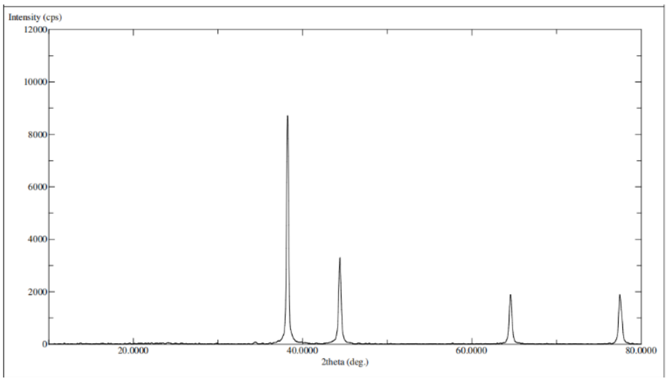


Figure 7. XRD data of Sunflower honey-AgNPs

XRD analysis of the biosynthesized ZnO, Cu&silver nanoparticles from sunflower honey were further confirmed by the characteristic peaks observed in the XRD image as shown in. The sharp peaks revealed the crystalline nature of ZnO, Cu&silver NPs. The XRD pattern for the silver nanoparticle showed 4 intense peaks in the whole spectrum of 2ϴ values ranging from 0–70 nm. Silver crystals showed peaks at 39.52°,42.58°,65.44°, and 78.44° which could be attributed to the crystallographic planes. The average crystalline size of silver nanoparticles was found to be around 20- 90 nm. Earlier workers reported similar results for Ag nanoparticles.

Copper Nanoparticle

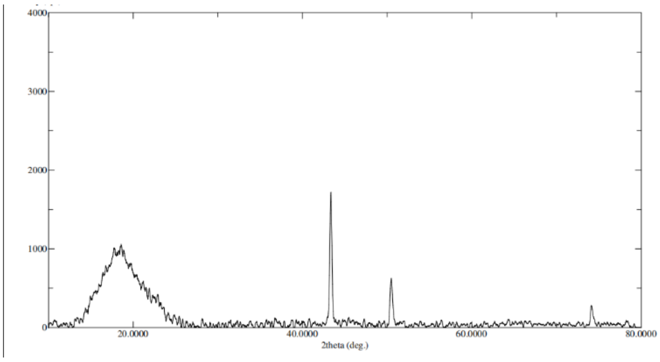


Figure 8. X-ray diffraction patterns of Cu-NPs: CuNPs with honey

XRD Analysis of copper nanoparticle: Highly pure copper from honey was obtained as depicted by XRD. The XRD graphs showed only the copper without other compounds including copper oxide. Both Cu-NPs had a similar diffraction profile and XRD peaks at 2θ of 43.30°, 50.40°, and 74.12° c. The XRD result was consistent without the peak of copper oxide which suggests the retained purity of copper is also reported in the same observation for copper nanoparticles

ZnO Nanoparticles

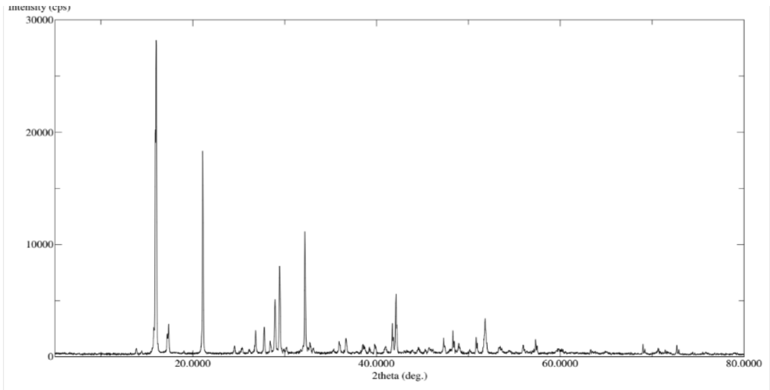


Figure 9. XRD pattern of ZnO NPs using Sunflower honey assisted combustion method

The XRD pattern for the ZnO nanoparticle shown is depicted in The XRD pattern of ZnO NPs prepared by using Sunflower honey. 21.08°,33.10°,57.32°,63.23°&70.68° The sharp peaks revealed the crystalline nature of ZnO NPs. This specifies the reliability of sunflower honey chosen as a capping and reducing agent in the green synthesis approach.

