ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

Synthesis, Characterization and Antimicrobial Assessment of Benzyl – Quaternized Ammomium Ester

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Publication Date: 2026/08/11

Abstract: Methyl 3- (N-benzyl-N, N-diethylammonium) propanoate bromide was synthesized via quaternization of methyl 3- (N, N-diethylamino) propanoate with benzyl bromide. The synthesized Benzalkonium quaternary ammonium compound possessing ester functionality was characterized by FTIR, ^{1}HNMR, ^{13}CNMR and GC-MS. The antimicrobial activity of the ester QAC was achieved by determining its minimum inhibitory concentration (MIC) value and minimum bactericidal concentration (MBC) value against strains of gram-positive Bacterial (S. Pneumonae, B. aureus, S. aureus) and gram-negative Bacterial (K. Pneumonae, E. Coli) Our findings revealed that the synthesized soft QAC containing a hydrolysable ester group primarily exhibit bactericidal and bacteriostatic mode of action, effectively inhibiting bacterial growth even at appreciable minimum inhibitory concentration value of 6.25mg/L.

Keywords: *Soft QAC, Bactericidal, Bacteriostatic, Quaternization, Synthesized, Benzalkonium, Hydrolysable.*

How to Cite: Chukwueke, A. K.; Iwu, I. C.; Akalezi, C.; Alisa, C. O.; Ndukwu, D. E. (2026) Synthesis, Characterization and Antimicrobial Assessment of Benzyl – Quaternized Ammomium Ester. *International Journal of Innovative Science and Research Technology*, 11(7), 3483-3490. https://doi.org/10.38124/ijisrt/26jul1447

I. INTRODUCTION

Quaternary ammonium compounds (QACs) are amphiphilic substances possessing a central nitrogen atom bonded to four *aryl* or alkyl (R) groups and a negatively charged ion, usually a halide (X) or other organic anion ($N^{+}R_4X^{-}$). Fedorowicz et al. 2024. The cationic and amphiphilic nature of QACs accords it as membranolytic agent, the cationic nitrogen of QACs disrupt microbial cell membrane which is achieved by the electrostatic interaction of the nitrogen atom with the negatively charged phospholipid bilayer followed by the insertion of the QACs hydrophobic substituents into the microbial matrix thereby inhibiting microbial DNA replication and transcription leading to cell lysis. Sprung et al. 2025.

The structure of QACs grants them significant versatility, not only as antimicrobial agents, also acts as disinfectants, antiseptic, preservatives, sanitizers, phase transfer catalysts and personal care products. Quaternary ammonium compounds possess antimicrobial activity against broad spectrum of gram-positive and gram-negative bacterial and some viruses. QACs are stable in acidic and basic media and are usually soluble in water.

The wide variety of chemical structures of QACs and its modification has been an evolution in their effectiveness in activity and expansion of its methods of application over the last century. Selection of application and methods of application has been shown to affect the activity of QACs. Gerba et al. 2015.

QACs are classified on the type of R groups which can include long carbon chain, branching of the carbon compound and the presence of aromatic groups. These variations normally affect the antimicrobial activity of QACs in terms of dose and action against different strains of microorganisms. Crncevic et al. 2025, sharma et al. 2020.

II. EXPERIMENTAL SYNTHESIS OF QUATERNARY AMMONIUM COMPOUND

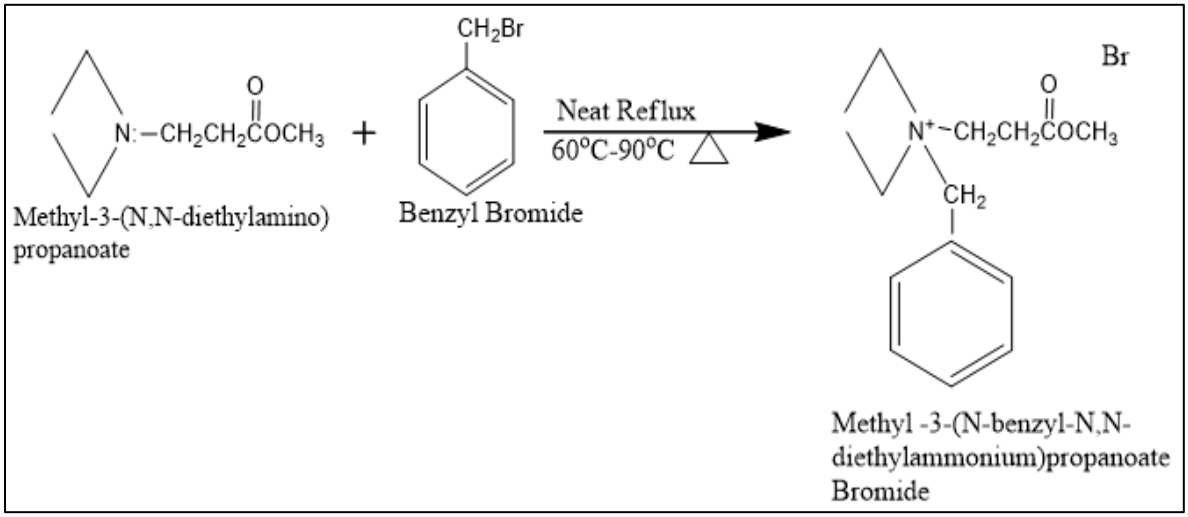
A 500cm^3 3-neck round bottom flask with a thermometer was immersed in a water pot and placed on a magnetic stirrer hot plate, 23g (0.1444 mol) methyl 3-(N,N-diethylamino) propanoate was added into the flask and

