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Full Length Research Paper

Physico-chemical analysis of *Ximenia americana*.L seed oil and structure elucidation of some chemical constituents of its seed oil and fruit pulp

A. E. M. Saeed[1]* and R. S. M. Bashier[2]

[1]Department of Chemistry, College of Science, Sudan University for Science and Technology, P. O. Box 407, Khartoum, Sudan.
[2]Department of Chemistry, College of Science, Zalingei University, Sudan.

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In this study, fixed oil was extracted from the seeds of *Ximenia americana*. The study covered the percentage yield, physical and chemical properties of the extracted oil. The highest oil obtained was 51% w/v. The physical properties of the oil were found to be reactive index (1.477), density (0.9376 g/ml), boiling point (157°C) and viscosity 42 at 70°C and 227.58 at 25°C. The chemical properties of the oil were : iodine value (47.59), acid value (0.2805), peroxide value (30), saponification value (11.43), ester value (9.82), and the ratio value (35.009). The molecular weight of the major component of the oil was 604. The major component of the oil ($C_{40}H_{76}O_3$) was identified as methyl-14,14- dimethyl – 18-hydroxy heptatriacont-27,35-dienoate [$CH_3OCO(CH_2)_{12}C(CH_3)_2(CH_2)_3CHOH(CH_2)_8CH=CH(CH_2)_6CH=CHCH_3$]. A compound was isolated from the fruit pulp, and purified using column and thin layer chromatography, with a molecular weight of 578, molecular formula ($C_{35}H_{62}O_6$) and was identified as dimethyl - 5- Methyl – 28,29 - dihydroxy dotriacont-3,14,26 - triendioate. These compounds were not reported before from *X. americana* seeds or fruit pulp [$CH_3OCOCH_2CH=CHCH(CH_3(CH_2)_8CH=CH(CH_2)_{10}CH=CH(CHOH)_2(CH_2)_2COOCH_3$].

Key words: *Ximenia americana*, seeds, oil, fruit pulp.

INTRODUCTION

In sudan *Ximenia americana* tree, family Olecaceae is widely distributed in different regions. It is found in Darfur (Jabal Marra, Radom); Blue Nile (Ingessena Hills); Kordofan (Nuba Mountains, Nuhud); Red Sea Hills (Erkwit); Bahar Ghazal, Upper Nile and Equatoria (Torit).

X. americana bark, fruit and leaves have many uses in local medicine for people and animals (Mwangi and Malii, 1994). The leaves and twigs are used for fever, cold, as mouth wash for tooth aches, as laxative and an eye lotion (Omer and Ali, 1998). The leaves are used for headaches and poison antidote (Feiberger and Vanderjagt, 1998). Roots treat skin aches and problems, headaches, leprosy, hemorrhoids, sexually transmitted diseases, guinea worm, sleeping thickness, oedema and act as an antidote to poison (Teo, 1997). The fruit is useful in treating habitual constipation. The bark is used dried, powdered and applied to skin ulcers (Kuroki and Conn, 1989). The fruits are eaten in large quantities and act as a vermifuge (Niemi et al., 2005*)* .

X. americana pulp, seed and fruit contain hydrocyanic acid (Benoit and Santillana, 2000). Bark contains approximately 17% oils, heartwood and flowers contain essential oils (Fatope and Adam, 2005). Fruits, fruits pulp, leaves, twigs and roots contain constituents used in folk medicines (Benoit and Santillana, 2000). It was reported

*Corresponding author. E-mail: ahmedelsadig@yahoo.com. Tel: 00249 912218596. Fax: 00249 183 769363.

Table 1. Viscosity data of *X. americana* seeds oil.

No	Temperature (°C)	Viscosity cp.	Shear stress	Torque (%)
1	70	42.00	0.00	-0.53
2	65	48.82	0.98	5.32
3	60	56.20	1.12	6.12
4	55	66.34	1.33	7.22
5	50	80.16	1.60	8.67
6	45	94.90	1.90	10.25
7	40	117.01	2.34	12.69
8	35	143.73	2.87	15.59
9	30	183.35	3.67	19.91
10	25	227.58	4.55	24.69

that *X. americana* seed oil contained oleic, linoleic, linolenic, arachidonic, eicosatrienoic, erucic and nervonic acids (Eromosele and Eromosele, 2002). The major constituents of the volatile oil of the leaves of *X. americana* were benzaldehyde, hydroxy benzyl cyanide and isophorone(Mevy et al., 2006). Mitei et al., 2009 reported the contents of α and β tocotrienol, campesterol, β- amyren and lupeol from the seed oil of *X. caffra* in Botswana.

