

Article

Synergistic Enhancement of Photoelectrochemical Hydrogen Evolution, Antimicrobial, and Cytotoxic Activities in a ZIF-8/*Aspergillus nidulans* Extract Nanocomposite

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Abstract

Pushing ahead in materials chemistry means building tiny substances that work hard and take into account clean power, planet care, life science all at once. From this effort comes a new direction: ZIF-8, a metal-linked cage structure, now fused with active components extracted from the common fungus *Aspergillus nidulans*. Not just mixed, but grown together with fungal extracts tucked neatly into the skeleton of the material while keeping its orderly shape intact. Three versions appeared—loaded at 2%, 4%, and 6 weight percent—and each one was mapped out using X-ray signals, sharp images from electron scans, and element tracing. A light flickered on and off during tests in which electricity flowed through these new composites set between three points, designed to split water and release hydrogen gas. Surprisingly, the ZIF-8@2% *nidulans* blend showed strong teamwork between electricity- and light-driven biology, creating a sharp spike in temporary current while cutting down reaction delay to just 310 mV/dec—pushing hydrogen release through a faster molecular handshake. In this mix, natural compounds from fungi act like tiny solar collectors, helping electrons move more freely, which is reflected in impedance scans as lower resistance. On top of that, higher doses of the material effectively blocked harmful microbes, thanks to ZIF-8 breaking cell walls and active fungal ingredients punching holes as well. Instead of relying on harsh chemicals, it uses a nature-inspired design in which molds meet synthetic frameworks, creating a single system with potential for sustainable energy generation and antimicrobial applications.



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Keywords: ZIF-8; *Aspergillus nidulans*; bio-hybrid nanocomposite; photoelectrochemical hydrogen evolution; Tafel kinetics; antimicrobial activity; solar energy conversion; multifunctional catalysts

1. Introduction

In the face of a finite supply of fossil fuels and growing environmental problems, the world's scientific community has turned to alternatives that are both sustainable

and environmentally friendly, with zero-emission options taking center stage [1]. The most promising clean energy carrier is hydrogen (H_2), with the highest gravimetric energy density of 142 kJ/g and an entirely harmless combustion product (water) [2,3]. Solar energy-based photoelectrochemical (PEC) water splitting and the hydrogen evolution reaction (HER) are elegant and green approaches for directly converting solar radiation into chemical fuel among various hydrogen production technologies [4–6]. The commercialization of PEC-HER is, however, greatly hindered by the scarcity of cost-effective, stable and highly active electrocatalysts that can induce charge separation and promote electron transfer at the interfaces [7,8]. Metal–organic frameworks (MOFs) are interesting porous crystalline materials composed of metal coordination centers connected by organic molecules, and have attracted considerable interest in the past decade for their use in energy conversion and storage [9,10]. In particular, the Zinc ion framework ZIF-8 (Zinc ions (Zn^{2+}) coordinated to 2-methylimidazole linkers) is widely recognized for its outstanding thermal stability, permanent porosity, large surface area, and easily tunable structural topology [11,12]. While there are many advantages, the pristine is severely limited when used in the direct application of photoelectrochemical systems. ZIF-8 has a very large electron band gap (3.3–3.4 eV), which renders it sensitive to a small portion of the solar spectrum (ultraviolet light) and has poor electrical conductivity, which produces high recombination rates of photo-excited electron–hole (e^-/h^+) pairs and low current densities [13,14].

To circumvent these drawbacks, researchers have focused on modifying the electronic structure of ZIF-8. Traditional strategies include doping the framework with foreign transition-metal ions (such as Cobalt, Iron, or Copper) or fabricating semiconductor heterojunctions with carbon-based structures such as graphitic carbon nitride ($g-C_3N_4$) or graphene derivatives [15,16]. Concurrently, another critical global challenge is the rising threat of antimicrobial resistance (AMR) among pathogenic microbes, which requires the urgent engineering of advanced biocidal agents [17,18]. Interestingly, nanoscale zinc-based frameworks naturally exhibit intrinsic antimicrobial activity through the slow, controlled release of localized Zn^{2+} ions, which induce oxidative stress and rupture of microbial cell walls. Consequently, designing a single bifunctional catalyst that simultaneously drives energy generation (PEC-HER) and exhibits strong antimicrobial efficacy represents a highly desirable, atom-economic objective.

Recently, the integration of biological entities with synthetic nanomaterials—often termed “bio-nanocomposites”—has emerged as a highly sustainable methodology [19,20]. Fungal species, particularly *Aspergillus nidulans*, are rich factories of diverse secondary metabolites, proteins, amino acids, and extracellular enzymes [21]. These bio-organic molecules possess complex functional groups (such as carboxyl, hydroxyl, and amine bands) that can act as natural reducing, stabilizing, and capping agents. More importantly, certain bioactive fungal extracts possess unique conjugated molecular chains that absorb visible light and act as organic charge-shuttling antennas when coupled with wide-bandgap inorganic materials [22]. Despite the extensive exploration of ZIF-8 modifications, several critical gaps remain in the current literature: Reliance on Toxic Synthetic Chemicals: Most classical methods used to sensitize or alter the band structure of ZIF-8 rely on harsh, toxic chemical reducing agents, surfactants, or expensive noble metals, which raise environmental concerns and limit scalability.

Underutilization of Biomass and Fungal Extract Co-catalysts: While green synthesis of simple metal oxide nanoparticles using plant extracts is common, the deliberate incorporation of crude or refined fungal extracts from *Aspergillus nidulans* directly into a crystalline MOF matrix as a functional photo-biochemical co-catalyst remains virtually unexplored. Lack of Dual-Functional Platforms: Most research decouples energy-conversion materials from biomedical agents. There is a profound scarcity of single-phase hybrid

materials engineered to simultaneously maximize PEC solar-hydrogen kinetics and deliver potent antimicrobial protection through a unified, synergistic mechanism. To address these challenges, this study explores the “closed-loop” valorization of biological extracts by introducing a novel, eco-friendly bio-hybrid nanocomposite framework. The primary objective is to evaluate the synergistic enhancement of both photoelectrochemical hydrogen evolution kinetics and antimicrobial performance achieved by anchoring *Aspergillus nidulans* extract within a ZIF-8 matrix. We systematically investigate the effects of extract loading (2%, 4%, and 6 wt%) on phase integrity, morphological transformation, interfacial charge storage (CV), charge-transfer resistance (R_{ct} via EIS), and transient photocurrent dynamics. Through this systematic approach, we establish the fundamental mechanism underlying this bio-inorganic coupling, optimizing the configuration to achieve high-efficiency solar-to-chemical conversion while maintaining reliable biocidal activity.

