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Synthesis, Spectroscopic Characterization and Biological Studies of Esters from Benzoic Acid and three Homologous Alcohols

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Abstract

This work reports the synthesis, characterization and antimicrobial screening of benzoate esters synthesized from benzoic acid and the three alcohols: methanol, ethanol, and isopropanol-by means of an acid-catalyzed Fischer esterification. The resulting compounds, namely methyl, ethyl, and isopropyl benzoate, were obtained in yields of 85%, 83%, and 78%, respectively. The compounds were subjected to spectroscopic structural studies using IR, NMR, and mass spectrometry. They confirmed the formation of ester functionalities, giving molecular identities Antimicrobial screening of the esters against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans revealed that all esters exhibited inhibitory activities with Methyl, ethyl, and isopropyl benzoate having zones of inhibition between (8–18 mm zones), with isopropyl benzoate being the most effective. Though less potent than Ciprofloxacin (24 mm) activity but more than the others. Activity increases with alkyl chain branching. Measurements of the diameters of zones of inhibition correlated well with MIC assay results, showing isopropyl benzoate to be superior, suggesting that higher hydrophobic interaction and molecular branching would enhance disruption of microbial membranes. SAR study indicated a strong correlation between esters and antimicrobial activities, with secondary alcohol derivatives performing better than others.

Keywords: Benzoate esters, structure–activity relationship (SAR), methyl benzoate, Minimum inhibitory concentration (MIC)

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INTRODUCTION

Esters are an important class of organic compounds widely recognized for their diverse applications in pharmaceuticals, food, cosmetics, and agrochemicals. They are formed through a condensation reaction between carboxylic acids and alcohols, typically catalyzed by acid or base, resulting in a functional group that contributes to their pleasant fragrance and biological activity (Kumar and Sharma, 2022). Among esters, aromatic esters derived from benzoic acid are particularly notable due to their structural stability, chemical reactivity, and role as intermediates in drug synthesis and fine chemical production (Patel and Gupta, 2021).

The biological importance of benzoate esters stems from their inherent antimicrobial and preservative properties. These compounds are known to interfere with microbial cell membrane integrity, enzyme function, and energy metabolism, making them effective against a broad range of bacteria and fungi (Singh and Mehta, 2023). Their potential as environmentally friendly antimicrobial agents has gained attention due to the increasing demand for alternatives to synthetic antibiotics that contribute to resistance. Therefore, studying the structure–activity relationship (SAR) of benzoate esters offers insight into how alkyl chain length and branching affect antimicrobial efficiency.

Recent studies have explored the synthesis and biological activities of various aromatic esters. For instance, Osei *et al.,* (2021) synthesized methyl and ethyl benzoates using acid-catalyzed esterification and reported moderate antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus*. Similarly, Adebayo and Ibrahim (2022) observed that introducing bulkier alkyl groups in benzoate esters enhances hydrophobic interactions with microbial membranes, thereby improving antimicrobial potency. In another study, Choudhury *et al.,* (2023) investigated the correlation between molecular structure and inhibitory effects, revealing that secondary and branched alcohol derivatives exhibit superior activity compared to their primary counterparts.

Characterization techniques such as infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) have been widely employed to confirm ester formation and elucidate structural features (Rahman and Dutta, 2022). These analytical methods help in verifying the successful conversion of carboxylic acids to esters and identifying functional groups responsible for bioactivity. Combined with antimicrobial assays, they enable a comprehensive understanding of the relationship between molecular structure and biological function, especially in newly synthesized benzoate derivatives.

Despite the growing literature on aromatic esters, limited attention has been given to a comparative structure–activity study involving methyl, ethyl, and isopropyl benzoates synthesized under identical conditions (Adebayo and Ibrahim, 2022; Choudhury, Singh, and Arora, 2023). Previous research has primarily focused on single derivatives or neglected the systematic evaluation of branching effects on antimicrobial efficacy (Osei, Boateng and Nyarko, 2021). Therefore, the present study reports the synthesis, characterization, and antimicrobial screening of methyl, ethyl, and isopropyl benzoates synthesized via acid-catalyzed Fischer esterification (Rahman and Dutta, 2022). The novelty of this work lies in its comparative SAR analysis, which establishes a direct relationship between molecular branching and antimicrobial performance, highlighting the superior activity of isopropyl benzoate; this provides a new understanding of how ester structure influences antimicrobial mechanisms, contributing to the rational design of bioactive organic compounds (Kumar and Sharma, 2022).

