



SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF POLYESTERAMIDE RESIN FROM *MORINGA OLEIFERA* SEED OIL (MOSO) FOR SURFACE COATING APPLICATION

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ABSTRACT

This paper describes the antimicrobial and corrosion inhibitive properties of synthesized polyesteramide resin from *Moringa oleifera* seed oil (MOSO). N,N'-bis (2-hydroxyethyl) *Moringa oleifera* oil fatty amide (HEMA) was synthesized via aminolysis from MOSO. The fatty amide obtained from aminolysis (HEMA) undergoes polycondensation reaction with adipic acid to form polyesteramide (MOPEA). The synthesized polyesteramide resin was characterized by Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (¹H NMR), Carbon 13 nuclear magnetic resonance (¹³C NMR) spectroscopic analyses and thermogravimetric analysis (TGA). Selected physico-chemical parameters of MOSO, HEMA and MOPEA were examined. Coating performance, thermal stability and antimicrobial properties of the cured resin (MOPEA) were evaluated.

Keywords: *Moringa oleifera*, aminolysis, thermal stability, antimicrobial, coating.

INTRODUCTION

The undoubted depreciation of the world crude oil reserves and the various environmental concerns caused by the use of petrol-based feed stock have driven researchers in the coating and bio-fuel industries towards harnessing sustainable resource materials as feed stocks for their productions (Aigbodion *et al.*, 2001; James *et al.*, 2011; Siyanbola *et al.*, 2011; Ge *et al.*, 2014). In recent years, the synthesis of polymeric materials from vegetable seed oils has paved way for the utilization of these natural renewable resource materials as functional base material for the production of resins (Siyanbola *et al.*, 2013). The fatty acid composition of seed oils plays a pivotal role in determining its appropriate polyol modifications and the eventual properties of the synthesized resins. Seed oils with high percentage of unsaturated fatty acids (that is having iodine value >130) are regarded as drying oils (Larock and Lu, 2009). These seed oils (sunflower oil, linseed oil, safflower oil, walnuts oil) are usually

preferred for coating systems because of their autoxidation ability that lead to the curing of coatings under ambient temperature (Porter *et al.*, 1995). Organic coatings can also be prepared from semi-drying and non-drying seed oils when appropriate formulations are carried out on the fatty acids of the oil (Siyanbola *et al.*, 2013; Akintayo *et al.*, 2011). The presence of unsaturated fatty acids double bond in the seed oils provide site for modifications, such as epoxidation (Sharmin *et al.*, 2007), hydroformylation (Guo *et al.*, 2002), ozonolysis (Kévin *et al.*, 2014), urethanation (Ahmad *et al.*, 2002), amidation (Zafar *et al.*, 2004; Siyanbola *et al.*, 2013) etc. These are the reactive precursors for resinification. The vast arable land mass of Nigeria is used for agricultural purposes. The tropical climatic condition of the country has provided suitable environmental conditions for the growth of wide and domestic plants and herbs bearing seed oils. *Moringa oleifera* commonly known as 'drumstick tree', 'benzoil tree' or 'horseradish tree' in English (USDA, 2014), 'Ewe Igbale' in Yoruba, 'Okwe oyibo' in Igbo and

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'*Habiwal*' in Hausa (Anwar *et al.*, 2007) is an example of such tropical domestic plant bearing rich seed oil. *Moringa oleifera* belongs to the family Moringaceae and is native to the foot hills of Himalayas. Over the years, its cultivation is widely seen in Africa, Central and South America, India, Bangladesh, Pakistan, Malaysia, Philippines and Sri Lanka (Ricardo, 2012). The maturation of the seedlings of *Moringa oleifera* is a function of its breed. Horticultural Research Station of Tamil Nadu Agricultural University (TNAU), India released a new variety of *Moringa oleifera* known as Periyakulam 1 (PKM 1). PKM 1 seedlings grow up to 4 meters in height within 12 months of planting the seed (Lalas and Tsakins, 2002). All the parts of *Moringa oleifera* tree (seed oil inclusive) have found medicinal use in African and South Asia folk medicine (Fahey, 2005; Ogbunugafor *et al.*, 2011). It's possible use for machine lubrication, bio-fuel, hair care and perfume products as also been reported (Anwar and Rashid, 2007; Tsaknis *et al.*, 1999). Since *Moringa oleifera* seed oil (MOSO) has reasonable percentage oil yield of about 45%, which is rich in oleic acid (approximately 73%) (Ricardo, 2012). It can thus be considered for the production of organic coating resins.

Over the years, the synthesis of various active antimicrobial organic coatings from seed oils as been on the increase (Siyabola *et al.*, 2013). Farooq *et al.* (2012) study, reviewed the antimicrobial properties of *Moringa oleifera*, his report shows that the oil extract of *Moringa oleifera* resist the growth of *P. Aeruginosa* and sensitive to *Bacillus stearothermophilus* (Farooq *et al.*, 2012; Ali *et al.*, 2004). The present study evaluates the antimicrobial properties of synthesized polyesteramide from *Moringa oleifera* seed oil (MOPEA) as well as its physico-chemical properties.

MATERIALS AND METHODS

Oil was extracted from *Moringa oleifera* seeds obtained from local market in Kaduna State, Nigeria. N-Hexane was used as a solvent. The fatty acid composition of the oil was carried out using a gas chromatography (GC; HP-1 ms, 30 m × 0.25 mm × 0.25 μm, FID detector). The diethanolamine, diethyl ether, adipic acid, xylene, 4-methyl pentan-2-one, anhydrous sodium sulphate was obtained from S.D. Fine Chemicals (Mumbai, India). Sodium methoxide (Merck, India).

The air dried seeds were cleaned by hand picking in order to get rid of foreign materials. The seed coats (pericarp) of the seeds were removed by cracking. Seeds were kept away from moisture prior to grinding (size reduction). The ground seeds were packed in the Soxhlet thimble and n-Hexane (solvent) was poured into the Soxhlet round bottom distillation flask. A condenser was connected to the top of the extractor. Then the heating mantle was

switched on and heat was supplied to the distillation flask. Vapourised solvent were generated and condensed as hot liquid solvent in the packed thimble. This process resulted in the extraction of the oil through the various siphoning of oil plus solvent into the distillation flask. The mixture of oil and n-hexane was separated by the use of rotary evaporator.

