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RESEARCH ARTICLE

Green Synthesis, Characterization, and Antimicrobial Activity of Carbon Nanoparticles Derived From Mustard Oil

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Highlights

- Using an environmentally friendly combustion technique, carbon nanoparticles were effectively produced from mustard oil.
- FTIR and XRD revealed that CNPs contain C=O, C-H, O-H, and C=C bonds.
- The CNPs showed antimicrobial activity against *Aspergillus niger*, *E.coli*, and *Bacillus subtilis*.
- Nanoparticles for antimicrobial applications can be created using green chemistry.

Abstract

In this study, carbon nanoparticles (CNPs) were made from mustard oil using the principles of green chemistry. Green chemistry employs a low-cost, straight forward combustion method that eliminates the need for potentially dangerous components. The characterization techniques used in this study were Fourier Transform Infrared (FTIR) Spectroscopy and X-ray diffraction (XRD). FTIR spectroscopy examination verified the presence of carbon nanoparticles by identifying the presence of C=O, C-H, O-H, C-O, and C-C bonds in the sample. The carbon nanoparticles produced were shown to be amorphous by XRD analysis. Nevertheless, the antibacterial activity of generated carbon nanoparticles was effective when tested on Bacillus subtilis, E. coli, and fungus.

Keywords: *CNPs, FTIR, Green Chemistry, Mustard-oil, XRD*

Introduction

Nanotechnology is a multidisciplinary domain of science that investigates the fundamental concepts, manufacturing, and structure of nanoparticles, as well as their properties and associated phenomena [1]. Nanoparticles are discrete clusters of atoms larger than an atom but smaller than the bulk substance, typically ranging in size from 1 to 100 nm [2]. These particles are well-known for their larger surface area and quantum effects, which distinguish them from bulk materials in terms of size, reactivity, energy absorption, and mobility. Such unique characteristics of nanomaterials offer remarkable physical, chemical, and biological properties, opening up many prospects for research, innovation, and application [2, 3]. Moreover, nanomaterials are being utilized widely in various fields such as medication, electronics, and energy harvesting [4].

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Carbon is one of the most prevalent non-metallic elements on the earth. There are various allotropes of carbon even at the nanoscale which include fullerenes, carbon nanodots, nanotubes, nanohorns, graphene, nanoribbons, and nanodiamonds [5]. Carbon nanomaterials have attracted huge interest among scientists due to their distinct electric, optical, and chemical properties. They have widespread applications in many fields such as electronics, biomedical, energy, hydrogen storage, water filtration, and others [6]. There is a consistent increase in the resistance of fungi and bacteria to the newly developed drugs despite the development of new medicines. Many carbon-based nanomaterials have shown bactericidal and fungicidal effects, which could be alternative ways in the fight against bacterial and fungal infections [7]. CNPs show a high ability to interact with bacteria, which is influenced by their size and surface area [8]. In addition, CNPs such as CNTs can be used as immunotherapeutic agents to combat coronavirus infections [9]. Recently, there has been a wide application of graphene-based nanomaterial in many fields of biomedical engineering because of their significant physical, chemical, and organic properties. Graphene and its derivatives also have been utilized to form nano-composite filters which are used in the purification of several respire germs present in the air [10]. Carbon-based materials like carbon dots possess exclusive properties of chemical stability and photochemical alteration, due to which they have been extensively studied nowadays [11]. In fluorescence sensors, the CNPs could be utilized for Cu^{2+} detection [12]. Moreover, Carbon nanoparticles doped with other chemicals showed enhanced properties in many fields. Antibacterial activity of doped CNPs is very effective in dental caries [13, 14]. By doping CNPs with various hetero atoms, the sensitivity of the electrochemical sensors can be advanced by tuning the electrochemical properties of CNPs [15].

The synthesis of carbon nanoparticles could be achieved by several techniques, including chemical vapor deposition, hot wire, laser ablation, and others. However, these techniques are more expensive, non-renewable, and hazardous [16]. Most of the problems can be tackled by accomplishing the creation of CNPs with a green approach. Green synthesis, which produces carbon nanoparticles using the green chemistry principle, is the most efficient way since it is inexpensive and uses harmful chemicals as minimum as possible [17]. Concerns about the environment, health, and economy are causing the practice of green synthesis to gain popularity since it offers greenly manufactured nanoparticles essential in creating a more sustainable and environmentally friendly world [18, 19]. In this study, a straightforward yet effective combustion process is used to create nanoparticles from mustard oil. This is the flame synthesis technique of combustion, which is a more economical and green approach than other methods. Concerns about the environment, health, and economy are causing this practice to gain popularity.

