OCCURRENCE AND SOURCES OF PENTACYCLIC TRITERPENOL METHYL ETHERS AND ALKANONES AS MOLECULAR INDICATORS IN SUSPENDED PARTICULATE MATTER FROM GREAT KWA RIVER, SOUTHEAST NIGERIA

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ABSTRACT

Occurrence and source identification of Pentacyclic Triterpenol methyl ethers and alkanones as molecular indicators in suspended particulate matter obtained during high and low tides from Great kwa River, South East, Nigeria were characterized using gas chromatography- mass spectrometry (GC-MS) technique. Pentacyclic Triterpenol methyl ethers (PTMEs), taraxer-12-en-3-one, taraxer-14-en-3-one, taraxerone, taraxerol, miliacin, β-amyrin methyl ether, α-amyrin methyl ether, β-amyrenyl acetate, α-amyrenyl acetate and friedelin were characterized in the suspended particulate matter. Alkanones such as 6,10, 14-trimethyl pentadecan-2-one, pentacosan-2-one, heptacosan-2-one, octacosan-2-one, nonacosan-2-one, triacontan-2-one, hentriacosan-2-one, dotriacotan-2-one and tritriacotan-2-one were also found. These distinct compounds are useful for assessing diagenetic transformation that occurs during transportation of organic detritus. PTMEs are biomarkers of specific higher plants species, while alkanones are indication of direct microbial oxidation of the n-alkanes derived from epicuticular vascular plant wax. The presence of taraxerol and its ketonic counterpart taraxerone in the sample was an indication of dominant inputs from the study area vegetation and also reflect the oxidation and direct biological inputs. The accumulation of these compounds during low tide than high tide was due to sedimentation and oxidative process of the organic matter.

Key words: *PTMEs, alkanones, sources, occurrence, SPM, molecular indicators*

INTRODUCTION

The biochemical composition of Suspended particulate matter is influenced by the season, nutrient supply, the water outflow and the weather conditions. Large amount of organic detritus are introduced into the marine environment by river water through diagenetic, biogenic, pyrolytic as well as geochemical sources (Orok et al; 2012). Pentacyclic triterpenoids are generally used as biomarkers to trace genetic sources of organic matter in sedimentary environments, petroleum exploration or paleoenvironmental reconstructions of biome changes that is responsible for climatic changes (Simoneit, 2002, Jacob et al; 2005). Aliphatic straight chain lipids such as n-alkanones, n-alkanes and n-alkanols in the range C_{20} – C_{36} have been shown to indicate contributions from terrestrial higher plants to marine environment especially sediments (Simoneit, 1978; Cranwell, 1982). Compounds classes such as oleananes, ursanes, fernanes, lupanes and their derivatives are distributed as the oxygenated forms in many varieties of higher plant species, (Simoneit, 2008, Jacob, 2007, Mahato and Sen, 1997). Among the various compounds in epicuticular waxes of leaves and roots, the n-alkanes and n-alkanones usually exhibit odd-to-even (o/e) carbon chain length predominances, while the fatty acids and alcohols exhibit even-to-odd (e/o) predominances. Under aerobic conditions, the transformation of plant-derived triterpenoids often involves oxidation, dehydration, hydrolysis, decarboxylation, ring opening and aromatization reactions (Simoneit, 1998). Plant wax analyses without hydrolysis do reveal triterpenol esters as part of the wax esters in epicuticular waxes (Elias et al; 1999; Pereira et al; 2002). Xie et al; (2003) reported that n-alkan-2-ones originate from direct incorporation of epicuticular plant waxes, microbial β-oxidation of n-alkanes and microbial β-oxidation and decarboxylation of fatty acids. The PTMEs are natural products which are resistant to environmental alteration and are good biomarkers. This present paper aimed at (i) characterization and source identification of PTMEs and alkanones in suspended particulate matter (ii) to determine the influence of tidal variations on the accumulation and distribution of these lipids in suspended particulate matter from Great Kwa River.

STUDY AREA

The Great Kwa River (Fig. 1) has its source from Oban hills of South Eastern Nigeria. The river flows southwards through an estimated 56km of thick forest before discharging into the Cross River estuary at Calabar. It is located between latitude $4^{0}53^{1}$ and $4^{0}50^{1}$N and longitude $8^{0}20^{1}$ and $8^{0}30^{1}$E. Its shallow depth (1.0-5.5m at flood tide) is navigable throughout the year by small boats (Nna, 2014 and CRBDA, 1982). The climate of the Great Kwa River is that of the rain forest belt of southern Nigeria. Average evaporation and run-off are estimate at $1,521.3 \times 10^{6}m^{3}$ and $3,439.7 \times 10^{3}m^{3}$ respectively. Municipal surface runoff water from the city of Calabar reaches the Great Kwa River estuary after drainage through fresh water and mangrove swamps.