2. Results & Discussion

The crystalline structure and phase purity of pristine ZIF-8 and the fabricated ZIF-8@*nidulans* nanocomposites with varying extract fractions (2%, 4%, and 6%) were evaluated via X-ray diffraction (XRD), as illustrated in Figure 1. Pristine ZIF-8 exhibits sharp, well-defined diffraction peaks at 2 theta values of approximately 7.30°, 10.35°, 12.70°, 14.70°, 16.40°, and 18.00°, which correspond to the (011), (002), (112), (022), (013), and (222) crystal planes of a sodalite-type cubic framework, respectively [23]. This profile is in perfect agreement with the simulated and reported literature for crystalline ZIF-8. Upon the integration of *Aspergillus nidulans* extract (2%, 4%, and 6%), the positions of all major characteristic peaks of the ZIF-8 host matrix remain virtually unaltered. This preservation indicates that incorporating the biological extract molecules does not collapse or severely distort the long-range crystalline framework of the metal–organic framework (MOF). A slight decrease in peak intensities and a subtle broadening are observed with increasing extract content, which can be attributed to the amorphous or highly disordered nature of the encapsulated organic biomolecules/peptides from the *A. nidulans* extract, as well as slight changes in domain size due to host–guest interactions.

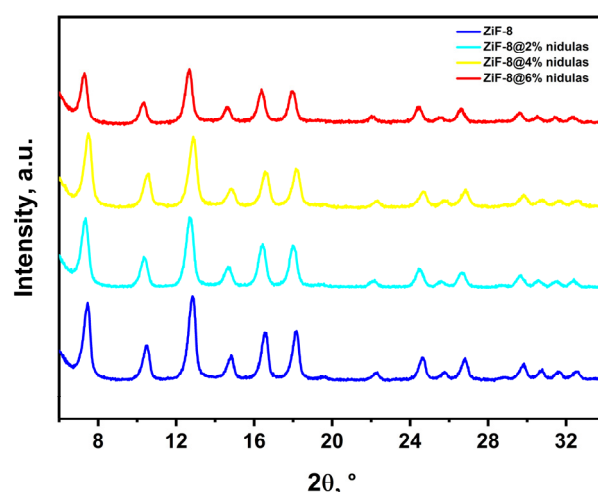


Figure 1. X-ray diffraction (XRD) patterns of pristine ZIF-8 framework and ZIF-8@*Aspergillus nidulans* extract nanocomposites prepared with varying extract weight fractions (2%, 4%, and 6%).

Field-emission scanning electron microscopy (FESEM) was employed to monitor morphological changes across the sample set, as shown in Figure 2. Figure 2a confirms that pristine ZIF-8 consists of uniform, nanoscale pseudo-spherical/polyhedral particles densely packed with well-defined grain boundaries, with an average size well below

100 nm. Figure 2b shows the amorphous, highly aggregated, and bulk-like matrix morphology typical of crude dried biological extracts (*A. nidulans*). For the ZIF-8@*nidulans* nanocomposites (Figure 2c,d), a progressive morphological transition is apparent. At optimal loading (Figure 2c, highlighted in yellow circles), the primary ultra-fine ZIF-8 nanoparticles remain distinct but are anchored onto or encapsulated by the thin organic sheets of the extract. At higher loading (Figure 2d), significant aggregation occurs; the excess biomaterial forms an overlying surface coating, transforming the particulate framework into larger, fused agglomerates. This dense organic capping layer directly correlates with the decreased surface accessibility and mass-transport bottlenecks identified in subsequent performance tests.

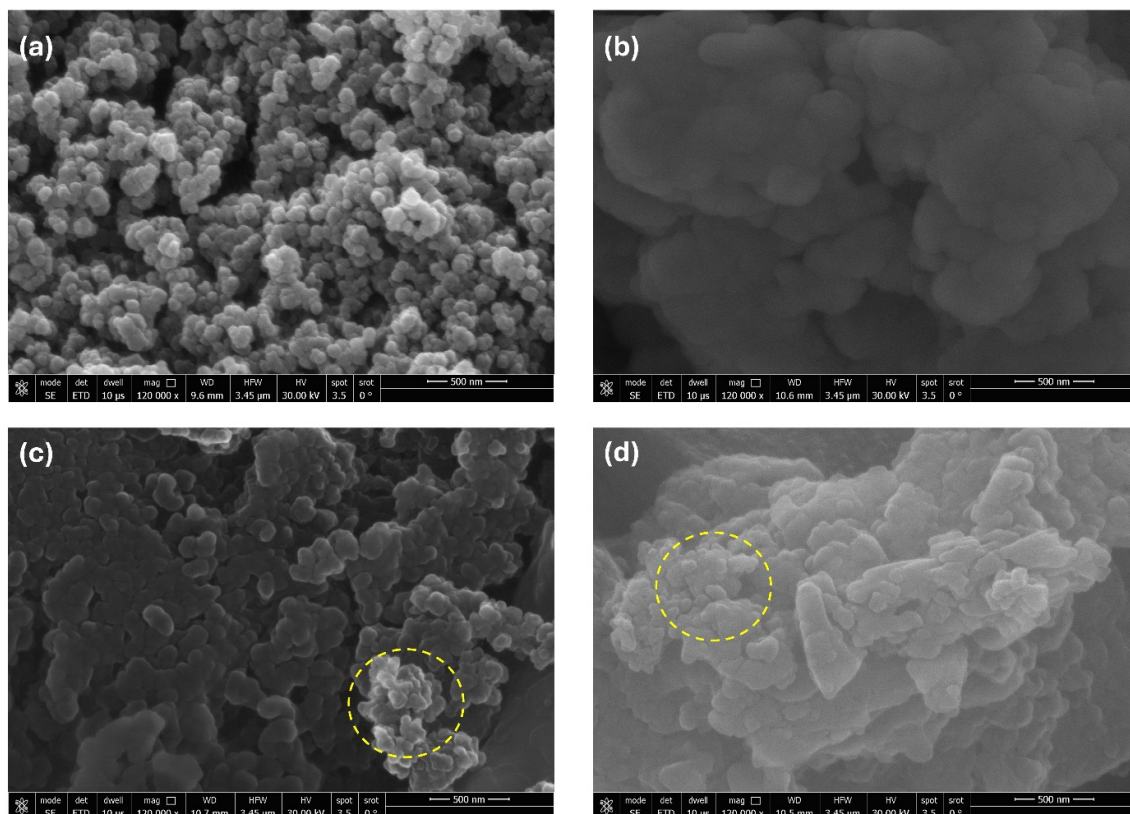


Figure 2. Field-emission scanning electron microscopy (FESEM) micrographs of (a) pristine nanoscale pseudo-spherical ZIF-8 particles, (b) bulk-like aggregated pure *Aspergillus nidulans* extract, (c) ZIF-8@2% *nidulans* nanocomposite displaying anchored particulate-matrix framework (yellow dashed circle), and (d) ZIF-8@6% *nidulans* nanocomposite showing heavy particle encapsulation and aggregation (yellow dashed circle). All scale bars represent 500 nm.

