

Antibacterial Activity of Ag@HKUST-1-Encapsulated Cinnamomum sintoc Essential Oil Against *Salmonella typhi*

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Abstract

Typhoid fever, caused by *Salmonella typhi* (*S. typhi*), remains a pressing public health issue in developing regions, due to rising antimicrobial resistance and poor sanitation. In response to this challenge, this study explores the antibacterial potential of *Cinnamomum sintoc* (*C. sintoc*) essential oil, a traditional medicinal plant used by indigenous Dayak communities, by encapsulating it into a silver-doped metal-organic framework, Ag@HKUST-1, and evaluates its antibacterial activity against *S. typhi*. *C. sintoc* oil was extracted via steam distillation, and its chemical profile was analyzed using GC-MS. The Ag@HKUST-1 composite was synthesized through solvothermal assembly of HKUST-1, followed by in situ incorporation of silver nanoparticles (AgNPs). Characterization via XRD, FTIR, nitrogen physisorption, SEM-EDS, TEM, and UV-Vis, confirmed successful formation and integration of AgNPs into the HKUST-1 framework. Encapsulation efficiency was highest at 1.5 mL of oil (16.82%), indicating an optimal loading threshold. Antibacterial assays using the agar disk diffusion method showed that the supernatant of the encapsulated system had the strongest inhibition at 1.5 mL (4.0 mm), while the solid fraction exhibited a sustained release effect, with stable inhibition up to 2.7 mm (3 mL). These results suggest that Ag@HKUST-1 serves as an effective carrier for volatile bioactives, enabling controlled delivery and enhanced antibacterial performance. This approach highlights a promising strategy for combating drug-resistant typhoid infections through MOF-assisted phytochemical delivery systems.

1. Introduction

Typhoid fever is a disease caused by the gram-negative bacterium *Salmonella Typhi* (*S. typhi*) and remains a formidable public health burden globally [1]. This pathogen not only poses severe threats to human health but also precipitates substantial economic losses. Infection is primarily transmitted through the ingestion of contaminated food or water, followed by invasion of intestinal epithelial cells, internalization within phagocytes, and systemic dissemination [2]. Crucially, following acute infection, *S. typhi* can colonize the gallbladder, where it persists long after symptoms subside, effectively serving as a chronic reservoir for further disease transmission.

While typhoid fever is historically treatable with antibiotics, the efficacy of standard therapeutic regimens is rapidly diminishing due to the escalating prevalence of antimicrobial resistance. Local surveillance data indicate that *S. typhi* strains exhibit a 44.6% resistance rate to ampicillin. This trend is exacerbated by antibiotic misuse in regions such as Central Kalimantan, Indonesia where over one-third of prescriptions deviate from recommended dosages and nearly half violate procedural guidelines [3]. Consequently, clinical practice has been forced into a premature shift toward third-generation antibiotics, highlighting the urgent necessity for alternative, sustainable antimicrobial strategies.

Given these challenges, natural products offer a promising avenue for developing novel antibacterial agents. Specifically, the essential oil of *Cinnamomum sintoc* (*C. sintoc*), traditionally utilized by the Dayak Deah and Linoh tribes for its medicinal properties, has garnered attention. Sumiwi et al. (2015) confirmed that *C. sintoc* essential oil possesses significant antibacterial, analgesic, anti-inflammatory,

and antioxidant activities [4]. However, the therapeutic application of essential oils in their unmodified form is severely limited by their high volatility and physicochemical instability under environmental conditions.

To address these limitations, advanced drug delivery systems are required to enhance stability and enable the controlled release of bioactive compounds. Metal-Organic Frameworks (MOFs), a class of materials characterized by metal ions coordinated with organic ligands to form highly porous networks, have emerged as ideal candidates. Among these, the copper-based MOF, HKUST-1 ($\text{Cu}_3(\text{BTC})_2$), is particularly notable for its high surface area, porous crystalline structure, and intrinsic antibacterial properties [5]. Furthermore, the functionalization of MOFs with AgNPs to form Ag@HKUST-1 composites offers a synergistic enhancement. AgNPs are potent antibacterial agents that induce cell death by generating reactive oxygen species (ROS) [6], [7]. As one of the most widely commercialized nanomaterials, AgNPs are favored for their established efficacy and relatively lower toxicity compared to other metallic nanoparticles [8].

Despite these advancements, the encapsulation of *C. sintoc* essential oil within an Ag@HKUST-1 composite for use against *S. typhi* has not been explored. Previous research has primarily focused on encapsulating thymol or cinnamon oils in AgNPs or MOFs to combat agricultural pathogens like *Botrytis cinerea* and *Colletotrichum musae*, or generic bacteria such as *S. aureus* and *E. coli* [9]–[12]. Furthermore, existing studies on Ag@HKUST-1 have primarily focused on applications in cancer theranostics and the degradation of chemical agents such as sulfur mustard, without addressing bacterial inhibition via essential oil delivery [13], [14]. Therefore, this study aims to bridge this gap by synthesizing and characterizing an Ag@HKUST-1 composite loaded with *C. sintoc* essential oil and assessing its antibacterial efficacy. By integrating traditional ethnopharmacological knowledge with cutting-edge materials engineering, this research seeks to provide a novel, effective, and sustainable strategy to combat *S. typhi*.

2. Materials and Methods

2.1 Reagents and Chemicals

All reagents and chemicals used in this study were of analytical grade and used without further purification. Copper(II) nitrate trihydrate [$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$], 1,3,5-benzenetricarboxylic acid (trimesic acid, H_3BTC), ethanol (EtOH), N,N-dimethylformamide (DMF), silver nitrate (AgNO_3), sodium borohydride (NaBH_4), and sodium chloride (NaCl) were purchased from Merck (Germany). Absolute ethanol was used as the solvent in synthesis and encapsulation steps. Deionized distilled water was used for all aqueous preparations. Essential oil of *Cinnamomum sintoc* was extracted in-house from fresh root material obtained from Palangka Raya, Central Kalimantan, Indonesia. Nutrient Agar (NA) media for antibacterial testing was obtained from Himedia Laboratories (India). Chloramphenicol antibiotic standard (1%) was used as the positive control in antibacterial assays.

2.2 Extraction and Characterization of *Cinnamomum sintoc* (Sintoc) Essential Oil

The essential oil was extracted from the roots of *Cinnamomum sintoc* using a steam distillation method with the following steps: (1) cleaning and cutting of plant samples, (2) distillation of 100 g of root sample using a sample-to-water ratio of 1:2 for 8 hours at $95 \pm 5^\circ\text{C}$, and (3) collection of essential oil in vials for yield calculation. The chemical composition of the extracted oil was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS).

2.3 Synthesis of HKUST-1

To synthesize HKUST-1, 0.7248 g of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and 0.2103 g of H_3BTC in a mixed solvent of ethanol and dimethylformamide (DMF) (40 mL total volume). The solution was stirred for 10 minutes, then subjected to solvothermal synthesis at 80°C for 20 hours. The resulting product was filtered and washed three times with an ethanol:water solution (1:1 v/v), followed by drying at 60°C for 12 hours to yield HKUST-1 in powder form.

2.4 Synthesis of Ag@HKUST-1

To prepare the Ag@HKUST-1 composite, 0.5 g of HKUST-1 powder was dispersed in 100 mL of ethanol and stirred to form a suspension. A solution of 0.1 g AgNO_3 in 15 mL distilled water was then added, followed by the dropwise addition of 0.105 g NaBH_4 dissolved in 3 mL distilled water. The mixture was stirred for 2 hours until a greenish-black solution formed, indicating the formation of silver nanoparticles within the HKUST-1 matrix. The composite was filtered, washed with ethanol, and dried at 60°C for 12 hours. In addition, pure AgNPs were synthesized separately by mixing 600 mL of 2 mM NaBH_4 solution with 200 mL of 1 mM AgNO_3 solution. The reaction was carried out at room temperature with constant stirring for 2 minutes, producing a yellow-orange colloid. This solution was dried at 80°C for 12 hours to obtain AgNPs powder.

2.5 Characterization of Materials

The synthesized materials were characterized using a variety of analytical techniques. Crystallinity was assessed with an XRD PANalytical EMPYREAN X-ray diffractometer, operating in the 2θ range of $5-50^\circ$ with Cu-K α radiation ($\lambda = 1.540593 \text{ \AA}$). The functional groups of the materials were identified using a Bruker Tensor II FTIR spectrometer equipped with the attenuated total reflectance (ATR) method, covering a spectral range of $4000 - 300 \text{ cm}^{-1}$. Ultraviolet-visible (UV-Vis) spectra were recorded on a Shimadzu S-600 spectrophotometer within the 400–800 nm wavelength range. Nanoparticle size distribution was analyzed using HR-TEM (Hitachi H-9500) at varying magnifications, and particle size was determined via image processing software (ImageJ). Morphological analysis was performed using scanning electron microscopes integrated with energy-dispersive spectrometer (SEM-EDS) using Thermo Fisher Phenom ProX. Nitrogen physisorption measurements were carried out using a NOVAtouch LX2 (BET Surface Area and Pore Size Analyzer) at 77 K after degassing the sample at 225°C for 6 hours.

2.6 Encapsulation of *C. Sintoc* Essential Oil into Ag@HKUST-1

A total of 3 mg of Ag@HKUST-1 composite was dispersed in ethanol, then mixed with 1 mL of *C. sintoc* essential oil. The mixture was stirred at room temperature for 24 hours to facilitate encapsulation. Following this, the mixture was centrifuged to separate the precipitate and supernatant. The chemical composition of the supernatant was analyzed using GC-MS to assess encapsulation performance. For optimization, the volume of essential oil used in the encapsulation process was varied from 0.5 to 3 mL. In each experiment, 3 mg of Ag@HKUST-1 was dispersed in absolute ethanol, followed by the addition of a specific volume of essential oil. The mixture was stirred for 24 hours, centrifuged, and the resulting supernatant was analyzed using UV-Vis spectrophotometry to obtain absorbance data, which was converted into encapsulation efficiency value using the equation below.

$$\text{Encapsulation Efficiency} = \left(\frac{m_1}{m_2} \right) \times 100\%$$

where m_1 is mass of encapsulated *C. sintoc* oil, and m_2 is total mass of oil used in the encapsulation process.

2.7 Antibacterial Assay of *Salmonella typhi* with Encapsulated Sintoc Oil in Ag@HKUST-1

The antibacterial activity of the essential oil-encapsulated Ag@HKUST-1 composite was tested against *S. typhi* using the agar disk diffusion method. Variations in essential oil volumes used for encapsulation included 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL. Sterile Nutrient Agar was used as the culture medium, autoclaved at 121°C for 20 minutes, and poured into petri dishes. A standardized *S. typhi* inoculum was prepared by suspending the bacteria in 2 mL of 0.9% NaCl solution to match the turbidity of 0.5 McFarland standard. A 100 μ L aliquot of bacterial suspension was spread evenly across the agar surface using a sterile spreader. Sterile paper disks (6 mm diameter) were impregnated with 100 μ L of each test sample and placed on the inoculated agar. Chloramphenicol 1% (filtered through a 0.2 μ m cellulose membrane) was used as a positive control, while distilled water served as a negative control. Plates were incubated at 37°C for 16 hours. Inhibition zones (in mm) around each disk were measured using a digital caliper to evaluate antibacterial activity against both the precipitate and the supernatant. All experiments were performed in triplicate to ensure reproducibility and reliability of the results.