Synthesis of N,N'-bis (2-hydroxyethyl) *Moringa oleifera* oil fatty amide (HEMA)

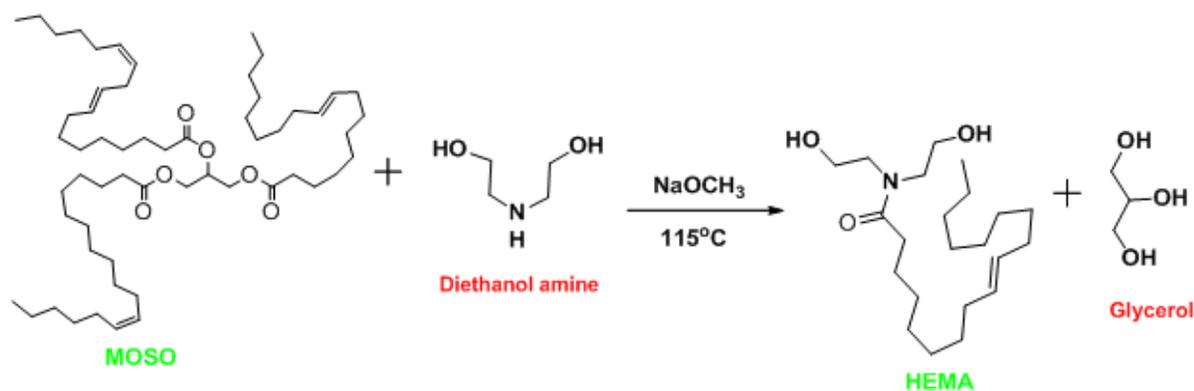
The preparation of HEMA was carried out in a four necked round bottom Pyrex flask containing diethanolamine and *Moringa oleifera* seeds oil and fitted with mechanical stirrer, condenser, and thermometer. The flask was submerged in an oil bath. With a molar ratio of 6:1 diethanolamine to oil in the presence of 2% sodium methoxide, the mixture in the four necked round bottomed flask was reacted at 115°C while stirring. The progress of the reaction was monitored by thin layer chromatography (TLC). At the completion of the reaction, the reaction mixture was allowed to cool and it was dissolved in diethyl ether in a separating funnel. The ethereal layer was washed with 5% aqueous hydrochloric acid. The ether layer was separated and washed with water and later dried over anhydrous sodium sulphate. Rotary evaporator was used in concentrating the ether layer (Adewuyi *et al.*, 2011; Siyanbola *et al.*, 2013).

Synthesis of *Moringa oleifera* polyesteramide (MOPEA)

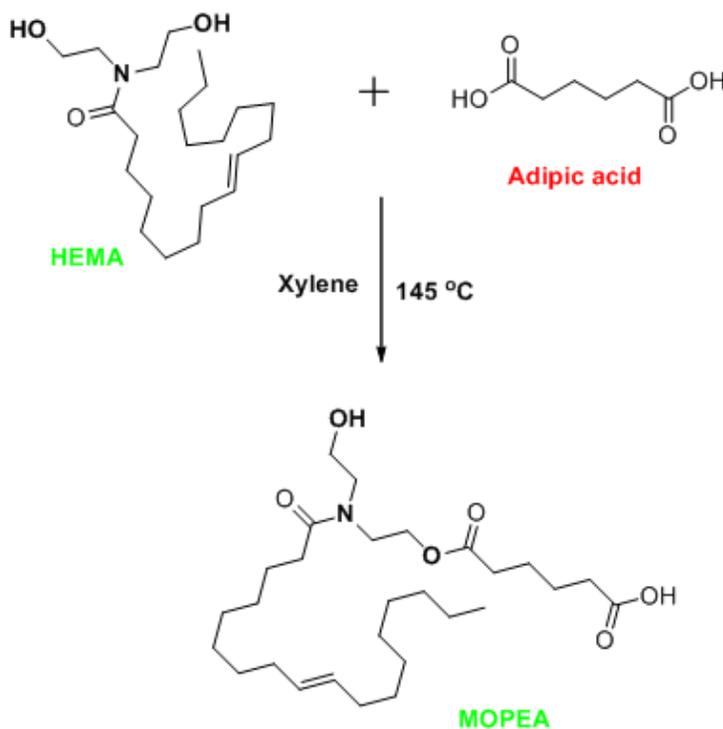
MOPEA was synthesized by reacting HEMA (0.046 mol) with adipic acid (0.046 mol) and 40 ml xylene as solvent in a four neck round bottom flask connected to a Dean stark, thermometer, mechanical stirrer, and a nitrogen inlet tube. The reaction mixture was refluxed at 145-160 °C until the theoretical amount of water was collected and the reaction was monitor by the determination of acid value at regular intervals (Misiev, 1991). At the end of the reaction the product (MOPEA) was taken out of the four neck round bottom flask and xylene (solvent) was withdrew from the compound using rotary evaporator under reduced pressure.

Antimicrobial analysis

Antimicrobial activities of *Moringa oleifera* seeds oil, pre-polymer (HEMA) and polyesteramide (MOPEA) was studied and tested against Gram-positive organisms and Gram-negative organisms. One ml of the seed oil extract was dissolved in 4 ml of 5% dimethyl sulfoxide. Suspension of micro-organisms was made in sterile normal saline and adjusted to 0.5 Macfarland's standards (108 Cfu/ml). From the stock of 282.5 mg/ml extract, serial dilutions were made to 200, 150, 100, 60 mg/ml and were dissolved. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed.



Structure 1. N,N'-bis (2-hydroxyethyl) *Moringa oleifera* oil fatty amide (HEMA).



Structure 2. Synthesis of *Moringa oleifera* poly(esteramide) (MOPEA).

A sterile cork borer of 5 mm diameter was used to make wells on the medium. 0.1 ml of the various dissolved extract concentration were dropped into each, appropriate labelled well. The Mueller Hinton Agar plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation.