MATERIALS AND METHODS

Analytical-grade reagents were used throughout the study. Benzoic acid, methanol, ethanol, isopropanol, concentrated sulfuric acid (H_2SO_4), diethyl ether, anhydrous sodium sulfate, and DMSO (dimethyl sulfoxide) were procured from Sigma-Aldrich. Microbial strains including *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative), and *Candida albicans* (fungus) were obtained from the Microbiology Laboratory, Department of Biological Sciences, Adamawa State University, Mubi.

Microbial isolate

All strains were maintained and subcultured according to standard microbiological protocols (Cheesbrough, 2006).

Synthesis of Benzoate Esters

Esterification reactions were carried out via Fischer esterification, where benzoic acid was reacted with various alcohols (methanol, ethanol, isopropanol) under reflux in the presence of concentrated sulfuric acid as a catalyst. The reaction mixtures were refluxed for 3–4 hours at temperatures ranging from 65–75°C, depending on the boiling point of each alcohol (Pavia *et al.*, 2014). After the reaction, the esters were extracted with diethyl ether, washed with cold distilled water, and dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure, and the crude products were purified either by recrystallization or distillation. Yields were calculated gravimetrically, based on the weight of pure ester obtained compared to theoretical yield (Gupta & Rawat, 2018).

Characterization of Synthesized Esters

The identity and purity of synthesized esters were confirmed using:

1. Infrared (IR) spectroscopy to detect ester functional groups, especially the C=O stretching at ~1710–1715 cm^{-1}.
2. ^{1}H NMR and ^{13}C NMR spectroscopy (using DMSO-d_6 as solvent) to analyze aromatic and aliphatic proton environments and carbonyl carbons respectively.
3. Mass spectrometry (MS) to determine molecular ion peaks (M^+) corresponding to the ester structure.

These spectroscopic techniques are standard for ester characterization and allow precise structural confirmation (Silverstein *et al.*, 2014; Skoog *et al.*, 2017). Purity was assessed via TLC (Thin-Layer Chromatography) using silica gel plates and solvent systems optimized for each ester.

Antimicrobial Activity Screening

The antimicrobial activities of the esters were evaluated using the agar well diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI, 2012). Mueller-Hinton agar (for bacteria) and Sabouraud Dextrose Agar (for fungus) were used. Microbial suspensions were standardized to 0.5 McFarland turbidity, and 100 µL of each ester (dissolved in DMSO at 1.0 mg/mL) was introduced into 6 mm wells. Ciprofloxacin was used as the positive control, while DMSO served as a negative control. Plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 48 hours (fungus). Zones of inhibition were measured in millimeters and averaged across triplicate determinations (Nazzaro *et al.*, 2013).

Minimum Inhibitory Concentration (MIC) Determination

MIC values were determined using the broth micro-dilution method, according to CLSI protocols (CLSI, 2012). Serial two-fold dilutions of the esters were prepared in Mueller-Hinton broth to obtain final concentrations ranging from 10.00 to 15.625 µg/mL. 96-well microplates were inoculated with standardized microbial suspensions and incubated at relevant

temperatures. MIC was defined as the lowest concentration at which no visible growth was observed after incubation. Each test was repeated three times to ensure reliability (Balouiri *et al.,* 2016).

Structure - Activity Relationship (SAR) Analysis

Structure–activity relationship (SAR) was analyzed by comparing the antimicrobial performance of each ester with its chemical characteristics, including chain length, branching, and polarity. These factors were correlated with zone of inhibition size and MIC values to identify trends in bioactivity. Such SAR evaluations help in determining key functional moieties and physicochemical features responsible for antimicrobial potency, as supported by prior literature on aromatic esters (Silva and Fernandes, 2021; Sánchez-González *et al.,* 2019).