To verify the homogeneous distribution of the biomolecules within the MOF, energy-dispersive X-ray spectroscopy (EDS) elemental mapping was performed on a selected composite zone (Figure 3). The elemental breakdown reveals a predominant Zinc signal (70 wt.% Zn K-shell emission) originating from the coordination centers of the MOF network. Concurrently, carbon (11%), nitrogen (9%), and oxygen (10%) are distributed with high uniformity throughout the mapped region. The prominent presence of oxygen (O K channel), which typically originates from amino acid residues, proteins, and secondary metabolites in the fungal extract, confirms the successful chemical integration of *A. nidulans* into the porous matrix of the ZIF-8 support.

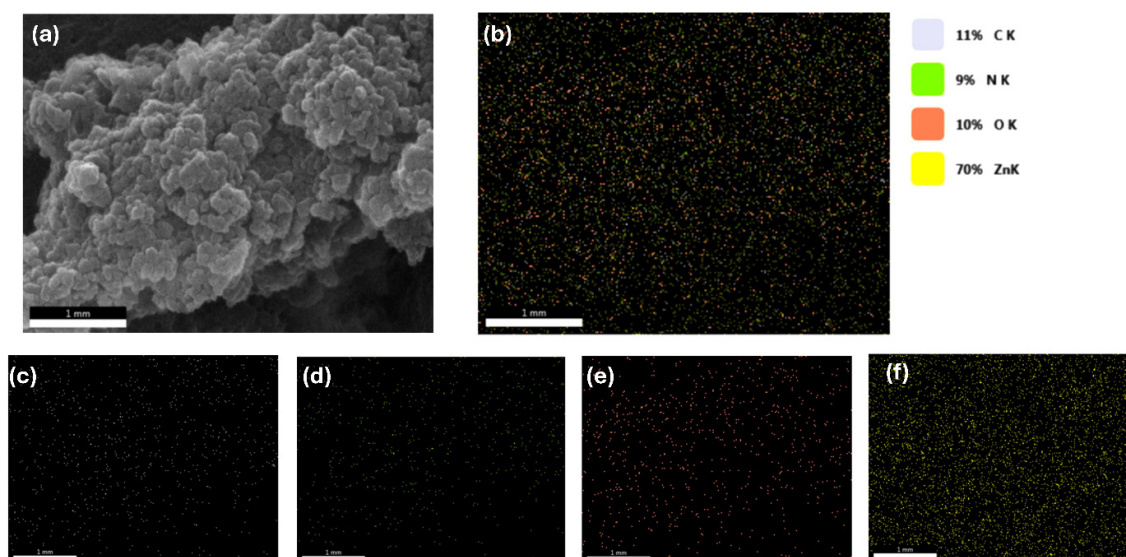


Figure 3. Energy-dispersive X-ray spectroscopy (EDS) elemental mapping analysis of the ZIF-8@nidulans nanocomposite: (a) selected FESEM scanning area, (b) combined elemental overlay map, and individual elemental distribution channels for (c) carbon (C), (d) nitrogen (N), (e) oxygen (O), and (f) zinc (Zn). Note: Insert weight percentages (wt.%) inside the panel (b) legend based on raw data calibration.

To explore the electrochemical profiles and charge-transfer behavior before evaluating gas evolution, Cyclic Voltammetry (CV) was recorded at scan rates of 10–100 mV/s (Figure 4). The CV loops across the samples deviate from the ideal rectangular shape of pure electric double-layer capacitors (EDLCs), demonstrating pseudo-capacitive/faradaic redox features. Both pristine ZIF-8 (Figure 4a) and pure *A. nidulans* (Figure 4b) show stable, widening current envelopes as scan rates ramp up, though their integrated current responses differ significantly. The ZIF-8@2% nidulans variant (Figure 4c) maintains broad redox-active current profiles, demonstrating optimized exposure of active surface sites. However, as the extract concentration increases to 4% and 6% (Figure 4d,e), the overall current density decreases significantly by nearly an order of magnitude (dropping down to peak currents near 0.0006–0.0012 A/cm²). This dramatic decline stems from the excessive insulating biopolymer layer that wraps the active sites, thereby restricting the transport of free ions at the electrode–electrolyte interface.

The performance of the fabricated electrodes for the photoelectrochemical hydrogen evolution reaction (PEC-HER) was systematically studied using linear sweep voltammetry (LSV), Tafel kinetic analysis, and transient photocurrent measurements under chopped-light conditions (Figure 5). Polarization Characteristics: As shown in Figure 5a, pure *A. nidulans* extract exhibits the highest cathodic current density, reaching approximately −0.15 A/cm² at −1.75 V vs. RHE, along with a favorable positive onset potential. Pristine ZIF-8 exhibits a lower current-density response. When introducing the extract to the MOF support, the ZIF-8@2% nidulans composite demonstrates a clear synergistic improvement over pristine ZIF-8. However, exceeding these thresholds (4% and 6%) leads to a progressive loss of current density and a negative shift in the onset potential, confirming that excessive organic bio-loading impedes the catalytically active centers. (Figure 5b,c) Tafel Kinetics and Overpotential Trends. To extract the reaction kinetics, Tafel slopes (vs. log J) were evaluated (Figure 5b,c). The estimated Tafel values for ZIF-8, pure nidulans, ZIF-8@2%, ZIF-8@4%, and ZIF-8@6% are 328, 328.3, 310, 422, and 371 mV/dec, respectively. The minimized Tafel slope for ZIF-8@2% nidulans (310 mV/dec) implies that the hydrogen

evolution mechanism at this optimum loading operates under the Tafel recombination pathway (where two adsorbed H intermediates recombine to liberate H_2).

