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Antimicrobial properties of chemically modified activated carbons derived from date palm seeds

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Abstract

The increasing prevalence of antibiotic-resistant microbes has intensified the search for alternative antimicrobial agents. This study explored the potential of activated carbon (AC) derived from date palm seeds (ACDS), a sustainable and readily available agricultural waste product, and its chemically modified derivatives as effective antimicrobial solutions. AC was modified with thiourea (ACDS@Tu) and dithiooximide (ACDS@DTO) to introduce functional groups capable of interacting with microbial cell components. The antimicrobial efficacy of ACDS, ACDS@Tu, and ACDS@DTO was evaluated against a panel of Gram-positive and Gram-negative bacteria as well as fungi using the agar well diffusion method. The results demonstrated that ACDS@DTO exhibited significantly enhanced antimicrobial activity compared with pristine ACDS and ACDS@Tu, particularly against Gram-positive bacteria, including *Bacillus subtilis* and *Streptococcus mutans*, with inhibition zones comparable to those of the standard antibiotic ampicillin. This enhanced activity is attributed to electron-donating functional groups (amide, thiol, and amine) on the surface of ACDS@DTO, which likely interact with the microbial cell walls and membranes, leading to disruption and cell death. FTIR analysis confirmed the presence of these functional groups, highlighting the successful modification of the ACDS. These findings suggest that chemically modified activated carbons, especially ACDS@DTO, hold promise as potential antimicrobial agents for various applications.

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Introduction

The global rise in antibiotic resistance poses a significant threat to public health, underscoring the urgent need to develop novel antimicrobial agents (El-Sadda *et al.*, 2021). Microbes' ability to resist antibiotics' effects is a complex phenomenon involving various mechanisms, including enzyme production, target modification, and efflux pumps (Rauf *et al.*, 2011; Ezati *et al.*, 2022; Travlou *et al.*, 2018). Recent studies have shed light on the genetic and molecular basis of resistance, highlighting the role of mutations, horizontal gene transfer, and efflux pumps in the development of multidrug-resistant strains (Zhang and Wang, 2022; Liu and Zhang, 2023). The urgency to address this growing crisis has prompted researchers to explore alternative antimicrobial agents that effectively combat resistant microbes (Khosravi and Mohammadi, 2021). Activated carbon (AC), a porous carbonaceous material with high surface area and adsorption capacity, has traditionally been used in water purification and air filtration (Ogungbenro *et al.*, 2020). Recent studies have explored its potential as an antimicrobial agent owing to its ability to adsorb and inactivate microorganisms. The antimicrobial properties of AC can be further enhanced by chemical modifications that introduce functional groups capable of interacting with microbial cell components, as confirmed by FTIR analysis, which demonstrated the presence of hydroxyl, carboxyl, and carbonyl groups on the AC surface. Louis *et al.* (2018) reported the development of multifunctional high-surface-area activated carbon from *Delonix regia*, which had antimicrobial properties and successfully reduced the growth of Gram-positive *B. subtilis* with a higher zone of inhibition (Louis *et al.*, 2018). This study focused on the utilization of date palm seeds as a sustainable precursor for AC production. Date palm seeds are agricultural waste products that are readily available and have been investigated for various applications, including AC production (Strelko *et al.*, 2004). By valorizing this waste material, we aimed to develop a cost-effective and environmentally friendly approach to produce antimicrobial agents. ACDS derived from date palm seeds were chemically modified with thiourea and dithiooximide, resulting in ACDS@Tu and ACDS@DTO, respectively. We hypothesized that these modifications would enhance the antimicrobial activity of AC by introducing functional groups that could interact with microbial cell walls and membranes. The antimicrobial efficacy of ACDS, ACDS@Tu, and ACDS@DTO was evaluated against a panel of Gram-positive and Gram-negative bacteria, as well as fungi, using the agar well diffusion method.

Materials and methods

Activated carbon preparation

KOH-activated porous carbon was prepared from date palm seeds following the method described by Ogungbenro *et al.* (2020). Briefly, crushed date seeds (CDS) underwent chemical activation using potassium hydroxide (KOH). A weighed mass of CDS was mixed with a KOH solution at an impregnation ratio of 4:1 (KOH:CDS). This mixture was heated at 90°C for 2 hours with continuous stirring. The resulting impregnated cake was dried at 100°C for 8 hours.