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

charged for 15 mins, 16.61g (0.1312 mol) benzyl bromide was slowly added. A reflux condenser is then installed and reaction was stirred for 7- 8hrs at temperature of 70-80°C. The reaction was quenched and allowed to cool to room temperature. The reflux condenser was removed.

8cm^3 dichloromethane (DCM) and 7cm^3 1.2M HCl was added to crude products in the flask and transferred to a separatory funnel. The separatory funnel was shaken vigorously and vented, the top aqueous layer was removed and poured into a beaker. The aqueous layer containing the quaternary amine and protonated tertiary amine was added 7cm^3 of 1.0M NaOH and 7cm^3 DCM. The DCM layer was removed leaving the QUAT in the aqueous layer which was transferred to a 250cm^3 beaker and gently heated at about 80°C to vaporize the water and residual DCM. An off-white product (26.10g) solid crystalline substance was obtained. The percent yield was calculated at 69.71% and the melting point of the crystalline product was recorded at 133°C.

➢ *Reaction Scheme 1 – Quaternization Reaction*



Scheme 1: Methyl -3- (N-Benzyl-N, N-Diethylammonium) Propanoate Bromide

➢ *Susceptibility Pattern /Zone of Inhibition of QAC*

The synthesized QUAT- methyl 3- (N-benzyl- N, N-diethyl ammonium) propanoate bromide was screened against three gram- positive bacterial strains (*Bacillus aureus, staphylococcus aureus, and streptococcus Pneumonia*) and two Gram-negative bacterial strains (*Escherichia coli and Klebsiella Pneumonia*). The bacterial suspensions were prepared in sterile saline from 24 hours cultures and standardized to obtain 0.5 McFarland's standard.

An inoculating loop was touched to five isolated colonies of the pathogen on an algar plate and used to inoculate a tube of culture broth which was incubated at 35-37°C until it became slightly turbid and was diluted to match the turbidity standard. The sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of the algar plate. After 5mins the appropriate antibiotic test disks which were prepared by dropping the test disks into appropriate antibiotic mixture (compound B or the antimicrobial compound) and placed with a multiple applicator device on the algar plate. The algar plate loaded with the compound B test disks was inoculated at 35-37°C 16-18 hours after which the diameters of inhibition zones (areas showing no microbial growth) were measured to the nearest mm (Lowry et al. 2006, Samore et al. 2005)

Minimum inhibitory concentration test (MIC) and minimum bactericidal concentration (MBC) were performed to determine the antibacterial activity of the quaternized ammonium ester. A bacterial susceptibility/zone of inhibition studies was also carried out to determine a pattern of bacterial growth inhibition by the quaternized ammonium ester. The susceptibility of the test microorganisms to the synthesized ammonium ester QAC was evaluated based on the diameter of the zone of inhibition. Larger zones of inhibition indicate greater sensitivity of the microorganisms to the anti-microbial compound, while smaller zones indicate reduced susceptibility.

➢ *Spectroscopic Procedure*

FTIR spectra of synthesized product was recorded from 400 to 4000cm^{-1} on Agilent Cary 630 ATR/FTIR spectrophotometer. The FTIR spectrum is shown in Figure 1.

^{1}HNMR AND ^{13}CNMR spectra were recorded on Nanalysis X685 60MHz NMR Spectrophotometer using D_2O as solvent. ^{1}HNMR AND ^{13}CNMR spectra of the ester QAC obtained as shown in Figures 3 and 2 respectively.