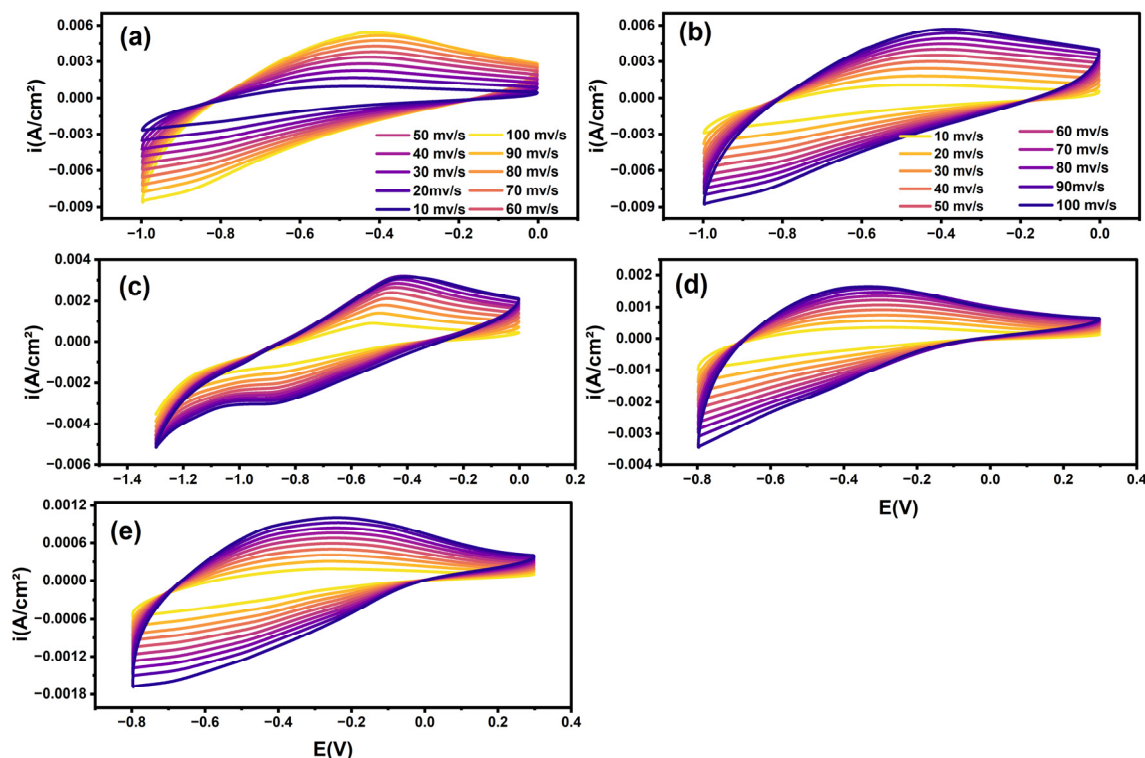


Figure 4. Cyclic Voltammetry (CV) profiles recorded at progressive scan rates $10\text{--}100\text{ mV s}^{-1}$ in a three-electrode configuration for: (a) pristine ZIF-8, (b) pure *Aspergillus nidulans* extract, (c) ZIF-8@2% nidulans, (d) ZIF-8@4% nidulans, and (e) ZIF-8@6% nidulans nanocomposites.

The low slope indicates an accelerated interfacial electron injection rate, triggered by the matching electronic bands between the bio-organic component and the MOF skeleton. Transient Photocurrent Inferences: The light-harvesting synergy was evaluated using transient photocurrent $i\text{-t}$ traces over multiple on/off illumination steps (Figure 5d). Pristine ZIF-8 shows a negligible photo-response due to its wide bandgap. In contrast, the pure *A. nidulans* extract shows powerful photocurrent spikes up to 0.06 A/cm^2 , highlighting its ability to capture light energy. Among the composite formulations, ZIF-8@2% nidulans yields the highest photocurrent. This confirms that the fungal extract functions as a built-in proposed mechanistic hypothesis system, effectively transferring photogenerated charge carriers into the ZIF-8 framework to suppress electron–hole recombination.

To decouple the internal resistance parameters and confirm the charge-transfer mechanism, Electrochemical Impedance Spectroscopy (EIS) Nyquist plots were obtained (Figure 6). The high-frequency intercept along the real axis (Z') represents the solution/equivalent series resistance (R_s), while the diameter of the arc reflects the charge transfer resistance (R_{ct}) across the electrode/electrolyte boundary. All samples show an incomplete, large capacitive arc extending into the low-frequency range, revealing combined charge transfer and diffusion control. Pristine ZIF-8 (black stars) possesses a highly restricted arc structure due to its poorly conducting framework. Strikingly, the ZIF-8@2% nidulans composite (blue stars) presents a tailored, linear-shifting arc slope that transitions efficiently across a wide impedance range. This demonstrates a reduced R_{ct} compared with the higher-loading counterparts (4% and 6%). At excessive loadings (4% and 6%), the arcs contract or present irregular impedance shifts, matching the CV drop-offs. This confirms that the 2% configu-

ration yields the most conductive pathways, facilitating rapid electron transport to feed the cathodic hydrogen evolution reaction.