3. Results and Discussion

3.1 Essential Oil Extraction and Chemical Characterization

The initial step in the exploration of natural products for therapeutic purposes involves the extraction and characterization of their bioactive components. In this study, essential oil from the roots of

Cinnamomum sintoc was extracted using steam distillation, yielding 0.5% from 10 kg of raw material. This yield is a fundamental parameter that provides a preliminary indication of production feasibility and serves as a basis for further phytochemical investigations.

The distilled *C. sintoc* oil was subsequently analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The analysis revealed approximately 30 peaks, each representing distinct chemical constituents of the essential oil (Fig. 1a). The major component identified was safrole, a phenylpropanoid compound, contributing the highest percentage (26.08%) of the total composition [4]. Other phenylpropanoids, such as methyl eugenol (3.43%) and estragole (0.15%), were also detected, though in smaller amounts. The GC-MS results are summarized in Table 1.

Hydrocarbon monoterpenes represented the most abundant group in the essential oil, accounting for approximately 56.76% of the total composition [15]. Notable compounds within this group included D-camphene (18.30%), α -pinene (12.46%), and β -pinene (10.82%). Other identified hydrocarbon monoterpenes included β -myrcene (1.87%), sabinene (2.20%), α -phellandrene (0.07%), and γ -terpinene (0.26%). Oxygenated monoterpenes, such as eucalyptol (7.31%) and linalool (1.27%), made up around 8.03% of the total composition. Collectively, phenylpropanoids (safrole, methyl eugenol, and estragole) contributed 30.56% of the oil content. Minor components, including sesquiterpenes and aromatic compounds, represented only 2.65% of the total. Examples of these minor compounds include caryophyllene (0.11%), (+)- δ -cadinene (0.14%), and α -calacorene (0.08%).

3.2 Characterization of Ag@HKUST-1

The synthesis of the Ag@HKUST-1 composite was successfully carried out by integrating AgNPs, reduced using NaBH_4 , into the metal–organic framework HKUST-1. The reduction of Ag^+ ions by NaBH_4 occurred rapidly, resulting in relatively uniform nanoparticles at the nanometer scale. The porous HKUST-1 framework served not only as a physical support but also as a growth-controlling matrix, effectively minimizing the aggregation of AgNPs. Characterization of Ag@HKUST-1 was performed using multiple analytical techniques, including XRD, FTIR, Nitrogen physisorption, SEM-EDX Mapping, TEM, and UV-Vis spectroscopy.

X-ray Diffraction (XRD) analysis was conducted to assess the crystallinity of the synthesized materials. The XRD patterns for AgNPs, HKUST-1, and Ag@HKUST-1 are shown in Fig. 2. The diffraction pattern of HKUST-1 exhibited peaks at $2\theta = 6.76^\circ, 9.42^\circ, 11.62^\circ, 13.46^\circ, 14.57^\circ, 17.51^\circ, 18.98^\circ,$ and 20.20° , which correspond to the Miller indices (200), (220), (222), (400), (331), (511), (440), and (600), respectively. These diffraction peaks are consistent with previously reported data for HKUST-1, confirming the formation of a face-centered cubic (fcc) crystal structure with space group Fm-3m and a unit cell parameter of $a = 26.34 \text{ \AA}$ [16]–[18]. The presence of sharp, well-defined peaks indicates that the synthesized HKUST-1 has high crystallinity and homogeneity. The absence of impurity phases such as CuO or Cu_2O confirms the purity of the sample [16]. AgNPs exhibits characteristic peaks at $2\theta = 38.1^\circ, 44.2^\circ, 64.4^\circ, 77.4^\circ,$ and 81.5° , corresponding to the (111), (200), (220), (311), and (222) planes of face-centered cubic silver [19], [20]. These results are consistent with the reference values reported in ICSD

No. 064994 [21]. Furthermore, the diffraction peaks of the Ag@HKUST-1 composite displays diffraction peaks corresponding to both HKUST-1 and AgNPs, indicating the successful incorporation of Ag into the MOF matrix. The presence of both sets of peaks verifies the co-existence of HKUST-1 and Ag within the composite, further confirming the successful synthesis of Ag@HKUST-1.

Fourier Transform Infrared Spectroscopy (FTIR) analysis further confirmed the functional group interactions. The FTIR spectrum of HKUST-1 (Fig. 3a) showed shifts compared to the spectrum of H₃BTC, such as broad O–H stretching bands (2886–3650 cm⁻¹), symmetric COO⁻ stretching at 1561 cm⁻¹, and a shift of the C = O stretching band from 1719 cm⁻¹ to 1619 cm⁻¹. These shifts indicate deprotonation and coordination of carboxylate groups with Cu²⁺ ions. Additional bands at 1440 cm⁻¹ (C–O), 1370 cm⁻¹ (C–C of benzene ring), and 725 cm⁻¹ (Cu–O) further validate the formation of the MOF framework. The FTIR spectrum of AgNPs (Fig. 3b) showed no strong organic stabilizer peaks, suggesting minimal capping agents. Peaks in the range of 1400 – 1000 cm⁻¹ are attributed to residual NO₃⁻ groups, B–H bonds, or surface interactions. The band at 1384 cm⁻¹ confirms the presence of nitrate precursors, while a broad peak around 1630 cm⁻¹ is associated with adsorbed water molecules. A weak band below 1000 cm⁻¹ corresponds to Ag–O bonding, indicating the formation of silver nanoparticles. In the FTIR spectrum of Ag@HKUST-1 (Fig. 3b), characteristic peaks of HKUST-1 are retained, while no distinct vibrational bands for Ag are observed in the 4000 – 400 cm⁻¹ range. This indicates that no new vibrational bonds formed between AgNPs and the HKUST-1 framework, suggesting that the incorporation of Ag does not disrupt the original MOF structure and provides insight into the interaction mode between Ag and HKUST-1.

Nitrogen Physisorption (BET) analysis was conducted to evaluate the surface area and porosity of HKUST-1 and the Ag@HKUST-1 composite using N₂ adsorption-desorption isotherms at 77 K (Fig. 4). HKUST-1 exhibited a Type I isotherm, characteristic of microporous materials, with a BET surface area of 407.9 m²/g, an average pore radius of 1.13 nm, and a total pore volume of 0.230 cm³/g. In contrast, the Ag@HKUST-1 composite displayed a Type IV isotherm with a small hysteresis loop, indicating mesoporous character. The composite showed a drastic decrease in surface area to 38.1 m²/g, an increase in average pore radius to 3.29 nm, and a reduced pore volume of 0.0628 cm³/g. This decrease in surface area and micropore volume suggests that the incorporation of AgNPs partially blocked the pores and disrupted the MOF structure. However, the larger pore size indicates that accessible cavities remain, potentially enhancing mass transfer and offering active sites from silver. Overall, the Type I isotherm of HKUST-1 confirms its microporous nature, suitable for adsorption and gas storage applications. Meanwhile, the Type IV isotherm of Ag@HKUST-1, which combines moderate porosity and the functional properties of silver, presents a promising candidate for catalysis, antimicrobial applications, and sensing platforms, where both surface area and silver content are crucial.

Scanning Electron Microscopy (SEM) analysis revealed clear morphological differences among HKUST-1, AgNPs, and the Ag@HKUST-1 composite. As shown in Fig. 5a, pristine HKUST-1 exhibited well-defined octahedral crystals with smooth surfaces and sharp edges, typical of highly crystalline MOFs. In contrast, AgNPs (Fig. 5b) appeared as irregularly shaped aggregates with rough surfaces, indicating a

tendency for agglomeration due to the reactive nature of silver surfaces. In the composite structure (Fig. 5c), Ag@HKUST-1 exhibited a more compact and rough surface morphology, deviating from the regular octahedral shape of pure HKUST-1. This suggests that silver nanoparticles were successfully integrated into the MOF framework, resulting in morphological changes and partial collapse of the crystal structure, supporting the formation of the composite. Energy-Dispersive X-ray (EDX) analysis confirmed the elemental composition of each sample. The EDX spectrum of HKUST-1 (Fig. 5d) showed only Cu, C, and O, consistent with its framework composition of BTC ligands and Cu²⁺ ions. In the AgNP spectrum (Fig. 5e), a strong Ag signal was observed, with minor peaks for C and O possibly due to residual reagents or substrate interference. For the Ag@HKUST-1 composite (Fig. 5f), strong signals for Cu, C, and O were observed alongside a distinct Ag peak, confirming the successful doping or dispersion of AgNPs into the HKUST-1 framework. Overall, the combination of SEM and EDX analyses confirms that silver nanoparticles were successfully incorporated into HKUST-1, resulting in significant morphological changes and the presence of Ag peaks in the EDX spectrum, further validating the successful synthesis of the Ag@HKUST-1 composite.

Elemental Mapping results are shown in Fig. 6 for HKUST-1 (a-d), AgNPs (e-h), and Ag@HKUST-1 (i-l). The mapping results are consistent with EDX data, confirming that silver (Ag) is evenly distributed throughout the HKUST-1 MOF matrix. These findings from both EDX and elemental mapping conclusively demonstrate that Ag has been successfully incorporated into the HKUST-1 matrix, and the Ag@HKUST-1 composite has been effectively synthesized.

Transmission Electron Microscopy (TEM) analysis was performed to investigate the structure and dispersion of AgNPs before and after integration into the HKUST-1 MOF framework. The TEM image in Fig. 7a shows that the synthesized AgNPs are small, with round to semi-round morphology, and display the characteristic dark contrast of metallic silver. Some particles appear to form dense, irregular agglomerates due to the highly reactive surface of silver. This agglomeration indicates the limited physical stability of pure AgNPs, which may reduce their effectiveness in antibacterial applications. The particle size distribution histogram indicates that the average diameter of the AgNPs is 17.37 ± 0.81 nm, with a relatively wide size range. This is consistent with the characteristics of AgNPs synthesized via wet-chemical methods. A small number of larger particles ($\sim 40\text{--}60$ nm) likely resulted from agglomeration during synthesis. Particle size variation is important as it influences specific surface area and reactivity; smaller particles possess a higher surface-to-volume ratio, which directly enhances catalytic properties, antibacterial activity, and plasmonic response [22].

In contrast, Fig. 7b presents the TEM image of the Ag@HKUST-1 composite, showing that AgNPs are evenly dispersed within the layered structure characteristic of the HKUST-1. The incorporation of silver particles into the pores or on the surface of HKUST-1 is clearly visible, with a significantly lower degree of agglomeration compared to pure AgNPs, indicating improved morphological stability. The particle size distribution histogram shows an average diameter of 21.56 ± 0.44 nm. Although slightly larger than pure AgNPs, the particle sizes in the composite are more uniform and homogeneous. This suggests that HKUST-1 not only acts as a carrier matrix but also regulates particle growth, prevents agglomeration, and

stabilizes the overall structure of AgNPs. Thus, the integration of AgNPs into the HKUST-1 framework via Ag@HKUST-1 composite formation effectively improves particle homogeneity and morphological stability. This combination strongly supports the composite's effectiveness in antibacterial applications, as it enables a more controlled and consistent release of Ag⁺ ions.