According to the different uses of *X.americana*, this work aimed at investigating the physical and chemical properties of the oil of *X. americana*, isolating and elucidating structure of some constituents of the seeds and fruit pulp of *X.americana* using different spectroscopic methods.

EXPERIMENTAL

X . americana fruits, brought from the west in Sudan (West Kordofan State), were used. The fruits were collected from the forest around Babanousa city, during the rainy season in July and August in 2007. The voucher specimens were identified and authenticated by the Taxonomists of Medicinal and Aromatic Plants Research Institute, National Research Center, P.O Box 2404, Khartoum, Sudan.

Thin layer chromatography was carried out by using silica gel type G for thin layer chromatography from sigma chemical Co, USA. Column chromatography was carried out by using silica gel type S for column chromatography particle size 0.063 - 0.2 mm from Riedel DeHAËN, Hannover.

Infrared (IR) analysis was carried out using 300 FT- IR spectrophotometer, Ultra violet spectroscopic analysis was carried out using the spectrophotometer model 6595 from Jenway limited. Nuclear Magnetic Resonance (H^1 – NMR) spectroscopic analysis was carried out with NMR Gemini 400 MHz instrument. Mass spectroscopic analysis (MS) was carried using the mass spectroscopic (MS) instrument , model QP 1000 EX.

Extraction of seed oil

The fruits of *X. americana* were freed from the coating layer for getting the pulp. The pulp was dried at room temperature. Seeds were obtained by breaking down the fruit into two parts. A weighted sample of the dry crushed seeds was repeatedly extracted with hot petroleum ether at 40 - 60°C. The solvent in the combined extracts was removed by rotatry evaporator to obtain the seed oil.

Physico-chemical analysis of seed oil

The chemical and physical properties of *X. americana* seed oil were analyzed according to AOAC (1990). Density was determined picnometrically according to AOAC. Refractive index was determined at 25°C with a Carl Zeiss Abbè refractometer. Viscosity was determined with Brookfield Rheometer, DV-III,PR57429,USA.

Methyl-14,14- dimethyl – 18-hydroxy heptatriacont-27,35-dienoate.

The seed oil extracted from *X. americana* seeds was subjected to chromatographic studies on TLC plates coated with silica gel. Chloroform :hexane 1:1 was used as a mobile phase and the major component was isolated.

Dimethyl - 5- Methyl – 28,29 dihydroxy dotriacont-3,14,26-triendioate

The dried yellow fruit pulps of *X. americana* were extracted with methanol in cold. The methanol extract was defatted with petroleum ether to remove the oil which may be extracted with the substance. This process was repeated until the colour of the sample changed from yellow to pale yellow semi solid. *X.americana* methanolic exract (2.4 g) was chromatographed over a column of 37.74 g silica gel. The sample was first eluted with 120 ml petroleum ether (60 - 80°C) followed by 120 ml aliquots of petroleum ether : chloroform in the ratio 50:50 then 100 ml aliquots of chloroform : ethyl acetate in the ratio 50:50, and finally 140 ml aliquots of ethyl acetate: methanol in the ratio 14:13 respectively. Fractions of 10 mls each were collected and the first 80 ml eluted from the column were discarded. Fractions containing similar TLC profiles were grouped together. The fractions isolated from *X. americana* pulp were subjected to chromatographic studies on TLC plates coated with silica gel. Chloroform :hexane :methanol 8:1:1 was used as a mobile phase.

RESULTS AND DISCUSSION

The physical and chemical properties of the oil of *X. americana* are shown in Tables 1and 2.

The oil recovered from 589.75 gm of seeds of *X.*

Table 2. *X. americana* seeds oil properties.

