CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF QUATERNARY AMMONIUM COMPOUNDS SYNTHESIZED FROM CANOLA AND COCONUT OILS

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) IN ORGANIC CHEMISTRY

SEPTEMBER, 2021

CERTIFICATION

This is to certify that this research work "**Characterization and antimicrobial evaluation of quaternary ammonium compounds synthesized canola and coconut oils**" was carried out by AMAEFULE ONYINYECHI .C. (Reg. No: 20154141658) in partial fulfillment of the requirement for the award of Masters of Science (M.Sc) degree in Organic Chemistry, at the Department of Chemistry, Federal University of Technology, Owerri, Imo State.

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DEDICATION

I dedicate this research work to my wonderful parents for their unconditional love towards me and also for their financial and moral support, may God bless and keep you.

ACKNOWLEDGEMENTS

I am forever grateful to the God Almighty, whom by His grace I was able to complete this work amidst all difficulties.

I remain grateful to my supervisors, Prof. (Mrs) C.E. Ogukwe and Dr. F.C. Onwumere, for their exceptional contribution and support to the successful completion of this work.

I thank Dr. C.O Akalezie, the Post-Graduate Coordinator, Chemistry Department for his assistance throughout my study.

I would also like to say thank you to the Head of Chemistry Department, Prof. Mrs. C. E. Ogukwe and the entire staff of the Chemistry Department for their contributions during the course of this study.

My special appreciation goes to my beloved parents Chief Sir & Lady Edmund Amaefule (KSM) and my beloved family, for their love, care, moral and financial support and encouragement towards my academic pursuits.

Finally, to my friends and fellow students, who in one way or the other have assisted me during this research work, thanks to you all.

ABSTRACT

Quaternary ammonium compounds were synthesized from Canola oil (*Brassica, napus*) and Coconut oil (*Cocos nucifera*) extracts by reacting the oils with N,N-diethylethylenediamine in the presence of potassium tertiary butoxide as catalyst. Fourier Transform Infrared (FTIR) spectrometric analysis of the starting materials, the intermediates and the final products were carried out and the results showed a disappearance of the carbonyl (C=O) stretch of the oil esters at 1744 cm^{-1} and appearance of an N-H band for an amide at 3433 cm^{-1}. Other functional groups such as C=C and C-H stretch of an aromatic compound and C=O stretch of an amide were also observed. Antimicrobial activity of the synthesized quaternary ammonium compounds showed excellent anti-bacterial activity with an inhibition diameter of 22 mm and 28 mm for *E.coli* by QAC from *Cocos nucifera and Brassica napus* oils respectively as compared to a standard antibiotic (Ampicillin).

TABLE OF CONTENTS

CHAPTER ONE

1.0 INTRODUCTION

Quaternary ammonium cations (QAC) also known as quats, are positively charged polyatomic ions of the structure R_4N^+, R being an alkyl group or an aryl group (IUPAC, Compendium of Chemical Terminology; 2006). Unlike the ammonium ion (NH^+_4) and the primary, secondary, or tertiary ammonium cations, the quaternary ammonium cations are permanently charged, independent of the pH of their solution and they are stable with a long shelf life (Smith, Michael, March, and Jerry, 2001). QACs comprise a quaternary nitrogen atom surrounded by four covalently bound organic moieties, whereby one permanent positive charge arises at the nitrogen atoms. Their charge is balanced by a counter ion, typically chloride or bromide, thus they exist as salts (Ines, Jan, Valeric, Wulf, Kornelia, and Sven, 2017).

The quaternary ammonium compounds are the products of a nucleophilic substitution reaction of alkyl halides with tertiary amines. Chemically, they have four carbon atoms linked directly to the nitrogen atom through covalent bonds, while the anion in the original alkylating agent becomes linked to the nitrogen by electrovalent bond. The general molecular structure for the quaternary ammonium compounds is represented in fig.1;

Figure 1: Basic Structure of QACs

R_1, R_2, R_3, and R_4 are alkyl groups that may be alike or different, substituted or unsubstituted, saturated or unsaturated, branched or unbranched, and cyclic or acyclic, and that may contain ether or ester or amide linkage; they may be aromatic or substituted aromatic groups. The nitrogen atom plus the attached alkyl groups form the positively-charged cation portion, which is the functional part of the molecule. The portion attached to the nitrogen by an electrovalent bond may be any anion, but is usually chloride or bromide to form the salt (Ines *et al.*, 2017). The four alkyl/aryl substituent in QACs also account for significant separation of R_4N^+ from its counter anion, especially in solution. It is believed that R_4N^+, often referred to as synthetic alkali metal, is completely dissociated in water solutions. As ionic substances, QACs are generally highly soluble in polar and protic solvents such as water and alcohols. However, their solubility decreases dramatically with increasing chain length, and QACs with R exceeding C_{14} have low solubility, or further are practically insoluble, in water. On the contrary, the solubility of QACs bearing long chains in non-polar solvents is substantially improved. QACs are generally solids, but their thermal properties can be modulated to a wide extent by the structure and length of the appended R-residues. Another notable feature of QACs is their ionic conductivity; their solutions are very good electrolytes (Bureš, 2019). Because of their structure comprising both polar (N+) and non-polar (R) terminuses, QACs are able to absorb on a surface or an interface, thus reducing their tension. This feature makes them a very popular group of surfactants. QACs exhibit a very wide range of biological and antimicrobial activity, which also determines their current application as bioactive agents. These unique physicochemical properties of QACs have resulted in their wide and diverse applications and their large industrial production. With production exceeding 500,000 tons/year worldwide, QACs have been included on the list of high-production-volume chemicals by the Organization for Economic Co-operation and Development (OECD, 2004).

Quaternary ammonium compounds are prepared by the alkylation of tertiary amines with a halocarbon. In older literature this is often called a Menshutkin reaction, however modern chemists usually refer to it simply as quaternization (Kuca, Kivala and Dohnal, 2004). The reaction can be used to produce a compound with unequal alkyl chain lengths; for example when making cationic surfactants one of the alkyl groups on the amine is typically longer than the others (Kurt, Hüls and Marl, 2000). A typical synthesis is for benzalkonium chloride from a long-chain alkyl dimethyl amine and benzyl chloride:

$$CH_3(CH_2)_nN(CH_3)_2 + ClCH_2C_6H_5 \rightarrow [CH_3(CH_2)_nN(CH_3)_2CH_2C_6H_5]^+Cl^-$$

QACs are further classified on the basis of the nature of the R groups, which can include the number of nitrogen atoms, branching of the carbon chain, and the presence of aromatic groups. These variations can affect the antimicrobial activity of the QAC in terms of dose and action against different groups of microorganisms. Examples of the structures of common QACs are shown in figure 2 (Charles P. Gerba, 2015). The length of the R groups affects the antimicrobial activity of QACs, having the alkyl chain lengths of C12 to C16 usually shows the greatest antimicrobial activity.

Centrimide Benzalkonium Chloride

Figure 2: Examples of some common QACs

Many antimicrobial products contain mixtures of QACs and other adjuvants to increase their efficacy or to target a specific group of organisms (Moore, Ledder, Gilbert, McBain, 2008). The wide variety of chemical structures possible with QACs has allowed an evolution of their effectiveness and an expansion of their applications over the last century as shown in Table 1 (Charles P. Gerba, 2015). It was noted that this increased efficacy while reducing costs and lowering toxicity.