GC-MS analysis was performed using Agilent 7890 Gas chromatography coupled to a mass selective detector. Analysis was carried out according to the principles of USEPA method 8270 for non- volatile to low-volatile organic compounds. Separation was achieved on an HP -5MS capillary column using helium as carrier gas at a flow rate of 1.0mL min^{-1}.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

III. RESULTS

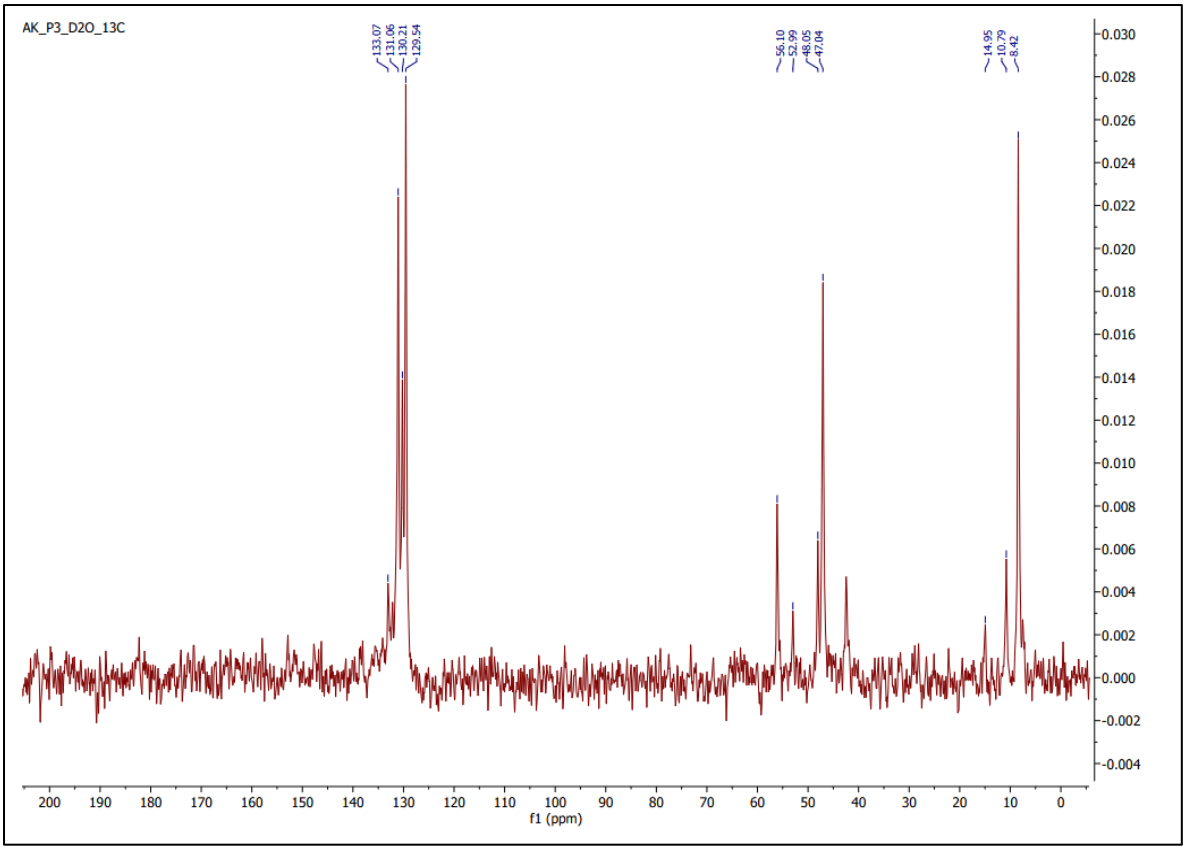


Fig 1 FTTR Methyl 3-(N-Benzyl-N,N – Diethyl Ammonium) Propanoate Bromide.