Colour	Yellow
Odour	Odourless
Taste	Tasteless liquid
State	Liquid
Solubility	Freely soluble in non polar solvents
Boiling point	157 C
Density	0.9376 g/ml
Viscosity 25 C	227.58
Viscosity 70 C	42.0
Refractive index	1.4770
Acid value	0.2805
Ester value	9.82
Iodine value	47.59
Peroxide value	30
Saponification value	11.43
Ratio value	35.009

americana is 302.77 gm which is equivalent to Yield 51.34% oil content on seed weight basis. The oil was yellow in colour, odourless and tasteless liquid. The viscosity data of *X. americana* seeds oil are given in Table 1. The viscosity of the oil and its temperature dependence in the range 25 - 70℃ suggested a potential industrial applica-tion of the oil as lubricating base stock. At 70℃, the reduction in viscosity of the oil was marked by over 80% of its value at 25℃. The viscosity value was found to be similar to those reported(Eromosele and Paschal, 2003). The value for the refractive index of *X. americana* oil was 1.4770. The observed value is higher than the values of some other seed oils (Abramovic and Abram, 2005). Acid value was used to determine the ability of the oil for resisting rancidity as the increase of acid value increases rancidity and *vice versa*. The acid value of *X. americana* seed oil laid in the range of arachis oil. The higher peroxide value beside the lower saponification and iodine values indicated that the *X. americana* seed oil is not edible oil. Values of 149.8 mg/100 g and 27.5 mequiv/kg for iodine and peroxide values, respectively were reported for *X. americana* seeds oil (Eromosele et al., 1994). However the reported titratable acidity of the *X.americana* fruit was more than 1 gm of malic acid in 100 g^{-1} F.W. (Mora et al., 2009).

The UV spectra of the major component of the oil which was extracted from *X. americana* seeds in chloroform showed an absorpation peak at (λ_{max}) 264 nm (ε = 3.146) and 270 nm (ε = 3.069). Simple ketones, acids, esters, amides and other compounds containing both π– systems and unshared electron pairs usually showed two absorption bands .One was a band of low intensity at wavelength above 300 nm due to π - π^* transition. The other was a band of high intensity at wavelength below 250 nm due to π - π^* transition.With conjugation, such as in case of α,β - unsaturated compounds (enones) the λ_{max} of $\pi - \pi^*$ band moved to a longer wavelength . This intense absorptivity may obscure the weaker band due to $n - \pi^*$.

The IR spectrum showed a broad absorption band extending from 3600 - 3200 cm^{-1} which clearly indicated the O- H stretching vibration (st.vib). The presence of the strong absorption at 1742 cm^{-1} can only give rise to C=O st.vib. of ester group .The C-O st.vib. appeared at 1161 cm^{-1}, and the C-H st .vib. of aliphatic saturated chains appeared at 2926, 2854 cm^{-1}. Bending vibration of these absorptions appeared at 1457 cm^{-1} at 1374 cm^{-1} which indicated CH_2 , CH_3 deformation and bending vibration , while C=C-H st.vib. appeared at 3100 cm^{-1} and C=C st.vib. at 1635 cm^{-1}. The mass spectrum (Figure 1) showed a molecular ion at m/e 604 corres-ponding to the molecular formula $C_{40}H_{76}O_3$. The base peak appeared at m/e 55 due to the allylic bond rupure (C_4H_7). Frag-mentation in C28 – C29 and C26 – C27 furnished ions at m/e 479 and 151 respectively. A peak appeared at m/e 73 is characteristic for the methyl ester and additional ions reinforced this entity appeared at m/e 31, 59 and 74 which was due to McLafferty rearrange-ment. The com-pound was given the name : methyl-14,14- dimethyl – 18-hydroxy heptatriacont -27,35-dienoate

$$CH_3OCO(CH_2)_{12}C(CH_3)_2(CH_2)_3CHOH(CH_2)_8CH{=}CH(CH_2)_6CH{=}CHCH_3$$

350 g of *X. americana* fruit pulps were used to yield 40 g of a semi - solid , dark brown methanol extract.

Using TLC (silica gel type G, methanol: acetone in ratio 2:3), a major compound, was isolated from methanolic extract of *X. americana* fruits pulp. However, different extracts by different solvents (ethanol, ethyl acetate, acetone, dichloromethane and chloroform) at room