The particle size of Zno, Cu&AgNO3 Nanoparticle

On the particle size analyzer, the particle size of Zno, Cu&AgNO3 Nanoparticle respectively 127.3nm,114.6nm&64.4nm are observed

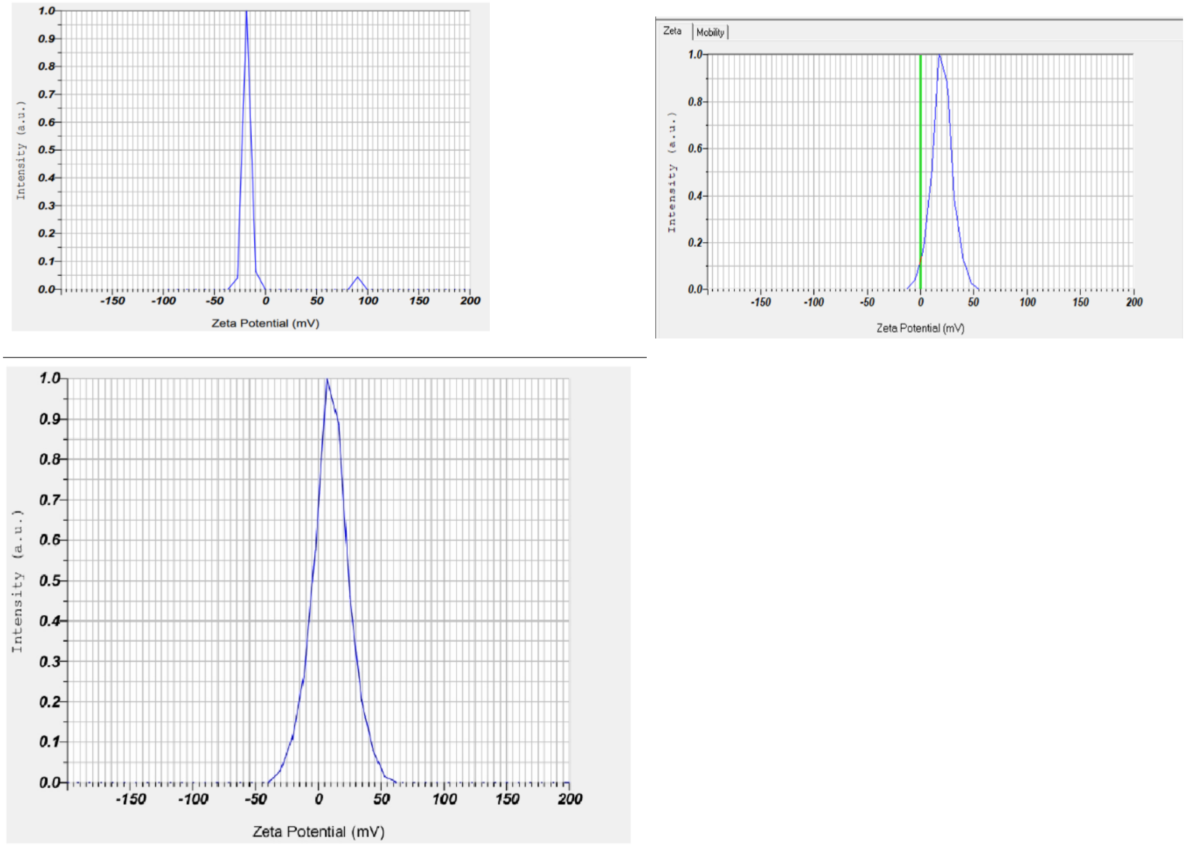


Figure 10. Zeta potential analysis of ZnO, Cu&AgNo3 NPs using Sunflower honey assisted green synthesis method.

Stability of ZnO, cu&AgNo3 NPs

Nanoparticle and colloidal suspension stability also depend on the surface load of the particles. In certain applications, stability is required and the effect of suspension conditions on electrostatic stabilization should be assessed. The dispersion stability of NPs can be calculated by the value of Zeta potential using the Zeta Potential Analyzer. Colloidal stability of ZnO NPs using honey-assisted methods. It indicates that ZnO NPs have a negative charge of − 18.3 mV, Cu NPs have a positive charge of +20 mV, and AgNo3 NPs have a positive charge of +16 mV, for the honey method used. The homogeneous surface morphology of ZnO NPs is evident from the great stability of ZnO NPs prepared with Sunflower honey. The uniformity in the distribution of the particles and the smaller size of this nanoparticle using Sunflower honey confirms greater colloidal stability.