UV-Vis Spectrophotometric Analysis was conducted to monitor the formation and stability of AgNPs. AgNPs were synthesized by mixing sodium borohydride (NaBH₄) and silver nitrate (AgNO₃), which resulted in a visible color change from colorless to yellow, an indication of AgNP formation due to surface plasmon resonance (SPR) [23]. The appearance of this yellow color corresponds to specific SPR absorption, which can be monitored using UV-Vis spectrophotometry [24]. This SPR band arises from the oscillation of conduction electrons in the nanoparticles. UV-Vis spectra were recorded in the wavelength range of 300–800 nm (Fig. 8).

3.3 Encapsulation of *C. Sintoc* Essential Oil into Ag@HKUST-1

A significant difference was observed in the composition of volatile compounds contained in *C. sintoc* essential oil before and after the encapsulation process. These changes are clearly evident in both the pattern and number of peaks recorded in the chromatogram generated by GC-MS analysis. Prior to encapsulation, the essential oil exhibited 30 chromatographic peaks. This number increased to 36 peaks after encapsulation, indicating a broader variety of chemical compounds detected in the oil once encapsulated within the Ag@HKUST-1 composite matrix, as shown in Fig. 1b.

Before encapsulation, the oil was dominated by various terpenoid compounds, including α-pinene, D-camphene, and β-pinene. Additionally, aromatic and phenylpropanoid compounds such as safrole and methyl eugenol were also present. Among all identified compounds, safrole stood out as the major constituent, contributing 26.08% of the total composition. The encapsulation process using the Ag@HKUST-1 composite led to notable changes in both the distribution and relative concentrations of these compounds. Specifically, the concentration of safrole decreased significantly, from 26.08% to 12.03%. In contrast, other compounds such as eucalyptol increased in abundance. In the encapsulated oil, eucalyptol contributed 12.19%, compared to only 7.31% before encapsulation. This phenomenon suggests a favorable or selective interaction between the Ag@HKUST-1 composite structure and oxygenated terpenoids such as eucalyptol. These compositional changes are detailed in Table 1.

Table 1
Comparison of *C. sintoc* essential oil compound profiles before and after encapsulation with Ag@HKUST-1 composite

No.	Compound	Group	Retention Time (min)	%Area	
				<i>C. sintoc</i>	<i>C. sintoc</i> Ag@HKUST-1
1	α -Tujena	Terpenoid (monoterpenoid hydrocarbon)	7.252	0.55	0.36
2	α -Pinene	Terpenoid (monoterpenoid hydrocarbon)	7.504	12.46	14.80
3	α -Pinene	Terpenoid (monoterpenoid hydrocarbon)	7.650	0.00	0.01
4	D-Camphene	Terpenoid (monoterpenoid hydrocarbon)	8.031	18.30	-
5	Sabinene	Terpenoid (monoterpenoid hydrocarbon)	8.901	2.20	2.60
6	β -Pinene	Terpenoid (monoterpenoid hydrocarbon)	9.107	10.82	11.78
7	β -Myrcene	Terpenoid (monoterpenoid hydrocarbon)	9.490	1.87	2.17
8	α -Felandrene	Terpenoid (monoterpenoid hydrocarbon)	10.185	0.07	0.10
9	α -terpinene	Terpenoid (monoterpenoid hydrocarbon)	10.619	0.17	-
10	m-Simene	Aromatic (aryl propanoid)	10.922	1.82	-
11	D-Limonene	Terpenoid (monoterpenoid hydrocarbon)	11.139	9.42	-
12	Eucalyptol	Terpenoid (monoterpenoid oxygenated)	11.265	7.31	12.19
13	Trans- β -Ocimene	Terpenoid (monoterpenoid hydrocarbon)	11.845	0.04	0.06

No.	Compound	Group	Retention Time (min)	%Area	
				<i>C. sintoc</i>	<i>C. sintoc</i> Ag@HKUST-1
14	γ -Terpinene	Terpenoid (monoterpenoid hydrocarbon)	12.361	0.26	0.28
15	Furanone	Aryl propanoid	12.927	0.11	0.41
16	3,4-Dimethylbenzyl alcohol	Aryl propanoid	13.550	0.13	-
17	D-Fenchone	Terpenoid (monoterpenoid hydrocarbon)	13.685	0.21	0.28
18	Linalool	Terpenoid (monoterpenoid oxygenated)	14.186	1.27	3.56
19	Isoborneol	Terpenoid (monoterpenoid hydrocarbon)	16.305	0.28	-
20	Sabinene	Terpenoid (monoterpenoid hydrocarbon)	17.872	0.10	-
21	α -Terpineol	Terpenoid (monoterpenoid oxygenated)	18.544	0.07	0.18
22	Estragole	Aryl propanoid	18.649	0.15	0.10
23	Safrole	Phenylpropanoid	22.855	26.08	12.03
24	α -Kubebene	Terpenoid (monoterpenoid hydrocarbon)	26.615	2.29	1.37
25	Methyl eugenol	Aryl propanoid	27.613	3.43	3.15
26	Drimenol	Terpenoid (monoterpenoid hydrocarbon)	27.865	0.17	-
27	Caryophyllene	Sesquiterpene	28.466	0.11	0.06
28	β -Selinene	Sesquiterpene	31.325	0.07	0.03
29	(+)- δ -Cadinene	Sesquiterpene	32.532	0.14	0.06
30	α -Calacorene	Sesquiterpene	33.398	0.08	0.04
31	Bornylene	Terpenoid (monoterpenoid	6.604	-	0.10

No.	Compound	Group	Retention Time (min)	%Area	
				<i>C. sintoc</i>	<i>C. sintoc</i> Ag@HKUST-1
		hydrocarbon)			
32	α -Gurjunene	Sesquiterpene	7.235	-	0.37
33	α -Fensene	Terpenoid (monoterpenoid hydrocarbon)	8.061	-	20.96
34	Cyclohexene, 4-ethenyl-1,4-dimethyl-	Aromatic (aryl propanoid)	11.161	-	9.76
35	β -Ocimene	Terpenoid (monoterpenoid hydrocarbon)	11.845	-	0.06
36	cis-2-Norbornanol	Terpenoid (monoterpenoid hydrocarbon)	15.549	-	0.02
37	Camphor	Terpenoid (monoterpenoid hydrocarbon)	16.293	-	0.35
38	4-Terpeneol	Terpenoid (monoterpenoid oxygenated)	17.858	-	0.20
39	Asifilene	Terpenoid (monoterpenoid hydrocarbon)	25.677	-	0.02
40	Cyclosativene	Terpenoid (monoterpenoid hydrocarbon)	26.284	-	0.03
41	(+)-Cycloisolongifol-5-ol	Terpenoid (monoterpenoid hydrocarbon)	27.862	-	0.10

A comparison of compound class abundance in free vs. encapsulated *C. sintoc* oil also reveals a marked shift after encapsulation (Table 2). In the unencapsulated oil, the monoterpene hydrocarbon group was the most dominant class, with compounds such as α -pinene, D-camphene, β -pinene, and safrole contributing significantly to its overall abundance. However, after encapsulation with Ag@HKUST-1, the abundance of this group decreased, though it remained dominant. Notably, D-camphene was no longer detected post-encapsulation, likely due to its interaction with the composite matrix, which may have altered its volatility or caused binding within the framework. In contrast, oxygenated monoterpenes showed increased abundance following encapsulation. This suggests that the Ag@HKUST-1 composite enhances the stability of oxygenated terpenoids, potentially leading to stronger bioactive effects in the encapsulated oil [25]. The aromatic group (arylpropanoids) also showed a significant compositional shift

after encapsulation. New compounds such as cyclohexene and β -ocimene were detected in the GC-MS profile of the encapsulated oil, indicating chemical interactions between the composite and the oil that resulted in a modified compound profile. A detailed comparison of compound class abundance is presented in Table 2.

Table 2
Comparison of compound class abundance in *C. sintoc* essential oil before and after encapsulation

Compound Group	% Area	
	Before	After
Monoterpen hydrocarbon	70.44	59.16
Monoterpen Oxygenated	9.55	16.77
Aryl Propanoid	7.99	15.72
Phenylpropanoid	26.08	12.03
Sesquiterpene	0.51	0.56

A particularly notable decrease was observed in the phenylpropanoid group, especially safrole, which dropped from 26.08% to 12.03%. Meanwhile, sesquiterpenes such as caryophyllene and β -selinene exhibited only minor decreases. These dynamics reflect the influence of encapsulation on the volatility and molecular retention within the Ag@HKUST-1 matrix. This matrix not only stabilizes volatile compounds but may also catalyze chemical transformations at room temperature [26], [27]. Furthermore, interactions with solvents and prolonged incubation during the encapsulation process can lead to degradation or oxidation, thereby altering the chromatographic profile [28]. Conversely, the increased proportion of certain compounds may reflect molecular size compatibility with the pore geometry of the Ag@HKUST-1 composite, which facilitates more efficient encapsulation [29].

3.4 Encapsulation Efficiency of Sintoc Essential Oil into Ag@HKUST-1

The encapsulation of *C. sintoc* essential oil into the Ag@HKUST-1 composite was evaluated based on varying volumes of added oil (0.5-3 mL). The UV-Vis spectral profiles of the essential oil before and after encapsulation showed slight differences. The unencapsulated oil displayed four absorbance peaks, while the encapsulated sample exhibited only three (Fig. 9). The optimal absorption wavelength was found at 282 nm. The absorbance data from Fig. 9 were converted into encapsulation efficiency.

The mass of encapsulated oil (m_1) was calculated using a linear regression equation derived from a standard calibration curve prepared with different volumes of *C. sintoc* oil. The standard curve with the regression equation ($y = 0,2703x-0,0286$), and the high R^2 value ($R^2 = 0,9964$) indicates a strong linear

relationship between the oil volume and absorbance, confirming the reliability and validity of this method for quantifying encapsulated oil and calculating encapsulation efficiency [30].

The encapsulation of *C. sintoc* essential oil within the Ag@HKUST-1 composite revealed a clear influence of oil volume on encapsulation efficiency. This parameter, defined as the percentage of active compounds successfully entrapped within the carrier matrix, varied significantly with the amount of oil used during the process [31]. The results of this evaluation are summarized in Table 3. Among the tested volumes, the addition of 1.5 mL of essential oil produced the highest encapsulation efficiency, reaching 16.82%. However, as the oil volume increased to 2.0 mL and beyond, a noticeable decline in efficiency was observed, falling to 12.84%, 10.29%, and 9.59% at 2.0, 2.5, and 3.0 mL, respectively. These findings suggest that increasing the amount of oil does not linearly translate into greater encapsulation; on the contrary, excessive volume appears to compromise the system’s overall efficiency.