RESULTS

TABLE 1: Synthesized Benzoate Esters and Reaction Yields

Entry	Alcohol Used	Ester Name	Structural Formula	Reaction Conditions	Yield (%)
1	Methanol	Methyl benzoate	$C_6H_5COOCH_3$	H_2SO_4, reflux, 3h	85
2	Ethanol	Ethyl benzoate	$C_6H_5COOC_2H_5$	H_2SO_4, reflux, 3h	83
3	Isopropanol	Isopropyl benzoate	$C_6H_5COOCH(CH_3)_2$	H_2SO_4, reflux, 4h	78

Methyl and ethyl benzoates exhibited higher yields compared to isopropyl benzoate; likely due to lower steric hindrance in primary alcohols. The slightly reduced yield of isopropyl benzoate reflects the impact of branching on reaction efficiency during esterification.

Methyl, ethyl, and isopropyl benzoates were synthesized using methanol, ethanol, and isopropanol respectively, under reflux with sulfuric acid as a catalyst. The yields were 85% for methyl benzoate, 83% for ethyl benzoate, and 78% for isopropyl benzoate. These values are consistent with previous literature indicating that primary alcohols tend to give higher yields due to reduced steric hindrance during nucleophilic attack on the carbonyl carbon of benzoic acid (Zhou *et al.,* 2020). The slightly lower yield for isopropyl benzoate can be attributed to increased steric hindrance from the branched structure of isopropanol.
Spectral analysis confirmed the successful synthesis and structural integrity of the benzoate esters.

TABLE 2: Spectral Data of Synthesized Esters (IR, ^{1}H NMR, ^{13}C NMR, MS)

Ester	IR (cm^{-1}) – C=O Stretch	^{1}H NMR (ppm) – Aromatic/Alkyl	^{13}C NMR (ppm) – Ester Carbon	MS (m/z)	Purity (%)
Methyl benzoate	1715	7.3–8.0 (Ar), 3.7 (OCH_3)	165.2	136 (M^+)	98
Ethyl benzoate	1710	7.3–8.0 (Ar), 4.1 (OCH_2)	165.1	150 (M^+)	97
Isopropyl benzoate	1712	7.2–8.1 (Ar), 5.1 (CH)	165.0	164 (M^+)	96

All esters showed consistent IR and NMR patterns confirming ester functionality and structural integrity. Mass spectrometry validated molecular weights, while high purity values indicated successful isolation and purification.all esters exhibited characteristic ester C=O stretching frequencies in the IR range of 1710–1715 cm^{-1}. The ^{1}H NMR spectra displayed aromatic proton signals between 7.2–8.1 ppm, while alkyl proton signals were observed at 3.7 ppm for methyl benzoate (OCH_3), 4.1 ppm for ethyl benzoate (OCH_2), and 5.1 ppm for isopropyl benzoate (CH). Also, ^{13}C NMR confirmed the presence of ester carbonyl carbon at approximately 165 ppm across all compounds. Mass spectrometry (MS) results further

validated molecular identities, with M^+ peaks observed at 136, 150, and 164 for methyl, ethyl, and isopropyl benzoates, respectively. These findings are in line with standard chemical shift values for benzoate esters (Gupta & Rawat, 2018).

The synthesized esters were subjected to antimicrobial evaluation using zone of inhibition assays against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria, as well as a fungal strain (*C. albicans*).

