

(RESEARCH ARTICLE)



Synthesis, characterization and antimicrobial evaluation of quaternary ammonium compounds from natural oils

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Abstract

Quaternary ammonium salts refer to any class of salt derived from ammonium in which the nitrogen atom is attached to four organic groups covalently, as in benzalkonium chloride (R_4N^+). Quaternary ammonium compounds were synthesized from Canola oil (*Brassic 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 analyses of the starting materials, the intermediates and the final products were conducted, the results showed a disappearance of the carbonyl (C=O) stretch of an ester (the oil) at 1744cm^{-1} and appearance of N-H band of an amide at 3433cm^{-1} , which resulted from the quaternization of benzyl chloride with the intermediates for both oils. Other functional groups such as C=C and C-H stretch of aromatic compound, C=O stretch of an amide were also observed. Mass spectrometric analysis of the final products were conducted and results showed the Molecular Ion peak (M^+) which is the molecular weights of the products. Vital fragments such as base peaks were observed for both quats, other fragmented radical cations were observed. Antimicrobial activity of the synthesized Quaternary ammonium compounds (QACs) exhibited excellent anti-bacterial activity covering a diameter of 22 mm and 28 mm of *E. coli* for QACs from *Cocos nucifera* and *Brassic anapus* oils respectively as compared to the standard antibiotic (Ampillicin).

Keywords: *Cocos nucifera*; *Brassic anapus*; Quaternary Ammonium; Antimicrobial; N, N-diethylethylenediamine; FTIR; Mass Spectrometry

1. Introduction

Quaternary ammonium salts with long alkyl chain length have been identified through synthetic process and have been reported to exhibit effective antimicrobial activity which may eliminate bacteria species resistance of the already available antimicrobial agents (Classissa *et al.*, 2007). Quaternary ammonium salts refer to any class of salt derived from ammonium in which the nitrogen atom is attached to four organic groups covalently, as in benzalkonium chloride (R_4N^+). These salts are cationic surface active compounds. Quaternary ammonium salts are among the high production volume chemicals (i.e. chemicals produced or imported in amount equal to or greater than one million pounds per year) as seen on the lists of United State Environmental Protection Agency and Organization for Economic Co-operation and Development (OECD,2004). Quaternary ammonium salts are positively charged polyatomic ions of the structure R_4N^+X with R being an alkyl or Aryl group and X represents a halo group (Henri and Warren, 2013), as shown below;

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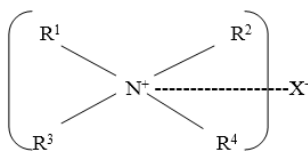
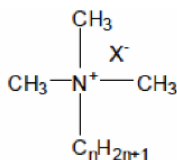
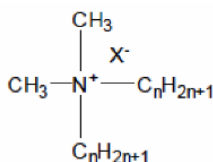


Figure 1 Basic structure of QAC'S

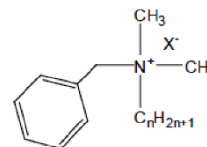
They are compounds of salts of quaternary ammonium cations with a counter anion. In oilfield parlance, quaternary ammonium compounds are called quaternary amines. Quaternary ammonium compounds including benzalkonium salts constitute an economically important class of industrial chemicals that are widely distributed among a diverse array of products and users from industrial to the household sector. Quats are stable with a long shelf life (Smith *et al*, 2001) Quaternary ammonium compounds can be classified in three major groups depending on the type of functional groups;



Monoalkonium halides



Dialkyl ammonium halides



Benzalkonium halides

Where n = length of alkyl chain and X = halide.

Benzalkonium chloride as an example of a quaternary ammonium salts are a mixture of alkyl benzyldiethyl ammonium chloride in which the alkyl group has various even numbered alkyl chain lengths. Depending on purity, benzalkonium chloride ranges from colourless to a pale yellow (impure).

Quaternary ammonium salts are readily soluble in ethanol and acetone. Aqueous solutions of quaternary ammonium salts are neutral to slightly alkaline. Quaternary ammonium solution foams when shaken. Concentrated solutions of Quaternary ammonium compounds have bitter taste and a faint almond-like odor. To prevent increase in viscosity or gel formation under low temperature condition, modifiers such as alcohol or glycols are incorporated into quaternary ammonium compound.

In Quaternary ammonium compound the organic radical is the cation and the halo group is usually the anion (chloride or bromine). Quaternary ammonium compounds are commonly called “quats” and have two different groups; hydrophobic alkyl group and a hydrophilic positively charged nitrogen atom which retains its cationic character at a very wide range of pH values. Quaternary ammonium may be freely soluble or insoluble water. The aqueous solubility of quaternary ammonium compounds decreases as the alkyl chain length increases (Prince *et al.*, 1999). Quats are large organic molecules having molecular weight typically between 300-500g/mole. The hydrophobic alkyl groups and the hydrophilic positively charged central nitrogen atom group confer distinct physical and chemical properties to quaternary ammonium compounds.

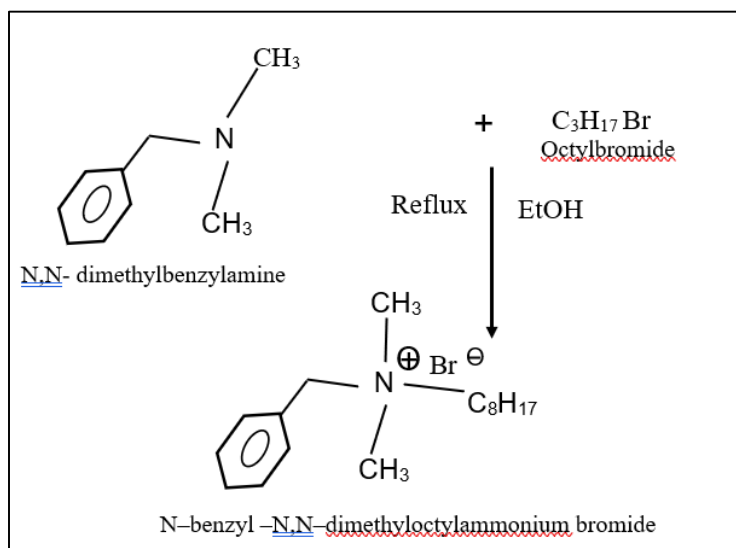
These compounds are extensively used in domestic, agricultural and health care settings. In industries they are used as surfactants, emulsifiers, fabric softeners, disinfectants, pesticides, corrosion inhibitors and antiseptics (Filip Bureš, 2019). In 2004, the annual consumption of quaternary ammonium compounds worldwide was reported as 500,000 tons (CESIO, 2004). and was expected to exceed 700,000 tons (Steinchen, 2001). Unlike the ammonium ion (NH_4^+) and the primary, secondary or tertiary ammonium cations, the quaternary ammonium cations are permanently charged over a wide range of pH of their solutions.