TABLE 1: Evolution of quaternary ammonium compounds

Generations (1916-1950)	**Compound(s)**
1st	Benzalkonium, alkyl chains, C12 to C18
2nd	Aromatic rings with hydrogen and chlorine, methyl and ethyl groups
3rd	Dual QACs; mixture of alkyl dimethyl benzyl ammonium chloride (lower toxicity)

4th	Dialkyl Methyl amines with two chains
5th	Synergistic combinations of dual QACs
6th	Polymeric QACs
7th	Bis-QACs with polymeric QACs

Quaternary ammonium compounds have been recognized as substances that can destroy living organisms with activity against a wide range of bacteria, viruses, yeasts and fungi (Charles Gerba, 2015). Hence, QACs include common disinfectants, antiseptics, preservatives, and sterilization agents used in households, hospitals, the textile/food industry, and water treatments. For instance, Domagk reported that benzalkonium chloride (BAC) was marketed as the antimicrobial agent 'Zephirol' as early as 1935. Christopher, Geoff, and Stephen in 2007, demonstrated that BAC and Didecyldimethylammonium chloride (DDAC) are traditional membrane-active antimicrobial agents with a similar minimum inhibitory concentration (MIC) against *Staphylococcus aureus* (0.4–1.8 ppm). Furthermore, Kevin, Megan, Jennings, Laura, Jacob, (2016) studied more than 200 QACs including paraquats (un)symmetrically *N*-disubstituted with various alkyl chains TMEDA derivatives, and further extended analogues with three and four heads. They noted that the MIC values of these new-generation QACs measured against standard panel of bacteria (Gram-positive and Gram-negative) ranged from 1 to 16 μM which proved that flexible and multi-headed QACs are less prone to microbial resistance

Because of the increasing microbial resistance towards these traditional QACs, new-generation QACs have recently been designed and developed like the ones synthesized in this work.

1.2 STATEMENT OF THE PROBLEM

Due to the variety of diseases caused by microorganisms and increasing drug resistance by bacteria to conventional antimicrobial agents, it is pertinent to synthesize modified QAC to combat such diseases and resistance.

1.3 AIM OF THE STUDY

To synthesize, characterize and evaluate the antimicrobial properties of QACs from Canola oil (*Brassica napus*) and Coconut oil (*Cocos nucifera*).

1.4 OBJECTIVES OF THE STUDY

The evaluation of QAC shall be achieved through the following objectives:

- Through the synthesis of tertiary amine compound by reacting the oils (canola and coconut oil extracts) with N,N-diethylethylenediamine in the presence of potassium tertiary butoxide (catalyst).
- Through the synthesis of QAC by quaternization of the tertiary amine (intermediate).
- Characterization of the synthesized quaternary ammonium chloride using Infrared spectroscopy
- To determine the antimicrobial studies of the different diameters of zone of inhibition of the synthesized compounds.

1.5 JUSTIFICATION OF THE STUDY

To find out if the presence of the amide functional group in the molecular framework will reduce and/or eliminate the bacteria resistance to conventional QACs.

1.6 SIGNIFICANCE OF THE STUDY

The presence of the amide functional group which resulted from the reaction of ester (coconut and canola oil) and the amine may eliminate the bacteria resistance to conventional QACs.

1.7 SCOPE OF STUDY

This research work focuses on reacting the natural oils with N,N-diethlyethylenediamine to form an intermediate(a tertiary amine), quaternizing the intermediate with benzyl chloride, FT-IR characterization of the quaternized compounds and anti-microbial evaluation of the synthesized compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PREPARATIONS AND CHEMICAL REACTIVITY OF QACS

In most cases, the synthesis of QACs is carried out from an amino compound, a nucleophile, and its exhaustive alkylation with a variety of electrophilic agents as shown in Scheme 1(Filip Bures, 2019).In principle, primary, secondary and tertiary amines can be used; however, the latter is the most common and convenient starting material. Quaternization of tertiary amines is also referred to as Menshutkin reaction. From the history, *N*-alkylation of amines involves Hofmann's reaction utilizing alkyl halides.

Where X (R_4X) can be Cl, Br, I, OTs, OH, OSO_3R, $O^+R_2BF_4^-$, H (alkene)/H+, or Organometal etc. R=alkyl Chain.

Scheme 1: General Synthetic route towards QAC starting from (a) Primary, (b) Secondary, and (c) tertiary amines.

2.2 QUATERNARY AMMONIUM COMPOUNDS AS DISINFECTANTS

Charles P. Gerba, 2015 noted the McDonnell proposal of the following series of events which occurs in the action of QACs against microorganisms: (i) QAC adsorption to and penetration of the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein), followed by membrane disorganization;(iii) leakage of intracellular lower-weight material; (iv) degradation of proteins and nucleic acids; and (v) cell wall lysis caused by autolytic enzymes. Soft antimicrobial agents bearing a labile linker may undergo facile non-enzymatic degradation into nontoxic building blocks (Thorsteinn, Már, Karl, Martha, Hilmar, Thorstenin, 2003). They noted that QACs with C12-C18 alkyl chains proved to be the most potent antibacterial agents. Terry, Vanessa, Haritha, Patricia, Jodi, Bill, Stephen, (2014), further reported that the modifications to alkyl chain length have been used to optimize cleaning and antimicrobial properties. They stated that through substitution of aromatic ring hydrogen with chlorine, methyl, and ethyl groups specifically, antimicrobial efficiency increases and improves detergent strength. Different generations of QACs have been generated as earlier noted in the previous chapter of this study. It has been reported that twin-chain or dialkyl quaternary QACs represent the newest generation which exhibit a wide spectrum of activity. These new synthetic polymeric QACs contain multiple positively charged amine centers that confer antimicrobial, anti-static, and surfactant properties in solution (Terry *et al*, 2014).

2.3 QUATERNARY AMMONIUM COMPOUNDS AS ANTIBACTERIAL

QAC exhibiting antibacterial properties are said to be surface-immobilized on nanoparticles, polymeric backbone, glass, or membrane (Mukherjee and Sirshendu, 2018). For example, it was reported by Yudovin-Farber, Nurit, Ervin, Abraham, (2010), that polyethyleneimine-based nanoparticles peripherally modified with

QACs having alkyl group of C4-C16, embedded in restorative composite resin prepared by simple quaternization as antibacterial agents. Antibacterial activity has shown to significantly depend on alkyl residue, with octyl (C8) derivative being the most potent compound. Ruowen, Guiqian, Dingcai, 2007 and Bekir, Mohamed, Lon, 2004 carried out a similar technique in preparing antibacterial polymers by utilizing methacrylic acid as polymerizable terminus appended to QAC core. They both group of authors concluded that chain length (R) and polymer size were the factors most significantly affecting the measured Minimum Bactericidal Concentration(MBC) values against Gram-positive/negative bacteria. Antonucci Zeiger, Tang, Sheng, Bruce and Lin, (2012) also synthesized asimilar dimethacrylate-QACs which was applied as precursors of antibacterial polymers used in Dentistry. Another useful polymeric backbone is the hyper-branched polyurea cationic QAC molecules which showed high effective contact-killing coating with a different mechanism of action than in solution (Lia, Mihaela, Steven, Yun, Oleksii, Petra, Joerg, Henny, Ton, Henk, 2013). A quaternary ammonium group containing hybrid coating by sol-gel process using tetraethoxysilane showed excellent antibacterial properties to glass surfaces (Muhammad, Jamil and Munawar, 2009).

McBain, Ledder, Moore, Catrenich, Gilbert, (2004) noted that Quaternary ammonium compounds (QACs) are amphoteric surfactants that are widely used for the control of bacterial growth in clinical and industrial environments. Broad-spectrum antimicrobial activity and surfactant properties have made QACs such as benzalkonium chloride the favored hygienic adjuncts in disinfectant cleansing formulations, and they have been increasingly deployed in domestic cleaning products over the last decade. They noted that the antimicrobial action of QACs involves perturbation of cytoplasmic and outer membrane lipid bilayers through association of the positively charged quaternary nitrogen with the polar head groups of acidic phospholipids. The hydrophobic tail subsequently interacts with the hydrophobic membrane core. At concentrations normally used for application to inanimate surfaces, QACs form mixed-micelle aggregates with hydrophobic membrane components that solubilize membrane and lyse the cells. Lethality occurs through generalized and progressive leakage of cytoplasmic materials (McBain *et al*, 2004).