Table 3
Encapsulation efficiency at various volumes of *C. sintoc* essential oil

Volume of <i>C. sintoc</i> oil (mL)	Encapsulation Efficiency (%)
0.5	8.21
1	8.8
1.5	16.82
2	12.84
2.5	10.29
3	9.59

A graphical representation of the relationship between oil volume and encapsulation efficiency (Fig. 10) shows a distinct bell-shaped trend, with a peak at 1.5 mL. Beyond this optimal point, efficiency declined, indicating a saturation threshold in the encapsulation system. This trend underscores the limited capacity of the Ag@HKUST-1 matrix to accommodate oil molecules, likely due to restrictions in pore volume and available active sites within the MOF framework. Thus, beyond a certain input level, additional oil cannot be effectively retained, resulting in significant loss and inefficiency.

This reduction in efficiency at higher volumes can be attributed to three key factors. First, the pore and active site capacity of Ag@HKUST-1 is inherently limited. As a porous crystalline material, HKUST-1 offers a fixed surface area and pore network capable of hosting only a finite quantity of guest molecules [31]. This capacity appears sufficient to achieve high loading at 1.5 mL. However, exceeding this volume likely causes oil molecules to remain unbound, either due to insufficient pore space or saturation of interaction sites, thus decreasing overall entrapment. Second, the hydrophobic nature of essential oils contributes to aggregation, especially at higher concentrations. In such cases, excess oil may form clusters outside the matrix rather than dispersing evenly within the carrier, leading to poor encapsulation

performance. Third, at elevated concentrations, competitive interactions among oil molecules may disrupt stable binding. The limited number of active sites within the MOF structure forces molecules to compete, which destabilizes the interaction dynamics between the oil and the composite framework. As a result, effective loading decreases, and encapsulation efficiency drops accordingly [12]. Together, these observations reinforce the importance of optimizing the oil-to-carrier ratio in the formulation of MOF-based delivery systems. Achieving a balance between the amount of active compound and the carrier's structural capacity is critical to ensure both high encapsulation efficiency and functional performance of the composite material.

3.5 Antibacterial Assay of *Salmonella typhi* with Encapsulated *C. Sintoc* Oil in Ag@HKUST-1

The antibacterial activity of encapsulated *C. sintoc* essential oil in the Ag@HKUST-1 composite was evaluated against *S. typhi* using the agar disk diffusion method, with variations in oil volume: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL. A 1% chloramphenicol solution was used as the positive control, and distilled water served as the negative control. The diameter of the inhibition zone was measured to assess antibacterial activity in both the precipitate and supernatant fractions. All tests were performed in triplicate for data validation (Fig. 11).

The assay was conducted on two fractions obtained from centrifugation of the encapsulated oil system: the solid (precipitate) and the supernatant. Both fractions were tested across a range of oil volumes (0.5–3.0 mL), and inhibition zones were observed as indicators of antibacterial efficacy. The Ag@HKUST-1 solid fraction exhibited a trend of increasing inhibition zone size with increasing volumes of essential oil, reaching its peak at 3.0 mL (approximately 2.7 mm). Despite slight fluctuations at intermediate volumes (2.0–2.5 mL), the overall pattern remained relatively stable and consistent (Fig. 12). This suggests that active compounds in *C. sintoc* oil are released gradually and in a controlled manner from the Ag@HKUST-1 solid matrix due to the porous structure of the MOF, making it a promising candidate for long-term antibacterial release applications. In contrast, the supernatant showed the highest inhibition zone at 1.5 mL (~ 4.0 mm), followed by a sharp decline at higher volumes (Fig. 12). The supernatant demonstrated strong antibacterial effects at specific volumes but lacked stability as oil volume increased. This instability may be attributed to saturation effects, compound antagonism, or degradation of active compounds in the liquid medium. These findings suggest that while the supernatant is effective for rapid antibacterial action, it may not be suitable for sustained use due to its limited duration and stability. Overall, the solid form of Ag@HKUST-1 produced more consistent antibacterial zones compared to the supernatant, although it did not reach the maximum inhibition levels observed with the latter. Thus, the solid form is better suited for slow and stable release applications, while the supernatant acts as a fast-acting antibacterial agent with limited long-term efficacy.

Furthermore, Fig. 13 illustrates the comparison of inhibition zones among the positive control (K⁺), negative control (K⁻), solid fraction, and supernatant. The positive control exhibited the largest inhibition zone (~ 11 mm), confirming the effectiveness of the standard antibacterial agent. In contrast, the

negative control showed no inhibition zone, verifying that the observed antibacterial activity originated from the encapsulated *C. sintoc* oil within the Ag@HKUST-1 composite, and not from the medium or solvent.

4. Conclusions

This study successfully demonstrated the synthesis and characterization of a novel Ag@HKUST-1 composite as a nanocarrier for encapsulating *C. sintoc* essential oil. The composite was prepared via solvothermal and chemical reduction methods, with structural confirmation provided by XRD, FTIR, nitrogen physisorption, SEM-EDS, TEM, and UV-Vis analyses. The encapsulation efficiency varied with oil volume, with the highest efficiency (16.82%) achieved at 1.5 mL, indicating an optimal oil-to-carrier ratio for loading performance. GC-MS analysis revealed significant differences in the volatile compound profiles before and after encapsulation, notably a decrease in safrole content and an increase in eucalyptol, suggesting selective interaction between the oil constituents and the Ag@HKUST-1 matrix. Antibacterial assays against *S. typhi* confirmed that both the solid and supernatant fractions of the encapsulated oil exhibited inhibitory activity, with the supernatant showing rapid action and the solid fraction demonstrating more stable, sustained antibacterial effects. Overall, the Ag@HKUST-1 composite proves to be an effective platform for essential oil delivery, combining the synergistic antibacterial properties of silver and the structural advantages of MOFs. These findings highlight the potential application of this system in developing alternative antibacterial agents, especially for combating drug-resistant pathogens such as *S. typhi*.

Declarations

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contribution

S.U.M.B: conceptualization, investigation, formal analysis, and drafting; E.P.T.: conceptualization, formal analysis, and resource management; J.M.R: formal analysis and visualization of the data; M.I.: validation, and editing of the manuscript; Z.N: Validation and manuscript review; M.R.K.: conceptualization, validation, manuscript review, supervision, and project administration. All authors have read and agreed to the published version of the manuscript.

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Figures

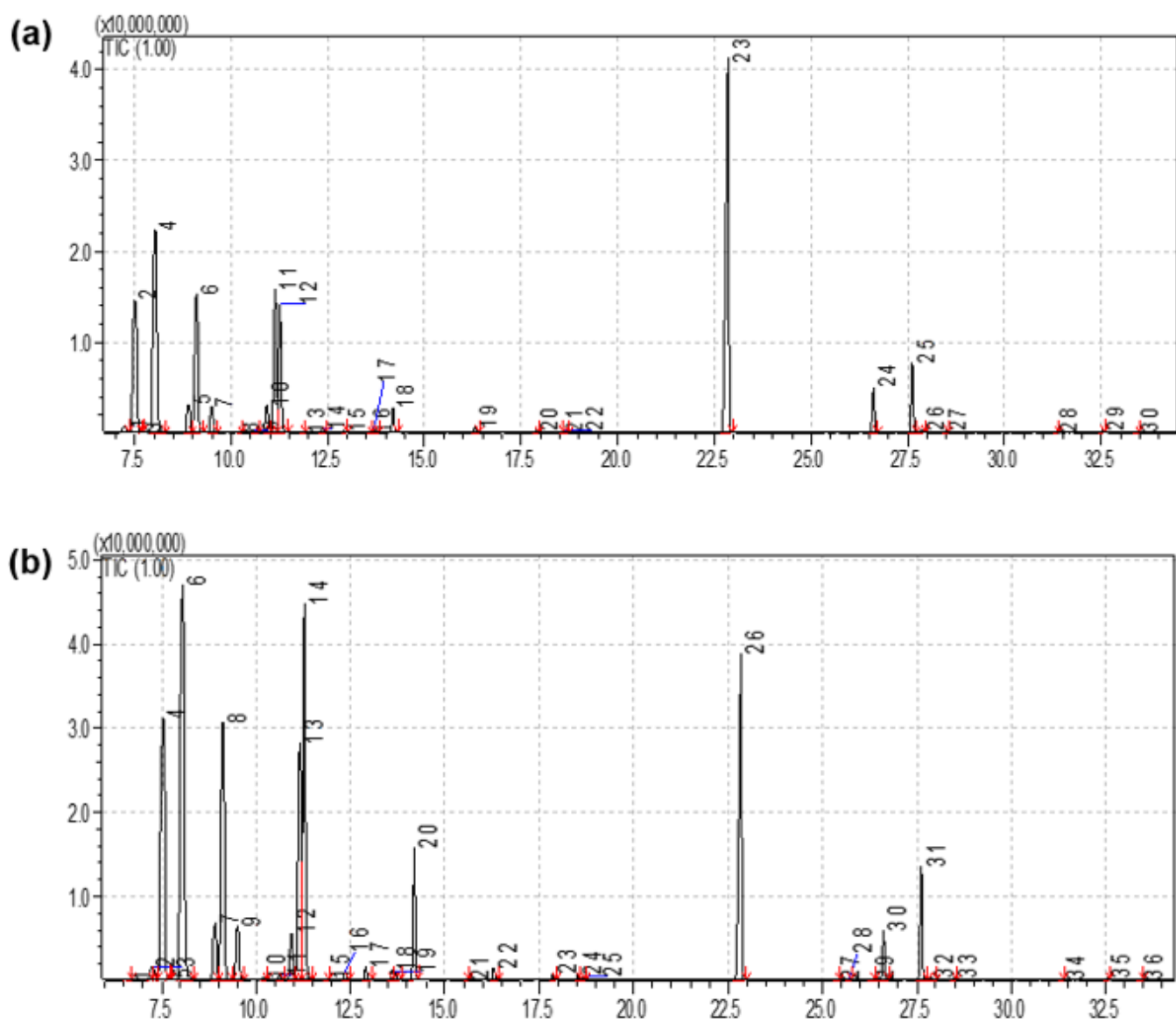


Figure 1

Chromatogram profiles of sintoc essential oil (a) before and (b) after encapsulation.

