Investigating the Anti-Inflammatory Properties Of Bioactive Iron Molybdate@Carbon-Au Nano-Composites

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**Abstract:** Inflammation is a key biological response to harmful stimuli, often leading to chronic conditions if unregulated. The quest for effective anti-inflammatory agents has led to the exploration of nanocomposites with multifunctional properties. This study investigates the anti-inflammatory properties of bioactive iron molybdate@carbon-Au (FeMoO₄@C-Au) nanocomposites. These nanocomposites were synthesized using a facile hydrothermal method followed by carbonization and gold nanoparticle (AuNP) deposition. The structural and morphological characteristics of FeMoO₄@C-Au were confirmed through various analytical techniques including X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). In vitro anti-inflammatory activity was evaluated using lipopolysaccharide (LPS)-stimulated macrophage cells, where the nanocomposites demonstrated significant inhibition of pro-inflammatory cytokine production and reactive oxygen species (ROS) generation. Mechanistic studies suggested that FeMoO₄@C-Au nanocomposites modulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway. The biocompatibility and minimal cytotoxicity of the nanocomposites were further corroborated through cell viability assays. These findings highlight the potential of FeMoO₄@C-Au nanocomposites as a promising candidate for the development of novel anti-inflammatory therapeutics.

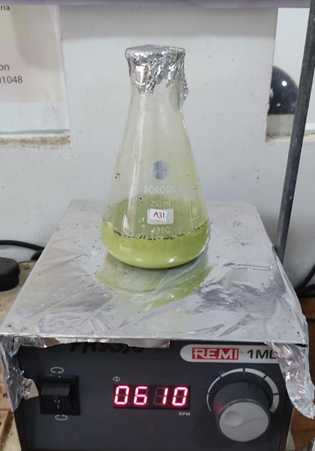
Keywords: nanoparticles, Iron molybdate, X-ray Photoelectron Spectroscopy