Subsequently, the dried cake underwent one-step chemical activation (combined pyrolysis and activation) in a Nabertherm furnace under a nitrogen atmosphere, at 700°C with a heating rate of 15°C/minute and a holding time of 1 hour. Post-activation treatment was conducted to remove residual chemicals. The KOH-activated carbons were treated with hydrochloric acid to neutralize excess KOH, washed with distilled water to eliminate remaining chloride ions, and finally, the ACs were dried at 150°C for 6 hours.

Preparation of modified activated carbons

ACDS@Tu and ACDS@DTO were synthesized via oxidation, Acylation, and Functionalization of ACDS. In the first step, AC was oxidized with 32.5% HNO_3 solution at 60°C for 24 hours to introduce carboxyl groups (AC-COOH) (Stöhr *et al.*, 1991). For instance, 4 g of activated carbon was put into 250 mL HNO_3 solution (32.5%) under magnetic stirring at 60 °C for 24 h, and then the AC-COOH was filtered, washed

with DI water, and then dried in an oven at 80 °C for 24 h. In the second step, Acylation was carried out by reacting AC-COOH with $SOCl_2$ in dichloromethane to convert carboxyl groups into acyl chloride groups (AC-COCl) (El-Ayaan and Alaa, 2005). 3 g of AC-COOH with 250 mL of a 4:1 mixture of dichloromethane and SOCl2 was refluxed for 24 h at 35°C in an N_2 atmosphere. The AC-COCl was immediately dried via rotary evaporation at 40°C and 300-400 mbar of vacuum to remove all solvents. Then, the AC-COCl was washed with ethanol and deionized water and dried overnight in the oven at 60 °C. Finally, Functionalization was done by reacting AC-COCl with either thiourea or dithiooximide in DMF to obtain ACDS@Tu or ACDS@DTO, respectively.

Material characterization

The surface morphology of the Activated carbon before and after modification was examined on the Scanning electron microscope (JSM-7610F) equipped with an energy-dispersive X-ray EDX. The phase structure was verified using an X-ray Diffractometer (D2 Phaser Bruker). The surface functional group on the surface of the materials was analyzed using the Fourier transform infrared spectrometry (FTIR) (Perkin Elmer Spectrum Two).

Antimicrobial assay

The antimicrobial activity of ACDS, ACDS@Tu, and ACDS@DTO was assessed using the agar well diffusion method against:

1. Gram-positive bacteria: *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans*
2. Gram-negative bacteria: *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella enterica* (ATCC 14028)
3. Fungi: *Candida albicans, Aspergillus niger*

Microbial suspensions were prepared and adjusted to a 0.5 McFarland standard, and agar plates were inoculated. Wells (6 mm diameter) were punched, and 100 µL of each test compound (15 mg/mL in DMSO) was added. Ampicillin (10 µg) and gentamicin (10 µg) served as positive controls for Gram-positive and Gram-negative bacteria, respectively, while nystatin (100 units) was used for fungi. DMSO was the negative control. After incubation at 37°C for 24 hours, the zones of inhibition were measured (Scott, 1989). Antimicrobial activity of ACDS, ACDS@Tu, and ACDS@DTO against various microorganisms. ACDS@DTO exhibited the highest antimicrobial activity among the tested materials. It showed potent activity against Gram-positive bacteria, particularly *B. subtilis* and *S. mutans*, with inhibition zones comparable to that of the standard antibiotic ampicillin. While ACDS and ACDS@Tu demonstrated some antimicrobial activity, it was significantly lower than that of ACDS@DTO

Statistical analysis

This experiment was carried out in triplicate, and inhibition zones were measured in mm. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p<0.05$).

Results and discussion

Characterization

The SEM image of ACDS, ACDS@TU, and ACDS@DTO is shown in Fig. 1.