Characterization of MOSO, HEMA and MOPEA

The Fourier transform infrared (FTIR) spectra were recorded on Elmer spectrum 100 spectrometer (PerkinElmer Inc., USA) by scanning eight times. The intensity and frequency of bands on the FTIR spectra were automatically obtained by using the instrument

software. ¹H NMR and ¹³C NMR were qualitatively recorded on Varian VXR-Unity 200 MHz spectrometer and Bruker UXNMR 400 MHz spectrometer respectively, using CDCl₃ and DMSO-d₆ as a solvent and tetramethyl silane as an internal standard. The thermal stability and curing behaviour of MOPEA was carried out on thermogravimetric analyzer (Perkin Elmer TGA 7, TA Instrument, USA) and differential scanning calorimetry (Perkin Elmer TA DSC Q100, USA) at a constant heating rate of 10°C min⁻¹ in nitrogen atmosphere respectively. The physico-chemical properties such as acid value, viscosity, refractive index, iodine value, and saponification value of MOSO, HEMA and MOPEA were also ascertained.



Fig. 1. Pictorial representation of MOSO, HEMA and MOPEA
 A = MOSO B = HEMA C = Solid MOPEA D = Liquid MOPEA

Table 1. Physico-chemical characterization.

Sample	Refractive Index at 40 °C	Specific gravity at 40 °C	Iodine Value gI ₂ /100g sample	Saponification nValue mg KOH/g sample	Acid Value mg KOH/g sample	Viscosity cSt at 40 °C
MOSO	1.450	0.924	80.1	180.31	3.5	60
HEMA	1.586	0.958	43.2	182.3	4.6	1463.1
MOPEA	1.693	0.986	26.2	166	8.2	-

Curing of resin

The non-drying nature of MOSO reflects on MOPEA as the resin did not cure at ambient temperature neither did it dry at 130°C but the resin was cured with curing agents at higher temperature of 175°C on mild steel plates (Mahapatra and Karak, 2004).

Coating preparation and testing

Different grades of silicon carbide papers were used for the preparation of the mild steel, which were initially rinsed with water, ethanol and acetone. The degreased mild strips were dried under vacuum for hours. The pristine and hybrid coatings were prepared by brush application of 60 wt% of resin in xylene on the mild strips. For chemical resistance test in water, acid (5 wt% HCl), alkali (5 wt% NaOH) taking in 3 inches diameter porcelain dishes standard sizes of mild strips of 30 × 10 × 1 mm³ were used.

RESULTS AND DISCUSSION

Structure 1 and 2 represent the reaction schemes for the synthesis of HEMA and MOPEA. The amination reaction between MOSO and diethanolamine in the presence of sodium methoxide (catalyst) leads to the formation of HEMA (Structure 1) while the synthesis of MOPEA was carried out by reacting aliphatic diacid (adipic acid) with HEMA in the esterification step (Structure 2). The solvent used during the formation of polyester resin was recovered by evaporation.

Physico-chemical characteristics

Different physical properties of the seed oil, its polyol and resin have been determined. The iodine value (I.V.) of MOSO (Table 1) confirms the non drying nature of the oil. However, there is a further decrease in I.V. as HEMA and MOPEA are been prepared from the oil. This observation may be associated with decrease in the percentage composition of unsaturated functional group, which culminates in increase in the molecular weight of the resin (MOPEA). On the other hand, the refractive index and specific gravity show increase in values as the polymer weight increases with increase in cross link density of polyester resin. Hydrogen bonding linkage between the terminal hydroxyl groups of the polyols may be responsible for the high viscosity observed in HEMA. Solubility test was carried out in different solvents namely; methyl isobutyl ketone, N,N'-dimethylformamide, diethyl ether, toluene, petroleum ether, carbon tetrachloride, xylene, dimethylsulfoxide, acetone, chloroform and ethanol. HEMA and MOPEA show about 95-100 wt% solubility in N,N'-dimethylformamide, dimethylsulfoxide, methyl isobutyl ketone, xylene, carbon tetrachloride and chloroform. The high solubility behaviour may be ascribed to the presence of long fatty acid chains in the sample. Figure 1 show the pictorial representation of the seed oil (MOSO), HEMA and MOPEA.

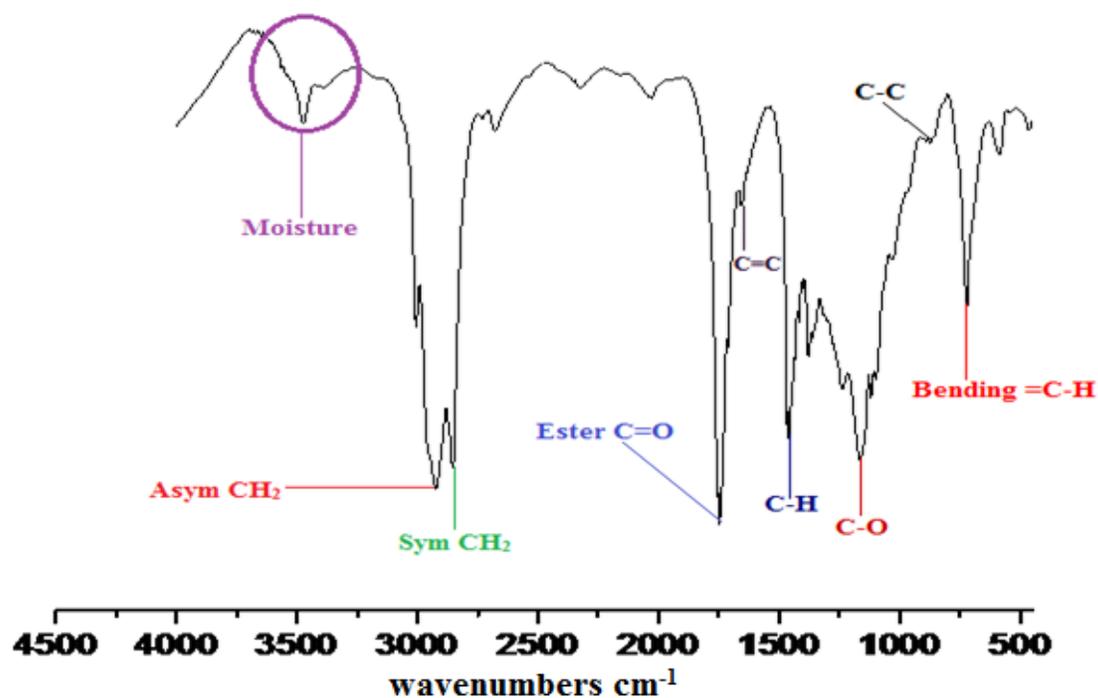


Fig. 2. FTIR Spectrum of MOSO.