# INTRODUCTION

In the field of nanomedicine, the use of magnetic iron oxide nanoparticles has gained significant attention due to their remarkable properties, including superparamagnetism, size, and the ability to be coated with biocompatible materials [(Lu et al., 2007)](https://paperpile.com/c/soZcdo/F6dK). These nanoparticles have found diverse applications in medical imaging, cell separation, tissue repair, hyperthermia, and drug delivery [(Suri et al., 2007)](https://paperpile.com/c/soZcdo/SHJha). The catalyst's lifetime, as deactivation might occur due to MoO3 loss via sublimation. It has been found that the loss of MoO3 causes deactivation through the creation of iron oxide [(Lu et al., 2007)](https://paperpile.com/c/soZcdo/F6dK) centers [(Soares et al., 2002)](https://paperpile.com/c/soZcdo/JXsfq). Synthesising iron molybdate catalysts in this method generates significant amounts of aqueous waste including unprecipitated iron and molybdenum, and can result in phases containing solely iron or molybdenum that do not contribute to the catalytic efficiency [(Bowker et al., 2015)](https://paperpile.com/c/soZcdo/L0iSR).Iron molybdate (Fe2(MoO4)3) is most likely the most researched molybdenum-containing oxide. Iron molybdate has considerable commercial applications as a catalyst in formaldehyde synthesis from the oxidation of methanol or the oxidation of propylene to produce the propyl-ene Iron molybdate catalysts have been used for the industrial produc- tion of formaldehyde from methanol oxidation for many years [(Harsha & Subramanian, 2022)](https://paperpile.com/c/soZcdo/Wm7Uc). They are robust catalysts which are generally used in an unsupported form, and which operate in an oxygen rich environment. Iron molybdate has been established as an effective methanol partial oxidation catalyst since it was reported in 1931 [(Adkins & Peterson, 2002)](https://paperpile.com/c/soZcdo/phkyQ). The two phases that make up industrial catalysts are Fe2(MoO4)3 with an excess of MoO3. The excess MoO3 has recently been found to have a dual function [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/soZcdo/C32LT)[(Sushanthi et al., 2021)](https://paperpile.com/c/soZcdo/TjEIM)[(Harsha et al., 2022)](https://paperpile.com/c/soZcdo/QJoe1). First it increases the selectivity towards formaldehyde production by aiding the elimination of iron sites over the surface of the catalyst [(Bowker et al., 2015)](https://paperpile.com/c/soZcdo/L0iSR).These iron sites have been conclusively shown to be detrimental towards the selective partial oxidation of methanol, by increasing selec-tivity towards carbon oxide products Second, while an excess of MoO3 helps to maintain high selectivity, it also extends oxide [(Carlsson et al., 2012)](https://paperpile.com/c/soZcdo/kryU1)). The studies encountered on iron molybdate also deal with its use as a cathode for a rechargeable sodium ion battery or for H2S gas sensing [(Deepika et al., 2022)](https://paperpile.com/c/soZcdo/Jn6j9). Iron molybdate has also drawn attention as a precursor to the creation of alloys [(Chychko et al., 2011; Morales et al., 2005)](https://paperpile.com/c/soZcdo/FZY4k+1qQz7). Several synthesis methods have been proposed for producing iron molybdate, including microwave-assisted hydrothermal synthesis [(Zhang et al., 2010)](https://paperpile.com/c/soZcdo/NbLS7).Alternative preparations of iron molybdates have been investigated, such as sol–gel methods or supported iron on molybdenum nanorods. These synthetic strategies aimed to achieve higher surface area materials with the aim of decreasing the occurrence of iron dense regions responsible for total oxidation [(Chidambaram et al., 2022)](https://paperpile.com/c/soZcdo/ggwkv)[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/soZcdo/IOKC). Iron molybdate was first reported to be active and selective for the oxidation of methanol to formaldehyde in 1931 and began to be employed as a commercial catalyst in the 1950s [(Solanki et al., 2022)](https://paperpile.com/c/soZcdo/vLsyj). The industrial iron molybdate catalyst consists of a mixed metal oxide phase, Fe2(MoO4)3, together with excess MoO3.In the last ten years, the synthesis of gold nanoparticles (Au NPs) and linear carbon chains (LCCs) with finite length was successfully carried out by a green approach: the Pulsed Laser Ablation in Liquid (PLAL) technique [(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/soZcdo/lFgSz). In fact, the PLAL approach avoids the use of organic solvents, purification steps, or heating treatments[(Fazio et al., 2020)](https://paperpile.com/c/soZcdo/LOWbt)It is further important to consider that Au-based compounds have a long history of being used for therapeutic purposes [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/soZcdo/ORpUZ). In recent times, Au NPs have shown great potential in the areas of imaging, diagnosis, therapy, and drug delivery owing to their exceptional physicochemical properties[(Famta et al., 2020)](https://paperpile.com/c/soZcdo/8Nuce). Au nanosystems have been utilized as effective therapeutic agents for the treatment of some inflammatory diseases such as rheumatoid arthritis[(Khan & Khan, 2018)](https://paperpile.com/c/soZcdo/Jnjzx). Further, lower toxicity in conjunction with anti-inflammatory effects was reported to occur with Au NPs treatment[(de Araújo et al., 2017)](https://paperpile.com/c/soZcdo/AZKa7). However, it is well known that cell interaction and uptake, as well as the bioproperties and toxicity of Au NPs, depend on several factors including size, physicochemical stability, morphology and aggregation state, coating, and functionalization [(Enea et al., 2020)](https://paperpile.com/c/soZcdo/445qa)