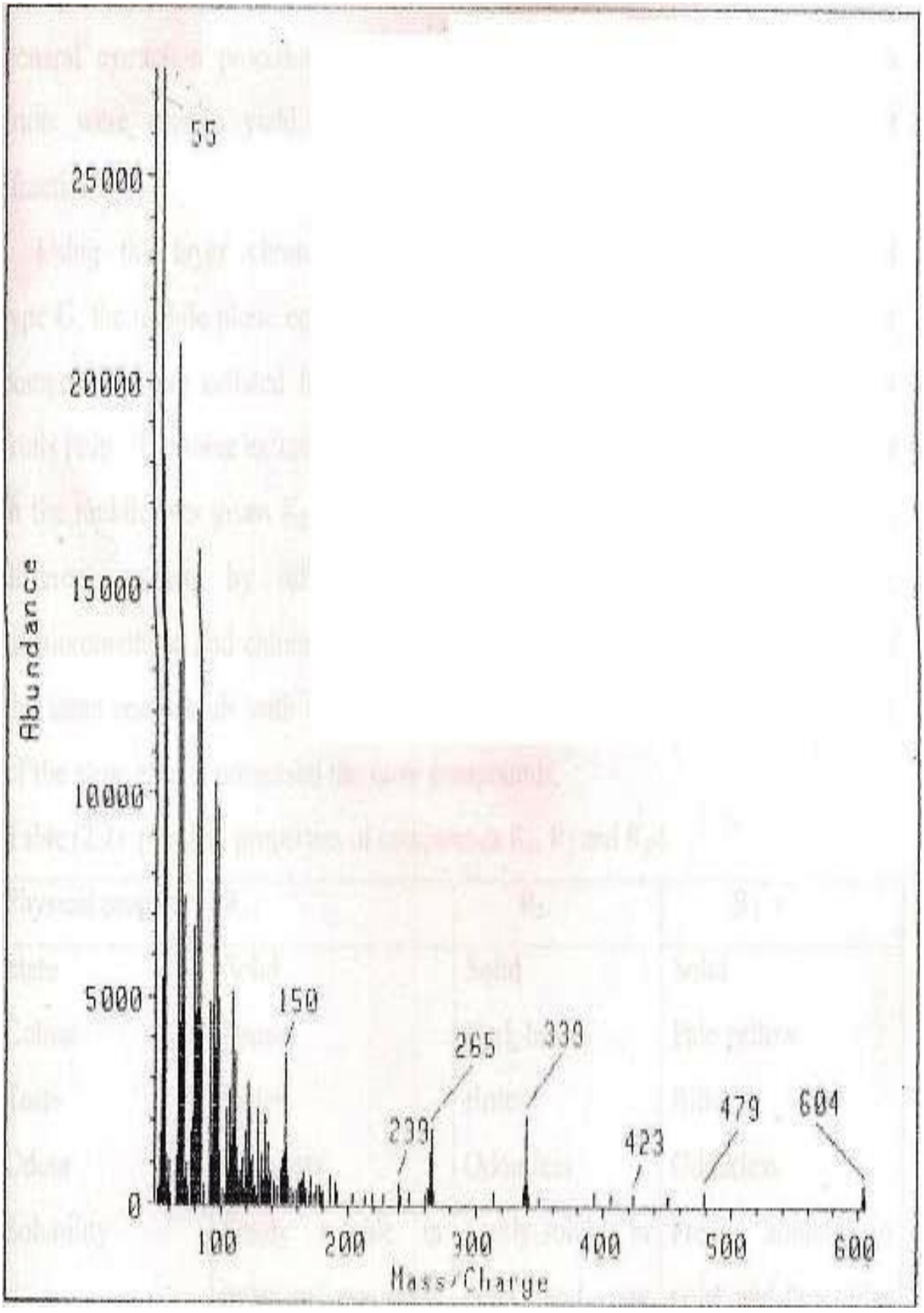

Figure 1. Mass spectra of methyl-14,14- dimethyl – 18-hydroxy heptatriacont-27,35-dienoate.