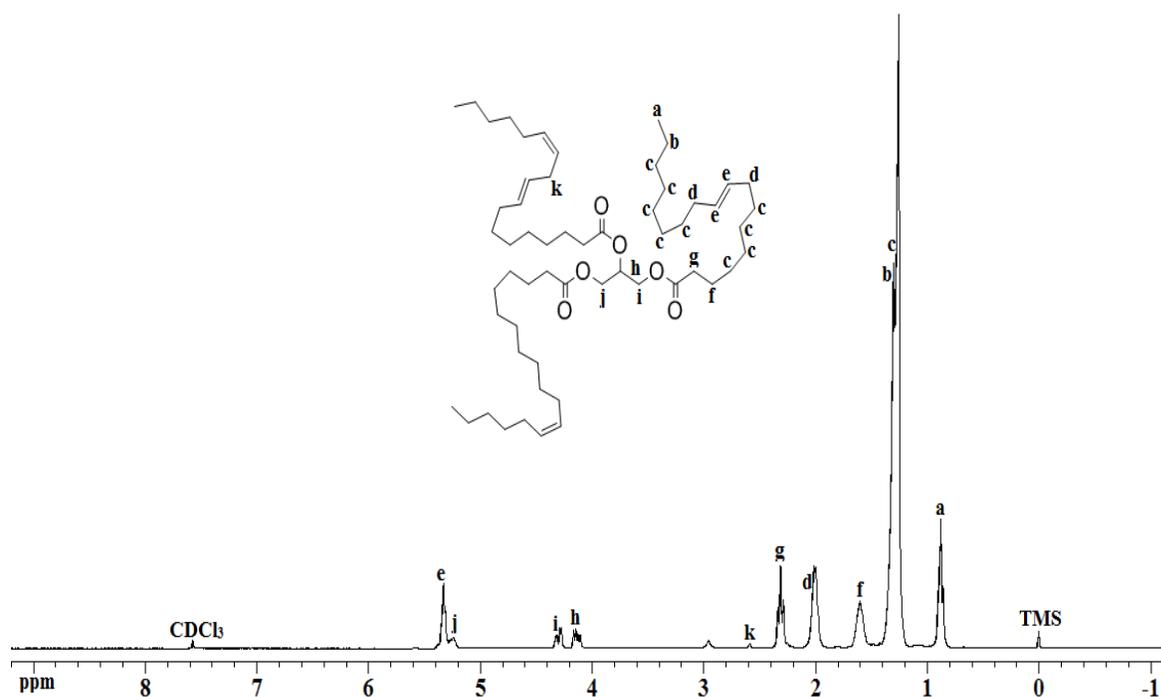


Fig. 3. ^1H NMR spectrum of MOSO.

Spectroscopic characterization of MOSO, HEMA and MOPEA

Figure 2 represents the FT-IR spectrum of *Moringa oleifera* seed oil. Bands such as stretching frequency of ester C=O and C-O were observed at 1745 cm^{-1} and 1162

cm^{-1} , respectively. While the presence of C-C and C=C stretching vibrations were noted at 869 cm^{-1} and 1657 cm^{-1} respectively. These observations corroborate the presence of saturated and unsaturated fatty acids in the oil.

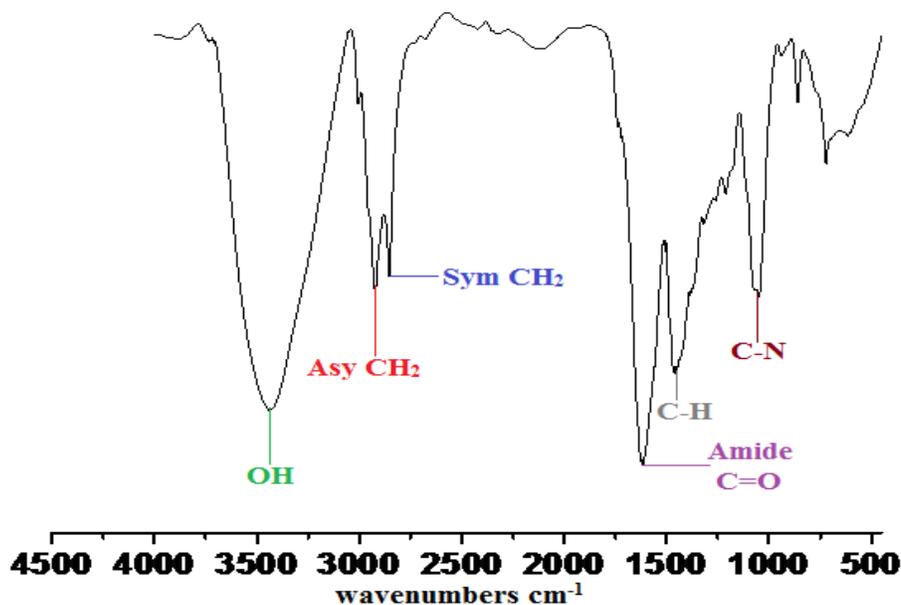


Fig. 4. FTIR Spectrum of HEMA.