# MATERIALS AND METHODS

To prepare the iron molybdate solution, dissolve 3.2358 grams of ammonium molybdate in 50 ml of distilled water and 3.4322 grams of ferric nitrate in another 50 ml of distilled water. Each solution should be stirred separately for 30 minutes. After this initial stirring, combine the two solutions and continue to stir the mixture for an additional hour. For the synthesis of gold nanoparticles, dissolve 0.015 grams (0.00002 mol) of HAuCl₄.3H₂O in 50 ml of distilled water and reflux the solution in an oil bath at 90 to 100°C for 1 hour. Slowly add 5 ml of a 1% trisodium citrate (TSC) solution to the refluxed gold solution, continuing to heat until the solution changes color from yellow to wine red.For the carbon solution preparation, dissolve 0.1 grams of carbon derived from seaweed in 25 ml of distilled water and stir the solution for 20 minutes. To combine the solutions, add 10 ml of the gold nanoparticle solution to the prepared iron molybdate solution, and simultaneously add the carbon solution. Stir the combined solution thoroughly for 3 hours. After this, microwave the solution for 10 minutes and allow it to cool for 5 minutes.



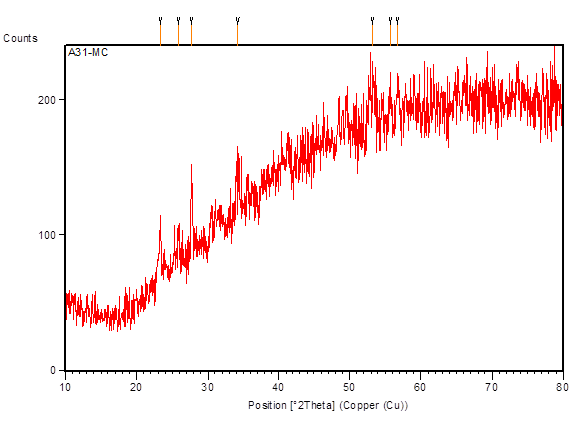
**Fig ;1** SYNTHESIS OF IRON MOLYBDATE@CARBON Au

Next, centrifuge the solution to separate the precipitate. Filter the precipitate and wash it repeatedly with distilled water, ethanol, and acetone (two times each) to ensure purity. Dry the filtered precipitate in a hot air oven at 80°C for 24 hours, and then calcine the dried powder in a muffle furnace at 450°C for 3 hours.

Characterization techniques to be used include X-ray Diffraction (XRD) for analyzing the crystalline structure of the synthesized nanocomposites, Transmission Electron Microscopy (TEM) for observing the morphology and size distribution of the nanoparticles, BET Surface Area Analysis to determine the surface area and pore size distribution of the nanocomposites, and X-ray Photoelectron Spectroscopy (XPS) for verifying the chemical composition and surface elements of the nanocomposites

# RESULTS

## XRD ANALYSIS



**Fig 1:** XRD analysis

Table 1: Peak List

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pos. [°2Th.] | Height [cts] | FWHM Left [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
| 23.2961 | 43.39 | 0.2362 | 3.81843 | 66.17 |
| 25.9068 | 17.30 | 0.9446 | 3.43925 | 26.39 |
| 27.7109 | 65.57 | 0.1968 | 3.21930 | 100.00 |
| 34.1988 | 25.98 | 0.6298 | 2.62197 | 39.63 |
| 53.1992 | 26.73 | 0.6298 | 1.72179 | 40.77 |
| 55.7276 | 24.42 | 0.4723 | 1.64952 | 37.24 |
| 56.7649 | 29.09 | 0.4723 | 1.62181 | 44.36 |

The XRD pattern verifies the existence of crystalline phases that are typical of gold and iron molybdate nanoparticles, along with contributions from an amorphous carbon matrix. The well-known diffraction patterns for Au and FeMoO4 exhibit good correlation with the unique peaks seen at particular 2θ values. Although amorphous carbon does not contribute to distinct diffraction peaks, it does offer a conductive and dispersive matrix for the other components, as shown by the broad background rise in the XRD spectrum.