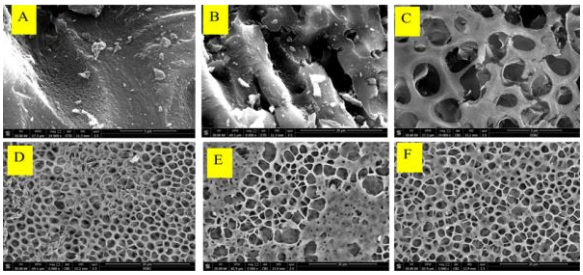

Fig. 1. SEM Image of A, B – ACDS, C, D – ACDS@TU E, F – ACDS@DTO

A comparison of the three activated carbons showed the presence of pores on ACDS@TU and ACDS@DTO. Pore structure development was started after the oxidation of ACDS. However, Fig 1. shows that the formation of the pores was well developed in the structure of ACDS@TU in comparison to ACDS@DTO. This may be because the functionalization of the AC using thiourea ruptured the pores, increasing its size compared to

functionalization with dithiooxamide, as seen in the ACDS@DTO.

FTIR spectra were collected to characterize the surface functional groups of the prepared carbon. The FTIR analysis revealed several significant absorption bands: a broad peak at 3404 cm^{-1} attributed to O–H stretching vibrations, peaks at 1576 cm^{-1} (C=C stretching in aromatic rings), and at 1704 cm^{-1} (C=O stretching in carbonyl groups) (Fig. 2). After modification of the ACDS, the peak 1576 cm-1 changed in frequency to 1589 cm-1 and reduced in intensity. It is to be noted that there was an appearance of peaks at 1092 and 660 cm-1 on the ACDS after modification ascribed to the characteristic stretching vibrations of the silanol Si–O–Si group and C-H bending vibrations from the Thiourea and Dithiooximide (Diagboya *et al.*, 2019). The broad peak around 3353 cm-1 is assigned to the N-H stretching vibration of DTO. The peaks observed at 1443 and 1091 cm-1 in the spectrum of ACDS-DTO correspond to the N-C-N stretching vibration. The absorption band at 1596 cm-1 is assigned to NH2 bending. All these observations confirm the formation of ACDS-DTO. These functional groups are vital for the antimicrobial activity of the modified AC.

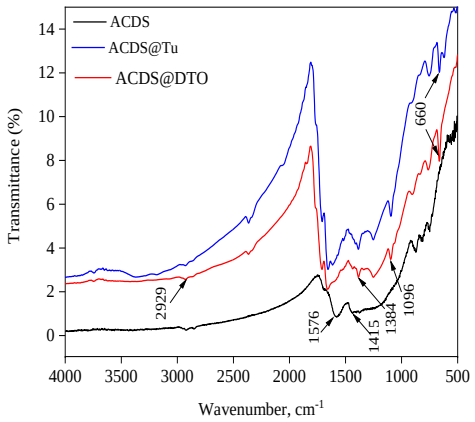

Fig. 2. FTIR spectra of ACDS, ACDS@TU, and ACDS@DTO showing the presence of functional groups

The materials' XRD pattern shows a major broad peak that can be indexed to amorphous carbon. The diffraction comes from the 002 plane (Rajbhandari *et al.*, 2012). The intensity of the peak (002) can be seen to increase with modification in ACDS@TU and ACDS@DTO, with ACDS @DTO having the highest intensity (Fig. 3).