1.1. Synthesis of Quaternary Ammonium Compounds

Quats are prepared by alkylation of tertiary amines with a halo carbon. This process by which a tertiary amine is alkylated with a halo carbon is called quaternization by modern chemist (Kuca *et al.*, 2005). Typically, one of the alkyl groups on the amine is longer than the other when making cationic surfactants (Kosswig, 2002).

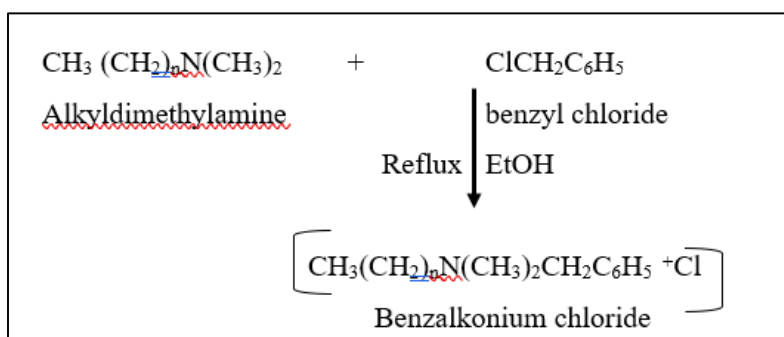
Two common methods of preparation of quaternary ammonium compounds are:

1.1.1. Preparation of Benzalkonium salt from *N,N*-dimethylbenzylamine and long chain *n*-alkylbromide as shown below (Debrececi and Mestyan, 2007).



Preparation of Benzalkonium bromide

1.1.2. Preparation of Quaternary Ammonium Salt from a Long-chain Alkyl dimethylamine and Benzyl Chloride



Preparation of Benzalkonium chloride

1.2. Reactions of Quaternary Ammonium Salts

Quaternary ammonium cations are unreactive towards strong electrophiles and acids. They are also stable towards most nucleophiles. Because of these, most unusual anions have been isolated as the quaternary ammonium salts. Permanganate can be solubilized in organic solvents when deployed as its NBu₄⁺ salt (Pine, 2011). They can undergo Stevens' rearrangement as well as dealkylation under harsh conditions. Quaternary ammonium cations containing N-C-C-H units can also undergo the Hoffmann elimination, that is quaternary ammonium salts with beta hydrogen can undergo elimination (Brasen and Hauser, 2008). Quaternary ammonium compounds have also been shown to have antimicrobial activity (Thorsteinsson *et al.*, 2008). Their antimicrobial activity depends on a changing length of the side *n*-alkyl chain. For a quaternary ammonium compound to have a high antimicrobial property, at least one of their alkyl groups must have a chain length in the range C₈ to C₁₈ (Viscardi, 2007). Quats can inhibit the growth of bacteria spores (Brill, 2006).

1.3. Applications of Quaternary Ammonium Compounds

Quaternary ammonium compounds found applications in the following ways;

1.3.1. As antimicrobials

Certain quaternary ammonium salts containing long alkyl chains are used as antimicrobials and disinfectants. Examples are benzalkonium chloride, cetylalkonium chloride, tetraethylammonium chloride. It is believed that quaternary

ammonium compounds act by disrupting the cell membrane. They are effective mostly in soft water at temperature up to 100°C and concentration of about 200ppm.

1.3.2. Plant growth retardant

Chlormequet chloride (cycocel) reduces plant height by inhibiting the production of primary plant hormone responsible for cell elongation (gibberellins)

1.3.3. Osmolytes

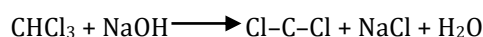
Quats are present in Osmolytes, specifically glycine butane which stabilizes osmotic pressure in cells (Sleator *et al.*, 2001).

1.3.4. As fabric softener

Example is Distearyl dimethylammonium chloride with low biodegradability now has been phased out. Diethylester dimethylammonium chloride (Zhishen, 2001).

1.3.5. Phase transfer catalyst

These are those catalysts that accelerate reactions between reagents dissolved in immiscible solvents. Dichlorocarbene is generated via phase transfer catalytic reaction of chloroform and aqueous sodium hydroxide



2. Materials and methods

All the materials used in this research are of analytical grade and was purchase from chemical store in Owerri, Imo State Nigeria

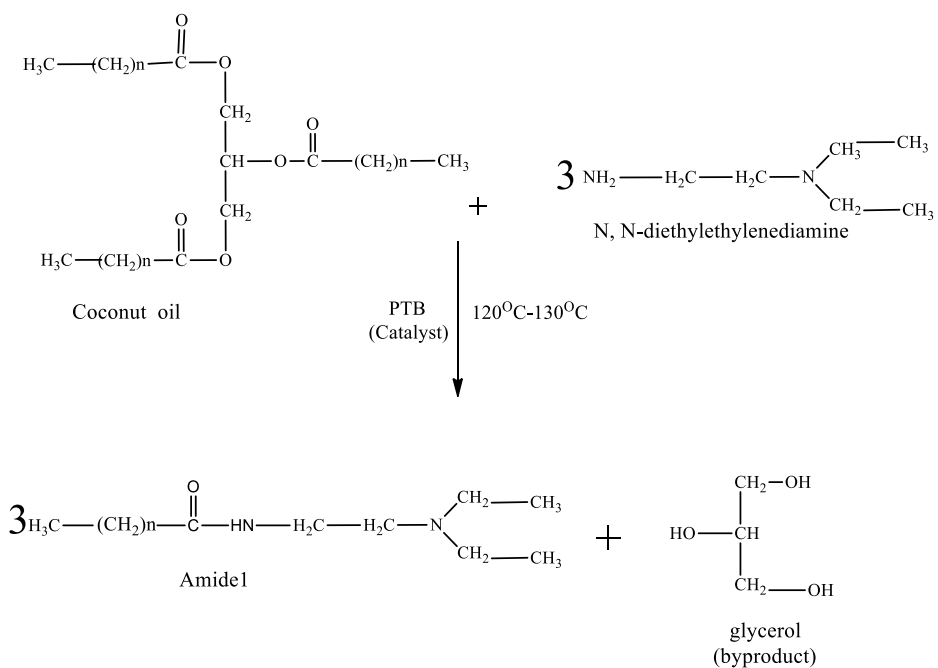
2.1. General methods for the synthesis of Quaternary Ammonium Compounds

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.