Ioannou, Geoff and Stephen (2007) noted the structural functionality of QACs, especially the role of chain length on activity against different bacteria. They reported that QACs with C16 chain length hydrophobic tail length affects the outer membrane of gram-negative bacteria more extensively than shorter-chain compounds, possibly due to the C16 chain interacting strongly with the fatty acid portion of lipid A. It has been reported that monoalkyl QACs bind by ionic and hydrophobic interactions to microbial membrane surfaces, with the cationic head group facing outwards and the hydrophobic tails inserted into the lipid bilayer, causing the rearrangement of the membrane and the subsequent leakage of intracellular constituents.

2.4 QUATERNARY AMMONIUM COMPOUNDS AS SURFACTANTS

Bureš (2019) described QACs as well-recognized cationic surfactants (surface active agents) featuring a positively charged and hydrophilic head (N+) and hydrophobic tail (long chain) (as shown in fig 2). In contrast to parent amines, QACs are permanent cationic surfactants regardless of the pH. Their amphiphilic nature is responsible for the fundamental mechanism of their action, which proceeds by reducing interfacial tension between two phases. Two fundamental parameters of each surfactant that must be considered before its application are surface activity and tendency to self-assemble in solution and form aggregates, micelles, and vesicles (critical micelles concentration, CMC) (Ramanatha, Lok-Kumar, Taizo, Qingmin, Jonathan and Katsuhiko, 2013).

Despite their lower industrial production as compared to anionic (sulfonate) surfactants, a broad application potential still exists for QAC surfactants, including emulsification, dispersion, wetting, foaming, stabilization, fabric softeners, antistatic agents, detergents, solid-state extractions, and many others (Filip Bureš, 2019). The fundamental physicochemical properties of QAC surfactants can be easily changed by the QAC structure and by adding other electrolytes into solution (Zia, Noor, Farman, Nasir and Hidayat, 2017). Sumit, Vinod and Kohlbrecher, (2014) studied the extent of interaction and stabilization of nanoparticles and proteins with cationic analogue, Dodecyltrimethyl Ammonium Bromide (DTAB) and compared it with anionic analogue, sodium dodecylsulfate(SDS), which revealed that the roles of the two surfactants were interestingly different, and the resulting three-component system can be easily tuned by the surfactant used.

2.5 QUATERNARY AMMONIUM COMPOUNDS IN ORGANIC SYNTHESIS

Bureš (2019) stated four general functions of QAC in organic synthesis and routine laboratory practices, that QAC may act as a (1) starting material, (2) reagent, (3) catalyst, (4) solvent. He noted that the utilization of QACs as starting materials is demonstrated in Scheme 2. Specifically, QACs have found useful application in Hofmann elimination reactions producing alkenes. However, this reaction is generally limited to tetraalkyl ammonium hydroxides. Therefore, most of the ammonium halides are converted to hydroxides by reacting them with silver oxide prior to thermal elimination (Filip Bureš, 2019). He further stated that another useful feature of this reaction is its possible extension to amines, which may be converted in situ into corresponding QACs by exhaustive alkylation with reactive methyl iodide. The methyl group has no β-hydrogens and thus cannot compete in the subsequent elimination reaction. The three-step reaction sequence is depicted in scheme 2.

Scheme 2: Conversion of amines/QACs into Alkenes

Michael, Manuel and Robert (2017) demonstrated QAC Hofmann elimination and utilization of the formed alkene as an alkylating reagent in C–H activation. The recent demonstration of Benzylic ammonium triflates which is similar to benzyltrimethylammonium hydroxide (Triton B) as useful starting materials for carbonylation reaction to amides via C-N bond activation was noted by Weijie, Shuwu, Fei, Tianxiang, Yan, Yuanyuan, Junkai and Tao, 2018. Stanley H. Pine (2011) and Li JJ (2014) reported that despite discrepancies in the mechanistic pathways of base-promoted QAC rearrangements, the Stevens rearrangement was found as a useful application in the preparation of tertiary amines. Jérôme, Maria-Héléna and Laurent, 2008 showed the use of a supra molecular asymmetric ion-pairing strategy which enabled the preparation of optically pure amines with enantiomeric excess (*ee*) up to 55% (shown in scheme 3).

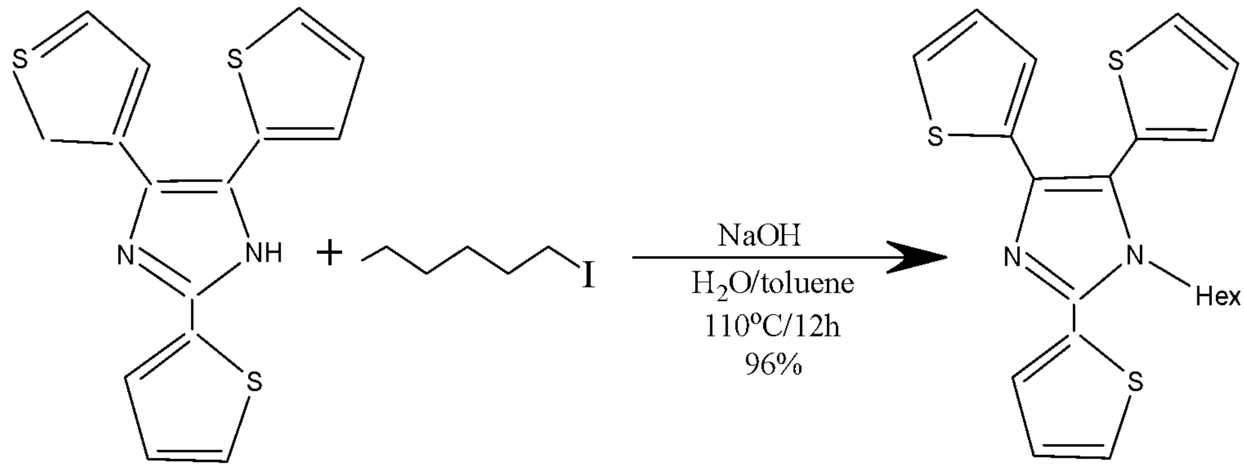
Scheme 3: Enantioselective 1,2-migration (Stevens rearrangements of QACs)

The straightforward synthesis of tetrahydrioisoquinoline alkaloids was recently utilized also by Stevens's rearrangement (Orejarena, Lahm, Opatz, 2013). Kivala and Diederich (2009) and Klikar, Solanke, Tydlitát and Filip (2016) reported that 1-Bromoalkynes proved to be another very useful starting materials but for the construction of modern t-conjugated material via cross-coupling reaction. Various QACs in the construction of 1,2,4-oxadiazoles with potential medicinal applications were examined by Otaka, Ikeda, Tanaka and Tobe in 2014. It was observed that the ring closure reaction of *O*-acylamidoximes could be smoothly and quickly get activated by Tetrabutylammonium hydroxide (TBAH) as represented in scheme 4.

Scheme 4: TBAH- Activated ring closure reaction towards 1,2,4- Oxadiazoles

2.6 QUATERNARY AMMONIUM COMPOUNDS AS PHASE-TRANSFER CATALYST

It is on record that since 1971, QACs have also been well known as Phase-Transfer Catalysts (PTC) which is capable of transporting one reagent across the interface to the other phase (Starks, Liotta and Marc, 1994). Water and a non miscible organic solvent are typical examples. This strategy allows the reaction of less polar organic substrates with a variety of inorganic and organic anions, generally not soluble in organic solvents. Examples of commercially available QACs used as PTCs are methyltrioctylammonium chloride (Aliquat) and benzyltriethylammonium chloride (TEBA). Filip Bures (2019) stated that the portfolio of PTC reactions is wide and includes displacement reactions, additions, oxidation/reduction, and hydrolysis. The recent developments and applications of chiral PTCs which allows stereoselective alkylation (e.g. synthesis of α-amino acids), aldol condensation, and epoxidation, as well as Michael, Mannich, and Strecker reactions was reviewed in 2007 by Maruoka and Hashimoto. The most efficient optically active QACs based on scaffolds include biphenyl/binaphthalene, cinchona alkaloid, and tartaric acid. Makosza(2000) reviewed the application of PTCs as a green methodology in organic synthesis, whereas Senthamizh, Nanthini and Sukanyaa, (2012) focused on the mechanistic aspects and important applications of PTCs. In general, imidazole undergoes *N*-methylation with MeI or dimethylsulfate smoothly in high yield. However, a similar alkylation of electron-rich derivatives, such as trithienyl imidazole, with higher iodoalkanes is sluggish and low-yielding (Bureš, 2019). Yung-Sheng, Jen-Shyang, Tse-Yen, Wei-I, Jiann and Ming-Chang, (2015) stated that using a TEBA/NaOH/H2O/toluene system trithienylimidazole can easily produce the *N*-hexylated derivative in 96% yield. (Scheme 5).