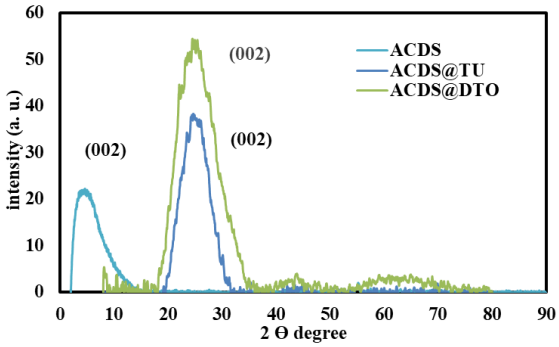

Fig. 3. XRD of ACDS, ACDS@TU and ACDS@DTO

Effect of modification on antimicrobial activity of ACDS

The materials ACDS, ACDS@DTO, and ACDS@TU were tested against the removal of Gram-negative bacteria, Gram-positive bacteria, and Fungi, and the zone of inhibition result is displayed in Table 1. The result reveals that overall; Modification of the AC improved antimicrobial activity for the ACDS@TU. The best antimicrobial effect was observed for the *Escherichia coli* and *Bacillus subtilis* by the ACDS@DTO in comparison to ACDS@TU. Some of the results showed that ACDS possesses antimicrobial properties without modification via oxidation and functionalization. For instance, modification of ACDS via thiourea had no effect on the antimicrobial activity against *Escherichia coli, Bacillus subtilis, Streptococcus mutans,* and *Candida albicans* (Fig. 4). The result revealed 9.0±1.0 vs. 9.0±1.0, 12.3±0.6 vs. 10.0±1.0, 11.3±0.6 vs. 10.3, and 12.0±1.0 vs. 10.0±1.0, for ACDS and ACDS@TU respectively. This could be a result of little or no interaction between the introduced electron-donating functionalities (–NH_2 and –CS) on the ACDS@Tu. Similar results have shown that the antimicrobial effect of activated carbon derived from almond shell tested against *S. aureus* showed a decrease in the microbial count from 10-6 to 10-4 cfu/ml, most probably because the conditions during the testing period did not provide growth optimum and there was no interaction between *S. aureus* and the activated carbon (Belcheva *et al.*, 2024).

Table 1. Antimicrobial activity of ACDS, ACDS@DTO, and ACDS@Tu against various microorganisms

Microorganism	ACDS (mm)	ACDS@DTO (mm)	ACDS@Tu (mm)	Standard antibiotic
Gram-negative bacteria				Gentamicin
Escherichia coli (ATCC:10536)	9.0±1.0	11.3±0.6	9.0±1.0	27.0±1.0
Klebsiella pneumoniae (ATCC:10031)	8.7±0.6	13.3±0.6	13.0±1.0	25.3 ±0.6
Salmonella enterica (ATCC: 14028)	NA	NA	NA	18.3±0.6
Gram-positive bacteria				Ampicillin
Staphylococcus aureus (ATCC:13565)	9.3	12.0±1.0	10.3±0.6	20.7±0.6
Bacillus subtilis (DSM:1088)	12.3±0.6	16.3±0.6	10.0±1.0	21.3±0.6
Streptococcus mutans (ATCC:25175)	11.3±0.6	25.0±1.0	10.3	26.3±0.6
Fungi				Nystatin
Candida albicans (ATCC:10231)	12.0±1.0	12.7±0.6	10.0±1.0	21.0±1.0
Aspergillus niger (ATCC:16404)	NA	NA	NA	19.3±0.6

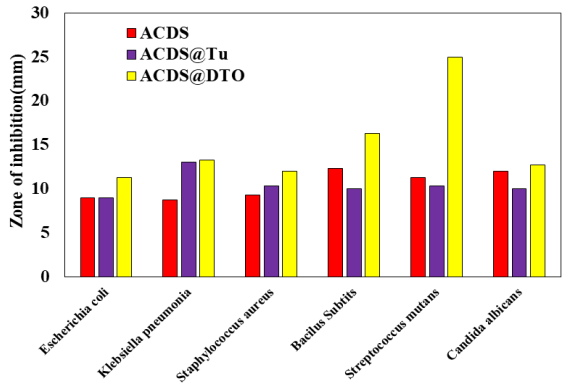

Fig. 4. Antimicrobial activity of ACDS, ACDS@TU AND ACDS@DTO against various microbes

The better performance of the ACDS@DTO in comparison to the ACDS and ACDS@TU can be explained using the structural difference and additional functional groups, such as the C=S functional group on the surface of the ACDS@DTO. The physicochemical properties of the Activated Carbon (ACDS), such as porous structure and high surface area, acted as a matrix for adsorption. The Surface functional groups like hydroxyls and carbonyls enhance its interaction with microbial cells, contributing to its inherent antimicrobial properties (Das *et al.*, 2019). The study by Das *et al.* (2019) supports this claim, demonstrating that ACs derived from various biomasses, even without metal incorporation, exhibit antibacterial activity against *E. coli*, primarily due to their porous structure and the presence of active functional groups. The FTIR analysis shown in Figure 2 illustrates these functional groups, confirming their role in the antimicrobial activity of AC.

Thiourea modification of ACDS introduces electron-donating functionalities (–NH_2 and –CS) onto the AC surface. These functional groups, particularly the N-H bonds, enable hydrogen bonding with microbial cell walls, enhancing the antimicrobial properties compared to pristine AC (Diagboya *et al.*, 2019). However, the overall effectiveness remains limited compared to DI@AC.