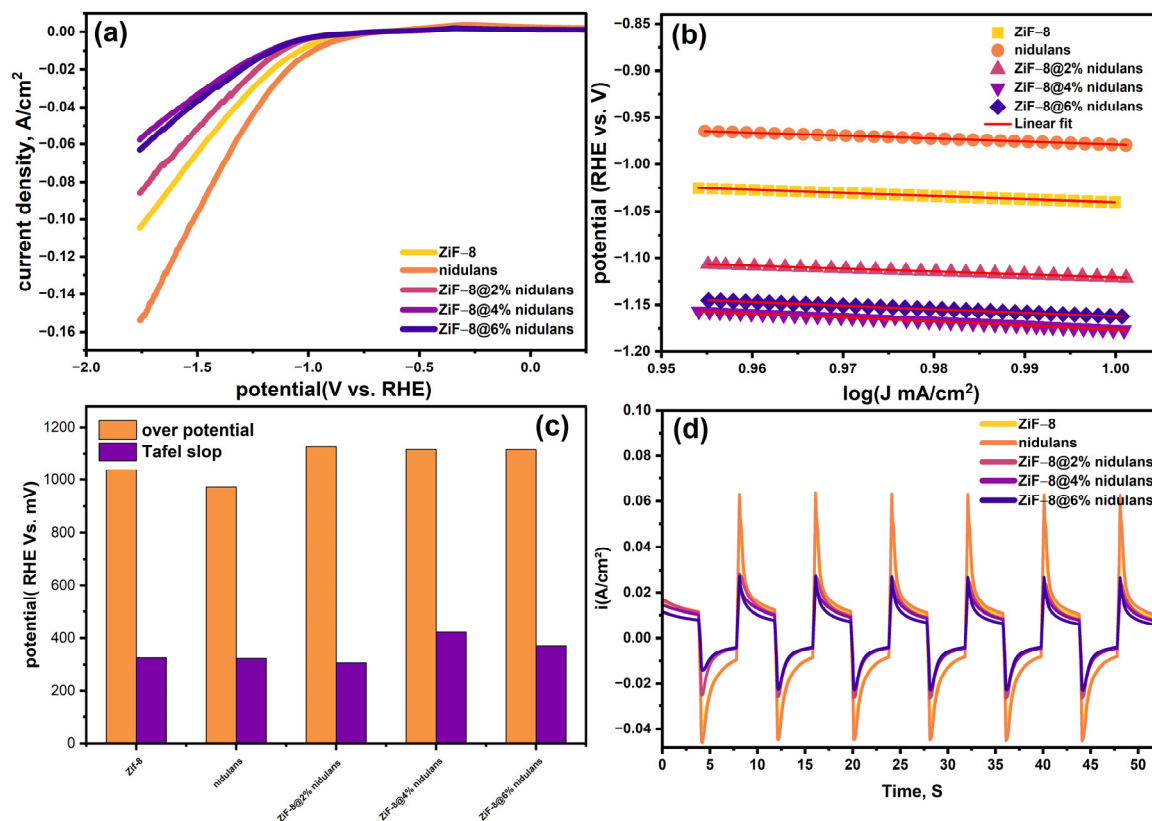


Figure 5. Photoelectrochemical hydrogen evolution reaction (PEC-HER) performance metrics: (a) Linear sweep voltammetry (LSV) polarization curves for pristine materials and ZIF-8@*Aspergillus nidulans* nanocomposites evaluated under illumination, (b) corresponding Tafel kinetic plots (Potential vs. $\log J$) derived from the polarization sweeps with linear fitting lines, (c) comparative bar chart displaying the required overpotential versus estimated Tafel slopes, and (d) multi-cycle transient photocurrent response ($i-t$) curves under chopped-light on/off illumination steps.

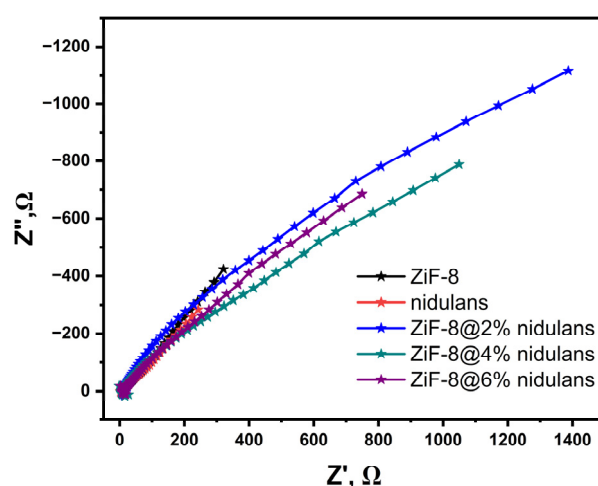


Figure 6. Electrochemical Impedance Spectroscopy (EIS) Nyquist plots showing the imaginary impedance (Z'') versus real impedance (Z') profiles for pristine ZIF-8, pure *Aspergillus nidulans* extract, and the ZIF-8@*Aspergillus nidulans* nanocomposite series (2%, 4%, and 6%).

Aspergillus nidulans ethyl acetate extract was analyzed for the chemical components using two-stage mass spectrometry to derive the molecular mass, molecular formula, and

characteristic fragment ions of the detected metabolites. Previously separated *Aspergillus* compounds were used to compare the obtained molecular formula with published data. Twenty chemical secondary metabolites were detected in negative ion mode and identified based on their precursor ions and MS2 fragmentation patterns, compared with the Competitive Fragmentation Modeling for Metabolite Identification online database. The mass spectrometric analysis of compounds extracted from various *Aspergillus* species reveals a striking diversity in molecular structures and fragmentation patterns, highlighting the complexity inherent in fungal metabolites. The compounds are arranged by retention time, accompanied by their previously reported occurrence in fungal species (Table 1, Figure 7).

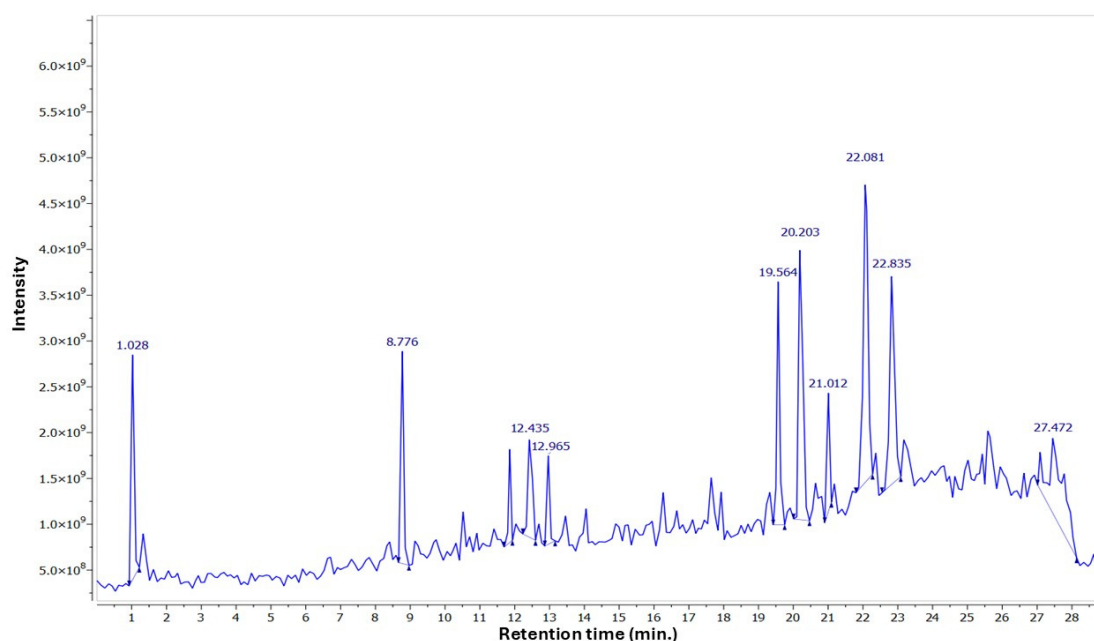


Figure 7. ESI-TIC negative mode chromatogram of *A. nidulans* ethyl acetate extract.