Scheme 5: N-Hexylation of trihienylimidazole usingTEBA as phase-transfer catalyst

QAC having ionic character makes them very popular reaction media, which may also be chiral. In addition to water and organic solvents, ionic liquids (ILs) represent a new frontier in materials science, capable of bringing polar and nonpolar compounds together and facilitating their reaction (Payagala &Armstrong., 2012). Fluorescein was recorded to be a recently developed QAC-derived ILs by Burés (2019). A combination of the ammonium cation substituted with relatively short C1–C4 alkyls and perfluoroalkyltrifluoroborate ($R^{F}BF3^{-}$) anion affords low melting, low-viscous and hydrophobic ILs (Hajime,Zhi-Bin and Kuniaki, 2005).

2.7 QUATERNARY AMMONIUM COMPOUNDS AS ELECTROLYTES

Elgrishi, Routree, McCarthy, Routree, Eisenhart, Dempsey, (2018) mentioned QACs like Tetrabutylammonium HexafluoroPhosphate (TBAHFP) and tetrabutylammonium tetrafluoroborate (TBATFB) as good electrolytes both in solution and as ionic liquids which have been used as supporting electrolyte for electrochemical measurement in particular cyclic voltammeter (CV). They noted that TBAHFP is sufficiently soluble in dichloromethane and acetonitrile which increases its conductivity, making it electrochemically inert and it can be easily purified by crystallization, and this therefore widens the electro active window for CV measurements significantly.

Masayoshi, Morgan, Shiguo, Kazuhide, Tomohiro, Kaoru, (2017) reported that QAC-derived electrolytes have been applied in lithium ion batteries, mostly as room-temperature ionic liquids. Stating that their reductive stability is significantly affected by the ammonium cation used, and various QACs have therefore been designed to date. Butyltriethylammonium bis(trifluoromethanesulfonyl)imide (N_{2224}TFSI) or cyclic ammonium salts such as *N*-alkylmethylpyrrolidinium (mpyr) or *N*-alkylmethylpiperidinium (mpip) was stated as examples of QAC as electrolytes for electrochemical measurements and batteries (Bures, 2019). It was reported that a solution of QAC and Lithium bis(trifluoromethanesulfonyl) imide (LiTFSI) lithium salt showed low viscosity and high conductivity in comparison with conventional organic electrolytes. Furthermore, the electrolyte was noted to be stable up to 5.7 V vs. Li without any signs of decomposition, is nonflammable, and showed high capacity retention (94%) after 75 cycles (Selvamani, Suryanarayanan, Velayutham and Gopukumar, 2016).

2.8 FATE OF QUATERNARY AMMONIUM COMPOUNDS IN THE ENVIRONMENTS

Zhang, Cui, Zeng, Jiang, Yang, Yu, Zhu and Shen (2015) currently reviewed the environmental occurrence of QACs, focusing strongly on wastewater and the aquatic environment including information on concentrations, fate, ecotoxicity, and analytical methods. Adding that considerable amounts of QACs reach agricultural fields by the application of manures, sewage sludge or wastewater for nutrient recycling. Charles P. Warren (2013) reported about the naturally produced QACs that may be encountered in soil solution and that can be taken up by plants, example Choline. Due to their hydrophobic cation-exchange characteristics, Xiaolin, Xiaojun, Bixian, Jingqin, Li, Shanshan (2014) reported that QACs tend to adsorb strongly on sludge and sediment, and that the sorption energies are controlled by both the hydrophobic effect of their long alkyl chains and favorable electrostatic interactions between the cationic ammonium head group of QACs and cation-exchange sites on the surface of natural particles. Extremely high levels of QACs have been reported in the municipal sewage sludge (up to 9200 mg/g) prior to a voluntary phase-out of dialkyldimethylammonium compounds (DADMACs) as the fabric softener ingredient in Europe during the early 1990s (Fernández, Valls, Bayona, Albaigés (1991), Gerike, Klotz, Kooijman, Mattijs, Waters (1994), and Martinez-Carballo, Gonzalez-Barreiro, Sitka, Kreuzinger, Scharf, Gans (2007).

2.9 TOXICITY AND ASSESSMENT OF QUATERNARY AMMONIUM COMPOUNDS

The toxicity of QACs has been reported towards various aquatic and terrestrial microorganisms including fish (Zhang *et al.*, 2015). Nalecz-Jawecki, Grabinska-Sota and Narkiewicz (2003) stated that the toxicity of QACs decreases as the alkyl chain length increases, since the hydrophobicity of QACs with longer alkyl chain length increases resulting in low bioavailability and high partitioning with organic or negative charged surfaces. Their toxicity may be as low as 0.1mg/l depending on the QAC structure. Guohua, Zuoming, and Jing (2012) studied a quantitative structure-activity relationship (QSAR) which showed a direct correlation between topological parameters and EC_{50} values of 13 aliphatic QACs with systematically extended R-groups. Identifying the alkyl chain length and molecular total connectivity index as the most important parameters affecting QAC acute toxicity. Despite their biodegradability, QACs remain toxic for a long period due to their high and fast sorption ability, thus resulting in significant accumulation in the environment. Bureš (2019) mentioned the frequently studied and environmentally most abundant QACs to include (di)alkyltri(di)methylammonium compounds of the general formula $RMe_3N^+X^-$ and $R_2Me_2N_+X^-$ examples are Cetyltrimethylammonium bromide/chloride (CTAB/CTAC), Benzalkonium chloride (BAC), Distearyldimethylammonium chloride (DSDMAC).Ismail, Ulas and Spyros (2010), Ruan, Shanjun, Thanh, Runzeng, Yongfeng and Guibin (2014), Xiaolin *et al*., 2014 and Guillaume, Marie, Kopferschmitt, Philippe, Gabrielle and Maurice (2017) detected the environmentally most abundant QACs as significant pollutants in municipal sludge, water and air worldwide. The widespread use of QACs allows humans to be exposed to them with almost all body surfaces and cavities having the potential of absorbing, inhaling and ingesting QACs into the body.

2.10 OTHER APPLICATIONS OF QUATERNARY AMMONIUM COMPOUNDS

The adsorption ability of QACs onto organic surfaces makes the use of QACs extremely important in the personal care industry (Charles P. Gerba, 2015). Skin care products and hair conditioners contain mainly alkyl QACs (including mono-, di- and tri- alkonium salts), ethoxylated and ester QACs in their formulations (Tang D, 2001). It has been reported that QACs are also used in paper processing to produce tissue paper or fluff pulp, which are used in diapers, towels, napkins and facial and toilette tissue products. It is known that QACs such as dialkonium salts are effective chemical bonding agents in paper. QACs interact with nature fibre-to-fiber bonding that occurs during the paper-making process. He further noted that the hydrophobic and hydrophilic moieties of QACs makes them interact with the fiber surface, reducing the inter-fiber bonding and forming a thin lubricant layer. Furthermore, it was noted that this reduction of the inter-fiber bonding, together with the lubricating effect of QACs gives a soft feel to the paper. In the mechanical fluff pulp process, QACs protects the fibers against damage and reduces the defibration energy needed. QACs are being increasingly incorporated into contemporary products that are utilized orally, such as mouthwash, applied to the skin or eyes or administered as a nasal spray (Hallen and Graf, 1995).