Materials and Methods

Materials and Reagents

The mustard oil was purchased from a local market in the Jhapa district of Nepal. Analytical-grade chemicals were used in this research. Nitric acid (HNO_3) was purchased from Merck whereas; neomycin, ketoconazole, Muller Hinton Agar (MHA), and Muller Hinton Broth (MHB) were obtained from Himedia, India. Glassware, including beakers, conical flasks, and funnel, was purchased from Borosil Company, India.

Synthesis of Carbon Nanoparticles

The incomplete combustion of mustard oil was used to synthesize the carbon nanoparticles. First, mustard oil was poured into the mud lamp after it had been cleaned with a fresh cotton cloth. The flame was created using a cotton wick immersed in mustard oil. The lamp was then encircled on all sides by bricks that rose 40 cm above the lamp wick, creating a canal-like structure that was airtight to prevent the soot from spreading and prevented insufficient soot from being collected. Due to a lack of air, this allowed for incomplete combustion to take place. The bricks' interior space measured 20 cm in width by 30 cm in length. To prevent the created soot from escaping and completely depositing on the foil, an aluminum foil was placed on top of the lamp at a height of 40 cm over the brickwork canal. Regular checks and refills were made to the lamp's oil, and any worn-out wick was replaced. The two-day experimentation was conducted. A nearly 1 cm thick layer of soot of about 4g was deposited on the aluminum foil. After that the prepared CNPs were stored in the preservative vial and its purification was performed by using concentrated nitric acid. Finally, the collected black soot particles were characterized by XRD and FTIR spectroscopy. A schematic diagram showing the synthesis of carbon nanopowder is shown in Figure 1.

Fig 1. Schematic Diagram of the Synthesis of Carbon Nano Powder.

Purification of Carbon Nanoparticles

Concentrated nitric acid was applied to the black shoot after it had been prepared by burning mustard oil to release the trace components. 40 ml of concentrated HNO_3 must be used to treat one gram of the material. As a result, 4g of the sample was added to 160 ml of concentrated HNO_3 in a conical flask. The conical flask was then refluxed for 30 minutes. The flask's mouth was then sealed with filter paper to make it airtight, and it was kept in a dark location for 48 hours. The moist sample was filtered after 48 hours using Whatman's filter paper and repeatedly rinsed with distilled water. As a result, the sample was clean and devoid of contaminants. In the end, the wet sample was dried for 4 hours by being kept in a 200°C oven. Finally, carbon nanoparticles that were devoid of contaminants were produced [20, 21].

Characterization Techniques

The synthesized CNPs were examined using X-ray diffraction (XRD) analysis and Fourier transform infrared (FTIR) spectroscopy, where XRD was used for phase identification of the materials as crystalline or amorphous, and FTIR was used to identify the functional group present in the sample as impurities. In XRD, the concentration data was gathered using a step scan mode over a range of 10-80° at the National Academy of Science and Technology (NAST) Nepal. The accelerating voltage of 20 kV and emission current of 10 mA was used and thus obtained spectrum was studied. In FTIR, 1 gram of carbon nanoparticle was taken in a small tube and FT-IR was carried out in the range of 4000 to 400 per cm wavelength. The obtained spectrum was studied.

Antimicrobial Assay of Carbon Nanoparticles

The antimicrobial activity of prepared carbon nanoparticles was studied against the Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli,* and fungi (*Aspergillus niger*). The agar-well diffusion method was used to detect the antimicrobial activity of the test organism of the sample. The agar used in this method was Muller Hinton Agar (nutrient agar).

For the antibacterial study, 1% dimethyl sulfoxide and Neomycin were used as a negative and positive control. Similarly, for antifungal activity ketoconazole was used as positive control and 1 % dimethyl sulfoxide was used as negative control. *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger* were first grown for 24 hours in liquid nutritional media. Then 100 µl culture broth of each strain was placed on a nutrient-agar plate and kept for 15 min at 37℃. The 10 µl sample was pipette and was kept in the well in the nutrient-agar plate containing microorganisms and incubated overnight at 37℃. The next day, the antimicrobial activities of the sample against *Staphylococcus aureus*, *Escherichia coli*, and fungi microbial strains were investigated by analyzing the zone of inhibition [22, 23]. Because they have more peptidoglycan in their cell membrane, gram-positive bacteria might be less vulnerable to NPs inhibitory effects [24].

Results and Discussion

Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 2 displays the FTIR spectrum of the sample.

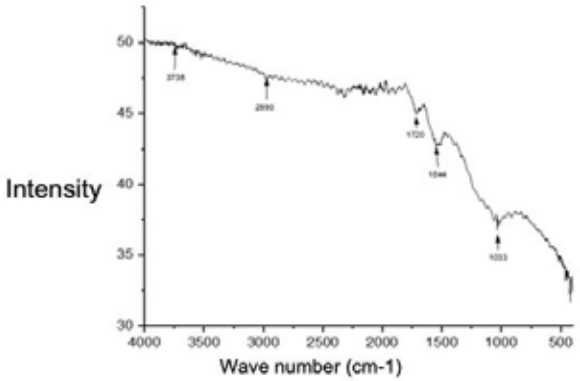

Fig 2. FT-IR Spectra of Carbon Nanoparticles Prepared from Mustard Oil.