Many of these previously reported compounds have shown promising biological activities. Acetophthalidin, derived from *A. fumigatus* BM923, was reported to exhibit inhibition of the mammalian cell cycle by [24]. Manniche et al. (2004) [25] demonstrated moderate antimalarial activity against *Plasmodium falciparum* by the Karanakafuran A and B isolated from *A. karnatakaensis* IBT 22154. Auroglaucine from *Eurotium repens* UM-031509 showed good binding affinity for the human cannabinoid receptor [26]. *Aspergillus wentii*-derived Aspewentin B displayed cytotoxicity against marine zoo- and phytoplanktons, as recorded by [27], and demonstrated cytotoxicity against HL-60 and K562 cell lines by 3-hydroxydiorcinol from *A. sydowii* FNA026. Aspergoterpenin B retrieved from *A. versicolor* produced antimicrobial activity on *Erwinia carotovora*. Aspericacid A, isolated from *Aspergillus* sp. LS78, presented significant antifungal activity as reported by [28].

Table 1. List of tentatively identified compounds from *A. nidulans* EtOAc extract analyzed by LC-MS-MS.

No.	t_R	M–H	MF	MS2	Compound Name	Reported Source	Reference
1	7.41	207.03	C ₁₀ H ₈ O ₅	189, 165, 149, 137, 123	Acetophthalidin	<i>Aspergillus fumigatus</i> BM923	[24]
2	7.69	245.12	C ₁₅ H ₁₈ O ₃	227, 201, 199, 183, 169, 155, 139	Aspergillusene B	<i>Aspergillus sydowii</i> PSU-F154	[29]
3	7.79	243.09	C ₁₁ H ₁₆ O ₆	197, 169, 153	Tensyuic acid F	<i>Aspergillus niger</i> FKI-2342	[30]

Table 1. Cont.

No.	t _R	M–H	MF	MS2	Compound Name	Reported Source	Reference
4	10.75	313.14	C ₁₉ H ₂₂ O ₄	295, 277, 257, 251, 223, 213, 171	Karnatakafuran A	<i>Aspergillus karnatakaensis</i> IBT 22154	[25]
5	11.64	229.11	C ₁₁ H ₁₈ O ₅	211, 183, 167, 141	(–)-9-hydroxyhexylitaconic acid	<i>Aspergillus aculeatus</i> CRI322-03	[31]
6	13.04	243.12	C ₁₂ H ₂₀ O ₅	225, 207, 199, 195, 181, 167, 153	9-hydroxyhexylitaconic acid-4-methyl ester	<i>Aspergillus aculeatus</i> CRI322-03	[31]
7	14.08	265.07	C ₁₃ H ₁₄ O ₆	219, 193, 179, 177, 165, 159	Ruakuric acid	<i>Aspergillus fumigatus</i>	[32]
8	14.85	295.13	C ₁₉ H ₂₀ O ₃	277, 251, 171, 155	Karnatakafuran B	<i>Aspergillus karnatakaensis</i> IBT 22154	[25]
9	16.44	297.15	C ₁₉ H ₂₂ O ₃	279, 265, 251, 243, 197	Auroglaucine	<i>Eurotium repens</i> UM-031509	[26]
10	19.60	309.06	C ₁₄ H ₁₄ O ₈	291, 277, 253, 237, 209	Dikojic acid B	<i>Aspergillus flavus</i> GZWMJZ-288	[33]
11	21.04	404.16	C ₂₃ H ₂₃ N ₃ O ₄	402, 357, 320	Asperdiazapinone C	<i>Aspergillus</i> sp. PSU-RSPG185	[34]
12	22.30	339.13	C ₂₀ H ₂₀ O ₅	293, 255, 163	Aurgosin I	<i>Penicillium</i> sp. JP-1	[35]
13	23.15	279.16	C ₁₆ H ₂₄ O ₄	249, 233, 207, 189, 163, 133	Decumbenone A	<i>Aspergillus sulphureus</i> KMM 4640	[36]
14	25.61	283.17	C ₁₉ H ₂₄ O ₂	241, 199	Aspewentin B	<i>Aspergillus wentii</i> na-3	[27]
15	2.28	195.02	C ₁₀ H ₁₂ O ₄	177, 151, 149, 133, 121	3,5-Dimethylorsellinic acid	<i>Aspergillus nidulans</i>	[37]
16	6.66	193.00	C ₁₁ H ₁₄ O ₃	165, 149, 147, 137, 121, 105	2-ethyl-4,6-dihydroxy-3,5-dimethylbenzaldehyde	<i>Aspergillus nidulans</i>	[37]
17	10.94	245.01	C ₁₄ H ₁₄ O ₄	138, 123, 109	3-hydroxydiorsinol	<i>Aspergillus sydowii</i> FNA026	[38]
18	14.08	265.07	C ₁₃ H ₁₄ O ₆	219, 193, 179, 177, 163, 153, 137	Antafumicin A/B	<i>Aspergillus niger</i> NH-401	[39]
19	14.85	295.13	C ₁₅ H ₂₀ O ₆	277, 251, 233, 155, 137	Aspergoterpenin B	<i>Aspergillus versicolor</i>	[33]
20	24.47	281.16	C ₁₆ H ₂₆ O ₄	263, 237, 209, 187	Asperic acid A	<i>Aspergillus</i> sp. LS78	[28]

The antibacterial results of the study demonstrated the efficacy of *A. nidulans* extract, MOF, and MOF + extract against *B. cepacian*, *E. coli*, and *S. typhimurium*. Notable variations among the tested agents were observed where MOF + extract at different concentrations exhibited the most potent antibacterial activity with inhibition zones of 19.33, 13.67, and 10.67 mm at concentration 10%, 5%, and 2.5%, respectively, compared with *A. nidulans* extract and MOF individually (Table 2). Meanwhile, no antibacterial activities were demonstrated against *E. coli* and *S. typhimurium* at all concentrations of the tested agents.

Table 2. The antibacterial activity, in mm, of *A. nidulans* extract, ZIF-8, and ZIF-8@*nidulans* against *B. cepacian* bacterial strain.

Test Agent	Concentration		
	10%	5%	2.5%
<i>A. nidulans</i> extract	13.33 ± 33 ^c	10.33 ± 33 ^c	7.67 ± 33 ^c
MOF	10.67 ± 33 ^d	9.33 ± 33 ^c	7.00 ± 00 ^c
MOF + Extract	19.33 ± 33 ^b	13.67 ± 33 ^b	10.67 ± 33 ^b
Chloramphenicol	31.67 ± 88 ^a	20.67 ± 33 ^a	12.33 ± 67 ^a

Data were presented as mean ± SE, and values accompanied by different letters are significant at $p < 0.05$.