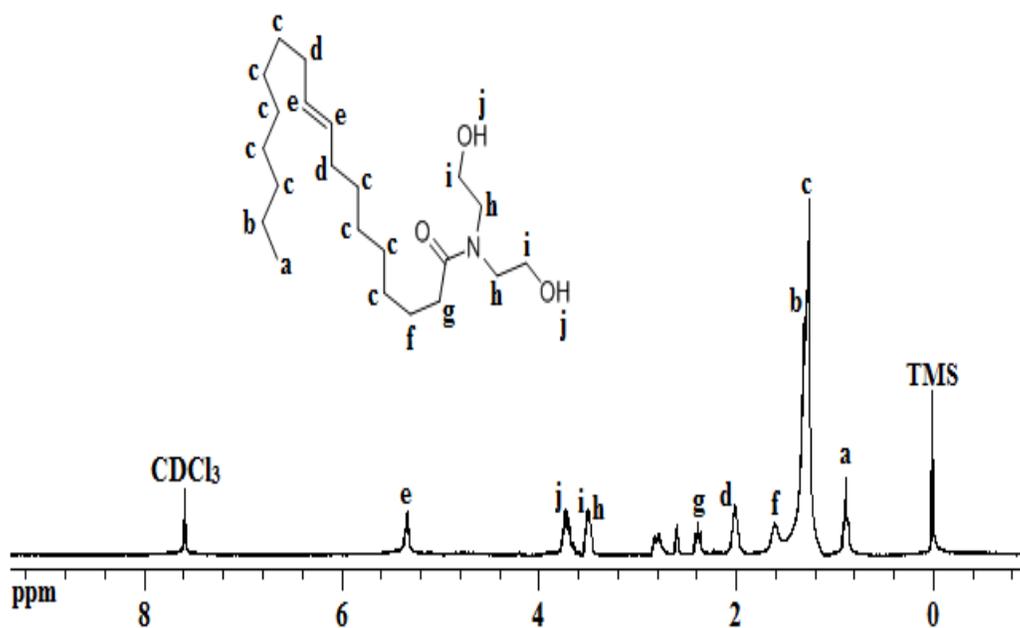


Fig. 5. ^1H NMR of HEMA.

The ^1H NMR spectrum of MOSO (Fig. 3) shows the terminal methyl protons ($-\text{CH}_3$) of the fatty acid chain was observed at $\delta = 0.88\text{-}0.90$ ppm. The chemical shifts between $1.26\text{-}1.31$ ppm accounts for internal protons ($-\text{CH}_2-$) present in the fatty acid chains, while the peaks at $\delta = 5.24\text{-}5.33$ ppm represent $-\text{CH}$ of glycerol backbone and $=\text{CH}$ of unsaturated carbons. Glycerol $-\text{CH}_2-$ protons appear at $\delta = 4.14\text{-}4.32$ ppm.

The FTIR spectrum of HEMA is illustrated in figure 4. HEMA has a characteristic $-\text{OH}$ broad band absorption at

3405 cm^{-1} . The CH_2 stretching bands for asymmetric and symmetric appear respectively at 2923 cm^{-1} and 2853 cm^{-1} , the strong $\text{C}=\text{O}$ stretching vibration of amide ($-\text{NCO}-$) appears at 1615 cm^{-1} while 1048 cm^{-1} band represent C-N stretching vibrations. C-H bending vibrations typical of that of alkane are observed at 1462 cm^{-1} .

Typical ^1H NMR spectrum of HEMA is shown in figure 5. The presence of the following peaks confirms the structure of HEMA polyol shown in Figure 1. The terminal protons ($-\text{CH}_3$) of the fatty acid chains resonate

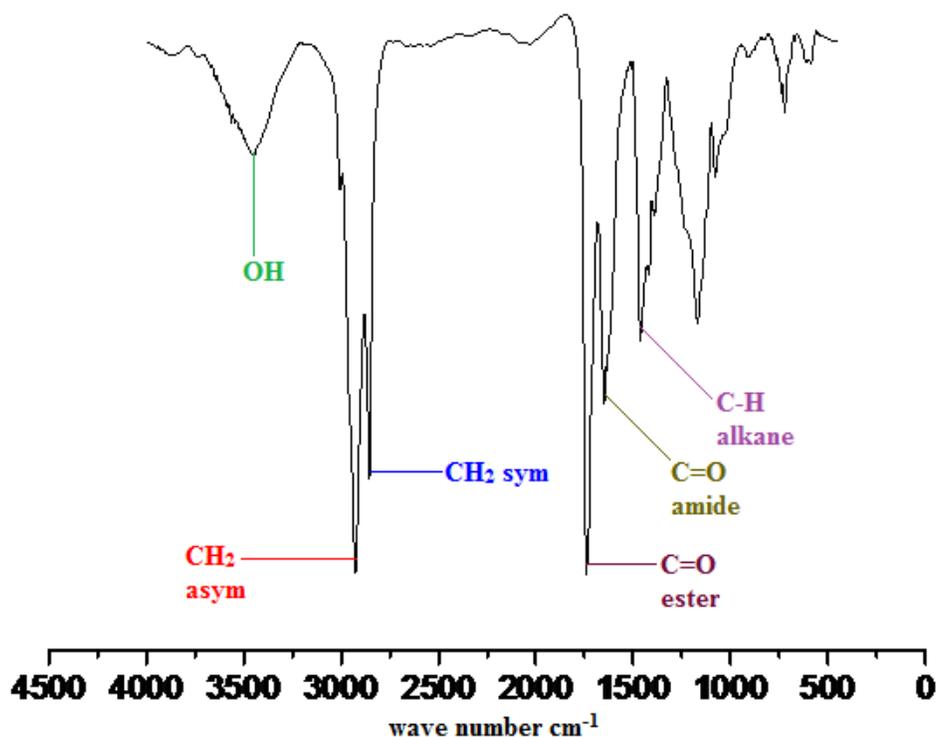
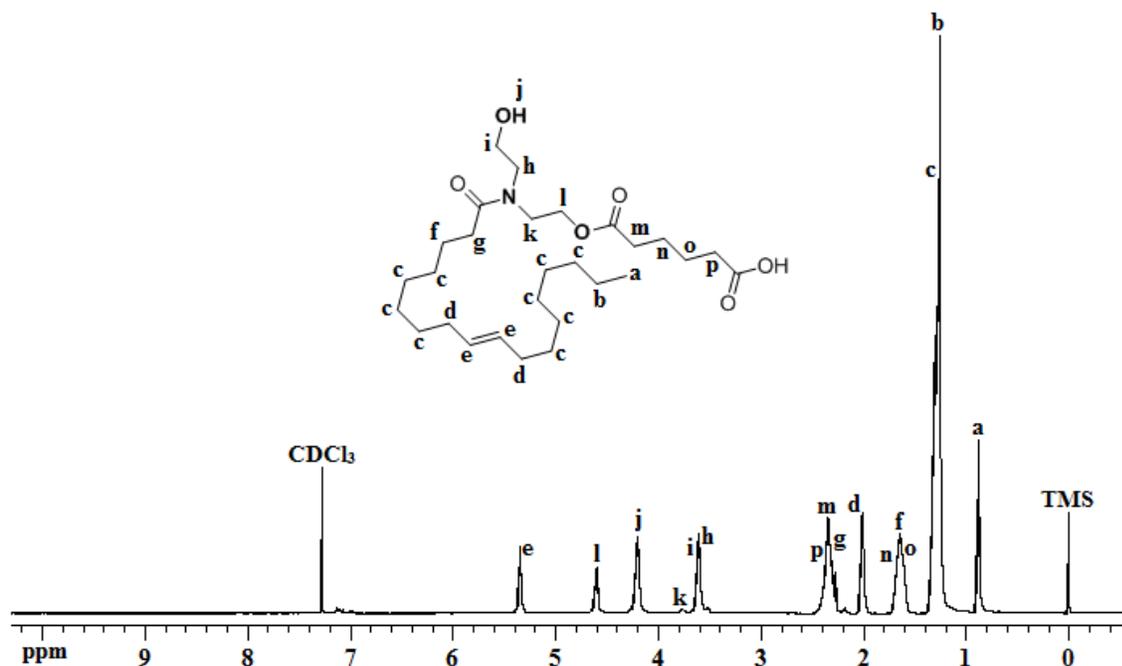