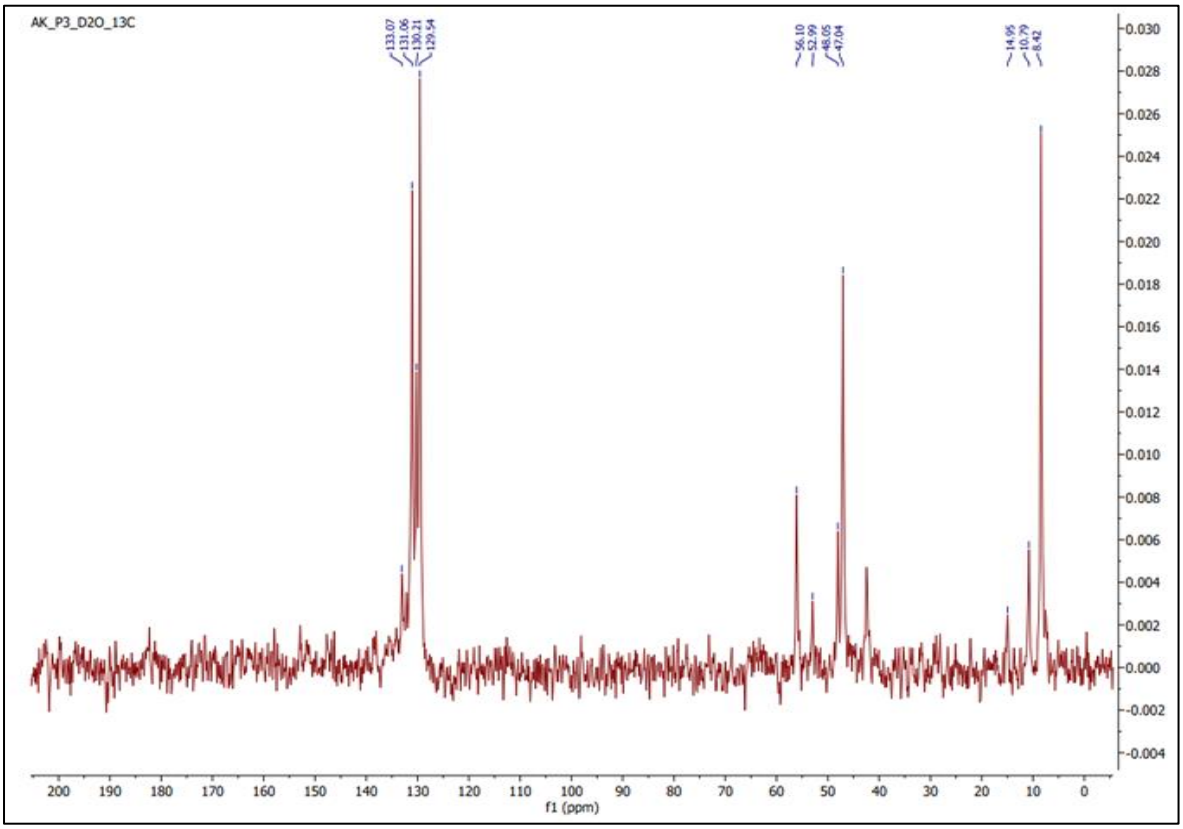
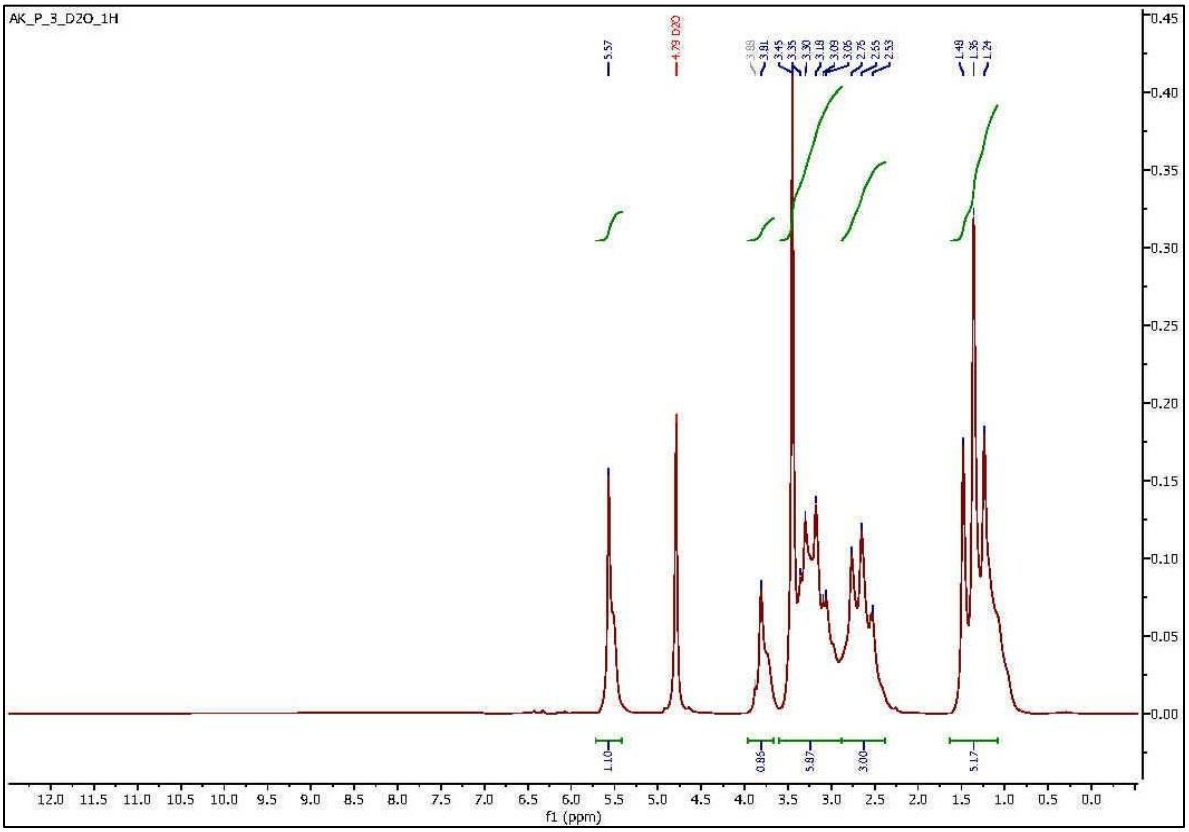