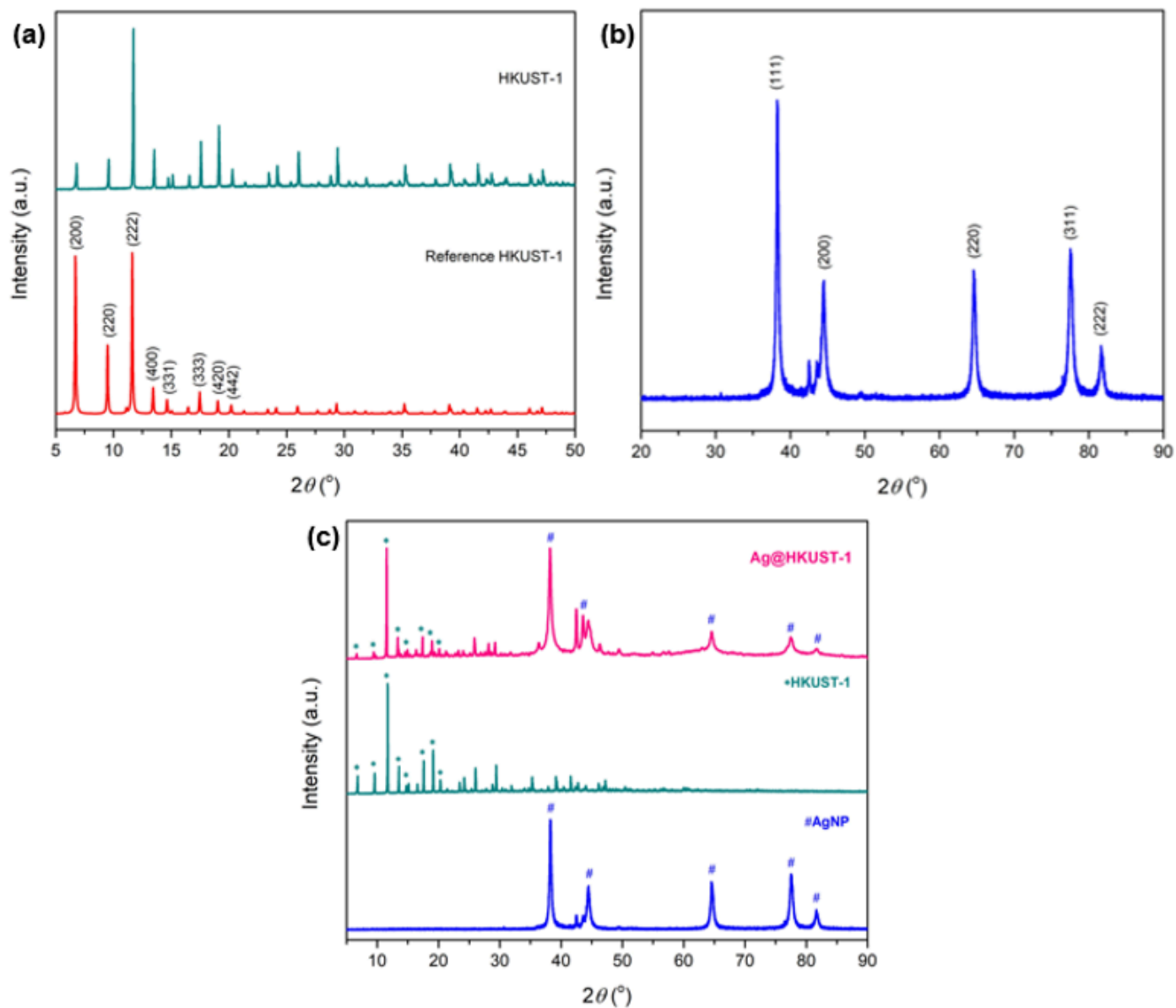


Figure 2

XRD patterns of (a) Reference and HKUST-1; (b) AgNPs; and (c) AgNPs, HKUST-1, and Ag@HKUST-1.

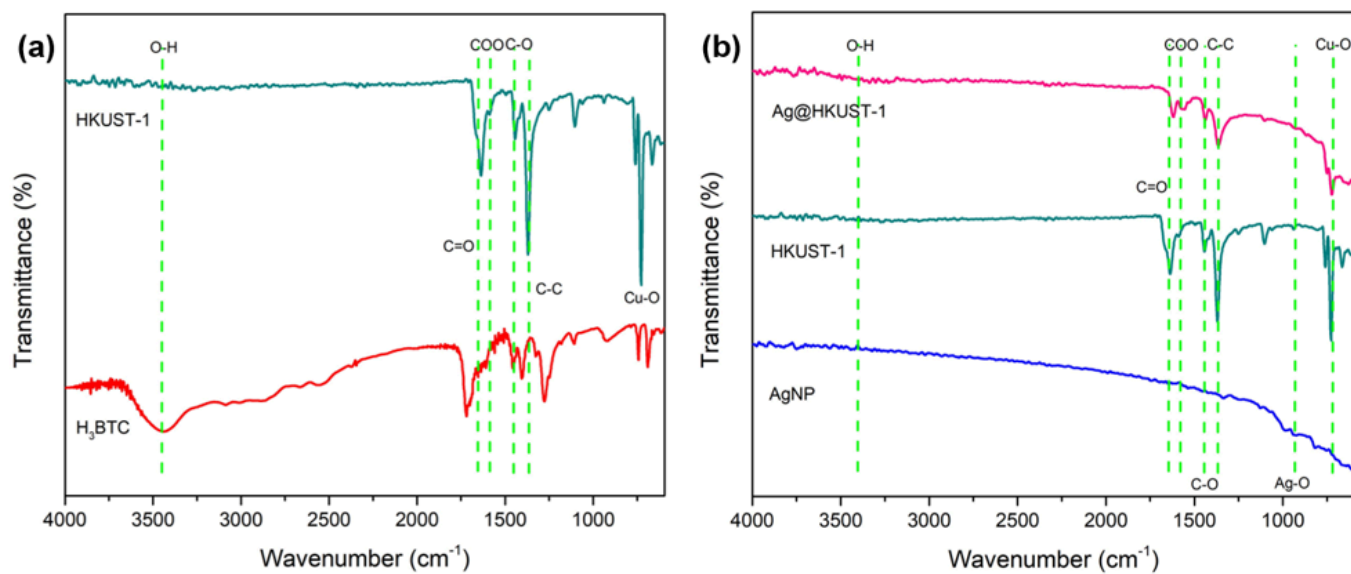


Figure 3

FTIR spectra of (a) H₃BTC and HKUST-1; and (b) AgNPs, HKUST-1, and Ag@HKUST-1.

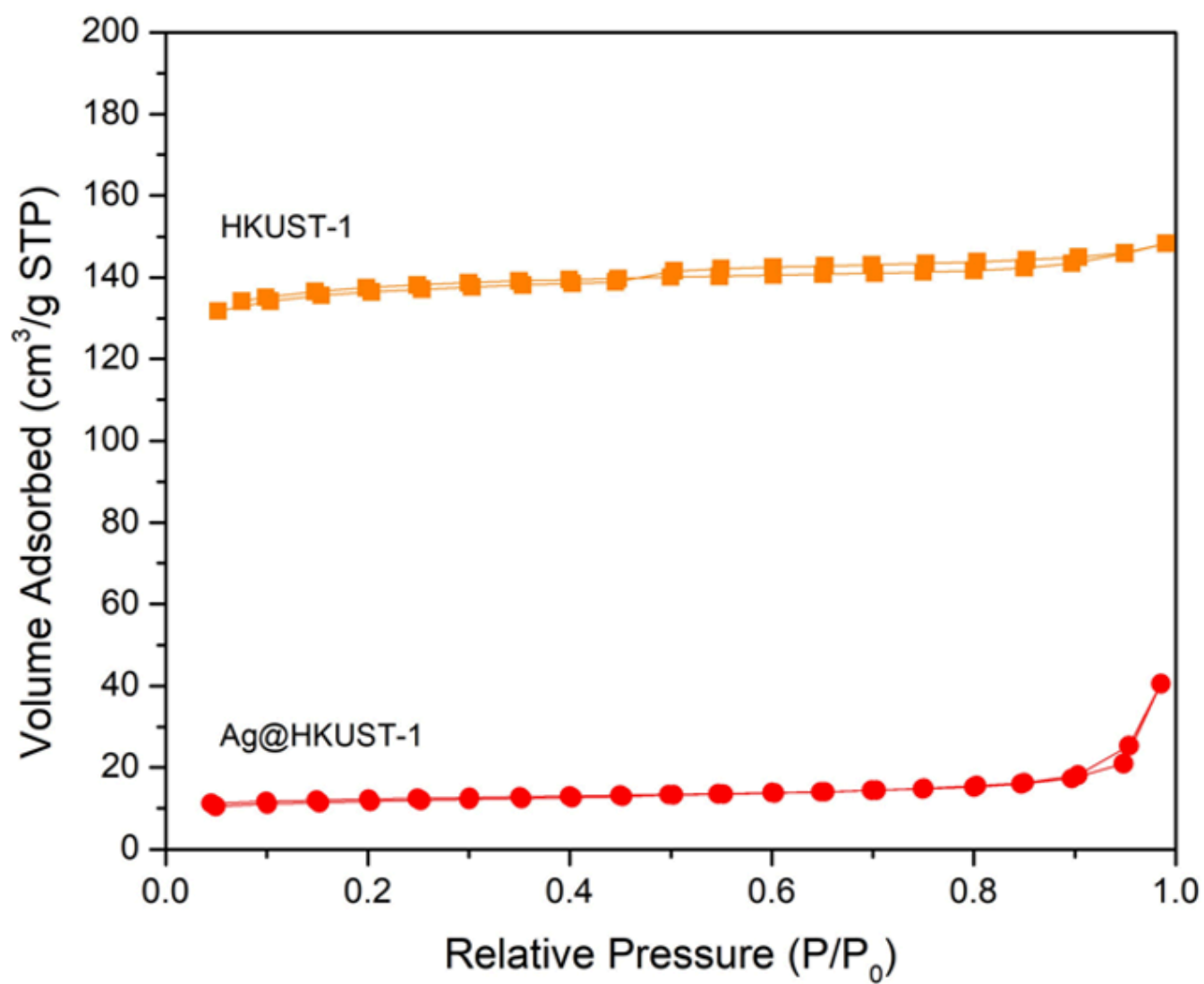


Figure 4

N₂ adsorption/desorption isotherms of HKUST-1 and Ag@HKUST-1.