Antibacterial activity of silver nanoparticles

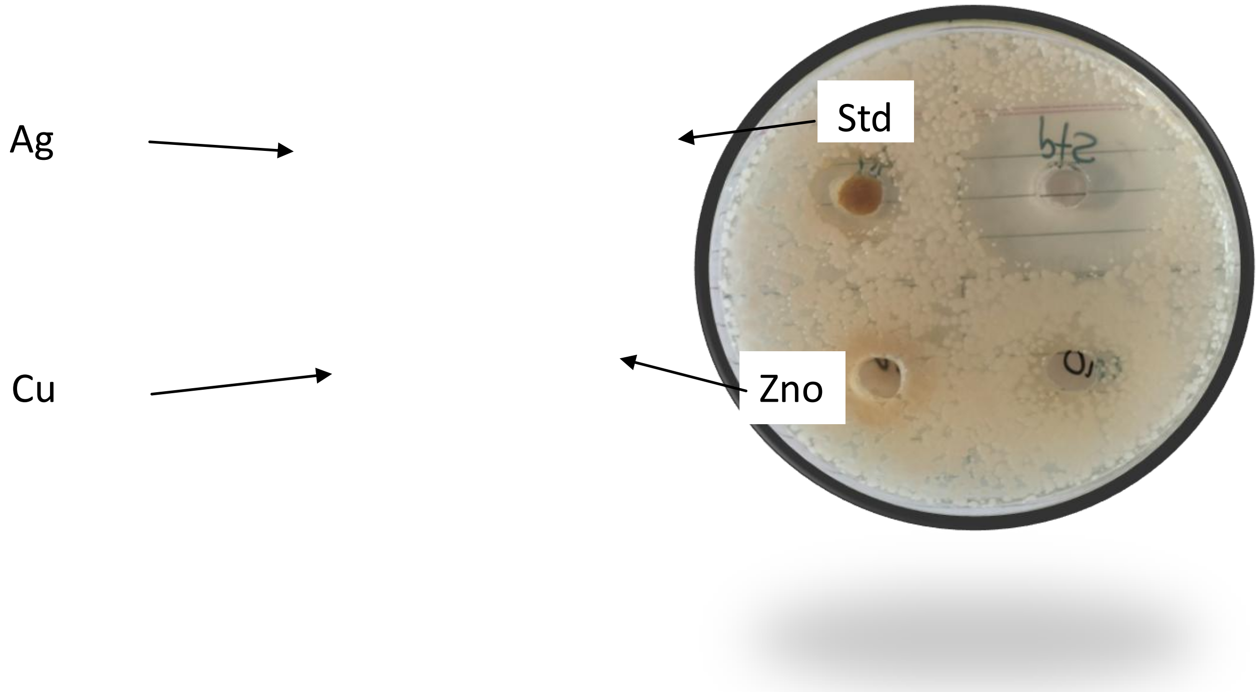


Figure 11. The antibacterial activity of silver, copper, and ZnO nanoparticles using sunflower honey was studied against gram-positive (S. aureus) and gram-negative bacteria (E. coli).

This antibacterial activity compares with standard streptomycin S. aureus showed the highest zone of inhibition. The antibacterial activity of ZnO, Cu&silver nanoparticles was studied against gram-positive (S. aureus) and gram-negative bacteria (E. coli). The synthesized ZnO, Cu& silver nanoparticles from honey showed varied inhibition zone against the tested microorganisms. Among all the studied against tested microorganisms, the maximum zone of inhibition was confirmed against S. aureus. AgNPs showed the highest zone of inhibition against S aureus (15mm±0.5), ZnO NPs zone of inhibition was confirmed against S. aureus(14mm±0.8) & CuNPs zone of inhibition was confirmed against S. aureus(11mm±0.7). AgNPs showed the Overall, antimicrobial activity of ZnO, Cu&silver nanoparticles showed the highest zone of inhibition against G+ve bacterial stains than the G-ve stains, due to the thick cell wall, the zone of inhibition was less against G-ve bacteria. so only AgNPs are considered to be having antimicrobial activity because the size of silver nanoparticles has a high surface area leading to effective interaction and penetration. In addition, the nano size of silver is capable to attack bacteria and penetrate easily inside the cell and make a severe effect on biomolecules. AgNPs can be used as effective antibiotics against E. coli, Salmonella typhimurium, Staphylococcus epidermidis, and Staphylococcus aureus. The effect of AgNPs as antimicrobial substances depends on the shape of nanoparticles, studied against E. coli and found that triangular-shaped nanoparticles are the most effective shape.