Fig. 6. FTIR Spectrum of MOPEA.

Fig. 7. ^1H NMR Spectrum of MOPEA.

at $\delta = 0.88$ ppm, the chemical shift at $\delta = 1.27$ - 1.30 ppm represent internal protons ($-\text{CH}_2-$) of the fatty acid chains. The $-\text{CH}_2-$ protons alpha to the double bond carbon atoms ($-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$) on the fatty acid chains is observed at $\delta = 2.03$ ppm while the olefinic protons

appears at $\delta = 5.32$ ppm. $\delta = 3.41$ ppm and $\delta = 3.50$ ppm respectively represent the alpha and beta $-\text{CH}_2-$ arising from the diethanolamide. The hydroxyl protons of the amide was observed at $\delta = 3.74$ ppm.

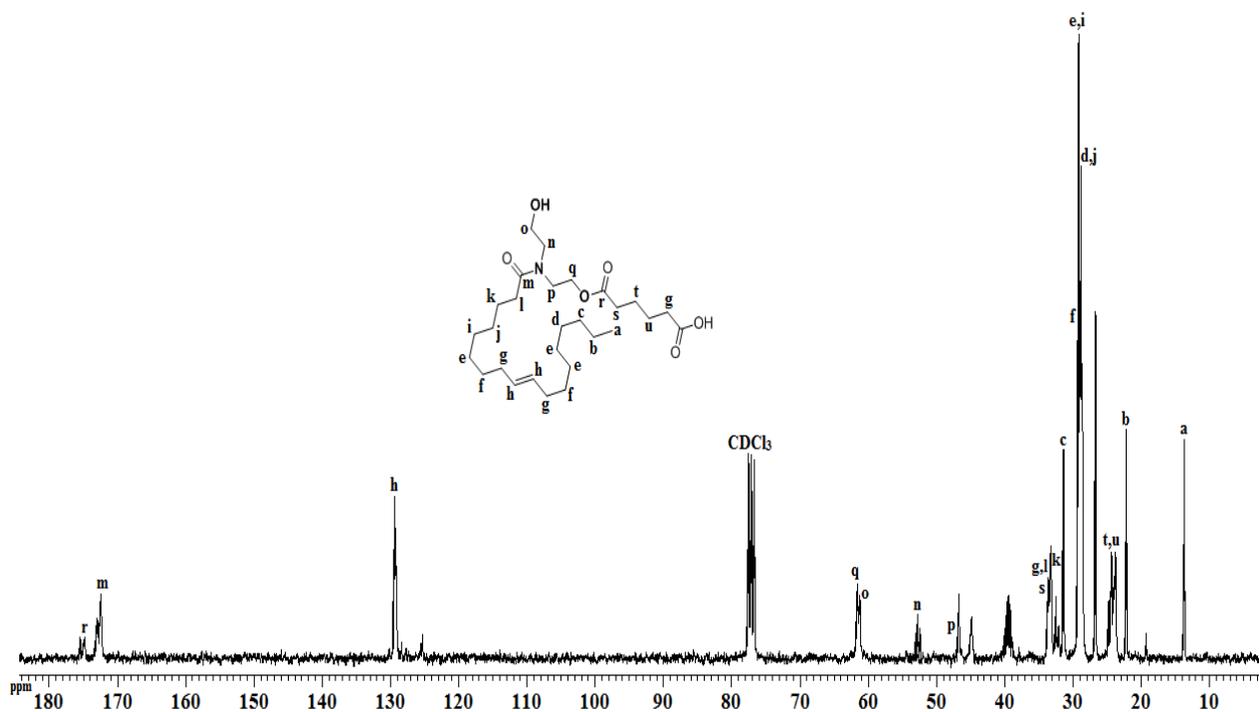


Fig. 8. ^{13}C NMR Spectrum of MOPEA.

Table 2. Antimicrobial activity of HEMA and MOPEA.

Sample	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. niger</i>
HEMA	++	+	++	++	++
MOPEA	+++	++	+++	+++	+++

+ = mild; ++ = moderately active; +++ = highly active

As represented in figure 6, the presence of the following functional groups on the spectrum confirms the formation of MOPEA. The hydroxyl ($-\text{OH}$) and ester carbonyl ($-\text{C}=\text{O}$) functional groups respectively shows a characteristic stretching absorption bands at 3434 cm^{-1} and 1736 cm^{-1} , while stretching absorption bands at 2854 cm^{-1} and 2925 cm^{-1} respectively represent symmetric and asymmetric $-\text{CH}_2$. The amide carbonyl ($-\text{NCOR}$) band was observed at 1646 cm^{-1} , the ester $-\text{C}-\text{O}$ stretching frequency appears at 1170 cm^{-1} and having the $\text{C}-\text{N}$ stretching absorption band at 1458 cm^{-1} . The ^1H NMR spectrum of MOPEA is presented in figure 7, this confirms the presence of the following protons as illustrated in reaction figure 2. Proton peaks at $\delta = 0.88$ ppm and $\delta = 1.30$ ppm respectively represent terminal methyl group and its alpha methylene group. The double bond protons and its immediate methylene group resonate at $\delta = 5.32$ ppm and 2.02 ppm respectively. The $-\text{CH}_2-$ attached to the amide $-\text{C}=\text{O}$ appears at $\delta = 2.38$ ppm while