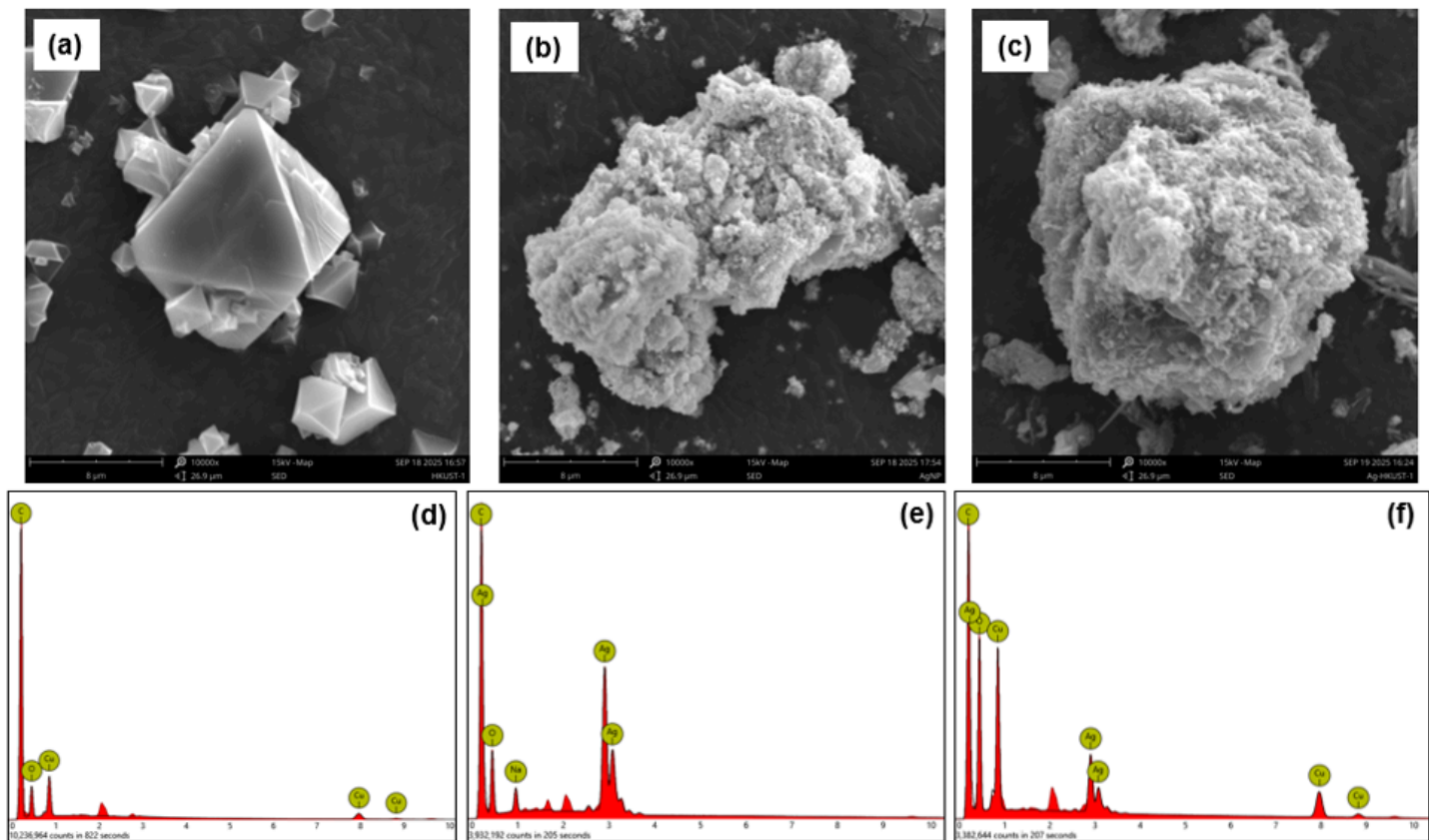


Figure 5

SEM images and EDX analysis of (a) and (d) HKUST-1; (b) and (e) AgNPs; and (c) and (f) Ag@HKUST-1.

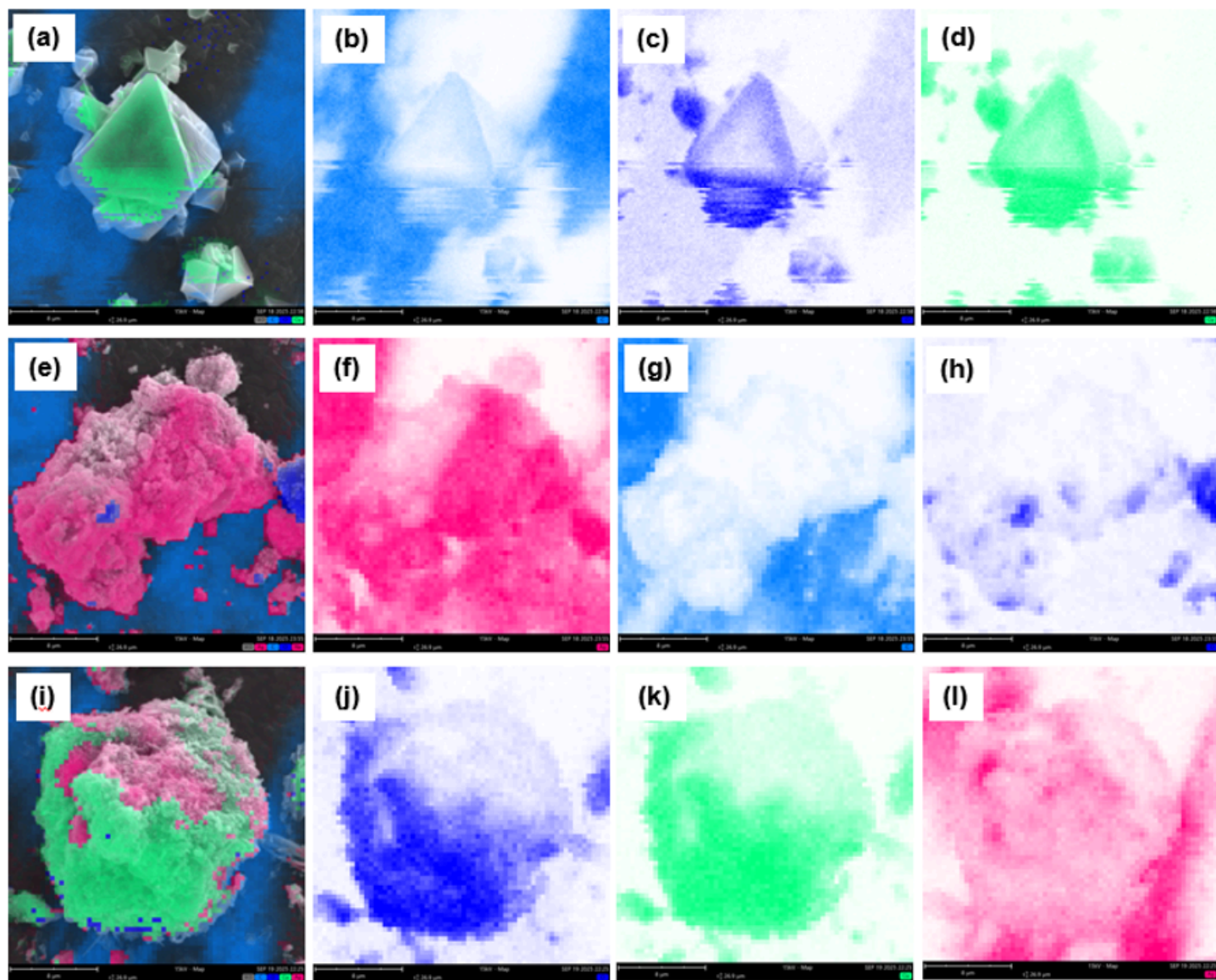


Figure 6

Elemental mapping analysis of HKUST-1 (a-d), AgNPs (e-h), and Ag@HKUST-1 (i-l).

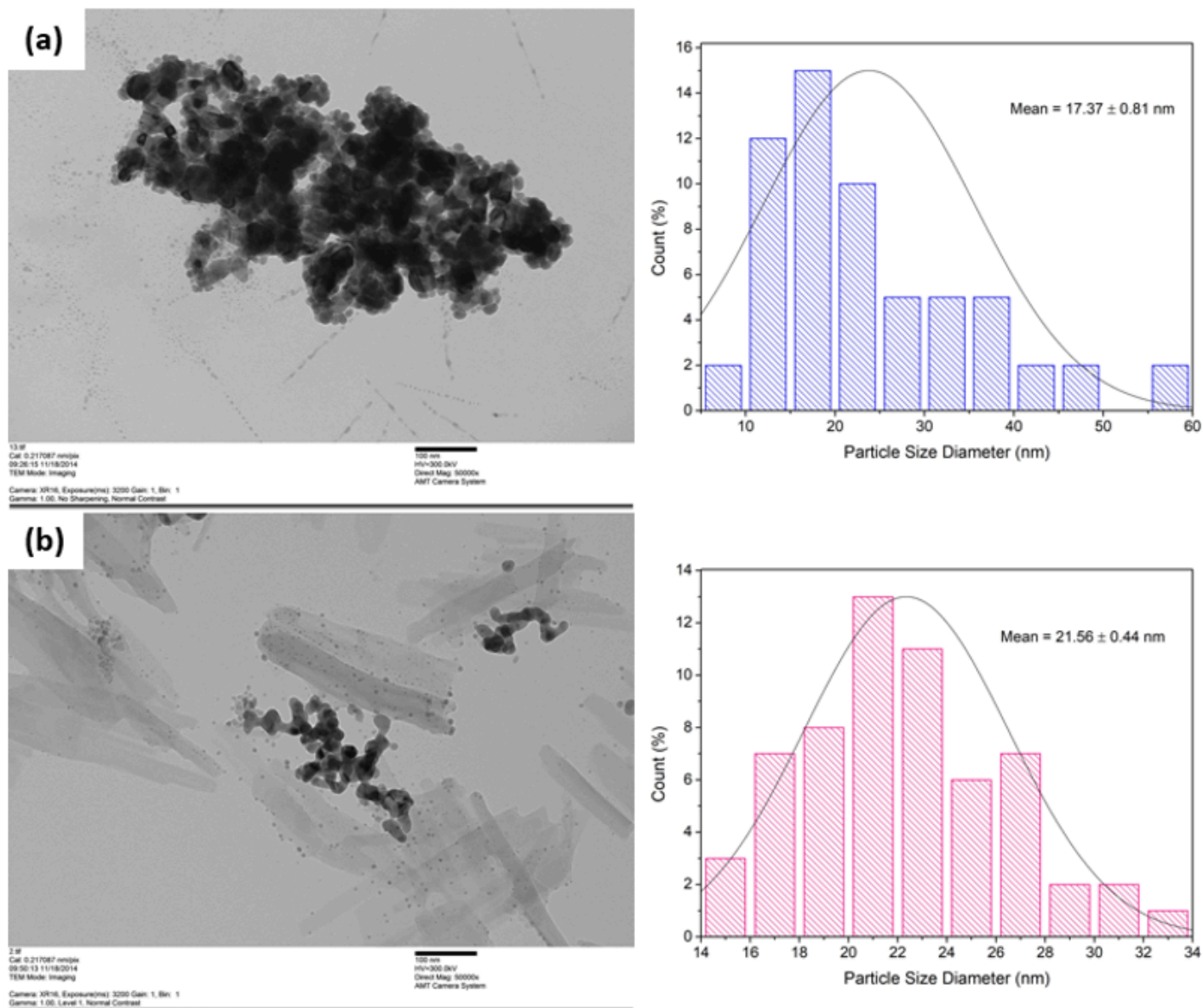


Figure 7

TEM images and particle size distribution histograms of (a) AgNPs and (b) Ag@HKUST-1.