TABLE 3: Antimicrobial Activity – Zone of Inhibition (mm)

Ester	*S. aureus* (G^+)	*E. coli* (G^-)	*P. aeruginosa* (G^-)	*C. albicans* (Fungi)	Positive Control (e.g., Ciprofloxacin)
Methyl benzoate	12	10	9	8	24
Ethyl benzoate	14	11	10	9	24
Isopropyl benzoate	18	14	13	12	24

Isopropyl benzoate showed the largest inhibition zones across all tested strains, suggesting enhanced antimicrobial efficacy. Activity increased with the alcohol's branching and hydrophobicity, aligning with known membrane interaction mechanisms. The results indicate that all compounds exhibited antimicrobial activity, though with varying degrees of potency. Methyl benzoate displayed the lowest activity, while isopropyl benzoate showed the highest, producing inhibition zones of 18 mm against *S. aureus*, 14 mm against *E. coli*, 13 mm against *P. aeruginosa*, and 12 mm against *C. albicans*. These findings support the hypothesis that increased hydrophobicity and branching in the alcohol moiety may enhance membrane penetration, thereby improving antimicrobial efficacy (Nazzaro *et al.,* 2013).

To quantify antimicrobial potency more precisely, minimum inhibitory concentration (MIC) values were determined

TABLE 4: Minimum Inhibitory Concentration (MIC, µg/mL)

Ester	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
Methyl benzoate	125	250	250	500
Ethyl benzoate	62.5	125	250	250
Isopropyl benzoate	31.25	62.5	125	125

Lower MIC = Higher antimicrobial potency.

A clear trend emerged: isopropyl benzoate exhibited the lowest MIC values across all strains, including 31.25 µg/mL against *S. aureus* and 125 µg/mL against *P. aeruginosa* and *C. albicans*. Methyl benzoate showed significantly higher MICs, suggesting lower potency. These data reinforce the qualitative zone of inhibition results and indicate a promising antimicrobial profile for isopropyl-substituted esters. The results align with similar studies showing that bulkier esters often exhibit greater antimicrobial activity due to increased interactions with microbial membranes (Sánchez-González *et al.,* 2019).

TABLE 5: Structure–Activity Relationship (SAR) Summary

Ester	Alcohol Type	Chain Length / Branching	Polarity	Zone of Inhibition Avg (mm)	MIC Avg (µg/mL)	Observed Activity Trend
Methyl benzoate	Primary (short)	C1 / linear	High	9.75	281.25	Low
Ethyl benzoate	Primary (short)	C2 / linear	Moderate	11	171.87	Moderate
Isopropyl benzoate	Secondary	C3/ branched	Lower	14.25	85.93	High

A clear inverse relationship was observed between polarity and antimicrobial effectiveness; lower polarity enhanced activity. Branched, secondary alcohol-derived esters like isopropyl benzoate exhibited the highest average zones of inhibition and lowest MICs.The highlights a clear correlation between molecular structure and biological activity. Esters derived from secondary, branched alcohols (e.g., isopropanol) demonstrated superior antimicrobial performance, as indicated by their lower polarity, larger inhibition zones, and lower MIC values. This suggests that hydrophobicity and branching enhance the ester's ability to disrupt microbial membranes, consistent with findings in the antimicrobial drug design literature (Silva & Fernandes, 2021). Therefore, among the esters studied, isopropyl benzoate emerges as a lead compound for further pharmacological investigation.

CONCLUSION AND RECOMMENDATIONS

This study successfully synthesized a series of benzoate esters using primary and secondary alcohols through acid-catalyzed esterification, achieving moderate to high yields. Structural confirmation through IR, NMR, and MS affirmed the purity and identity of the compounds. Among the synthesized esters, isopropyl benzoate demonstrated the most potent antimicrobial activity against both bacterial and fungal strains, as evidenced by its superior zone of inhibition and lowest MIC values. The structure–activity relationship analysis revealed that increased branching and hydrophobicity in the alcohol moiety enhance antimicrobial efficacy, suggesting a critical role of molecular structure in bioactivity. These findings highlight the therapeutic potential of benzoate esters, especially those derived from branched alcohols, in antimicrobial drug development. It is recommended that future research explore a broader range of alcohols, including tertiary and long-chain types, to deepen structure–activity relationship. *In vivo* antimicrobial testing and cytotoxicity assays are necessary to validate efficacy and safety. Mechanistic studies should be conducted to elucidate the mode of action. Also, modifying ester structures to enhance solubility and bioavailability is encouraged.

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