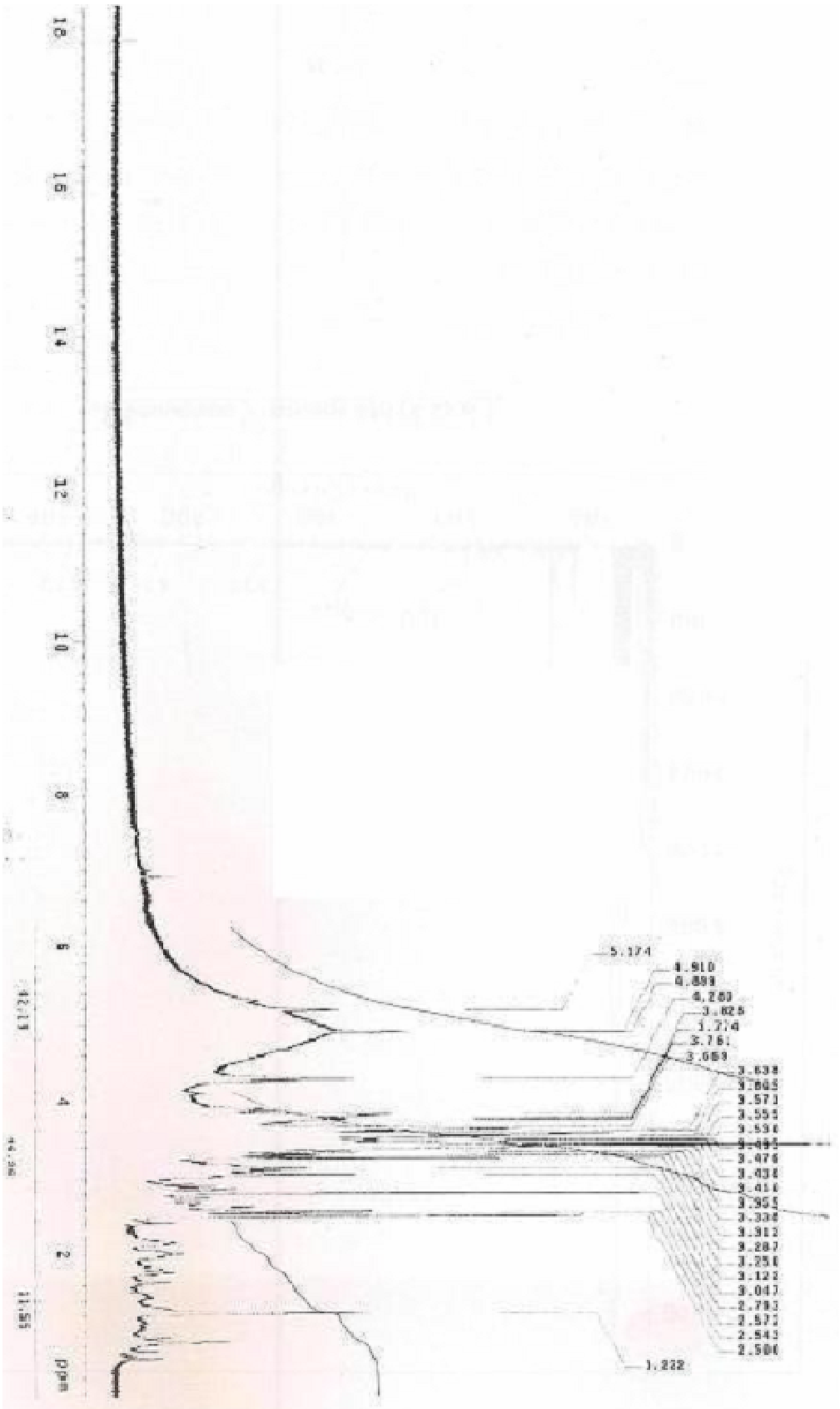

Figure 2. 1HNMR spectra of dimethyl - 5- Methyl - 28,29 dihydroxy dotriacont-3,14,26-triendioate ($DMSOd_6$).