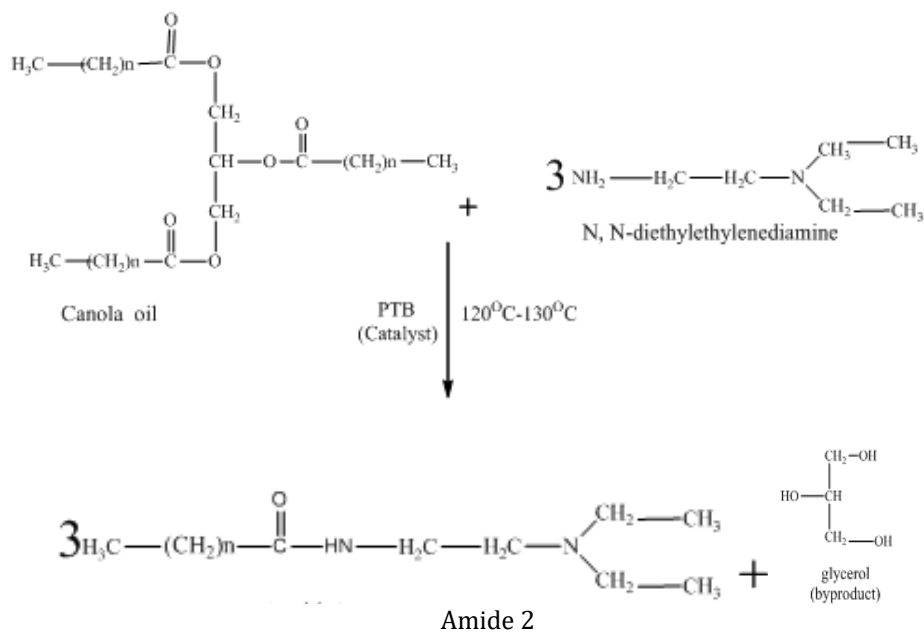
About 20 g of coconut oil and 16 g of N,N-diethylethylenediamine were measured into a beaker then poured into a three neck round bottom flask. 1.0 g of potassium tertiary butoxide was added directly into the flask containing the mixture. The reaction flask containing the mixture with the condenser inserted 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 between 120°C to 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 1-4.

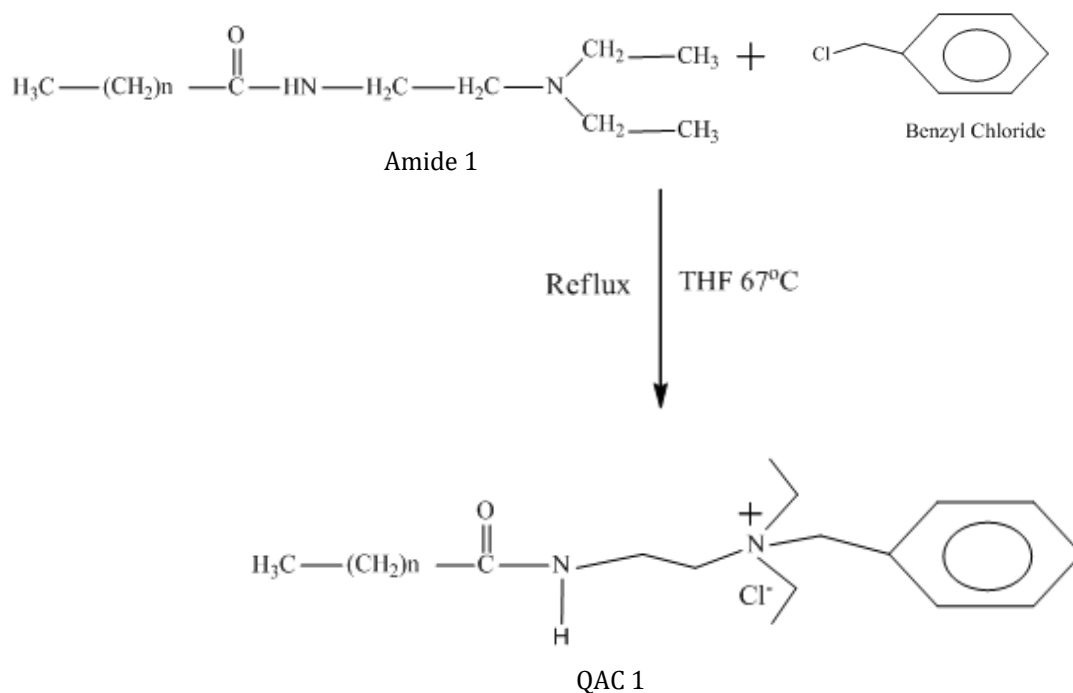


Scheme 1 Reaction Scheme for the aminolysis of coconut oil (*Cocos nucifera*) fatty acid.