DISCUSSION

Nanotechnology is one of the most fascinating and modern areas of scientific study. Interest in green synthesis techniques that don't produce dangerous molecules as byproducts is increasing since environmental synthetic protocols are required for the manufacture of nanoparticles. As a result, we discussed a straightforward, environmentally friendly, and practical approach for synthesizing Ag, Cu, and ZnO nanoparticles in the

Maharashtra region of (India) by using native sunflower honey. that at least 200 compounds, including proteins, enzymes, amino acids, minerals, vitamins, and polyphenols, can be found in honey[17]. This study used Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) bacterial strains to investigate their impact on growth and bacterial output. UV-visible (UV-vis) absorption spectroscopy in the scan range of 200-800 was used to observe ZnO, Cu, and AgNo3-NPs. Because of the surface plasmon resonance (SPR) of ZnO, Cu, and AgNo3-NPs, the efficient synthesis of ZnO, Cu, and AgNo3-NPs with honey was confirmed by UV-vis absorption, where peaks were detected at 372, 613, and 450 nm, respectively. ZnO exhibits a distinctive absorption peak in the spectrum at a wavelength of 372 nm, which can be attributed to the material's inherent band-gap absorption caused by electron transitions from the valence band to the conduction band[18]. The honey contains Cu-NPs. The optimal absorption peak at 613 nm was seen, showing that the size of the nanoparticles had decreased. This might be due to the fact that honey includes monosaccharides and protein, which act as stabilizing factors and prevent the Cu-NPs from aggregating. Visual examination of a solution mixture's color change was used for the initial characterization of AgNPs. The optimal absorption peak was observed at 450 nm, and this absorption is attributable to the nanoparticles' surface plasmon resonance (SPR) caused by the reduction of the Ag+ ions in an aqueous solution of sunflower honey.[20] Through UV-visible spectroscopy, it was discovered that the organic compounds in Sunflower Honey interact with Ag+ ions, leading to an absorption peak at a shorter wavelength. The particle size analyzer showed that Zno, Cu, and AgNO3 nanoparticles had sizes of 127.3nm, 114.6nm, and 64.4nm, respectively. The study found that silver nanoparticles had a smaller size compared to ZnO and Cu nanoparticles, and all three nanoparticles showed great thermal stability. XRD analysis confirmed the biosynthesis of ZnO, Cu, and silver nanoparticles from sunflower honey, through characteristic peaks observed in the XRD. The sharp peaks revealed the crystalline nature of ZnO, Cu&silver NPs, and full width at half maximum (FWHM) decreased. From the XRD analysis, we observe that The sharp peaks revealed the crystalline nature of ZnO, Cu&silver NPs. The peaks seen in the analysis of synthesized ZnO, Cu, and AgNPs indicated that the samples had a crystalline size in the nanoscale. This was confirmed by FESEM images of ZnO, Cu, and AgNo3-NPs at various magnifications. The achievement of such small sizes resulted in a satisfactory outcome. The size of the nanoparticles with honey was smaller. The prepared ZnO–NPs morphology by FESEM analysis. The nanoparticles possess a spherical and uniform shape in size about 20nm. The shape of CuNPs with honey was consistent This may suggest that the agglomeration of Cu-NPs with honey might occur slower. The size of the nanoparticle of cooper nanoparticle is 20nm. This could support that honey can act as a capping agent to prevent oxidation. Amounts of silver nanoparticles with spherical size were very few. The aggregation of particles due to the presence of cell components on the surface of silver nanoparticles as a capping agent and the spherical size of AgNPs 19nm provide the ability to penetrate into microbial cells and carry out their bactericidal properties. This outcome firmly establishes Honey's potential to function as a reducing and capping agent in the synthesis of silver nanoparticles. We observe that the FESEM analysis verifies the approximately spherical nature of NPs and the uneven form of the nanoparticles. Compared to ZnO and Cu NPs, the silver nanoparticle has a narrower range of particle sizes. The FTIR analysis has been employed to detect the biomolecules presented in honey ZnO, Cu&AgNPs In general, the FT-IR spectrum is used to determine the functional groups present in the sample. the FTIR spectra of ZnO nanoparticles synthesized using honey. large range of 3437 cm-1 samples indicates the presence of O-H stretch and hydrogen bonded. Peaks are associated with alkyne-C-C stretching vibration at around 2920 cm-1. The tensile vibration of the C=O hydroxyl (or) carboxyl groups on the sample surface is indicated by high absorption peaks at 1630 cm to 1640 cm-1. The peak of about 1382 cm shows the nitrate ions' asymmetric stretching vibration. The synthesized ZnO NPs' stabilization and capping agent may be due to the coordination of ZnO NPs with OH and C=O groups. FTIR measurement aims to identify the possible biomolecules responsible for capping and stabilizing the Cu-NPs synthesized using honey. the IR spectra of the honey, The peaks observed between 3100 and 3500 cm might be due to the hydroxyl group (-OH) and primary amine -NH bonded group of carboxylic acid -COOH. Next, the peak for the alkane C-H stretching was observed around 2928 cm for the Cu-NPs. A peak was seen for Cu-NPs around 2887-2906 cm^1 which might be due to the Cu-H bonding. Meanwhile, the peaks were observed between 1780 cm and 1607 cm-1 for the Cu-NPs with honey respectively which denote the C-C stretching aromatic rings, N-H vibration of amine,1417 cm-1 C-H bending in CuNPs with honey, and for 1047 cm-1 for C-O stretching for CuNPs with Honey. The peaks were observed at varying frequencies in the FTIR spectroscopy. The peaks corresponded to the presence of an amino group of proteins, aromatic compounds, aldehydes, unsaturated compounds like alkenes, primary amines, amides, and stretching of amines. The above spectrum depicts the presence of active functional groups in the infrared spectrum of different AgNPs obtained from sunflower honey