2.11 INSTRUMENTATION

METHODOLOGY OF FOURIER TRANSFORM INFRARED SPECTROSCOPY.

Fourier transform infrared spectroscopy (FTIR) is a largely used technique to identify the functional groups in the materials (gas, liquid, and solid) by using the beam of infrared radiations (Khan Shahid Ali, Sher Bahadar Khan, Latif Ullah Khan, Aliya Farooq, Kalsoom Akhtar, and Abdullah M. Asiri, 2018). The typical FTIR spectrometer consists of an IR light source, interferometer, sample compartment, detector, amplifier, and computer. The light source generates radiation which strikes the sample passing through the interferometer and reaches the detector. Then the signal is amplified and converted to digital signal (interferogram) by the amplifier and analog-to-digital converter, respectively. Eventually, the interferogram is translated to spectrum through the fast Fourier transform algorithm. Michelson interferometer is the main core of FTIR spectrometer. The interferometer consists of a beam splitter, fixed mirror, and a moveable mirror that translates back and forth, very precisely. The beam splitter is made of a special material that transmits half of the radiation striking it and reflects the rest half of the radiation. It works on the basis of principle that the light from the source is collected by collimating mirror and made its rays parallel, which strikes beam splitter and consequently splits into two beams. One beam is transmitted through the beam splitter to the fixed mirror, and the second is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beam splitter. Accordingly, both of these reflected radiations are recombined at the beam splitter, resulting in one beam that leaves the interferometer and interacts with the sample and strikes the detector (Khan *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Reagents: N,N-diethylethylene diamine, potassium tertiary butoxide, coconut oil, canola oil, benzyl chloride, Tetrahydrofuran, Silicon oil, distilled water.

3.1.2 Apparatus: Beakers, Three-neck round bottom flask, Thermometer, Cotton wool, Stop watch, Retort stand, Condenser, Magnetic Stirrer.

3.1.3 Equipment: FTIR spectroscopy, Model; Agilent Technology carry 60 FTIR

3.1.4 General methods for the synthesis of Quaternary Ammonium Compounds

Generally reacting an amine with an ester will produce an amide (Kuca *et al*., 2004). In this study, Quaternary Ammonium Compounds were synthesized by reacting N,N-Diethylethylenediamine with canola and coconut oils with potassium tertiary butoxide to yield an amide, which upon reaction with benzyl chloride produced the quaternary ammonium compounds as described below.

About 20g (0.138molar eqv) of coconut oil and 16 g (0.138 eqv) of N,N-diethylethylenediamine were measured into a beaker then poured into a three neck round bottom flask. 1.0 g of potassium tertiary butoxide was added directly into the flask containing the mixture. The reaction flask containing the mixture with the condenser inserted from one end of the flask was placed in an oil bath. The reaction mixture was stirred continuously using a magnetic stirrer, with a thermometer inserted in the oil bath. The mixture was refluxed for 6 hours at 120°C-130°C to allow for the formation of the amide (an intermediate). The mixture was allowed to cool for 24 hours. To separate the amide from the byproducts (glycerol and Potassium tertiary butoxide) saturated aqueous solution of salt was added to the flask containing the mixture as described by Ronald E. Majors (2018). Then the mixture was poured into a separatory funnel and the solution was allowed to settle for 24 hours. Afterwards, two layers were formed in the funnel, the upper layer being an organic phase while the lower layer is the aqueous phase. The aqueous layer was drained off while the organic phase was drained into a clean test tube. The amide, **amide1** was sent for analysis.

Same procedure was repeated for canola oil and the intermediate obtained from the organic phase (amide), **amide2**.

About 3.0 g of the intermediate, 1.3 g of benzyl chloride and 14.2 g of tetrahydrofuran (solvent) were added to another clean round bottom flask. The mixture was refluxed for 8 hours at 67 °C. The solvent was distilled off leaving behind a gel like solid the QAC namely, **QAC1** and **QAC2** from coconut oil and canola oil respectively as shown in Scheme 6-9.

Scheme 6: Reaction Scheme for the aminolysis of coconut oil (*Cocos nucifera*) fatty acid.

Scheme 7: Reaction Scheme for the aminolysis of canola oil (*Brassica napus*) fatty acid

Alkylation of the amide1 (intermediate) with benzyl Chloride to yield QAC1 from coconut oil.

Scheme 8: Reaction scheme for quaternization of coconut oil fatty acid

Alkylation of the amide2 (intermediate) with benzyl Chloride to yield QAC2 from canola oil.

Scheme 9: Reaction Scheme for quaternization of canola oil fatty acid

CHAPTER FOUR

4.1 RESULTS AND DISCUSSION

The results obtained from the synthesis, characterization and antimicrobial evaluations of the quaternary ammonium compounds are shown in the tables and figures below;

Table 4.1: Confirmation test results for the formation of quaternary products

Compounds	**Appearance**	**Solubility in water**
QAC1	Dark brown viscous liquid	Foaming solution
QAC2	Dark brown viscous liquid	Foaming solution

The above table 4.1 shows the Solubility tests conducted on the final products, the QAC1 and QAC2. Results obtained shows the final products were soluble in water as well as foaming which is a confirmatory test or physical evidence for the formation of a quaternary product.

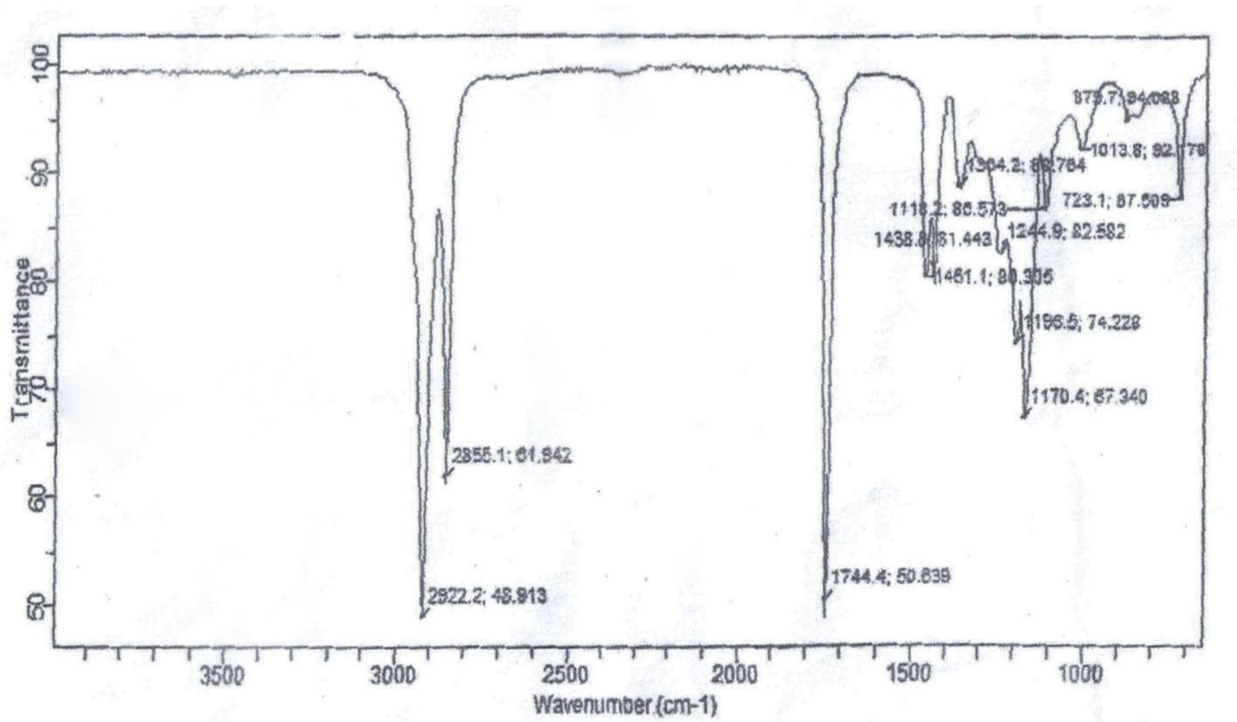
Table 4.2: Antimicrobial test results showing the zones of inhibition (mm) of the QACs from the two oil samples (coconut oil and canola oil) as against the standard antibiotic (**Ampicillin**)