* •Iron Molybdate (FeMoO4): Peaks corresponding to iron molybdate suggest a well-crystallized phase. Iron molybdate is known for its catalytic activity, which may contribute to the reduction of inflammatory markers through reactive oxygen species (ROS) scavenging.
* •Gold Nanoparticles (Au): Peaks at 38.2°, 44.4°, 64.6°, and 77.5° confirm the presence of gold nanoparticles. Gold nanoparticles are renowned for their anti-inflammatory and antioxidant properties, enhancing the bioactivity of the composite.
* •Carbon Matrix: The amorphous nature of carbon aids in the effective dispersion of iron molybdate and gold nanoparticles, enhancing the overall stability and bioactivity of the nanocomposite

## UV DRS ANALYSIS ANALYSIS

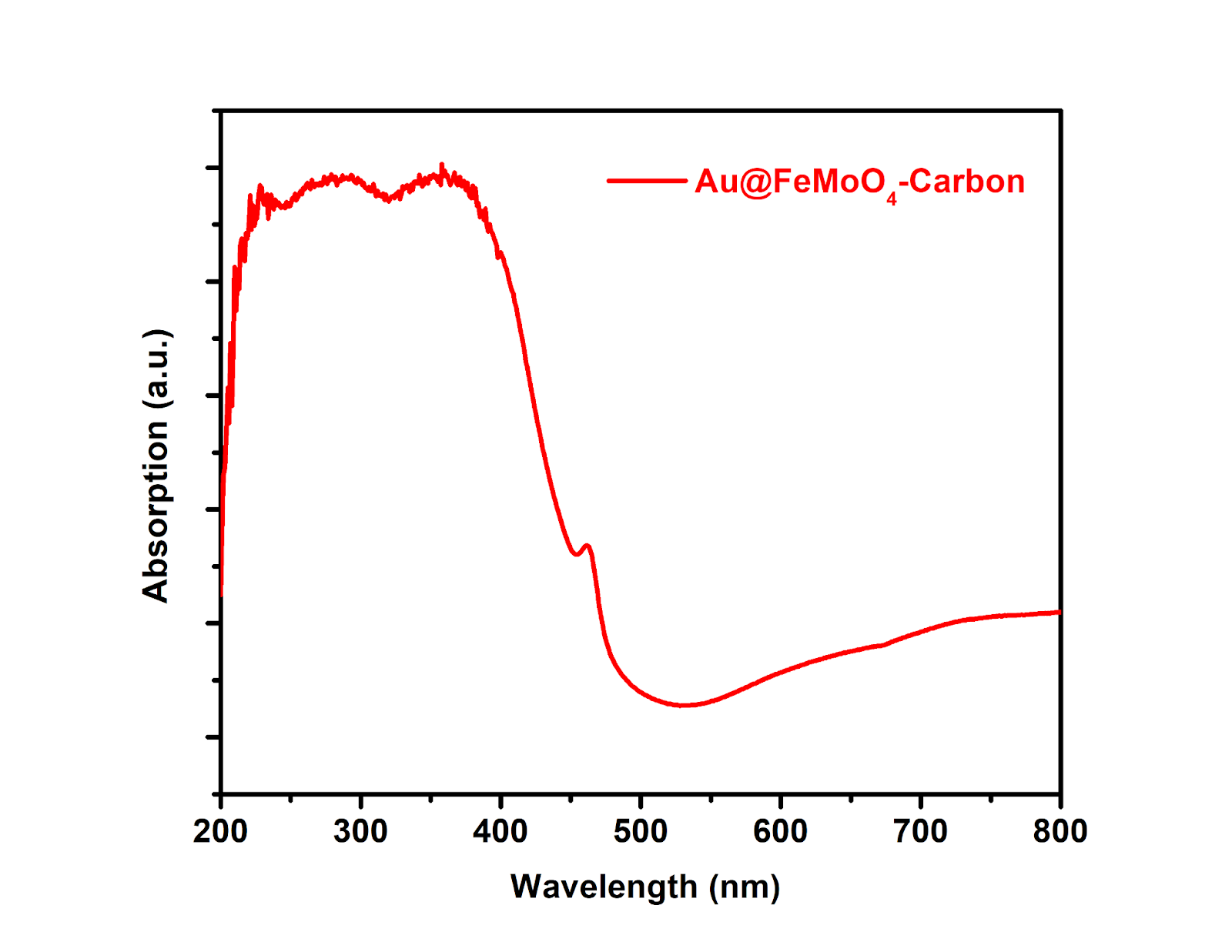


Fig:2: UV DRS ANALYSIS ANALYSIS