$-\text{CH}_2-$ attached with nitrogen resonate at $\delta = 3.73$ ppm and 3.62 ppm may be attributed to $-\text{CH}_2-$ attached to hydroxyl functional group. The peak observed at $\delta = 1.27$ ppm is represent the internal methylene protons of the fatty acid chains. However, the structure of MOPEA was further confirmed with the ^{13}C NMR spectroscopy (Fig. 8). The methylene carbon atoms ($-\text{CH}_2$) attached to free hydroxyl, amide nitrogen and amide carbonyl respectively resonate at $\delta = 60.9$ ppm, $\delta = 52.7$ ppm; $\delta = 46.9$ ppm and $\delta = 33.5$ ppm. The $\delta = 172.3$ ppm represent carbonyl carbon ($\text{C}=\text{O}$) attached to nitrogen (this peak affirms the binding of the fatty acids to the nitrogen of diethanolamine) whereas the methylene carbon atom preceding the ester linkage ($-\text{CH}_2-\text{COOCH}_2-$) appears at $\delta = 61.3$ ppm. Olefinic carbons ($-\text{CH}=\text{CH}-$) of the fatty acids resonate at $\delta = 129.3$ ppm while the internal methylene carbons of the fatty acid and adipic acid chains appears at $\delta = 33.5-22.1$ ppm. The terminal methylene carbon shows peak at $\delta = 13.6$ ppm.

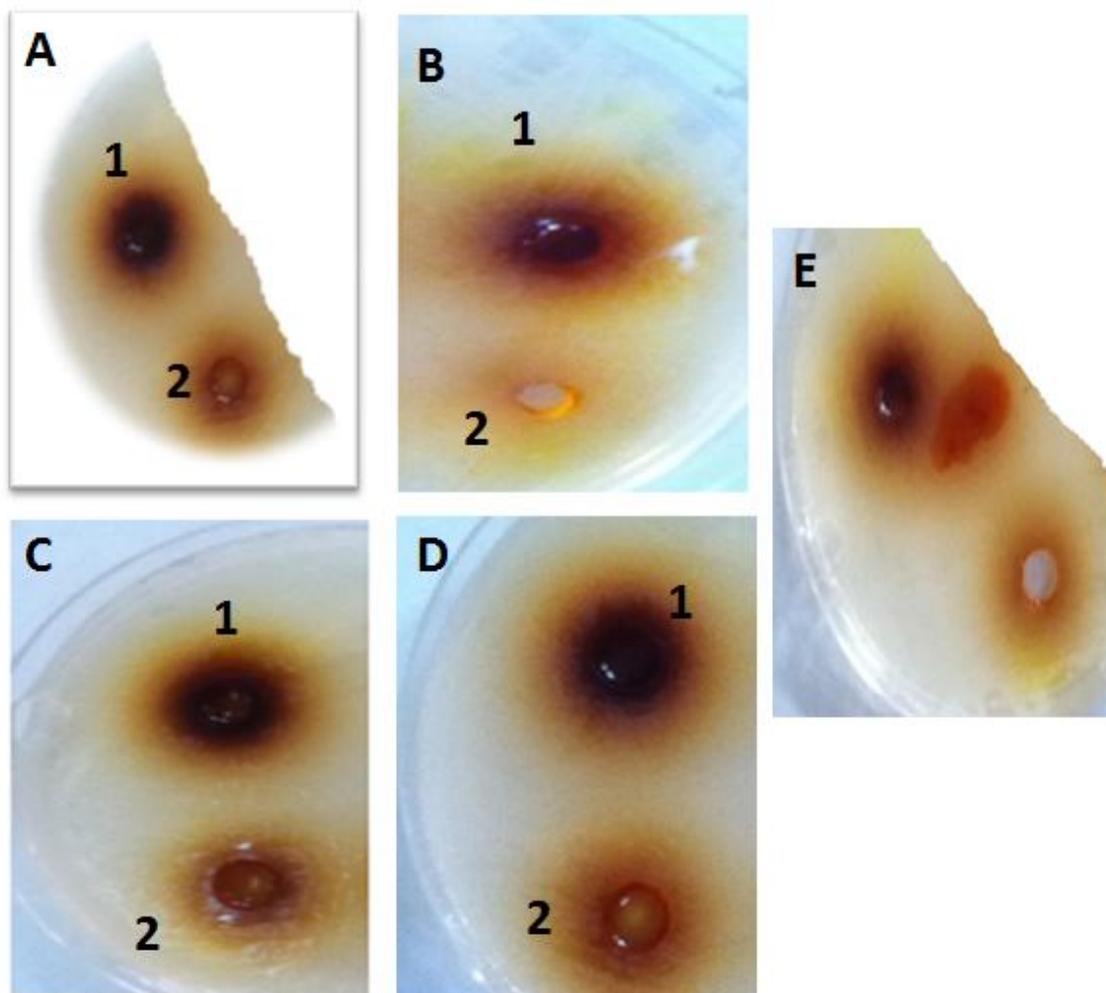


Fig. 9. Antimicrobial studies of HEMA and MOPEA.

A = *E. coli*, B = *B. subtilis*, C = *P. aeruginosa*, D = *S. aureus*, E = *A. niger*

Solubility test

The solubility property of HEMA and MOPEA were observed in various solvents namely; dimethylformamide (DMF), DMSO, ethanol, trichloromethane, toluene, diethyl ether, acetone, methanol, MIBK, and xylene. HEMA and MOPEA were highly soluble (about 100%) in the solvents tested but were sparingly soluble in methanol and ethanol. The solubility behaviour of the samples can be due to the presence of long fatty acid chains as well as the polarity of the groups.

Antimicrobial evaluations

In table 2 and figure 9, the antimicrobial screening tests of HEMA and MOPEA is respectively presented based on the zone of inhibition of microbial growth around the well. Since both samples were soluble in xylene, the antimicrobial screenings were carried out in xylene. This had no effect on the growth of media (Zafar *et al.*, 2007). Inhibition activities were showed as high, moderate, and

mild. Both samples show good inhibitive property especially MOPEA. This indication show that MOPEA will further show antimicrobial activity when the resin is further processed into other materials like urethanes and/or composites.