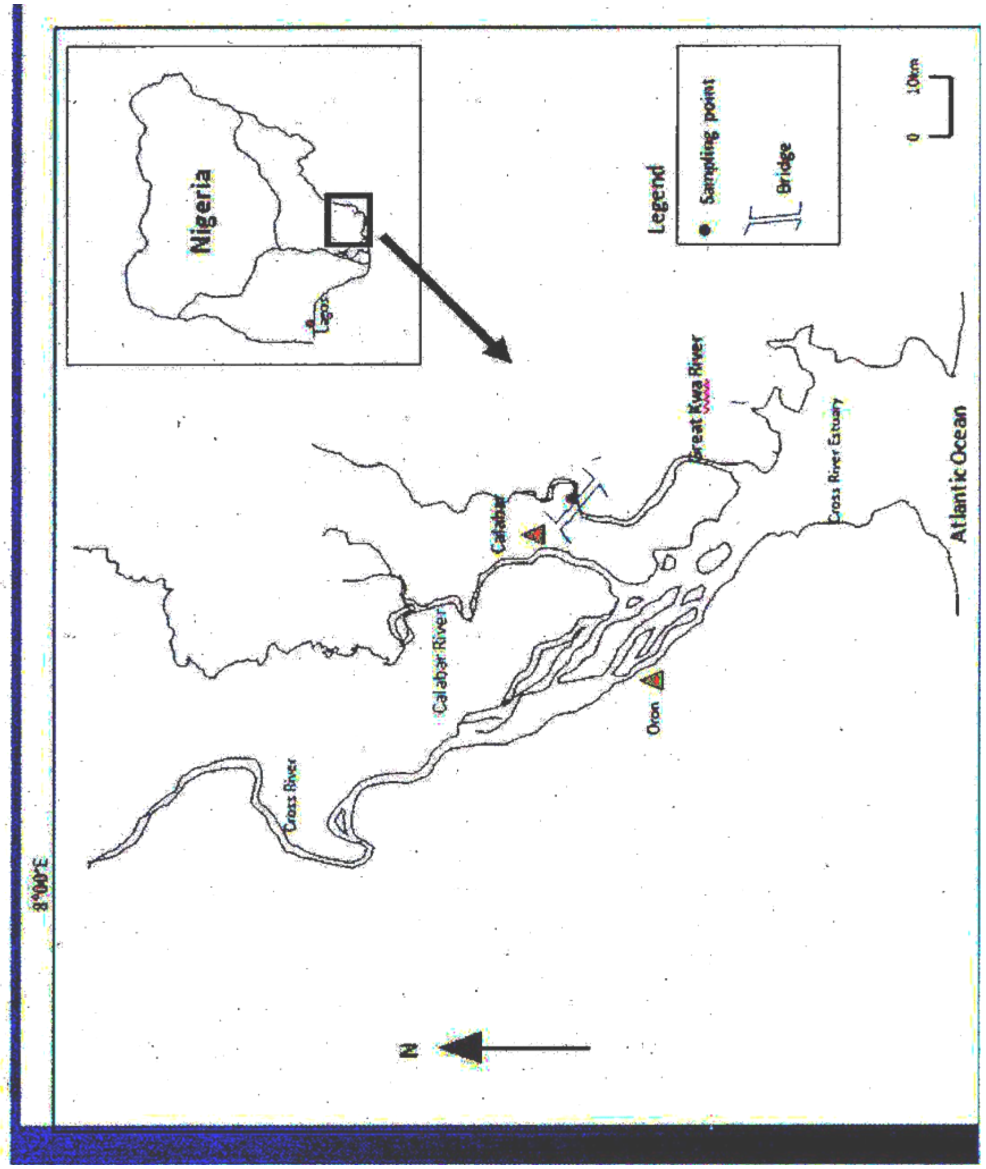

Figure 1. *Map of study area showing the sample point*

EXPERIMENTAL METHODS

Sample Collection and Preparation

Eight (8) surface water samples were collected at hourly intervals from a boat anchored at one location in the middle of the river (Fig.1) once a month from April – July (2011). The sampling periods for water covered both ebb and flood tidal phases between the hours of 8:30am and 4:15pm. This station was chosen so that homogeneous water samples could be obtained due to turbulent mixing. A 4 litre Nansen-type water sampler (made of plastic materials) was used after thorough rinsing with the water. Water samples were filtered under pressure (3 bar, purified N_2) through a thin polycarbonate membrane filter (Nuclepore) with a 0.4∞m pore size. Total suspended matter concentrations were determined gravimetrically. Samples of total suspended matter, collected on Nuclepore filters, were weighed on a Cahn model 4700 Electrobalance before and after filtration. Extraction of the SPM samples for SOM was carried out using a soxlet apparatus (EPA method 3540). The thimbles and the glass wool used in the extraction were soxhlet-extracted with dichloromethane for 20minutes on a water bath. 50g of SPM was placed in the soxhlet-extracted thimble with the glass wool and then filled with dichloromethane and extracted for 18hours. Extracts were desulphurised by addition of 30g activated copper (copper immersed in 20ml of 0.1M concentrated hydrochloric acid for 10mins) into the round-bottom flask during extraction. Extracts obtained were evaporated to near dryness using vacuum evaporator. The weights of extracts were determined as a measure of the amount of extractable or soluble organic matter (SOM) made up of asphaltenes and maltenes. Precipitation of asphaltenes from the SOM was carried out following the procedure described by Schoell et al (1983). 20ml of a mixture of dichloromethane/petroleum ether was added to 10ml of the extract and centrifuged at 3000rpm for about 20 minutes. The asphaltenes precipitated from SOM were discarded after weighing. The maltenes obtained from the extract were further separated by liquid chromatography (Column 30 x 1.2cm) into aliphatic, aromatic and hetero-fractions.