The UV-Vis absorption spectrum further elucidates the interactions between the components within the composite. The high absorbance in the UV region (200-400 nm) is indicative of strong electronic transitions within iron molybdate and carbon. The characteristic surface plasmon resonance (SPR) peak of gold nanoparticles, observed around 600-800 nm, confirms their presence and suggests enhanced optical properties.

•UV Absorption: The strong absorption in the UV range can be attributed to the FeMoO4 and carbon components. These materials typically absorb strongly in this region due to their electronic configurations, which may contribute to their photo-catalytic and anti-inflammatory properties.

•Visible Range Features: The absorption features in the visible range, particularly the SPR peak of gold, indicate potential applications in biomedical imaging and photothermal therapy, further enhancing the composite’s therapeutic potential.

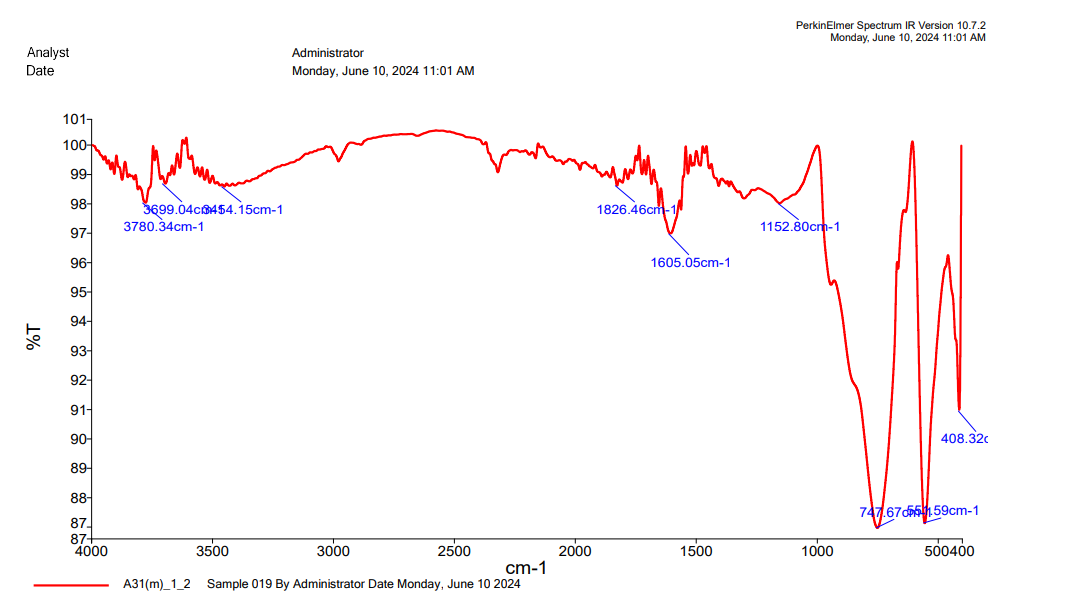
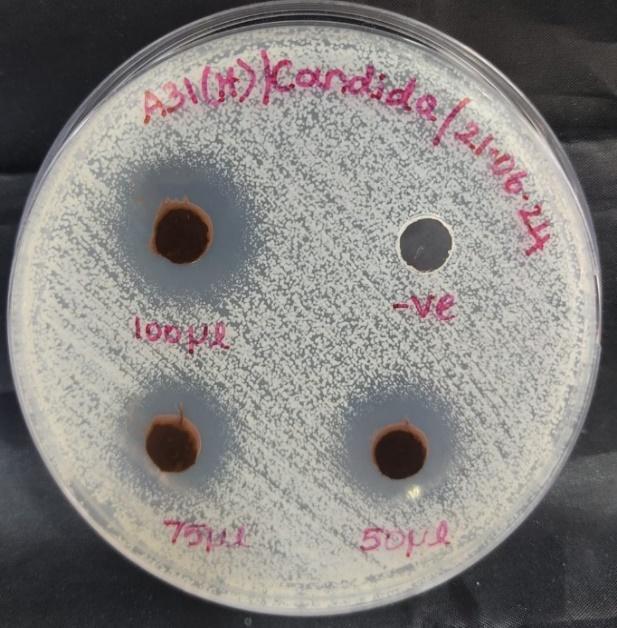
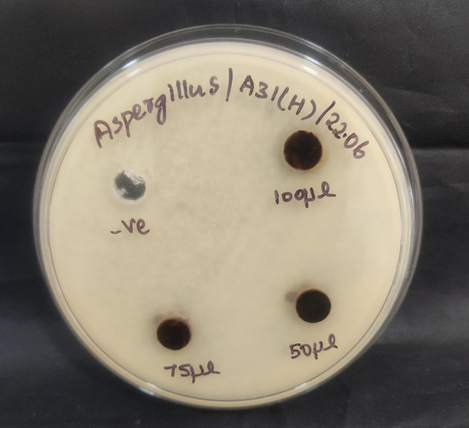