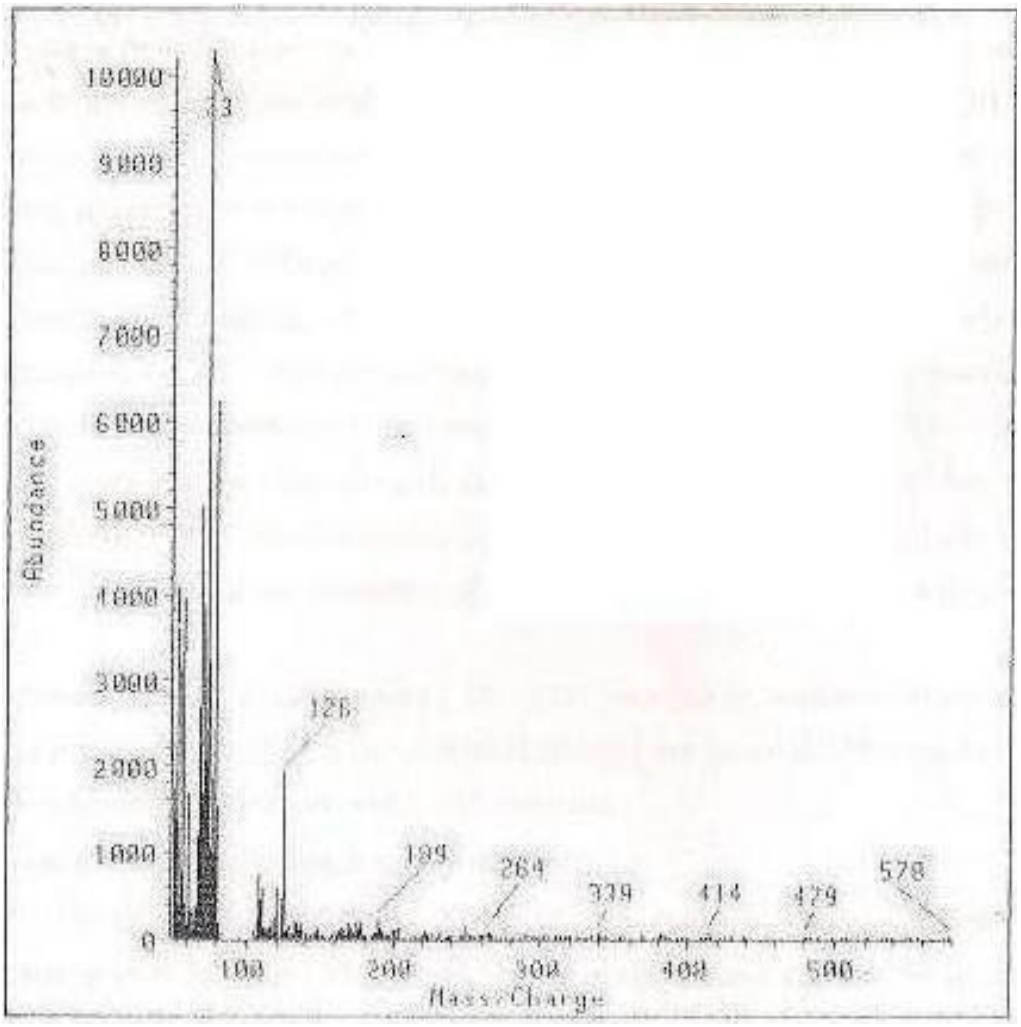

Figure 3. Mass spectra of dimethyl - 5- Methyl - 28,29 dihydroxy dotriacont-3,14,26-triendioate.

temperature resulted in isolation of the same major compound with variable intensities. The compound was solid, brown, odourless, bitter acid and had a melting point of 141 - 143°C.The IR spectra showed bands at 3500 cm^{-1} (O-H st . vib), 1731 cm^{-1} (C=O st. vib), 1235 cm^{-1} (C - O st . vib), 2993 (C-H st . vib . of aliphatic saturated chains), 1404 ,1380 cm^{-1} (CH_2 and CH_3 deformation and bending vibration) and (1645 C=C st.vib) which indicated an unsaturated ester.

The UV spectra in methanol showed an absorption peak at 210 nm (ε = 0.00886) and 280 nm (ε = 0.00212), as a forbidden transition was indicative of n - π* transition.The ^{1}H-NMR spectra in $DMSOd_6$ (Figure 2) showed a multiplet of overlapping peaks that appeared in the region of δ = 2.00 - 4.00 ppm which was highly suggestive of the nature and the richness of an aliphatic skele-ton in this compound. No peaks were seen above δ- value 6.50 ppm which gave evidence for the total absence of any aromatic ring. Further more, this conclusion can be extended to the presence of unsaturated aliphatic skeleton due to the appearance of a multiplet at δ5.64 - 5.83 ppm .

The mass spectra (Figure 3) showed a molecular ion at m/e 578 corresponding to the molecular formula $C_{35}H_{62}O_6$. The base peak appeared at m/e 73 ($C_3H_5O_2$) which is a characteristic for the methyl ester and additional ions reinforced this entity appeared at m/e 31, 59 and 74 which was due to McLafferty rearrangement. An ion at m/e 479 was due to [M - $C_5H_7O_2$] and an ion at m/e 339 is due to the cleavage C13 - C14. The ions that appeared at m/e 126 and 265 were due to ($C_7H_{10}O_2$) and [M – $C_{17}H_{28}O_2$] respectively. The compound was given the name dimethyl - 5- Methyl - 28,29 dihydroxy dotriacont-3,14,26-triendioate.

$CH_3OCOCH_2CH{=}CHCH(CH_3)(CH_2)_8CH{=}CH(CH_2)_{10}CH{=}CH(CHOH)_2(CH_2)_2COOCH_3$

The isolated compounds from the seeds or fruit pulp of *X. americana* were not reported before. Eromosele and Eromosele (2002) reported the isolation of other

unsaturated fatty acids from the seed oil of *X. americana*.

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