Diameter of Zones of inhibition (mm)

Samples (10mg)	*Lactobacillus Spp.*	*Salmonella enterica*	*Klebsiella spp.*	*E.Coli*	*Staphylococcus aureus*
1) QAC1 from coconut oil	20	22	18	22	19
2) QAC2 from canola oil	18	25	17	28	18
Ampicillin	16	20	15	20	18

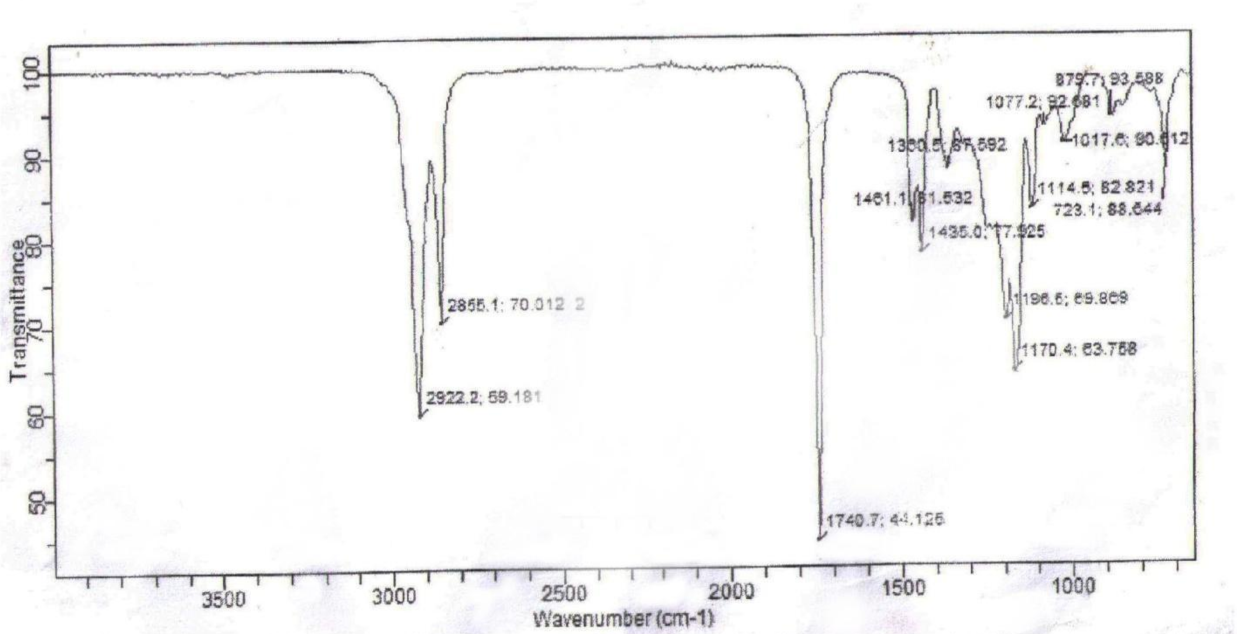
Table 4.2 shows that the two QACs from coconut and canola oil exhibited excellent anti-bacteria activity, especially on gram-negative bacteria (*Escherichia Coli, Lactobacillus spp and Salmonella enterica*) in comparison with the standard antibiotic (Ampicillin). This is as a result of the long alkyl chain length of the two quaternary ammonium compounds synthesized.

Fig 4.1: Infrared Spectrum of Coconut oil sample.



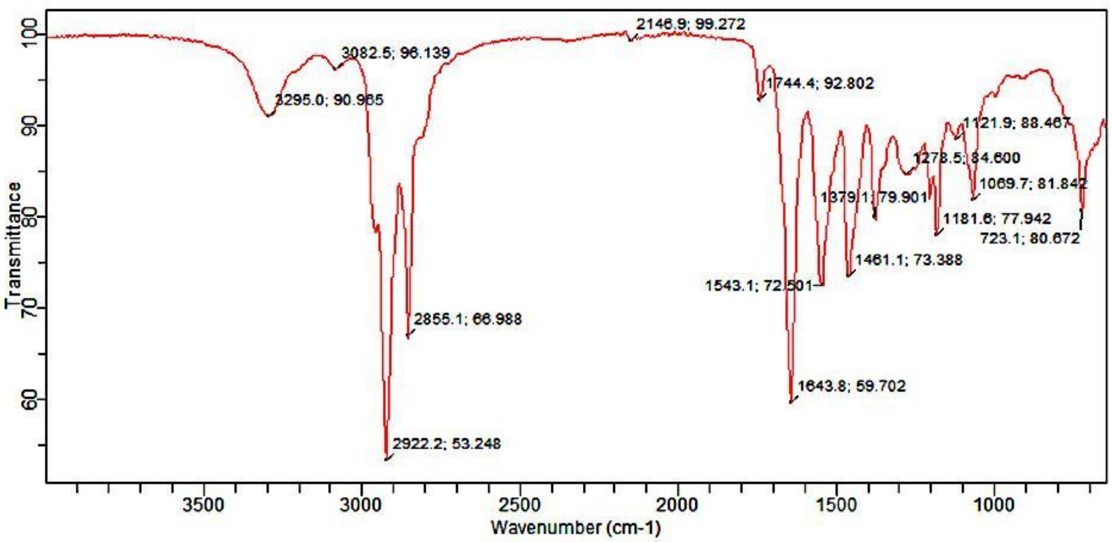
The above figure 4.1 is showing peaks of different functional group present in coconut oil. Peak at 1744 cm^{-1} is indicating absorption of carbonyl (C=O) stretch of an ester (coconut oil). Peaks at 2855 cm^{-1} and 2922 cm^{-1} are for C-H stretch of the methylene group of aliphatic hydrocarbon, sharp peak at 1170 cm^{-1} indicates the absorption of C-O stretch of alkyl ether.

Figure 4.2: Infrared Spectrum of Canola Oil sample



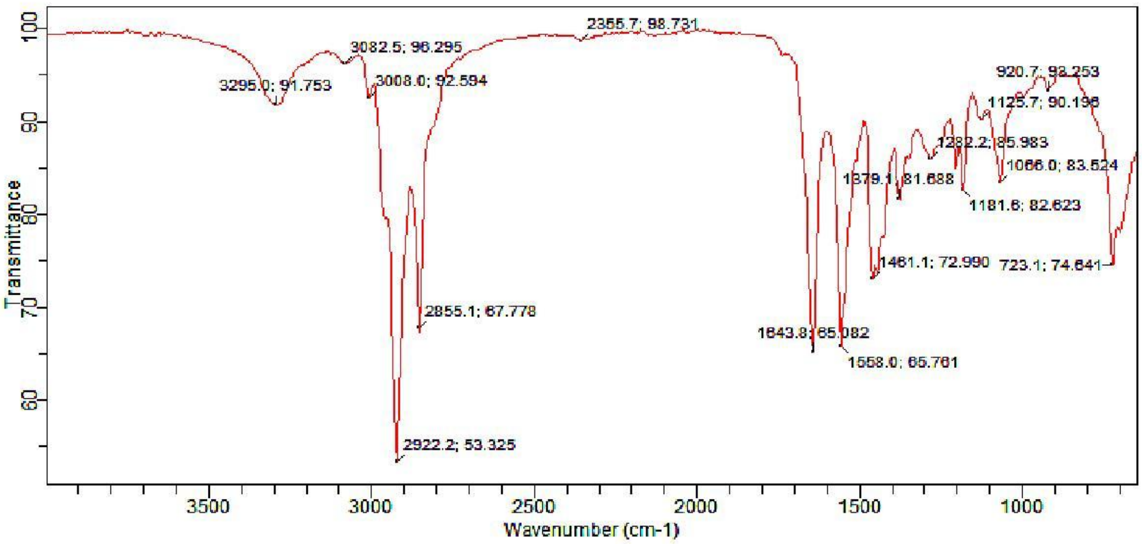
The above figure 4.2 is showing peaks of different fuctional group present in canola oil. Peak at 1740 cm^{-1} is indicating absorption of carbonyl (C=O) stretch of an ester (canola oil). Peaks at 2855 cm^{-1} and 2922 cm^{-1} are for C-H stretch of the methylene group of aliphatic hydrocarbon, sharp peak at 1170 cm^{-1} indicates the absorption of C-O stretch of alkyl ether.