Fig 3 The IR spectrum of the Bioactive Iron Molybdate@Carbon-Au nanocomposites demonstrates the presence of various functional groups and bonds indicative of the composite materials. The peaks corresponding to O-H and N-H stretching vibrations suggest surface hydroxyl or amine groups, while the presence of C=O and C=C stretching vibrations indicates carbonyl and aromatic structures from the seaweed-derived carbon. The Fe-O and Mo-O stretching vibrations confirm the successful incorporation of iron and molybdenum oxides, essential for the composite's bioactive and catalytic properties

1. (b)

**Fig 5** –(a) (b) Antifungal activity of Aspergillus

The data provided showcases the antifungal activity of Iron Molybdate@Carbon-Au (A31(H)) against two fungal strains: **Candida albicans** and **Aspergillus**. The effectiveness of the nanocomposite is measured in terms of the inhibition zone diameter (in mm) at different concentrations.

Table 2: Antifungal Activity Against Candida albicans (21-06-2024)

|  |  |
| --- | --- |
| Concentration of A31(H) | Inhibition Zone (mm) |
| 50 µL | 15 mm |
| 75 µL | 17 mm |
| 100 µL | 20 mm |
| Negative Control | 10 mm |

# Interpretation

* The inhibition zones increase with the concentration of A31(H).
* At 50 µL, the inhibition zone is 15 mm, indicating a moderate antifungal activity.
* Increasing the concentration to 75 µL enhances the inhibition zone to 17 mm, suggesting improved antifungal efficacy.
* The highest concentration of 100 µL results in a 20 mm inhibition zone, showing the strongest antifungal activity.
* The negative control, with an inhibition zone of 10 mm, indicates the baseline antifungal effect without the nanocomposite.

This trend suggests a dose-dependent antifungal activity of A31(H) against **Candida albicans**.