Fig 2 ^{13}CNMR X685 60MHz

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447



Nanalysis X685 60 MHZ

Fig 3 [1]HNMR Spectrum of Methyl 3-(N-Benzyl-N,N – Diethyl Ammonium) Propanoate Chloride

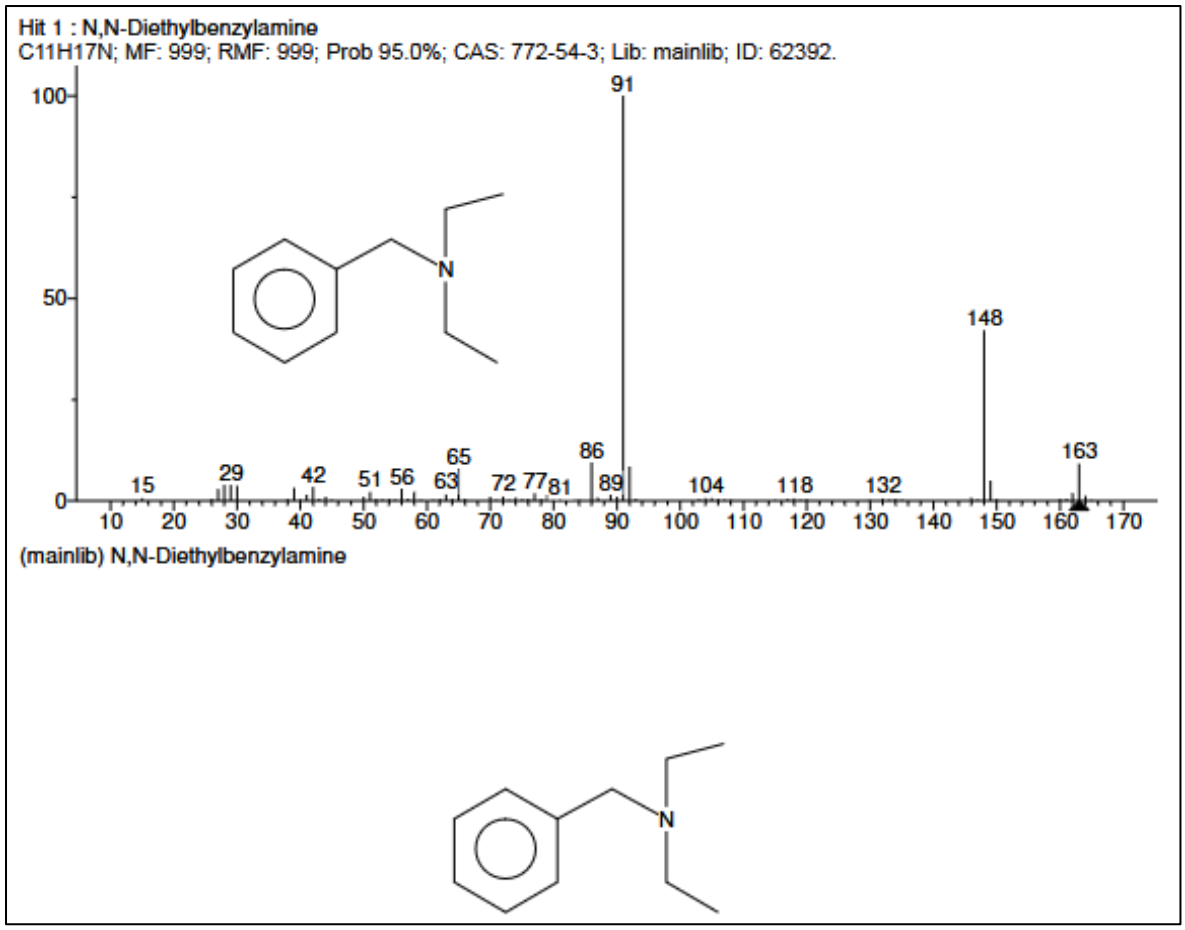


Fig 4 GC-MS

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

➢ *Spectral Data*

- ^{13}CNMR (D_2O, δ): 170 - 175 (C,1) C=O, 50 - 55 (C,1) OCH_3 of Ester, 55 - 60 (C,1) Benzylic (-CH_2), 45 - 48 (C,1) N^+-CH_2, 8 - 10 (C,1) CH_3 of ethyl group, 40 - 43 (C,1) β-amino ester chain: CH_2 carbonyl, 50 - 65 (C,1) β – amino ester chains carbon: CH_2 near quaternary nitrogen, 125 – 140 (C,4) Aromatic carbons.