The presence of amine and amide group phenolic compounds, and a carbonyl group(-coo-) in silver nanoparticles from the honey play an important role in reduction while. presence of 3282 cm-1 phenolic compounds O-H supports the antimicrobial activities in silver nanoparticles from sunflower honey. The binding of honey proteins to AgNPs using the amino acid residue's free amine groups and carboxylate ions. The silver Nanoparticles are 2378 cm-1. These functional groups demonstrate the complexity of Sunflower Honey's composition, which may be the cause for the conversion of $AgNO_3$ to AgNPs. Through free amine groups or carboxylate ions of amino acid residue present in the honey solution, the proteins can attach to Ag nanoparticles. Additionally, it has been proposed that proteins serve as the capping material and that glucose may be present in honey as a reducing agent and a stabilizing agent. The results of FTIR indicated the importance of honey constituents in maintaining the stability of the produced nanoparticles within the matrix. In this FTIR study, we conclude that all the functional groups are present in the nanoparticle. The antibacterial activity of Sunflower honey-ZnO, Cu&AgNPs against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). In our antibacterial study, we note that the silver nanoparticle exhibits a greater zone of inhibition when compared to ZnO, &Cu NPs and that a higher concentration of AgNPs resulted in a stronger suppression of fungal growth. It has been observed that the inhibition level of both strains increases with the increment of AgNP concentration at all different conditions. Although most previous studies have evaluated the antibacterial activity of AgNPs, the antifungal activity of metal nanoparticles against filamentous fungi is still limited. Biosynthesized Ag NPs demonstrated excellent antibacterial activity against bacteria, Gram-Positive (S. aureus) and Gram-Negative (E. coli). A COMPARATIVE STUDY OF GREEN SYNTHESIS, CHARACTERIZATION, AND ANTI-MICROBIAL ACTIVITY OF ZnO, Cu, AND $AgNO_3$, NANOPARTICLES OF INDIAN SUNFLOWER HONEY, we draw the conclusion from our research that the silver nanoparticle is superior to the ZnO and CU nanoparticles made from sunflower honey. This study is also useful for researchers working on these three metal NPs syntheses from Sunflower honey for their formulation and various herbal synthesis products to select the suitable NPs in their work according to the size of NPS, characteristics fit, and antimicrobial activity**.**

Conclusion

green synthesized NPs have simplicity in preparation economic and environmental, advantages, So this study compares three potential green synthesized NPs of Indian sunflower honey (Maharashtra region)As honey itself acts as a reducing, capping, and stabilizing agent, NP formation becomes more easily efficient, by the green synthesis method This study compares Zn, Cu & Ag NP3 of honey in Particle size formation UV, FTIR spectrum, SEM, XRD data & antibacterial data, In conclusion, Ag NPs show the lowest Particle size, better crystal formations of more antibacterial activity as compare the ZnO& Cu NPs of Indian sunflower Honey.

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