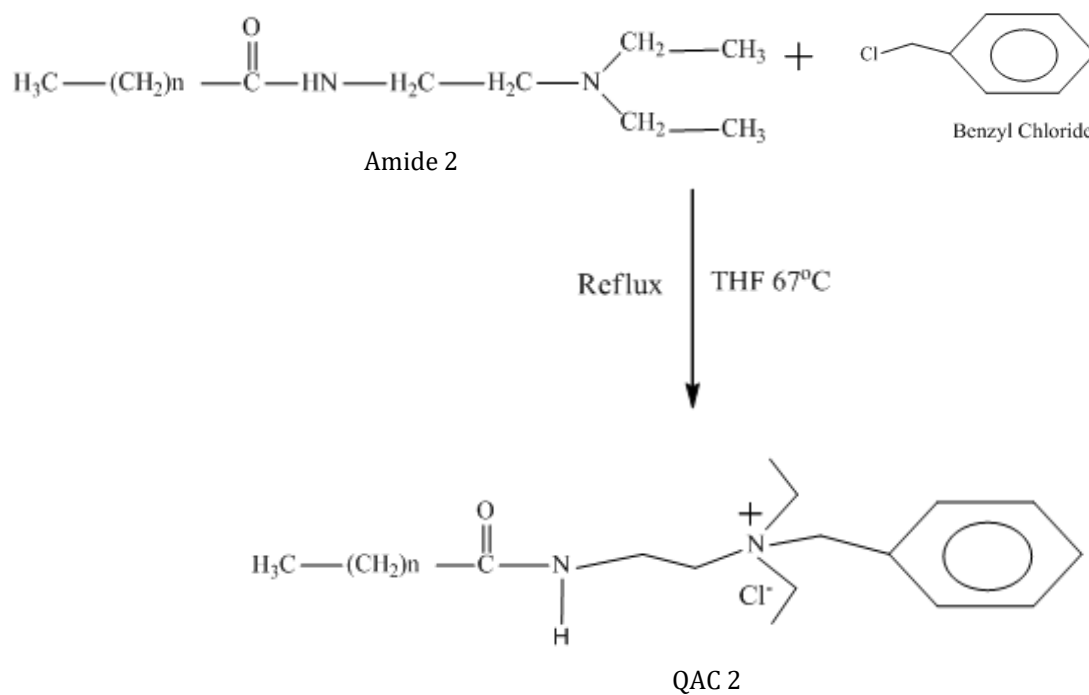
Scheme 2 Reaction Scheme for the aminolysis of canola oil (*Brassica napus*) fatty acid.

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



Scheme 3: Reaction scheme for quaternization of coconut oil fatty acid.

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



Scheme 4 Reaction Scheme for quaternization of canola oil fatty acid

2.2. Antibacterial Test

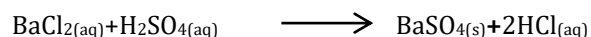
2.2.1. Collection of test samples

The pure bacterial stains such as *Salmonella enterica*, *Echerichia coli*, *Staphylococcus aureus*, *Klebsiella spp.* and *Lactobacillum spp.* used for this analysis were collected from the Microbiology Laboratory Federal University of Technology Owerri, Imo State.

2.2.2. Preparation of Macfarland's turbidity standard

A 1% v/v solution of sulphuric acid was prepared with a 1% v/v solution of dehydrated barium chloride. 0.6 ml of barium chloride solution was added to 99.4 ml of the prepared sulphuric acid solution with mixing. A quantity of the turbid solution formed was transferred into a capped tube of the same type used for preparing the test and control inoculums. The bacterial population used in this test was standardized using 0.5 *Macfarland's* standard.

Equation of reaction



A bacterial population equal to the turbidity of 0.5 *Macfarland's* standard was used for the study.

2.2.3. Antibacterial studies

The bacterial colonies were picked using a sterile wire loop to make a suspension of the test organism in a 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 inoculums and used to streak the surface of the agar. A sterile cork borer was then used to produce wells of 8mm allowing 30mm between adjacent wells and the petri dish. Sterile syringes were used to introduce fixed volumes of test compounds into the wells. The plates were incubated at 30°C for 24 hours. After the periods of incubation, the diameter of the zones of inhibition were measured in mm (Cheesbroug,2000). Ampicillin 10 mg/L was used as control.

2.3. Instrumentation

2.3.1. Fourier transform-infrared spectroscopy

The samples were dissolved in 98% dimethylsulfoxide (1 mL). The samples were molded into pellets. The source emits infrared radiation spanning a wide frequency range. A wavelength selector permits only a particular wavelength of radiation transmitted by the sample to fall on a detector which measures the intensity of radiation and a plot of this intensity for each wavelength is traced on paper by a recorder.

2.3.2. Mass spectroscopy

The samples were vapourized and introduced into an evacuated chamber, where it was bombarded with high-energy electrons (70 ev, 1600 kcal/mol) that strip an electron from the molecule. The resulting positive ions were attracted to & accelerated by the negative accelerating plates into a bent chamber that is surrounded by a magnetic field perpendicular to the plane of the paper. Varying the strength of the magnetic field changed the radius of the curvature of the different ions of different molecular weight going through the chamber, so that the spectrum of ions was gradually swept past the detector slit. The spectrum was recorded on graph paper as a plot of mass (actually mass/charge ratio) versus intensity. Neutral molecules were not detected & were removed from the system by several vacuum pumps.

3. Results and discussion

The results obtained from the synthesis, characterization and antimicrobial activities of quarterized fatty amides are shown in tables and Figures below.

Table 1 Confirmation test results for the formation of quaternary products

Compounds	Appearance	Solubility in water
Coconut oil	Slightly yellow coloured oily liquid	Insoluble
Canola oil	Slightly yellow coloured oily liquid	Insoluble
N,N-diethylethylene diamine	Colourless liquid	soluble
Amide 1	Light brown viscous liquid (oily)	Dispersible emulsion
Amide 2	Light brown viscous liquid (oily)	Dispersible emulsion
Benzyl chloride (C ₇ H ₇ Cl)	Colourless liquid	Insoluble
QAC 1	Dark brown viscous liquid (Foamy)	Foaming solution
QAC 2	Dark brown viscous liquid (foamy)	Foaming solution

Table 1 above shows the solubility tests conducted on the starting material (Canola oil, coconut oil and N,N-diethylethylenediamine), intermediates (amide 1 and amide 2) and the final products, the QACs (QAC 1 and QAC 2). Results obtained shows that N,N-diethylethyleneamine is soluble in water while the final products (QACs) were soluble in water as well as foaming which is a confirmatory test or physical evidence for the formation of a quaternary product.