- ^{1}HNMR (D_2O, δ): 1.2 t(6H), 2.62 t(2H), 3.2 - 3.6 q(4H), 3.89 s(3H), 2.62 t(2H), 4.5 s(2H), 5.5 – 6 s(5H). The yield of the synthesized product was 69.71%.

- FTIR : 3129 cm^{-1} (Broad small peak, C – H stretching vibration benzylic group), 2820-2926 cm^{-1} (Broad sharp peak, Aliphatic C – H stretching vibration CH_2/CH_3 groups), 1676 cm^{-1} (Broad band, C = O absorption band of a saturated ester), 1461 cm^{-1} (Intense peak, C – H stretching band from aromatic ring), 1372 cm^{-1} (Small sharp band, C – N stretching vibration of aliphatic amine), 1299 cm^{-1} (C – O stretching vibration of a saturated ester. Ester C – O – C)

Table 1 GC-MS

M/z	RELATIVE INTENSITY %	FRAGMENT	STRUCTURAL IMPORTANCE
163	18	Benzyl ammonium Ester Fragment	Retains portion of the molecular structure
148	50	Derived from M/z 163 after loss of CH_3	Indicates the ester-containing side chain
91	100 (Base Peak)	Tropylium ion ($C_7H_7^+$)	Characteristic fragment of the benzyl group
86	-	Diethylammonium fragment	Confirms the N, N-diethyl-amino group.
72	-	Aminoethyl fragment ($C_2H_5^+$)	Further fragmentation of the diethylammonium unit
77	-	Phenyl ion ($C_6H_5^+$)	Aromatic ring fragment

Table 2 Susceptibility Pattern/Zone of Inhibition of Synthesized Ester QAC against Test Organisms

SAMPLE	Concentration mg/L	Streptococcus Pneumonia mm	Staphylococcus Aureus mm	Bacillus Aureus mm	Escherichia Coli mm	Klebsiella Pneumonia mm
Methyl 3-(N-benzyl-N,N-diethyl Ammonium) Propanoate Bromide	100	26.5	31	32	44	45
	50	23.5	41	30	36	41
	25	22	35	27	32.5	38.5
	12.5	20.5	34	23.5	28.5	36.5
	6.25	18.5	23	22.5	21.5	28