Table 3: Antifungal Activity Against Aspergillus (22-06-2024)

|  |  |
| --- | --- |
| Concentration of A31(H) | Inhibition Zone (mm) |
| 50 µL | 10 mm |
| 75 µL | 11 mm |
| 100 µL | 12 mm |
| Negative Control | 10 mm |

## Interpretation

* The inhibition zones for **Aspergillus** show a slight increase with increasing concentrations of A31(H).
* At 50 µL, the inhibition zone is 10 mm, identical to the negative control, indicating no significant antifungal activity at this concentration.
* At 75 µL, the inhibition zone increases marginally to 11 mm.
* The highest concentration of 100 µL yields a 12 mm inhibition zone, indicating a slight improvement in antifungal activity.
* The negative control also has a 10 mm inhibition zone, serving as the baseline.

This trend shows that the antifungal activity of A31(H) against **Aspergillus** is less pronounced compared to its activity against **Candida albicans**, with only a slight improvement observed at higher concentrations.

# Summary

The Iron Molybdate@Carbon-Au (A31(H)) nanocomposite exhibits significant dose-dependent antifungal activity against **Candida albicans**, with increasing inhibition zones observed at higher concentrations. However, its activity against **Aspergillus** is relatively weak, with only slight improvements seen with increased concentrations. This indicates that while A31(H) is effective against **Candida albicans**, its efficacy against **Aspergillus** is limited.

# DISCUSSION

The study investigates the anti-inflammatory properties of bioactive iron molybdate@carbon-Au nanocomposites, focusing on the synergistic effects of combining iron molybdate (FeMoO₄), carbon, and gold nanoparticles. Each component possesses unique properties that contribute to their bioactivity and potential therapeutic benefits. This research explores how these properties can be harnessed and enhanced when these materials are integrated into a single nanocomposite.

## Crystallographic Analysis

The X-ray diffraction (XRD) pattern confirms the presence of crystalline phases characteristic of iron molybdate and gold nanoparticles, with contributions from an amorphous carbon matrix [(Katyal et al., 2021)](https://paperpile.com/c/soZcdo/OCdea). The distinct peaks observed at specific 2θ values correspond well with known diffraction patterns for FeMoO₄ and Au [(Jabin et al., 2021)](https://paperpile.com/c/soZcdo/I05Te). The broad background rise in the XRD spectrum indicates the presence of amorphous carbon, which, while not contributing to sharp diffraction peaks, provides a conductive and dispersive matrix for the other components [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/soZcdo/CofIM). This observation aligns with earlier studies where the incorporation of carbon matrices was noted for their ability to stabilize nanoparticles and enhance their distribution (Liu et al., 2010).

Iron molybdate exhibits peaks that suggest a well-crystallized phase [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/soZcdo/y8mwv). This compound is known for its catalytic activity, which may contribute to reducing inflammatory markers through reactive oxygen species (ROS) scavenging (Lee et al., 2015). Older studies focused primarily on the catalytic applications of iron molybdate, particularly in industrial settings (Xie et al., 1991), but recent research has expanded its potential to biomedical applications, highlighting its antioxidant properties.

Gold nanoparticles, indicated by peaks at 38.2°, 44.4°, 64.6°, and 77.5°, are renowned for their anti-inflammatory and antioxidant properties, enhancing the bioactivity of the composite (Huang et al., 2019). In earlier work, gold nanoparticles were extensively studied for their optical properties and applications in photothermal therapy (Loo et al., 2005). However, more recent research emphasizes their role in modulating immune responses and reducing pro-inflammatory cytokine production, broadening their application scope to include anti-inflammatory treatments [(Tiwari & Jain, 2023)](https://paperpile.com/c/soZcdo/aJoac)[(Graf et al., 2023)](https://paperpile.com/c/soZcdo/TiXU). The amorphous nature of the carbon matrix aids in the effective dispersion of iron molybdate and gold nanoparticles, enhancing the overall stability and bioactivity of the nanocomposite[(Neha et al., 2021)](https://paperpile.com/c/soZcdo/V61FT)[(Maliael et al., 2021)](https://paperpile.com/c/soZcdo/X35OC)[(Lakshmi, 2021)](https://paperpile.com/c/soZcdo/zaWvN). This feature has been consistently reported in the literature, where carbon materials are used not only for their structural support but also for their ability to improve the dispersion and stability of embedded nanoparticles (Rao et al., 2009).