Table 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 Samples (mm)	<i>Lactobacillus spp</i>	<i>Salmonella enterica</i>	<i>Klebsiella spp.</i>	<i>E.coli</i>	<i>Staphylococcus aureus</i>
Coconut oil	-	-	-	-	-
Canola oil	-	-	-	-	-
N-N diethylethylenediamine	-	-	-	-	-
Amide 1	-	-	-	-	-
Amide 2	-	-	-	-	-
QAC 1 from coconut oil	20	22	18	22	19
QAC 2 from canola oil	18	25	17	28	18
Ampicillin	16	20	15	20	18

Table 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. The oils and the intermediate compounds were not active against the microbes.

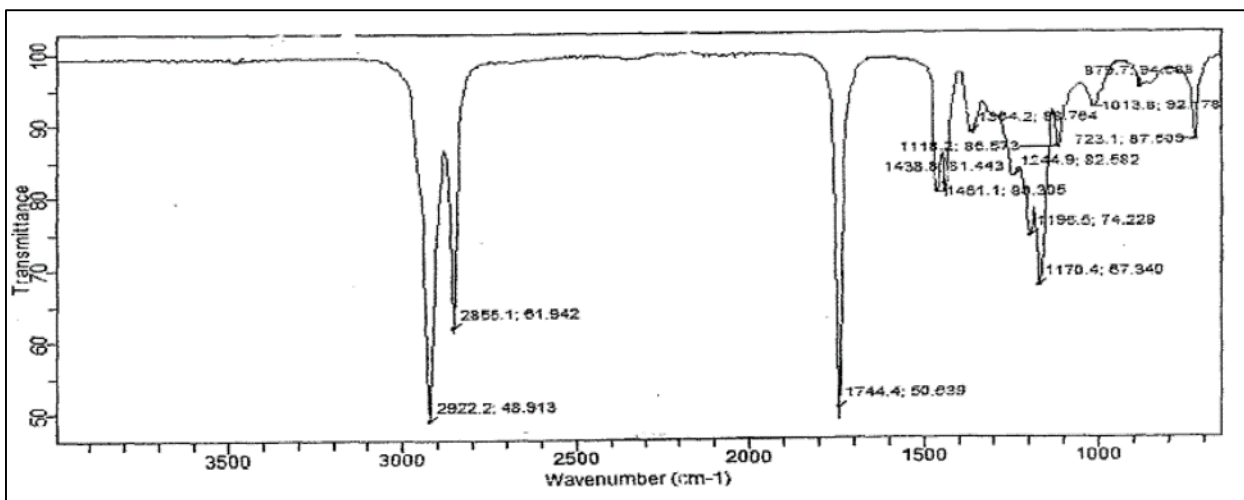


Figure 2 Infrared spectrum of coconut oil sample

The above figure 2 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.

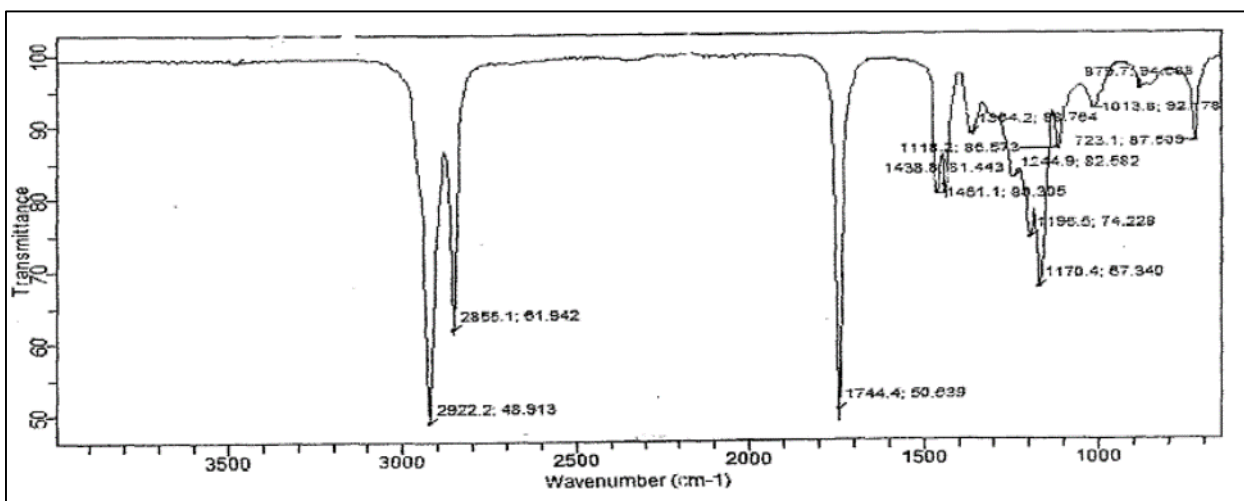


Figure 3 Infrared spectrum of canola oil sample

The figure 3 above shows peaks of different functional groups present in canola oil. Peak at 1740 cm⁻¹ indicate absorption of carbonyl (C=O) stretch of 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.