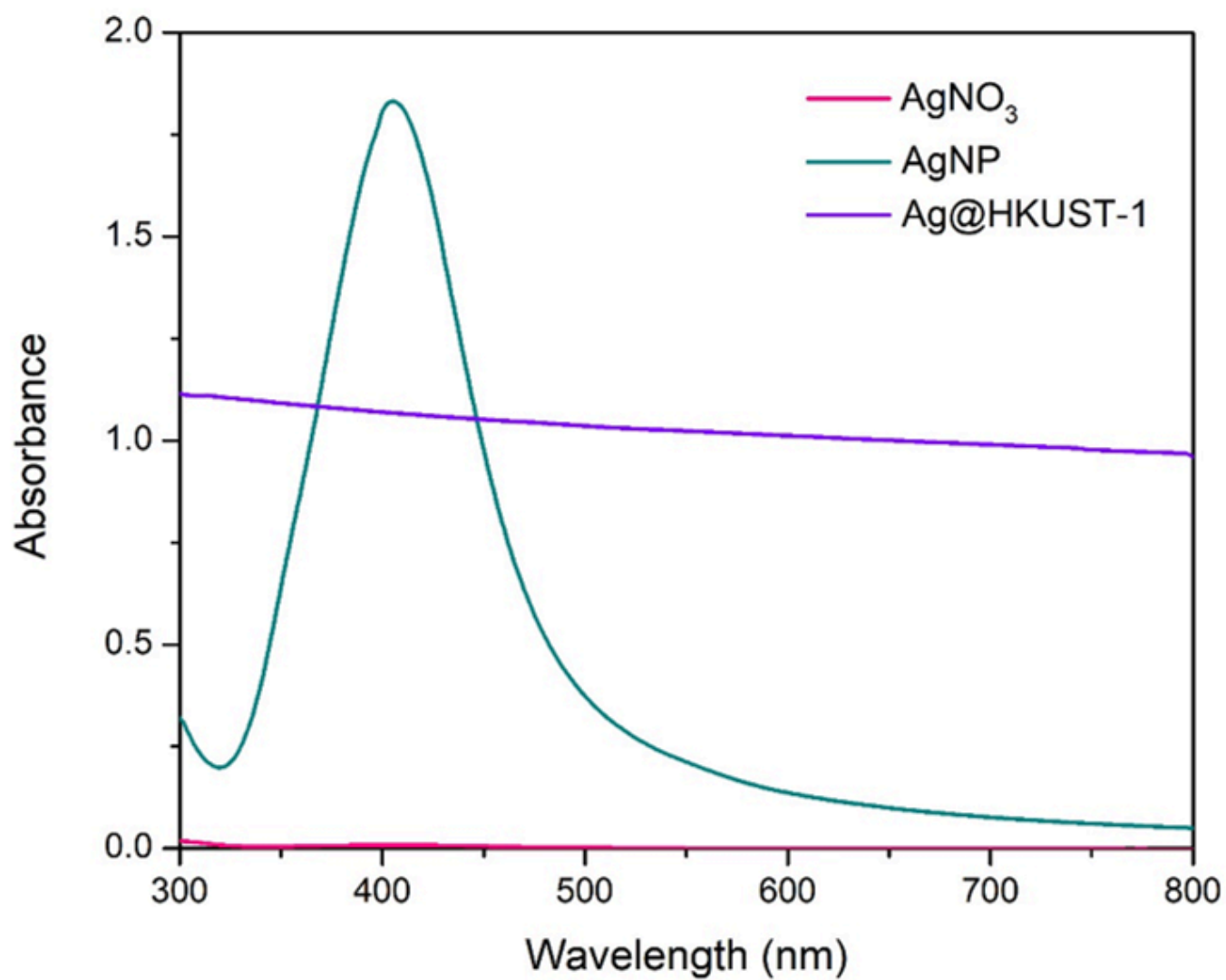


Figure 8

UV-Vis spectra of AgNO₃, AgNPs, and the Ag@HKUST-1 composite.

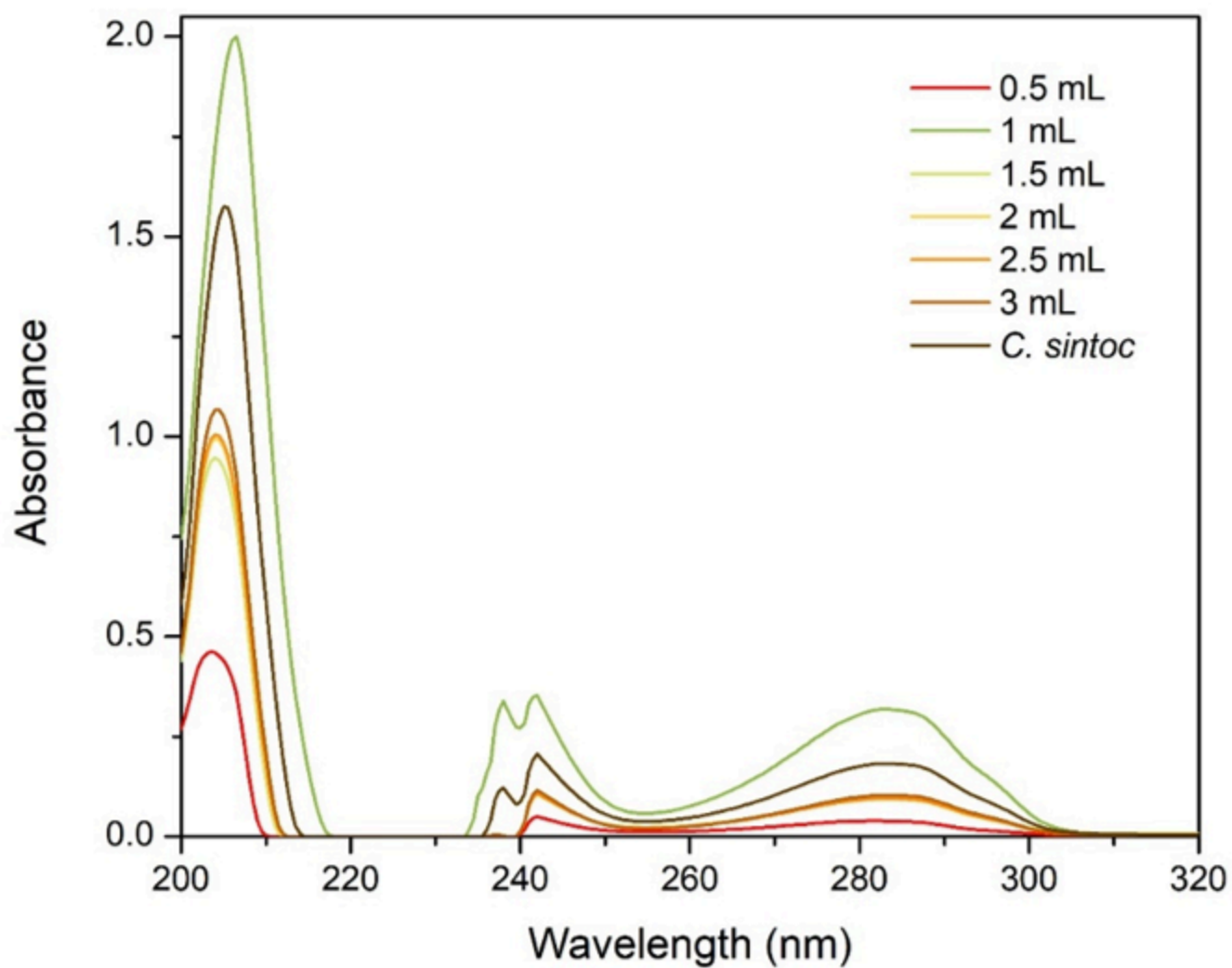


Figure 9

Spectral profiles of essential oil before and after encapsulation with Ag@HKUST-1.

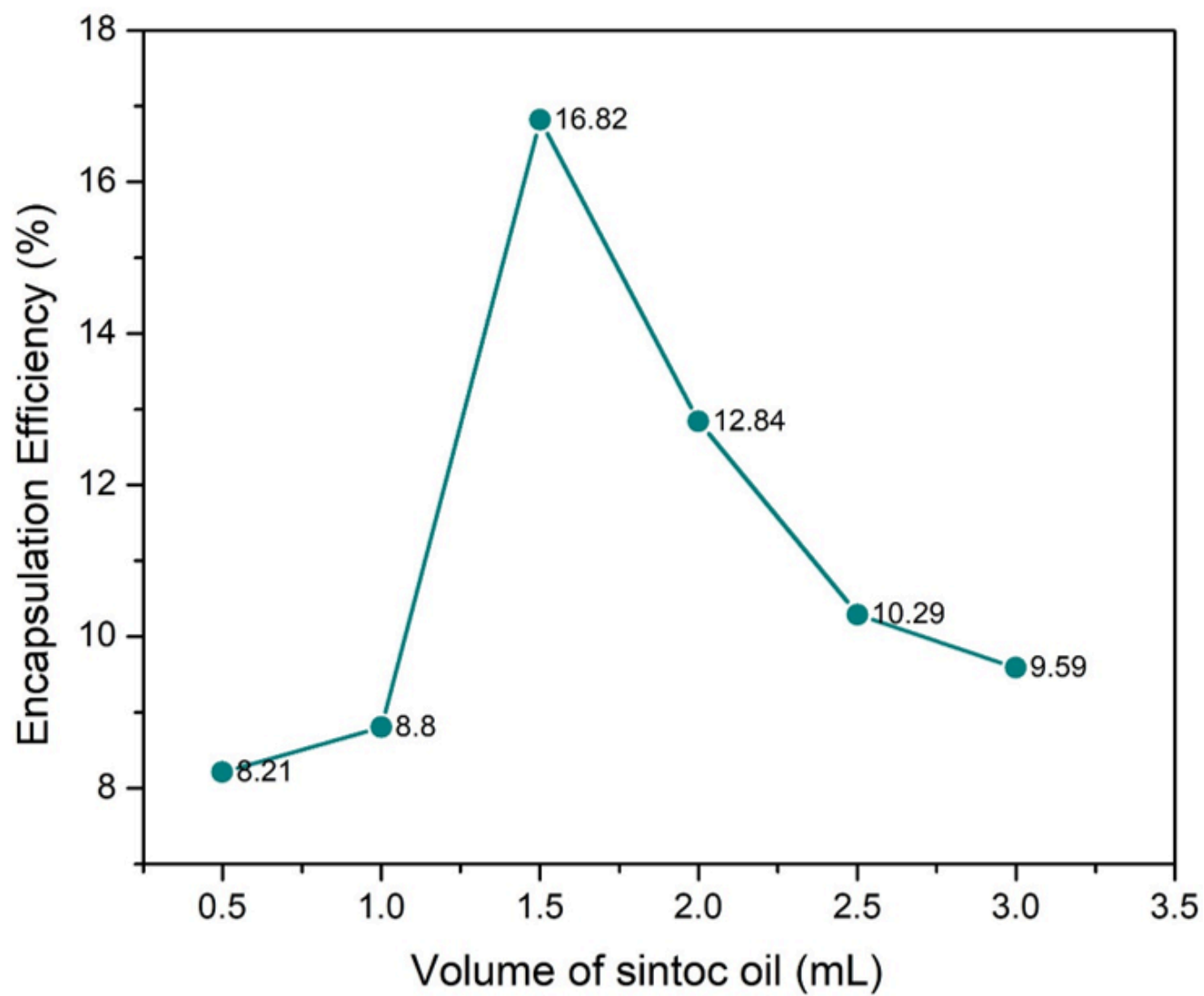


Figure 10

Graph of the relationship between *C. sintoc* oil volume and encapsulation efficiency