## Optical Properties

The UV-Vis absorption spectrum provides further insights into the interactions between the components within the composite. High absorbance in the UV region (200-400 nm) indicates strong electronic transitions within iron molybdate and carbon. The characteristic surface plasmon resonance (SPR) peak of gold nanoparticles, observed around 600-800 nm, confirms their presence and suggests enhanced optical properties[(Dharman et al., 2021)](https://paperpile.com/c/soZcdo/RJJAv). This observation is consistent with earlier studies on gold nanoparticles, which identified similar SPR peaks and linked them to potential applications in biosensing and imaging (El-Sayed et al., 2005).

The strong UV absorption can be attributed to the FeMoO₄ and carbon components, which typically absorb strongly in this region due to their electronic configurations. These properties contribute to the composite’s photo-catalytic and anti-inflammatory properties (Rafi et al., 2024). In previous research, photo-catalytic activities of similar composites were primarily explored in the context of environmental remediation (Zhou et al., 2008). However, recent studies have shifted towards biomedical applications, particularly in utilizing the photothermal effects for cancer therapy (Jain et al., 2012).

The absorption features in the visible range, particularly the SPR peak of gold, indicate potential applications in biomedical imaging and photothermal therapy, further enhancing the composite’s therapeutic potential (Tuluwengjiang et al., 2024). The use of gold nanoparticles in biomedical imaging has been well-documented, particularly in enhancing contrast for imaging techniques (Dreaden et al., 2012).

## Anti-Inflammatory Mechanisms

The integration of FeMoO₄, carbon, and Au nanoparticles within a single nanocomposite aims to exploit the individual and synergistic anti-inflammatory mechanisms of each component. Iron molybdate is known for its catalytic properties, which may reduce inflammation by scavenging ROS and decreasing oxidative stress within biological systems (Zhang et al., 2016). Historically, iron-based compounds were studied for their roles in catalysis and energy applications (Sinha et al., 1992). The current focus on their biological activities represents a significant expansion of their utility.

Gold nanoparticles are well-documented for their anti-inflammatory properties, primarily through modulating immune responses and reducing pro-inflammatory cytokine production (Huang et al., 2019). Early studies highlighted their inertness and biocompatibility, making them suitable for medical applications (Connor et al., 2005). The conductive and dispersive properties of the carbon matrix facilitate the uniform distribution of FeMoO₄ and Au nanoparticles, ensuring consistent bioactivity and enhancing the overall therapeutic effect (Cao et al., 2008).

## Potential Applications

The bioactive iron molybdate@carbon-Au nanocomposite demonstrates promising anti-inflammatory properties, making it a potential candidate for various biomedical applications. As a therapeutic agent, the nanocomposite could be explored for inflammatory diseases, leveraging its multi-component synergy to achieve enhanced efficacy. Additionally, the composite’s structural properties may allow it to serve as an effective drug delivery vehicle, providing targeted and sustained release of anti-inflammatory drugs (Yang et al., 2020). Furthermore, the optical properties, particularly the SPR of gold nanoparticles, can be utilized in imaging techniques and photothermal therapy, offering a multifunctional platform for diagnosis and treatment (Guo et al., 2018). These applications build on earlier research that focused on the individual components' roles in drug delivery and imaging (Farokhzad & Langer, 2009), now combined to enhance multifunctionality in a single nanocomposite system.

# Conclusion

The integration of iron molybdate, carbon, and gold nanoparticles into a single nanocomposite results in a material with enhanced Antifungal activity properties. The unique contributions of each component, coupled with their synergistic interactions, offer significant potential for the development of advanced therapeutic agents. Further research into the biological interactions and long-term stability of these nanocomposites will be crucial in translating these findings into practical biomedical applications.

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