Thermal property

The TGA thermogram of MOPEA is shown on figure 10, while figure 11 represents the derivative of thermogravimetric (DTG) analysis of MOPEA at a rate of $10^{\circ}\text{C min}^{-1}$, studied under nitrogen atmospheres. The TGA thermogram shows somewhat two degradation steps. The initial 5% weight loss at 165°C corresponds to entrapped solvent and moisture. The first step degradation is associated with 30% weight loss at 329°C , which corresponds to the decomposition of ester and amide. The faint second degradation with 82% of weight loss at 528°C corresponds to decomposition of the fatty acids hydrocarbon chains.

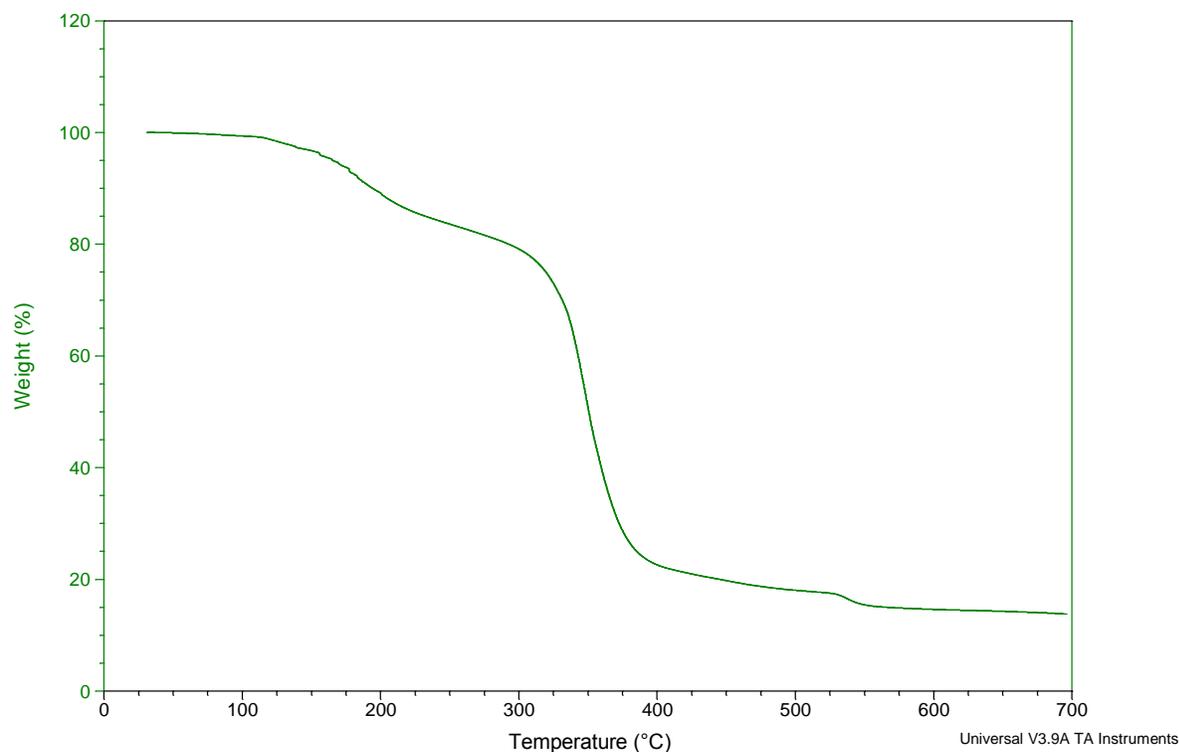


Fig. 10. TGA curve of MOPEA.

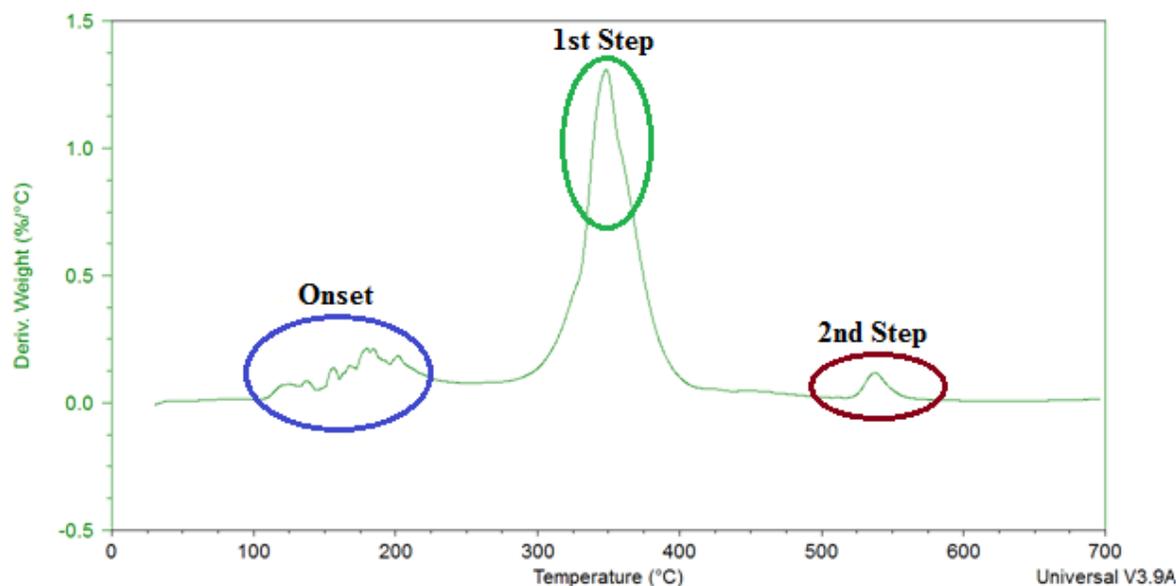


Fig. 11. Derivative of TGA of MOPEA.

CONCLUSION

Polyesteramide (MOPEA) of MOSO was successfully synthesized and characterized. The resin and its polyol showed excellent antimicrobial inhibitive properties. The remarkable solubility property of MOPEA in most tested solvents reveal and its thermal stability sows that this compound can be further processed easily. The thermal

stability profile is also an indication of how promising the resin will become when applied in the coating industry.

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