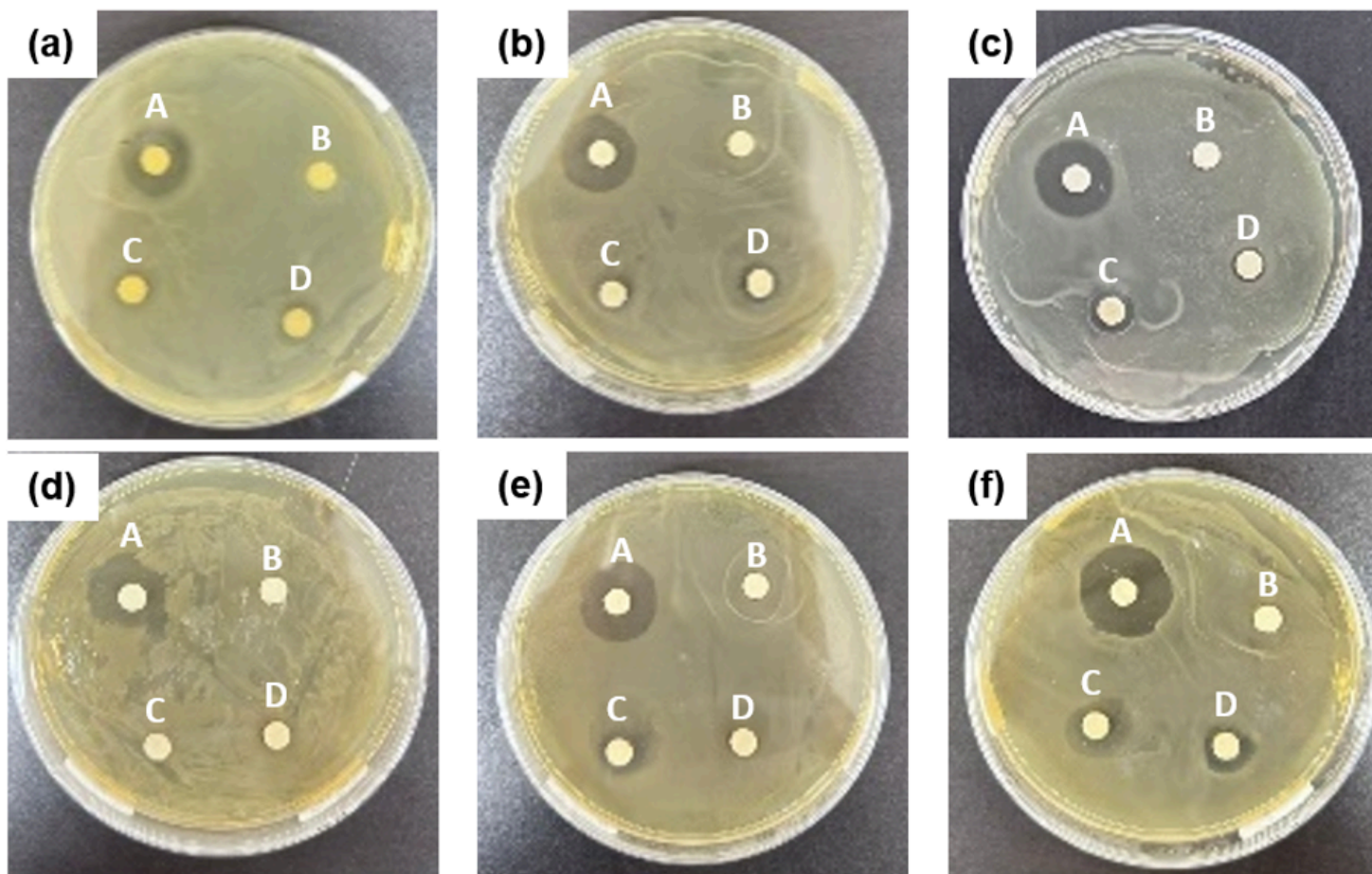


Figure 11

Results of antibacterial assay against *S. typhi* using *C. sintoc* oil encapsulated in Ag@HKUST-1 composite, with varying volumes of *C. sintoc* oil (0.5; 1; 1.5; 2; 2.5; and 3 mL). Reading sequence: A (Positive control); B (Negative control); C (Precipitate); and D (Supernatant).

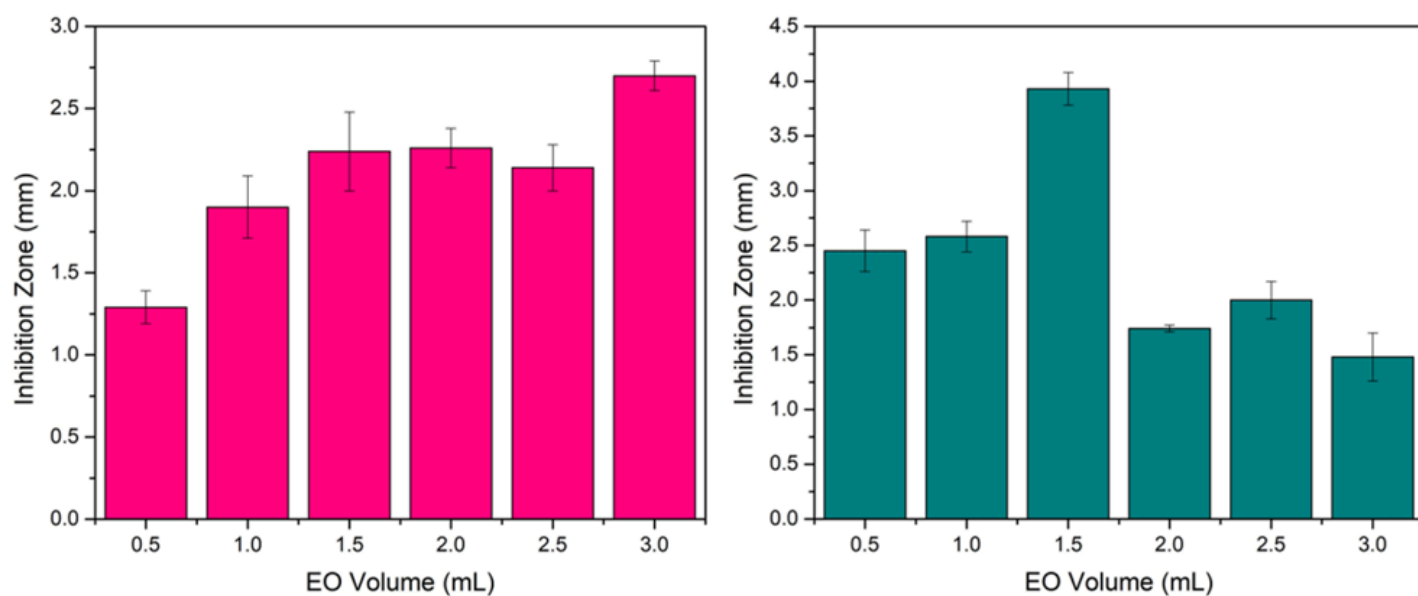


Figure 12

Relationship between *C. sintoc* oil volume and the inhibition zone of *S. typhi* by encapsulated *C. sintoc* oil in the Ag@HKUST-1 composite: solid fraction (left) and supernatant (right).

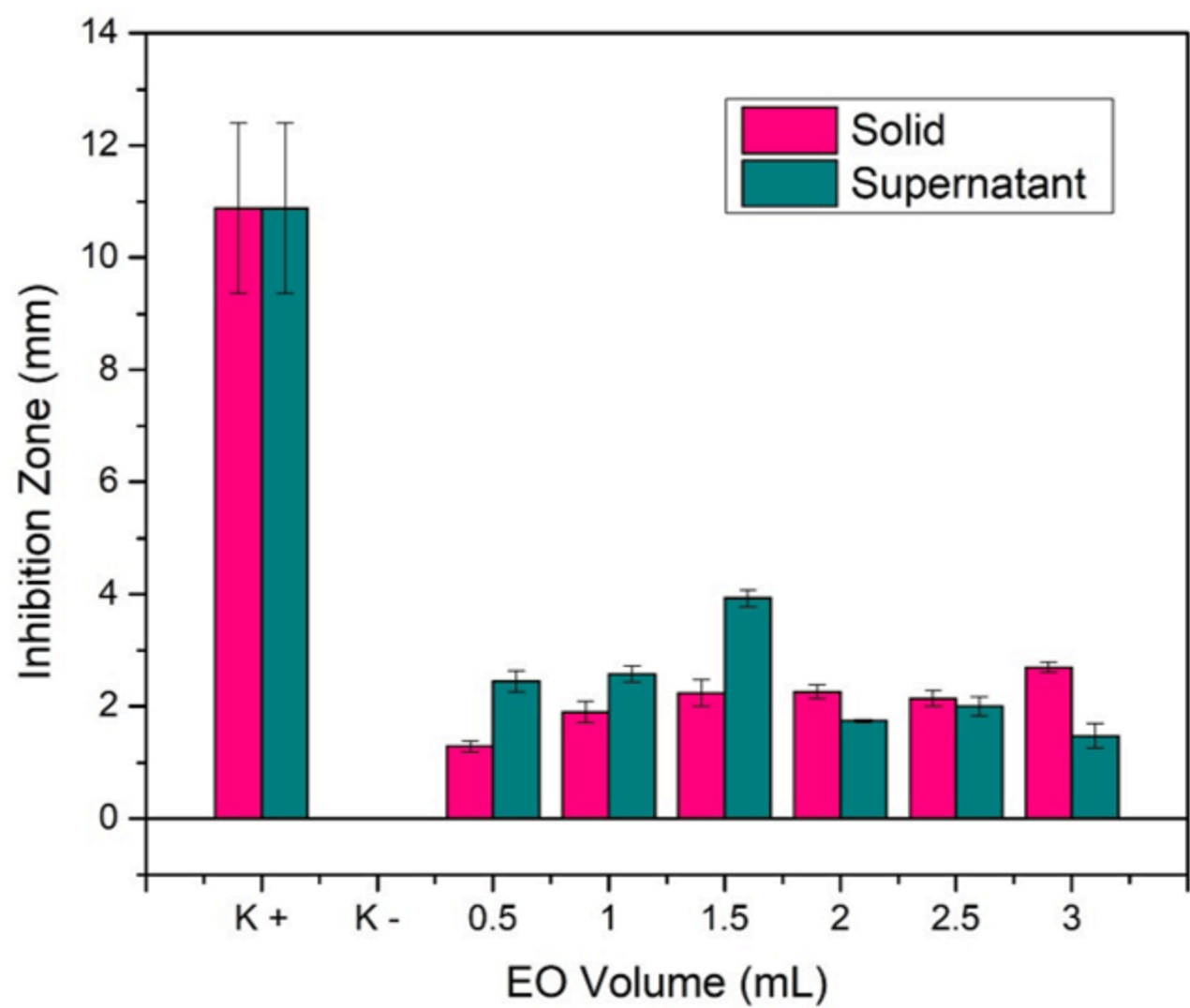


Figure 13

Comparison of *S. typhi* inhibition zones by positive control (K⁺), negative control (K⁻), precipitate, and supernatant of *C. sintoc* oil encapsulated in the Ag@HKUST-1 composite.