Figure 4.3: Infrared Spectrum of amide1 (intermediate)



The above spectrum is for the intermediate gotten from coconut oil, which is showing a strong sharp peak at 1643 cm-1 indicating the absorption of C=O of an amide. Peak at 2855cm-1 indicates C-H stretch of methyl group (-CH3) of aliphatic hydrocarbon, the C-H stretch of methylene group (-CH2) of aliphatic hydrocarbon is indicated with the peak at 2922 cm^{-1}. A singlet sharp peak at 3298cm-1 indicated N-H peak for an amide.

Figure 4.4: Infrared Spectrum of amide2 (Intermediate)



The above spectrum is the intermediate obtained from canola oil, which is showing a strong sharp peak at 1643 cm-1 indicating the absorption of C=O of an amide. Peak at 2855cm-1 indicates C-H stretch of methyl group (-CH3) of aliphatic hydrocarbon, the C-H stretch of methylene group (-CH2) of aliphatic hydrocarbon is indicated with the peak at 2922 cm-^{1}. A singlet sharp peak at 3295cm-1 indicated N-H peak for an amide.

Figure 4.5: Infrared Spectrum of QAC1

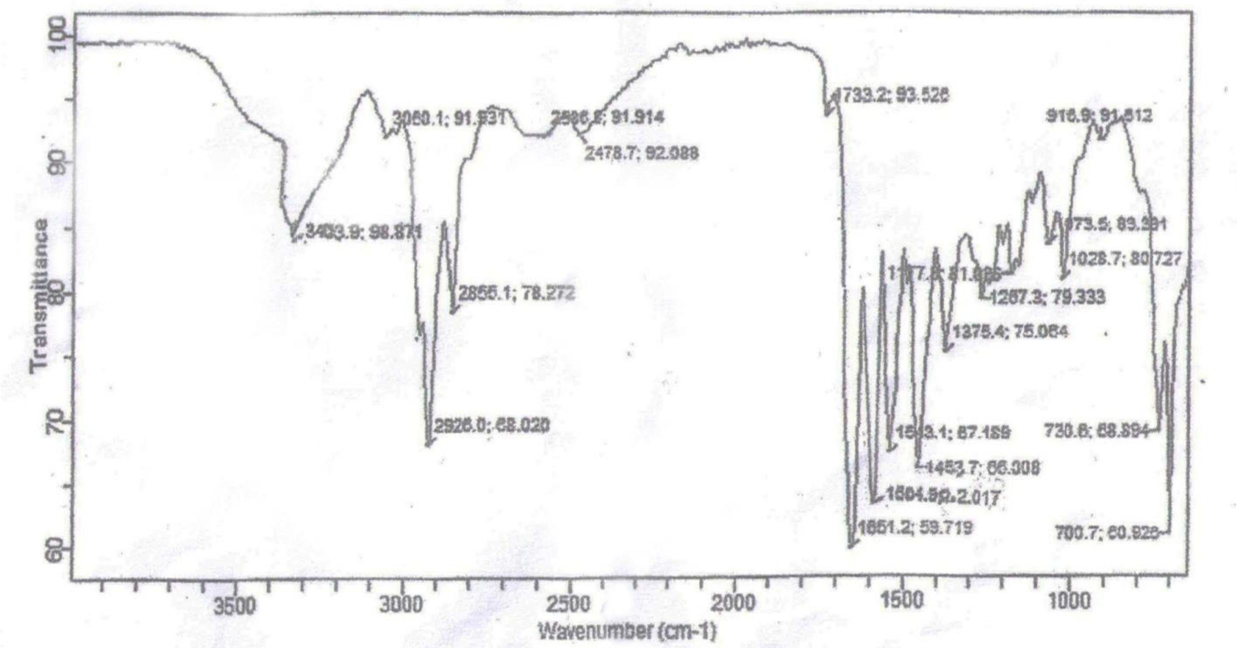


Figure 4.5 above shows the infrared spectrum of the QAC1 gotten from Coconut oil, notice a strong intense peak at 1651cm^{-1} indicating an absorption of carbonyl group (C=O) of an amide. A singlet sharp peak at 3403 cm^{-1} shows absorption of N-H band of an amide. Peak 2926 cm^{-1} indicates C-H stretch of methylene group of an aliphatic compound, at 3060 cm^{-1} is a peak for C-H stretch of aromatic compound, intense sharp peak at 1604 cm^{-1} and 1543 cm^{-1} are absorption of C=C of an aromatic compound. The sharp peak showing at 700 cm^{-1} and 730 cm^{-1} indicates C-N absorption in the quaternary fatty amide.

Figure 4.6: Infrared Spectrum of QAC2.

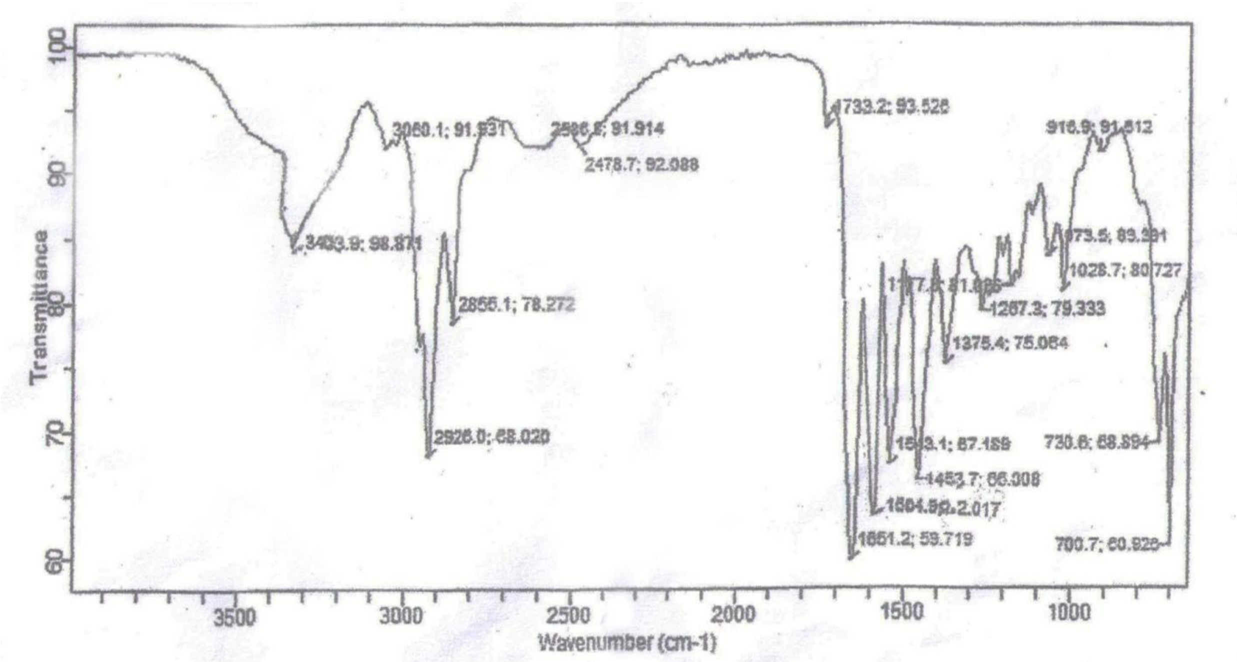


Figure 4.6 above shows the infrared spectrum of the QAC2 obtained from Canola oil, the strong intense peak at 1651cm^{-1} indicating an absorption of carbonyl group (C=O) of an amide. A singlet sharp peak at 3403 cm^{-1} shows absorption of N-H band of an amide. Peak 2926 cm^{-1} indicates C-H stretch of methylene group of an aliphatic compound, at 3060 cm^{-1} is a peak for C-H stretch of aromatic compound, intense sharp peak at 1604 cm^{-1} and 1543 cm^{-1} are absorption of C=C of an aromatic compound. The sharp peak showing at 700 cm^{-1} and 730 cm^{-1} indicates C-N absorption in the quaternary fatty amide.