➢ *Concentration – Response Curve*

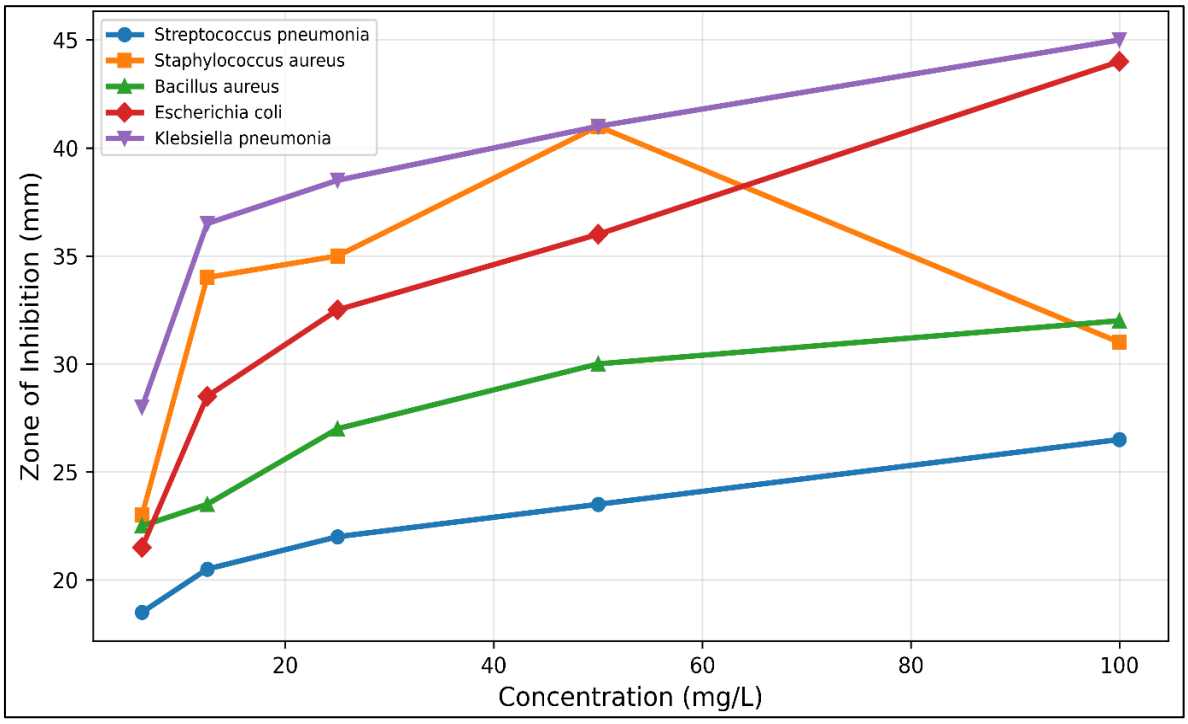


Fig 5 Concentration- Dependent Antimicrobial Activity of the Synthesized Ester Quaternary Ammonium Compound Against Selected Bacterial Strain

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

Table 3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of QAC

Sample	Ester QAC Concentration (mg/L)	Test Organisms				
		Streptococcus Pneumonia	Staphylococcus Aureus	Bacillus	Escherichia coli	Klebsiella pneumonia
Methyl-3-(N-benzyl-N,N-diethyl Ammonium) Propanoate Bromide	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
MIC		6.25	6.25	6.25	6.25	6.25
MBC		-	-	-	-	-

IV. DISCUSSION

➢ *Antimicrobial Assessment*

Quaternary ammonium compounds (QACs) are an important class of cationic antimicrobial agents generally recognized for its membrane- active properties against broad spectrum of microorganisms. Their antimicrobial efficacy is due to disruption of the cytoplasmic membrane and distortion of DNA/protein synthesis which leads to cell death.

The antimicrobial assessment of the synthesized quaternary ammonium compound, methyl 3-(N, N-diethyl ammonium) propanoate bromide (table 2) showed a strong activity against selected broad spectrum of gram positive and gram negative bacterial tested at different concentration values of 100mg/L, 50mg/L, 25mg/L, 12.5mg/L and 6.25mg/L. The observed inhibitory effect shows that the synthesized compound possesses antimicrobial activities capable of bacteriostatic action against bacterial. Studies have provided evidence that antimicrobial activity of quaternary ammonium compounds are due to the type of alkyl groups attached to the nitrogen atom indicating that longer alkyl chain length, presence of benzene substituents enhances interactions with microbial phospholipid membranes (Schnurch et al. 2025, Pardal et al.2002, Othman et al. 2014, Yousseff et al. 2013.).

The antimicrobial analysis data (table 2) shows that the synthesized ester QAC exhibited larger inhibition zones at MIC value of 6.25mg/L against two gram-negative bacterial (E. Coli, K. Pneumonae) despite the double membrane structure associated with gram-negative bacterial. This results are consistent with the synthesized ester quaternary ammonium compounds (Lindstedt et al. 1990) and Diethylaminoethyl methacrylate quaternary ammonium compound (Mohammed et al. 2010). These findings reveal that at higher concentration – (higher MIC values) ester quaternary ammonium compounds possess capability to inhibit bacterial growth preferably due to their greater susceptibility to E. Coli and K. Pneumonae respectively which also suggest a strong interaction with the negatively charged phospholipid bilayer of bacterial membrane. Our data (table 3) shows that the synthesized QAC exhibited same MIC and MBC values of 6.25mg/L against pathogenic bacterial tested. This identical MIC/MBC values shows that the concentration values required to inhibit bacterial growth were also enough to kill pathogenic bacterial. This study has shown that benzyl quaternized ammonium ester possess antimicrobial properties and identifies the importance of structural modification in improving the biological activity of quaternary ammonium compounds which provides basis for further development of effective, efficient biodegradable and safer handling of antimicrobial agents.