In the current study, the *A. nidulans* extract, ZIF-8, and ZIF-8@*nidulans*, in addition to standard Doxorubicin, were tested for their effect on the cell viability of human colon cancer (HCT-116) cell line using SRB assay, and the IC₅₀ values were determined from dose–response curves of different concentrations. In comparison with doxorubicin

($IC_{50} = 0.24 \mu\text{g/mL}$), the cytotoxicity assay of *A. nidulans* extract, ZIF-8, and ZIF-8@*nidulans* indicated IC_{50} values of 23.82, 23.12, and 6.11 $\mu\text{g/mL}$, respectively, thus exhibiting potent cytotoxicity of MOF + extract against the HCT-116 cell line (Figure 8). At different tested concentrations, Figure 9 shows the effects of *A. nidulans* extract, MOF, and MOF + extract on the HCT-116 cell line compared with the control and doxorubicin treatment.

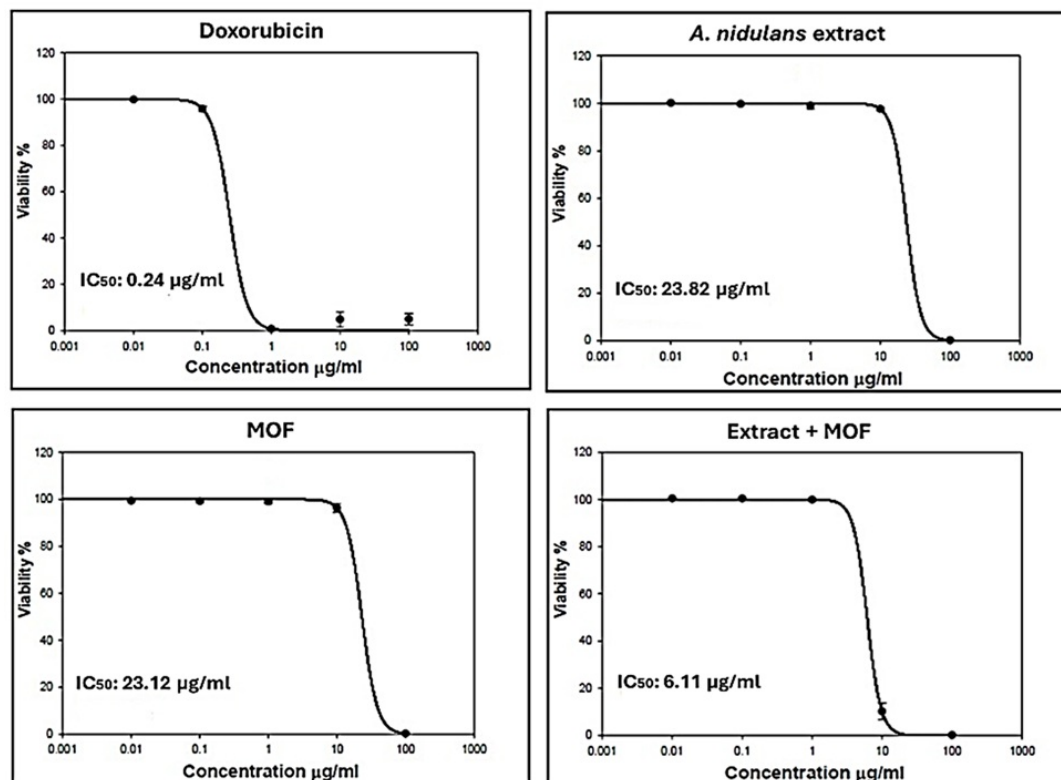


Figure 8. Curves of IC_{50} dose–response of cytotoxic activities of Doxorubicin, *A. nidulans* extract, ZIF-8, and ZIF-8@*nidulans* against the HCT-116 cell line.

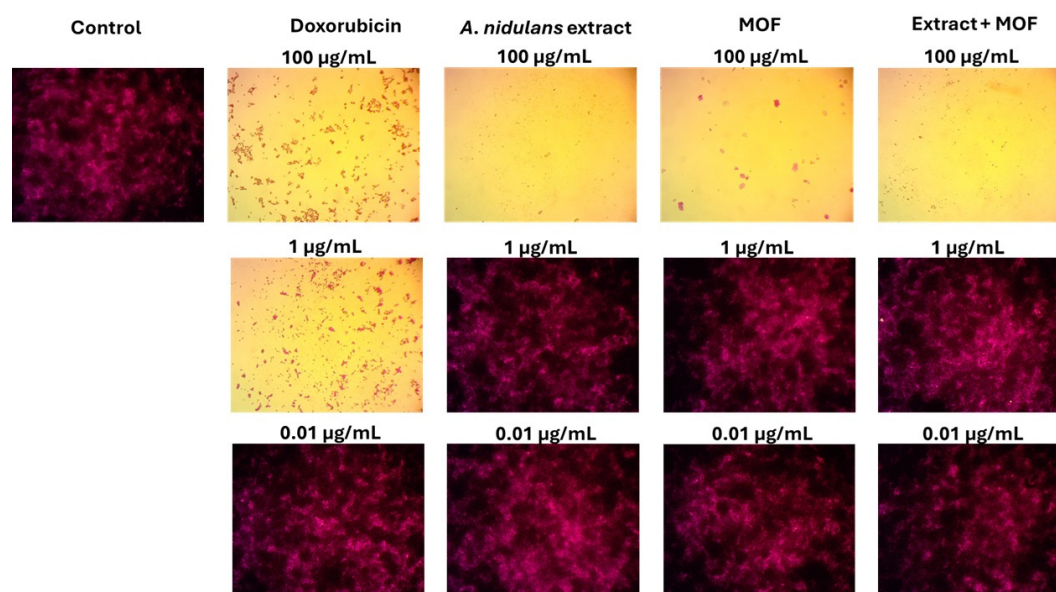


Figure 9. Optical microscope images of cytotoxicity assay of Doxorubicin, *A. nidulans* extract, ZIF-8, and ZIF-8@*nidulans* against HCT-116 cell line, magnification power: X100.

3. Experimental Work

3.1. Fungal Extract Preparation

Aspergillus nidulans AUMC 15444 (GenBank accession no. OR064351) was utilized to prepare fungal extract by fermentation on rice medium for one month at 30 °C [40]. After that, extraction by ethyl acetate (EtOAc) was carried out, defatted with n-hexane, and then the filtrate was evaporated to obtain a dry extract [41].

3.2. LC-ESI-MS/MS Analysis of *A. nidulans* Extract

The SCIEX Triple Quad 5500+ MS/MS system (Agilent Technologies, Waldbronn, Germany) is equipped with an electrospray ionization (ESI) negative mode for ion detection. The experimental condition and tentative identification of compounds were conducted using the reported protocol used by [42]. A blank control was included in the LC-MS/MS analysis, and the blank peaks were filtered and excluded using MSdial software version 4.92.