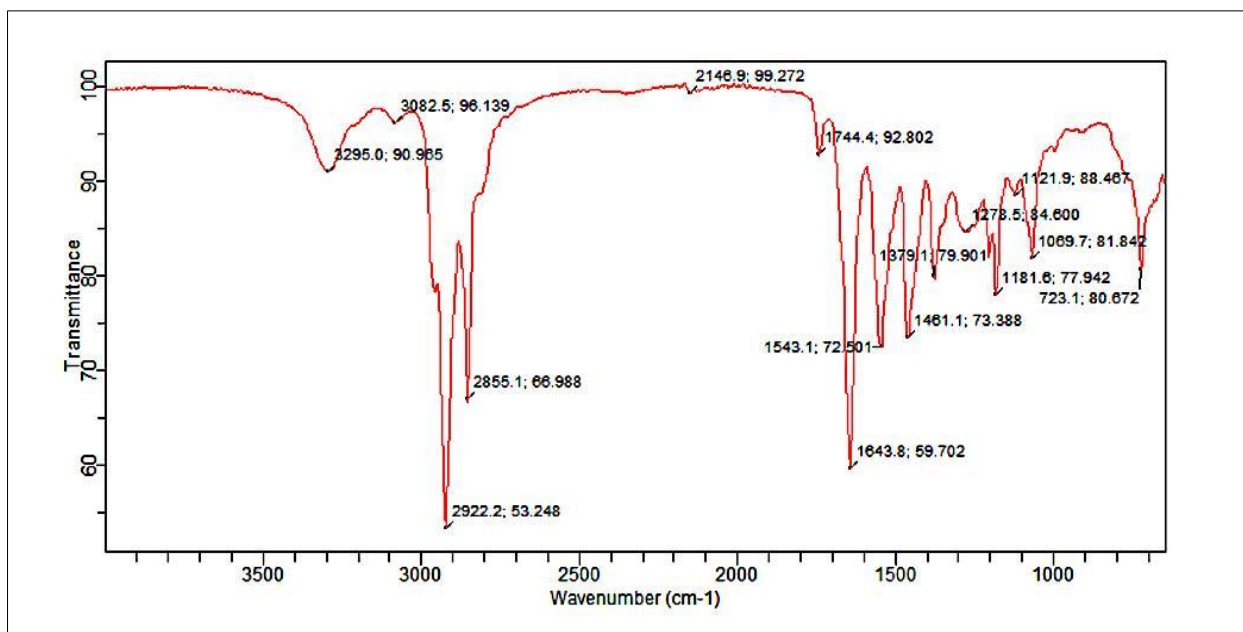


Figure 4 Infrared Spectrum of Amide 1 (intermediate)

The above figure 4 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 2855 cm⁻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 3298 cm⁻¹ indicated N-H peak for an amide.

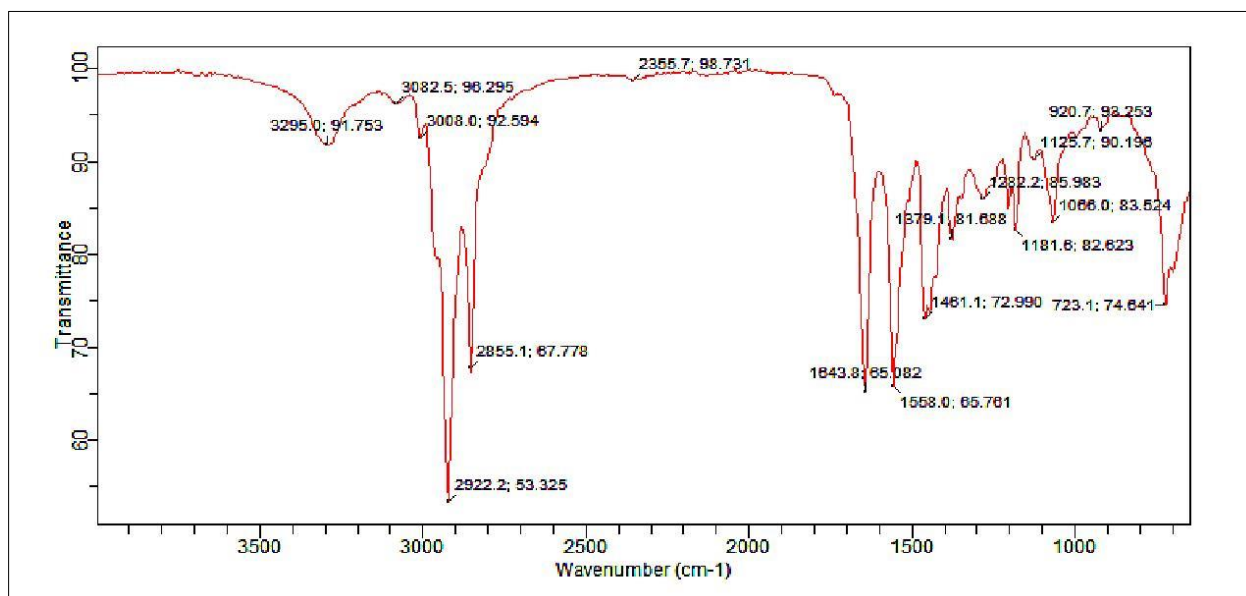


Figure 5 Infrared Spectrum of Amide 2 (intermediate)

The above figure 5 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 2855 cm⁻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 3295 cm⁻¹ indicated N-H peak for an amide.

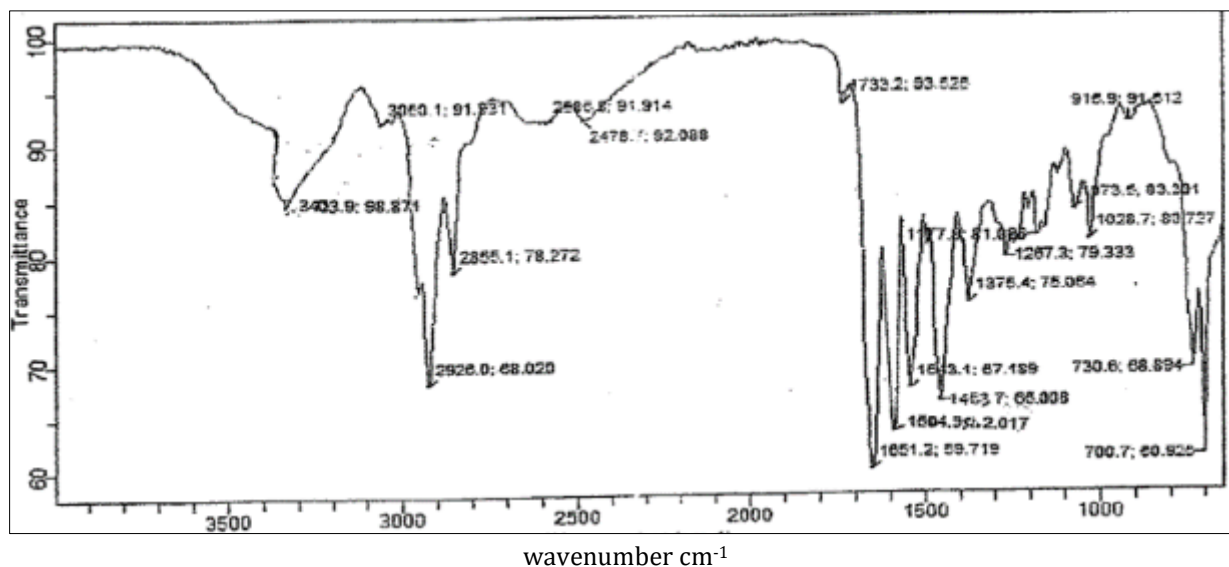


Figure 6 Infrared spectrum of QAC 1

Figure 6 above shows the infrared spectrum of the QAC 1 gotten from Coconut oil, notice a strong intense peak at 1651 cm^{-1} indicating an absorption of carbonyl group ($\text{C}=\text{O}$) 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 $\text{C}=\text{C}$ of an aromatic compound. The sharp peak showing at 700 cm^{-1} and 730 cm^{-1} indicates the monosubstituted nature of the benzene ring. Peak at 1267 cm^{-1} is for absorption of C-N band of an amine.