➢ *Characterization*

The synthesized quaternary ammonium compound - Methyl 3- (N-benzyl-N,N-Diethylammonium) propanoate bromide was obtained as an off-white crystalline solid , its FTIR spectrum (fig.1) confirmed the presence of essential functional groups needed for the ester QAC. The absence of N—H and O – H stretching bands was consistent with the quaternized ammonium ester, a broad peak at 2820- 2926 cm^{-1} confirms the aliphatic C—H stretching vibration of CH_3 group, this is consistent with the presence of an absorption band at 2865-2979cm^{-1} of Diethylamino ethyl methacrylate ammonium ester (Makhloufia et.al.2010) which confirms a saturated C-H stretching vibration band. Also, the C-H stretching band of Linear polyisocyanate quaternary ammonium salt (Fu et.al.2021) which appeared at 2880-2963cm^{-1} confirms a saturated alkyl chain length. The presence of a carbonyl (C=O) stretching vibration at 1672cm^{-1} and the C—O stretching band at 1299cm^{-1} provides evidence of an ester functionality of a saturated compound, this is consistent with the structure of Diethylamino ethyl methacrylate ammonium ester (Makhloufia et. al. 2010) with a carbonyl band at 1720cm^{-1}. The carbonyl vibrational band observed at 1697cm^{-1} for the structure of Linear polyisocyanate quaternary ammonium compound (Fu et. al. 2021) confirms the ester functionality of the synthesized ester QAC. The synthesized ammonium ester showed an intense peak at 1461cm^{-1} and a sharp peak at 1372cm^{-1} corresponding to C—H stretching band from aromatic ring and C—N stretching band of an aliphatic amine respectively. The broad peak at 888cm^{-1} and 722cm^{-1} of the synthesized ammonium QAC is due to the bromide ion and monosubstituted benzene C-H out of plane vibration of the benzylic group respectively, This is consistent with the C-H stretching band of a disubstituted benzene structure of LPOIQAC (Fu et. al.2021).

The signals obtained from ^{1}HNMR spectrum (fig. 3) of the synthesized ester QAC provided complete confirmation for the structure elucidation of the compound. The methoxy

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul1447

(-OMe) protons of the ester group appeared as singlet at (δ3.89) integrating for three protons which confirms the presence of methyl ester functionality, this is consistent with the 1HNMR spectrum of Diethylamino ethyl methacrylate ammonium ester (Makhloufia et. al.2010) and the 1HNMR for the structure of Linear polyisocyanate quaternary ammonium compound (Fu et. al.2021) methoxy (-OMe) group which were integrated for three protons and appeared as singlets respectively. The methyl groups of the β- amino ester chain appeared in the expected aliphatic chain region as triplets and integrated for two protons. This is also consistent with the spectrum of Diethylamino ethyl methacrylate (Makhloufia et.al 2010) which showed that the methylene groups of the β- amino ester chain appeared as triplets and integrated for two protons. The benzylic group of the synthesized ester QAC appeared as singlet and integrated for two protons is consistent with the benzylic group ^{1}HNMR of Linear polyisocyanate QAC (Fu et.al 2021) which were integrated for two protons and appeared as singlet. The aromatic peak of the synthesized ester QAC shifted a little downfield as a monosubstituted benzene, it appeared as a singlet and integrated for five protons, this is consistent with the 1HNMR spectrum of LPOI QAC aromatic signals for disubstituted benzene which integrated for four protons and appeared as singlet (Fu et.al. 2021). The observed chemical shifts of the synthesized ester QAC and integration were consistent with the quaternization of nitrogen atom and supported the proposed quaternary ammonium structure

The ^{13}CNMR spectrum of Methyl 3- (N-benzyl -N, N-diethylammonium) propanoate bromide confirmed its structure. (fig. 2)

Th ester carbonyl carbon with a weak signal appeared in the expected carbonyl region confirming the presence of hydrolysable ester functional group. The methoxy (-OMe) carbon which confirms methyl ester appeared at the expected region (δ50-55). The signals associated with the β-amino ester chain carbons were present at regions (δ 40-43) , – CH_2 near ester CH_2COO- and CH_2 near quaternary nitrogen. The signals for N, N- diethyl substituents were observed at region (δ8-10) , The benzylic carbon (CH_2) attached to quaternary nitrogen were observed at region (δ 60) gave strong evidence that quaternization occurred. The observed resonances were in agreement with the proposed molecular structure of the synthesized ester QAC.

The GC-MS spectrum (fig. 4) gave more confirmation of the molecular structure of the synthesized QAC ester by its fragmentation pattern.

The base peak at M/z 91 was assigned to the tropylium ion, a characteristic fragment normally observed from the benzylic substituent, this fragment is highly stabilized by resonance and usually the most intense peak in benzyl – containing compounds. Other fragmentation ions at M/z 86, 72, and 58 are due to the cleavage of the diethylammonium group. The M/z 148 is important because it is 50% as intense as the base peak, it is likely one of the stable fragments from the ester containing part of the molecule. (cleavage of the methoxy group of the molecule). The observed fragmentation pattern was consistent with the presence of the benzyl, ester and diethylammonium groups of the molecule.

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