3.3. Antibacterial Investigation

The antibacterial efficacy of the samples was assessed by the well diffusion method [43], on Muller–Hinton agar medium, against *Burkholderia cepacia* ATCC 25416, *Escherichia coli* ATCC 8739, and *Salmonella typhimurium* ATCC 14028 strains at three concentrations (10, 5, 2.5%). Chloramphenicol (1%, 0.5%, and 0.25%) was used as a standard (positive control), while sterile distilled H₂O and dimethyl sulfoxide were used as negative controls. The diameter of the inhibition zone around the well (6 mm) was considered positive activity.

3.4. Cytotoxicity Assay

The cancer cell lines were obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). HCT-116 (Human colon cancer) cells were cultured in RPMI medium amended with 100 units/mL penicillin, 100 mg/mL streptomycin, and 10% heat inactivated fetal bovine serum and incubated in a humidified 5% CO₂ atmosphere at 37 °C. Different concentrations of the samples were tested to determine the IC₅₀ in addition to Doxorubicin as a standard. The Sulforhodamine B (SRB) assay was employed to estimate cancer cell viability, and a BMG LABTECH FLUOstar Omega microplate reader (Ortenberg, Germany) was utilized to measure absorbance at 540 nm [44].

3.5. Synthesis of Pristine ZIF-8 and ZIF-8@*nidulans* Nanocomposites

Pristine ZIF-8 was prepared via a modified, eco-friendly room-temperature chemical co-precipitation method. In a typical procedure, Zn(NO₃)₂·6H₂O and 2-methylimidazole were dissolved separately in deionized water at a predetermined molar ratio [45]. The 2-methylimidazole solution was rapidly poured into the zinc nitrate solution under vigorous magnetic stirring for 2 h. The resulting white precipitate was isolated via high-speed centrifugation, washed three times with fresh water/ethanol to remove unreacted precursors, and dried in a vacuum oven at 60 °C overnight. For the synthesis of the ZIF-8@*nidulans* nanocomposites, a physical–chemical encapsulation technique was adopted. Varied weight fractions (2%, 4%, and 6 wt.%) of the dried *Aspergillus nidulans* extract powder relative to the theoretical mass of ZIF-8 were pre-dissolved in the ligand solution prior to the addition of the zinc salt precursor. The subsequent crystallization, washing, and drying protocols were identical to those used for pristine ZIF-8, yielding light-colored composite powders labeled ZIF-8@2% *nidulans*, ZIF-8@4% *nidulans*, and ZIF-8@6% *nidulans*.

3.6. Statistical Analysis

Every experiment was run three times. The data were reported as the mean ± standard error (SE) and determined below the 0.05 level of significance using multiple comparison tests

(Duncan) and analysis of variance (one-way ANOVA) using the SPSS program, version 16 (IBM, Armonk, NY, USA).

3.7. Material Characterization Techniques

The crystalline phases and structural configurations were investigated using an XRD LANScientific equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 5° to 35° . Surface morphologies and microstructural features were visualized using a FESEM operating at an accelerating voltage of 30.0 kV. Localized elemental distribution and chemical composition metrics were collected via an EDS elemental mapping system attached to the FESEM framework.

3.8. Electrochemical and Photoelectrochemical Measurements

Electrode Preparation: The working electrodes were prepared by dispersing 5 mg of the synthesized material (pristine ZIF-8 or ZIF-8@*nidulans* nanocomposite) in 1 mL of a mixture containing deionized water, isopropanol, and 5 wt.% Nafion[®] solution (3:1:0.05 v/v). The suspension was ultrasonicated for 30 min to ensure uniform dispersion. Then, 10 μL of the resulting ink was drop-cast onto a pre-cleaned FTO glass substrate (active area: 0.25 cm^2) and dried at 60°C for 2 h under ambient conditions. Prior to deposition, the FTO substrates were sequentially cleaned with acetone, ethanol, and deionized water in an ultrasonic bath for 15 min each, followed by drying under nitrogen flow. The mass loading was controlled to be approximately 0.2 mg cm^{-2} for all electrodes.

All electrochemical tests—including Cyclic Voltammetry (CV), linear sweep voltammetry (LSV), Electrochemical Impedance Spectroscopy (EIS), and transient photocurrent responses (i-t)—were recorded using a Corrtest Cs305 electrochemical workstation. A conventional three-electrode cell configuration was employed, containing the synthesized material deposited onto a conductive substrate (e.g., FTO glass or glassy carbon) as the working electrode, a Pt wire as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode, immersed in a suitable aqueous electrolyte. All potentials were converted and calibrated against the Reversible Hydrogen Electrode (RHE) scales via the standard Nernst relation:

$$E_{\text{vs. RHE}} = E_{\text{measured}} + E_{\text{reference}} + 0.059\text{pH}$$

The transient photocurrent measurements (i-t) were carried out under chopped-light on/off cycles using a solar simulator source with a calibrated light intensity. EIS curves were recorded over a frequency span from 100 kHz to 0.1 Hz at an open-circuit potential under an amplitude of 5 mV. Newport 91160-1000 solar simulator equipped with a 1000 W Xenon lamp and an AM 1.5G filter (Irvine, CA, USA).

4. Conclusions

In summary, this study successfully demonstrates the design, characterization, and deployment of an innovative, dual-functional bio-hybrid platform based on a ZIF-8 metal-organic framework integrated with bioactive *Aspergillus nidulans* extract. Detailed structural and elemental analyses (XRD, FESEM, and EDS mapping) confirmed that the biological extract was uniformly incorporated throughout the MOF host without altering its underlying crystalline framework. Photoelectrochemical characterizations confirmed that the loading of the fungal extract strongly dictates the interfacial kinetic pathways and charge-transport rates. The ZIF-8@2% *nidulans* composite emerged as the optimal formulation, demonstrating excellent transient photocurrent stability, reduced charge-transfer resistance (R_{ct}), and a minimized Tafel kinetic slope of 310 mV/dec , indicating that hydrogen generation proceeds via efficient Tafel recombination. This marked enhancement stems from a unique photobio-

chemical synergy, in which the fungal extract serves as an integrated proposed mechanistic hypothesis that suppresses charge recombination and accelerates electron injection into the ZIF-8 structure. Concurrently, the material exhibited strong antimicrobial properties, driven by the structural synergy between the components. Ultimately, this work offers an eco-friendly approach to developing high-efficiency, multi-functional nanomaterials from biological extracts, paving the way for advanced applications in solar energy harvesting and biomedical technologies.

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