4.2 DISCUSSION

Comparative tables of different Infrared spectrum of major functional groups present in the oils, the intermediates and the synthesized QACs from coconut and canola oils are represented in tables 4.2 and 4.3 respectively below.

Table 4.3 FTIR Absorption peaks of Coconut Oil, Intermediate and Synthesized QAC

IR Frequency cm^{-1}	Functional Group	Coconut Oil	Intermediate	Synthesized QAC
1744	C=O stretch of an ester	Present	Absent	Absent
2855 and 2922	C-H stretch of methylene group	Present	Present	Present
1170	C-O stretch Alkyl ether	Present	Absent	Absent
1643-1651	C=O amide stretch	Absent	Present	Present
3295-3403	N-H band of amide	Absent	Present	Present
1543 and 1604	C=C band of an aromatic compound	Absent	Present	Present
700 and 730	C-N band of quaternary Fatty amide	Absent	Absent	Present

The above table shows the presence or absence of functional groups in coconut oil, amide1 (intermediate) and QAC1 . Notice that the C=O stretch of ester at 1744cm^{-1} and C-O stretch of alkyl ether at 1170 cm-^{1} which was present in the coconut oil were absent in the intermediate and the synthesized QAC indicating that aminolysis reaction took place. This resulted to the formation of N-H band of the amide at 3295 cm^{-1} and C=O amide stretch at 1643cm^{-1} for the intermediate, which equally appeared in the spectrum of the synthesized QAC at 3403cm^{-1} for N-H band of the amide and at 1651 cm^{-1}for C=O amide stretch. The quaternization of the amide with benzyl chloride resulted to the formation of C=C peak of an aromatic compound at 1604cm^{-1} and 1543cm^{-1} and C-N peak of quaternary Fatty amide at 700cm^{-1} and 730cm^{-1}, these peaks were all absent in the coconut oil spectrum. This shows that there was a formation of a Quaternary Ammonium compound named QAC1 from coconut oil.

Table 4.4 FTIR Absorption peaks of Canola Oil, Intermediate and Synthesized QAC

IR Frequency cm^{-1}	Functional Group	Canola Oil	Intermediate	Synthesized QAC
1740	C=O stretch of an ester	Present	Absent	Absent
2855 and 2922	C-H stretch of methylene group	Present	Present	Present
1170	C-O stretch Alkyl ether	Present	Absent	Absent
1643-1651	C=O amide stretch	Absent	Present	Present
3295-3403	N-H band of amide	Absent	Present	Present
1543 and 1604	C=C band of an aromatic compound	Absent	Present	Present
700 and 730	C-N band of quaternary Fatty amide	Absent	Absent	Present

The above table shows the presence or absence of functional groups in canola oil, amide2 (intermediate) and QAC2. Notice that the C=O stretch of ester at 1740cm^{-1} and C-O stretch of alkyl ether at 1170 cm-^{1} which was present in the canola oil were absent in the intermediate and the synthesized QAC indicating that aminolysis reaction took place. This resulted to the formation of N-H band of the amide at 3295 cm^{-1} and C=O amide stretch at 1643cm^{-1} for the intermediate, which equally appeared in the spectrum of the synthesized QAC at 3403cm^{-1} for N-H band of the amide and at 1651cm^{-1} for C=O amide stretch. The quaternization of the amide with benzyl chloride resulted to the formation of C=C peak of an aromatic compound at 1604cm^{-1} and 1543cm^{-1} and C-N peak of quaternary Fatty amide at 700cm^{-1} and 730cm^{-1}, these peaks were all absent in the coconut oil spectrum. This shows that there was a formation of a Quaternary Ammonium compound named QAC2.

CHAPTER FIVE

5.1 CONCLUSION

Quaternary ammonium compounds were synthesized from coconut and canola oil. Fourier transform infrared (FTIR) spectroscopic analysis was carried out on the starting materials (canola and coconut oil) and the final product. The disappearance of the carbonyl (C=O) stretch of an ester (the oils) and appearance N-H band of an amide, which resulted from the quaternization of benzyl chloride with the intermediate was obtained. Other functional groups such as C=C and C-H stretch of aromatic compound, C=O stretch of an amide were also observed.

The antimicrobial evaluation of the Quaternary Ammonium Compounds synthesized from the coconut oil and canola oil exhibited excellent anti-bacteria activity with the following zones of inhibition as compared with the standard antibiotic (Ampillicin); Lactobacillus spp with diameter of 20 mm for QAC from coconut oil and 18 mm for QAC from canola oil as compared to diameter of 16 mm for ampillicin, Escherichia Coli with diameter of 22 mm for QAC from coconut oil and 28 mm for QAC from canola oil as compared to diameter of 20 mm for ampicillin, Staphylococcus aureus with diameter of 19 mm for QAC from coconut oil and 14 mm for QAC from canola oil as compared to diameter of 14 mm for ampicillin and that of Klebsiella spp showed diameter of 18 mm for QAC from coconut oil and 17 mm for QAC from canola oil as compared to diameter of 15 mm for ampicillin.

5.2 RECOMMENDATION

This research work should be subjected to Nuclear Magnetic Resonance Spectroscopy to consolidate the QAC structure. Also there should HPLC analysis to isolate the different components present in the synthesized QACs and each of the components will then be quantized.

5.3 CONTRIBUTION TO KNOWLEDGE

This study has demonstrated that synthesizing QAC from renewable sources (canola and coconut oils) is more economical than using synthetic sources.

I. The efficacy of the synthesized compounds were comparable to known antibiotics and could possibly be used as replacement for those,

APPENDIX

TEST FOR ANTIMICROBIAL ACTIVITY

Collection of Test Organisms

The bacteria strains (*Lactobacillus spp*, *Salmonella enterica*, *Klebsiella spp*, *Escherichia coli*, *Staphylococcus aureus*) used for this analysis were collected from Microbiology Laboratory, Federal University of Technology Owerri, Imo State.

Preparation of Macfarland's Turbidity Standard

1% $BaCl_2$ was prepared by dissolving 1g of $BaCl_2$ in 99 ml of distilled water. Also, 1% of H_2SO_4 was prepared by dissolving 1ml of conc. H_2SO_4 in 99 ml of distilled water. The bacterial population used in this study was standardized using 0.5 Macfarland's standard prepared by reacting 0.6 ml of 1% $BaCl_2$ and 99.4 ml of 1% H_2SO_4 to form $BaSO_4$ precipitate.

$$BaCl_{2\,(aq)} + H_2SO_{4\,(aq)} \longrightarrow BaSO_{4(s)} + 2HCl_{(aq)}$$

A bacterial population equals the turbidity of the 0.5 Macfarland's standard was used for the assay.

Antibacterial Assay

The bacteria colonies were picked using a sterile wire loop to make a suspension of the test organism in the sterile Bijou bottle. The turbidity of the test suspension was compared against the turbidity of the prepared test standard. A sterile swab stick was dipped into the inoculum and used to streak the surface of the agar. A sterile cork borer was then used to produce wells of 8 mm allowing 30mm between adjacent wells and petri dish.

Sterile syringes were used to introduce fixed volumes of test compounds into the wells. The plates were incubated at $30^{o}C$ for 24 hours. After the period of incubation, the diameter of the zones of inhibition was measured in millimeters (mm). Ampicillin 10 mg/ml was used as control, as described by Cheesbroug (2000).

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