The introduction of dithiooxamide on the surface of ACDS significantly improves antimicrobial activity. The presence of multiple functional groups (amine, thiol, and C=S) on the modified AC enhances its interaction with microbial components. The C=S bond is particularly crucial, as it can disrupt microbial membranes and generate reactive oxygen species (ROS), leading to cell death. This observation aligns with the findings of Das *et al.* (2019), where the sharp edges observed in AC samples were suggested to have a puncturing effect on bacterial cell walls, contributing to their antimicrobial activity.

On the other hand, the enhanced activity of ACDS@DTO can be attributed to the synergistic

effects of its functional groups. The amine and thiol groups can interact with negatively charged cell membranes, facilitating penetration and disruption. In contrast, the thiourea-modified AC, while beneficial, does not exhibit the same level of interaction due to the absence of the more reactive C=S bond found in dithiooximide. Given the mechanisms by which microbes develop resistance to antibiotics, the findings of this study are particularly relevant. By presenting alternative antimicrobial agents, such as ACDS@DTO, this research contributes to the fight against antibiotic resistance. The ability of ACDS@DTO to disrupt microbial membranes and generate reactive oxygen species offers a promising strategy to combat resistant strains, potentially reducing reliance on traditional antibiotics and mitigating the risk of further resistance development (El-Sadda *et al.*, 2021; Ezati *et al.*, 2022). Using date palm seeds as a precursor for AC production further adds to the appeal of this approach, offering a sustainable and environmentally friendly solution.

Table 2. Comparison of the antimicrobial effect of various activated carbon

Material	Microbe	Zone of inhibition	Reference
Zn-Ac	Gram-positive bacteria	4 mm	(Saravanan *et al.*, 2016)
Pb-AC	Gram-positive bacteria	8 mm	(Saravanan *et al.*, 2016)
(Ag-Ac)	Gram-positive bacteria	12 mm	(Saravanan *et al.*, 2016)
Activated carbon (DRP) AC- *Delonix regia*	*B. subtilis*	18 mm	(Louis *et al.*, 2018)
ACDS	*Bacillus subtilis*	12.3±0.6 mm	This study
ACDS@DTO	*Bacillus subtilis*	16.3±0.6 mm	This study
ACDS@Tu	*Bacillus subtilis*	10.0±1.0 mm	This study

Comparison of result with literature

This study highlights the antimicrobial potential of chemically modified activated carbons, explicitly focusing on the relationships between activated carbon (ACDS), thiourea-modified (ACDS@Tu), and dithiooximide-modified (ACDS@DTO). The result of this study is compared with the antimicrobial effect of other materials against gram-positive bacteria (Table 2). The comparison reveals that the (ACDS), (ACDS@Tu), and dithiooximide-modified (ACDS@DTO) shows good antimicrobial effect, which suggests that the ACDS@DTO is a suitable material and can be applied for disinfection applications.

Conclusion

This study demonstrates the antimicrobial potential of activated carbon derived from date palm seeds (ACDS) and its chemically modified forms, ACDS@Tu and ACDS@DTO.

Characterization of the materials using SEM, FTIR, and XRD revealed significant structural and chemical enhancements in the modified carbons. SEM analysis showed well-developed pores, with ACDS@DTO having the most defined pore structure. FTIR analysis confirmed the presence of functional groups such as hydroxyl, carbonyl, and C=S bonds, which play a crucial role in antimicrobial activity. XRD patterns indicated that all materials exhibited amorphous carbon structures, with the intensity of the (002) plane increasing in the modified samples, particularly ACDS@DTO. Among the tested materials, ACDS@DTO showed the highest antimicrobial activity against a broad range of Gram-positive and Gram-negative bacteria and fungi. This superior activity can be attributed to the introduction of dithiooximide, which provided additional functional groups that interacted more effectively with microbial cell walls, disrupting their structure and leading to cell death. While ACDS and ACDS@Tu also exhibited antimicrobial properties, their efficacy was comparatively lower, particularly against certain bacterial strains.

The findings of this study highlight the potential of using agricultural waste, such as date palm seeds, to produce sustainable and environmentally friendly antimicrobial agents. The chemical modifications, especially with dithiooximide, significantly enhanced the antimicrobial performance of the activated

carbon, offering a promising alternative to traditional antibiotics. These materials, especially ACDS@DTO, could be applied in water treatment, disinfection, and medical fields, contributing to efforts to combat antibiotic resistance.

Recommendation(s)

Future research should focus on scaling up production and testing these materials' long-term stability and safety in practical applications.

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