FTIR spectroscopy analysis was used in this experiment to determine the chemical composition of the carbon particles and the presence of any functional groups. The existence of absorbed moisture or water molecules with the -OH group is indicated by the narrow-spectrum peak at 3736 cm^{-1} in the sample. Stretching of the C-H bonds, both symmetrically and asymmetrically, is the cause of the faint absorption peak at 2990 cm^{-1}. C=O stretching vibrations are responsible for the peak at 1720 cm^{-1}. C=C stretching vibrations are responsible for the peak at 1544 cm^{-1}. The stretching of the C-O bond is responsible for the peak at about 1033 cm^{-1}. Therefore, it may be deduced from the measured peaks that the sample contains carbon nanoparticles with a significant amount of oxygen.

X-ray diffraction (XRD)

The broad XRD peak obtained suggested the presence of amorphous carbon nanoparticles. The size of the materials was determined from the full width at half maximum (FWHM) of an intense peak from XRD spectra after removing instrumental broadening using Debye-Scherrer's equation [25]:

$$D = \frac{K\lambda}{\beta\cos\theta}$$

D = Average diameter of nanoparticles

λ = Wavelength of Cu Kα radiation

β = Full width at half maximum (FWHM)

θ = Angle of diffraction

The size of the as-prepared material was found to be 9.5 nm corresponding to the intense peak at around 2θ value 25 degrees which was calculated by using the Debye-Scherer equation. XRD spectra of synthesized carbon nanoparticles are shown in Figure 3.

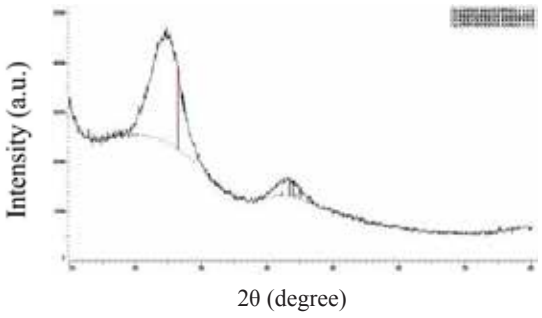

Fig 3. XRD spectrum of the synthesized carbon nanoparticles.

Antimicrobial Activity

The zone of inhibition of the sample after the incubation for 48 hours is observed as 0.5mm, 0.6mm, and 0.7mm for the test organisms *E. coli, Bacillus subtilis,* and *Aspergillus niger* (fungi) respectively. For *E.coli* and *Bacillus subtilis,* the zone of inhibition for positive control neomycin was determined to be 16mm and 14mm, respectively. Similarly, for the negative control, the 1% dimethyl sulfoxide zone of inhibition was found to be 0.0mm for both bacteria. Zone of inhibition for *Aspergillus niger* for positive control ketoconazole was found to be 1.1 mm and 0.0mm for negative control 1% dimethyl sulfoxide. From these results, it can be concluded that nanoparticles show very minimal antimicrobial activity due to the occurrence of low values of the zone of inhibition. Figure 4 shows the antimicrobial activity of a sample against *E. Coli* whereas Figure 5 shows the antimicrobial activity of a sample against *Bacillus subtilis*. In Figure 6, we show the antimicrobial activity of samples against *Aspergillus niger*.

Fig 4. Antimicrobial Activity of Sample against *E. coli*.

Fig 5. Antimicrobial Activity of Sample against *Bacillus subtilis.*

Fig 6. Antimicrobial Activity of Sample against *Aspergillus niger*.

Conclusions

Green synthesis of carbon nanoparticles was carried out successfully from incomplete combustion of edible quality mustard oil in a humble inexpensive and eco-friendly method and analyzed by using FTIR and X-ray diffraction spectroscopy. The FTIR result indicates the presence of compounds containing -OH, C-H, C=O, C=C, and C-O bonds in prepared carbon nanoparticles. X-ray diffraction analysis confirms the presence of the amorphous nature of carbon nanoparticles having a size of 9.5 nm. Carbon nanoparticles prepared by this method show very poor antimicrobial activity on *Bacillus subtilis, E. coli,* and fungi (A*spergillus niger*) due to the occurrence of small zones of inhibition.

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Author Declarations

There is no conflict of interest among the authors. All the figures and data presented in the manuscript are ours. Besides, the figures and images, which are not ours, have been given permission for re-publication attached to the manuscript. Regarding the Ethical Clearance, the project was approved by the local ethical committee of Tribhuvan University.

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