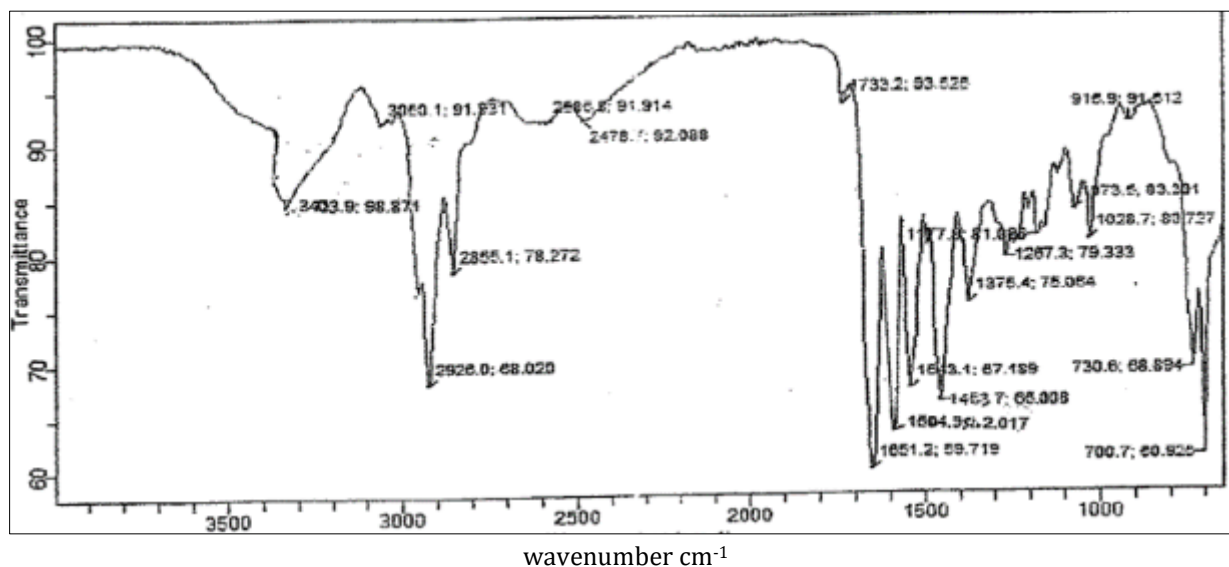


Figure 7 Infrared spectrum of QAC 2

Figure 7 above is the infrared spectrum of QAC 2 from canola oil, showing an intense strong sharp peak at 1651 cm^{-1} indicates an absorption of the carbonyl group ($\text{C}=\text{O}$), peak at 3404 cm^{-1} showed an absorption of N-H band of an amide. Peak at 2926 cm^{-1} indicates C-H stretch of methylene group ($-\text{CH}_2$) of an aliphatic hydrocarbon. Sharp peak at 3060 cm^{-1} is a peak for C-H stretch of an aromatic compound. Intense sharp peak at 1604 cm^{-1} and 1543 cm^{-1} are peaks for absorption of $\text{C}=\text{C}$ of an aromatic compound. A sharp peak at 2855 cm^{-1} is for ($-\text{CH}_3$) of an aliphatic hydrocarbon. Peaks at 730 cm^{-1} and 700 cm^{-1} are peaks for mono substituted nature of the benzene ring. Peak at 1267 cm^{-1} is for absorption of C-N band of an amine.

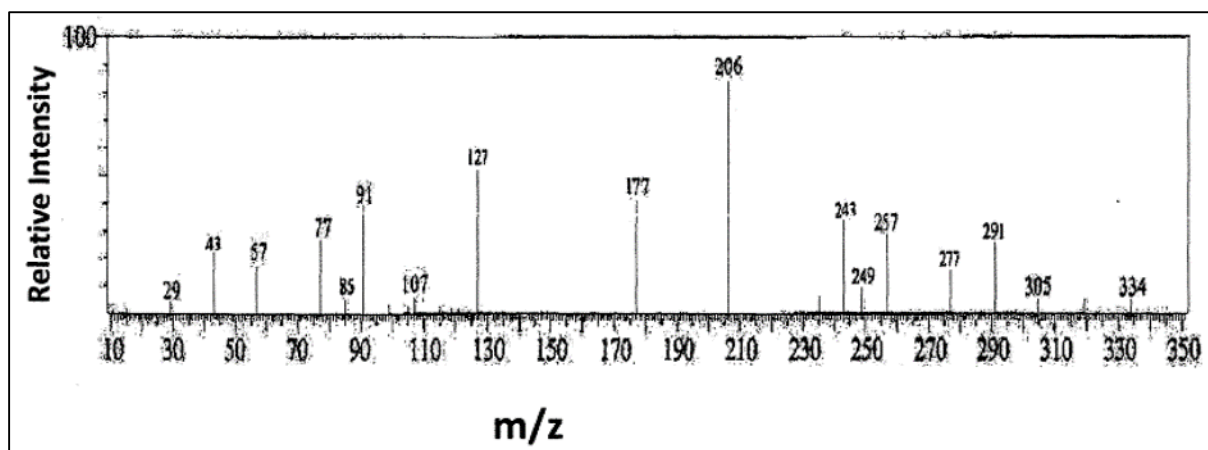


Figure 8 Mass Spectrum of QAC 1

Figure 8 above showed the result of mass spectrometric analysis of the QAC 1 from coconut oil. The following M/Z values for different fragment were observed

M/Z 334 (M^+) which is the molecular ion peak. M/Z 77 ($C_6H_5^+$), M/Z 43 ($+C_3H_7$) M/Z 57 ($+C_4H_9$), M/Z 99 ($C_7H_{15}^+$), M/Z 91 ($C_6H_5CH_2^+$), M/Z 15 ($+CH_3$)

M/Z 127 ($C_7H_{15}C(=O)^+$), M/Z 177 ($C_6H_5CH_2-N^+(C_2H_5)_2CH_2$)

M/Z 319 ($C_6H_{12}C(=O)-NHCH_2CH_2CH_2N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 305 ($C_5H_{10}C(=O)-NH-C_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 277 ($C_3H_6C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 257 ($C_7H_{15}C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2$)

M/Z 243 ($C_7H_{15}C(=O)-NHC_2H_4N^+(C_2H_5)_2$)

M/Z 235 ($C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 206 ($C_6H_5CH_2N^+(C_2H_5)_2CH_2CH_2-NH$) which is the base peak.

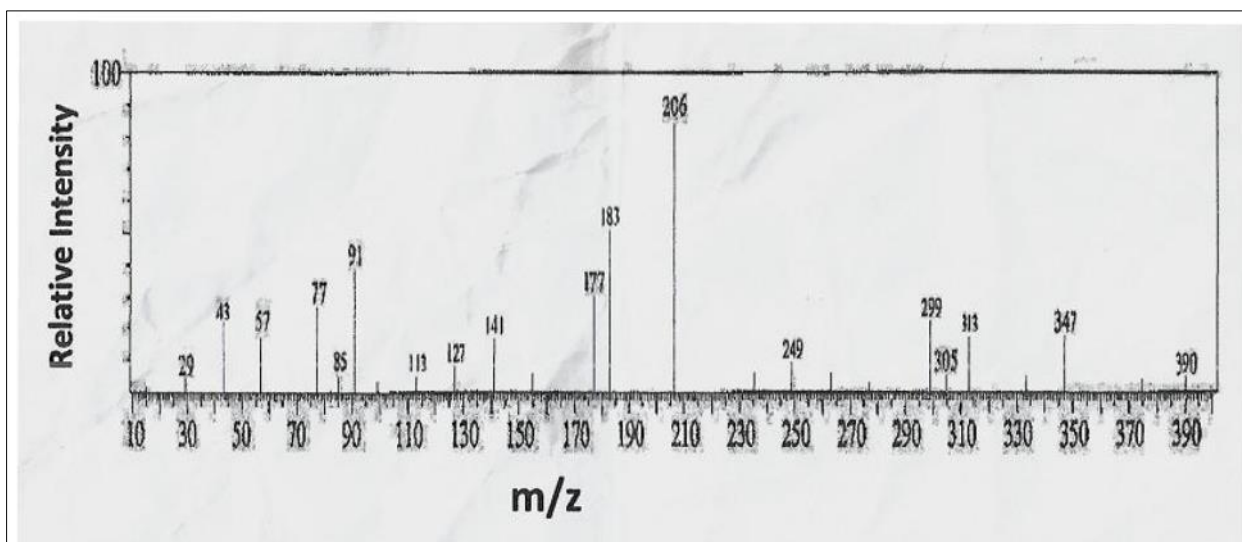


Figure 9 Mass Spectrum of QAC 2

Figure 9 showed the result of mass spectrometric analysis of QAC 2 from Canola oil. The following M/Z values for different fragment were observed;

M/Z 113 ($C_8H_{17}^+$), M/Z 43 ($C_3H_7^+$), M/Z 29 ($C_2H_5^+$), M/Z 57 ($C_4H_9^+$), M/Z 77 ($C_6H_5^+$), M/Z 91 ($^+CH_2C_6H_5$), M/Z 85 ($^+CH_2CH_2CH_2CH_2CH_2CH_3$), M/Z 127 ($C_9H_{19}^+$), M/Z 141 ($^+C_{10}H_{21}$)

M/Z 177 ($C_6H_5CH_2-N^+(C_2H_5)_2CH_2$) M/Z 183 ($C_{11}H_{23}C^+$)

M/Z 277 ($C_3H_6C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 299 ($C_{11}H_{23}C(=O)-NHC_2H_4N^+(C_2H_5)_2$)

M/Z 305 ($C_5H_{10}C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 313 ($C_{11}H_{23}C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2$)

M/Z 347 ($C_8H_{16}C(=O)-NHC_2H_4N^+(C_2H_5)_2CH_2C_6H_5$)

M/Z 206 ($C_6H_5CH_2-N^+(C_6H_5)_2CH_2CH_2NH$) Which is the base peak.

M/Z 390 (M^+), this is the molecular ion peak.

4. Conclusion

Quaternary ammonium compounds were synthesized from Coconut oil and Canola Oil. FTIR analysis was conducted on the starting materials, intermediates (amide 1 and amide 2) and the final products (QAC 1 and QAC 2). Results obtained showed the disappearance of C=O and C–O peaks of an ester during aminolysis. Other vital functional groups such as C=C of aromatic compound, C–N for quaternary fatty amine and -CH₃ of an aromatic hydrocarbon were observed in the products. Mass spectrometric analysis of the final products (QAC 1 and QAC 2) were conducted and results showed the Molecular Ion peak (M⁺) which is the molecular weights of the products. Vital fragments such as base peaks were observed for both QAC, other fragmented radical cations were observed.

Anti-microbial studies of the starting materials, the intermediate and the final products were evaluated. Based on the results obtained, we have been able to establish that QAC 1 and QAC 2 exhibited excellent anti-bacterial activity on various tested micro-organisms as compared with the standard antibiotics (ampicillin). It also showed that both QAC with a longer side alkyl chain length has much higher anti-microbial activity towards gram-positive and gram-negative bacteria.

Finally, inclusion of amide group which breaks the long side alkyl chain length and the positive nitrogen center did not deactivate the anti-microbial activity of these quaternary ammonium compounds.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Authors' contributions

All the authors made valuable contributions; in the laboratory work and in the facilitation of the development of synthesis, scripting and editing of the study report.

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