Instrumental Analysis

Gas chromatography –mass spectrometry (GC-MS) analyses of the isolated fractions were performed on a Hewlett-Packard Model 6890 GC coupled to a Hewlett-Packard Model 5973 quadrapole MSD. Separation was achieved on a DB5-Ms column (30m x 0.25mm i.d, 0.25mm film thickness). The GC operating conditions were as follows. Temperature holds at 65^0C for 2minutes, increases from 65 to 300^0c at a rate of 6^0Cmin^{-1}, and with final isothermal holds at 300^0c for 20minutes. Helium was used as carrier gas. The sample was injected in the splitless mode with the injector temperature at 300^0C. The mass spectrometer was operated in the electron impact mode at 70eV ionization energy and scanned from 50 to 650 Dalton. Data were acquired and processed using chemstation software. Compounds were identified by comparison with literature data and interpretation of mass spectrometric fragmentation patterns.

RESULTS AND DISCUSSION

The results are presented in Table 1. Pentacyclic triterpenol methyl ethers (PTMEs) monitored and identified in the samples from SPM at different tidal phases were taraxer-12-en-3-one, taraxer-14-en-3-one, taraxerone, tarexerol, miliacin, β_ Amyrin methyl ether, ∝-amyrenyl acetate and Friedelin. Representative iron chromatograms showing the occurrence of PTMEs in aromatic fraction of extracts from SPM is typified in Fig. 2 while Fig. 3, 4 and 5 shows the mass spectra of taraxerone, miliacin and taraxerol identified in the study area. The various concentrations of PTMEs identified and quantified in this study are presented in Table 1. SPM for samples collected at high and low tides. The total concentrations of PTMEs in SPM collected at low tide and high tides were 5.80mg/kg and 0.070mg/kg respectively. The results in the table show that the PTMEs were not detected in some high tide SPM samples (SH_1, SH_2, and SH_3).

The normal alkan-2-ones detected in SPM from the study are ranged from C25 – C32. Representative extracted ion chromatogram (m/z 59) showing the occurrence of normal alkanones in aromatic fraction of extracts from SPM at low tide in GRK is shown in Fig. 6. The mass spectrum of 6, 10, 14 trimethyl pentadecan-2-one (phytone) in aromatic fraction of extracts from SPM collected at low tide is shown in Fig. 7. The concentration of the n-alkanones identified and quantified in the SPM samples at both high and low tides are presented in Table 2.

Under aerobic conditions, the transformation of plant-derived triterpenoids often involves oxidation, dehydration, hydrolysis, decarboxylation, ring opening and aromatization reactions (Simoneit, 1998). For instance, in coal forming environments, higher plants triterpeniods generally undergo aromatizaton starting from ring A, triggered by the elimination of the oxygenated functionality at C-3 and proceeding to D/E (Sandison et al; 2003). The detection of these PTMEs in relatively few SPM samples may be linked to variation in environmental conditions such as the tidal changes which have been reported (Jacob et al; 2005) to determine the biosynthesis of these compounds in specialized tissues of certain species of higher plants. The ultimate sources of PTME in this river may be from monocotyledonous plants belonging to the Gramineae, on the basis of taxanomic identifications in the vicinity of the study area. The persistence of these PTMEs in these samples may reflect their relative stability to aerobic degradation. There is no evidence that these PTMEs have undergone diagenetic transformations to the 3α-PTMEs (Jacob et al; 2005) in the marine environment. Teraxerone detected in samples SL1 and SL3 is a product of aerobic oxidation of teraxerol mainly of mangrove origin (Oyo-Ita et al; 2010). The presence of the acetates in samples collected at low tide may indicate a close input source to that area since acetates such as the wax esters are susceptible to hydrolysis during river transportation. Therefore, their source may be traceable to litter and terrestrial detritus from grasslands and the deciduous forests. Taraxerol is found mostly in mangrove environments (Koch et al; 2011) especially in mangrove leaf wax (Simoneit et al, 2009). Its abundance in addition with its ketonic counterpart taraxerone indicates dominant inputs from the vegetation.

PTMEs are natural products and serve as good biomarkers useful for inferring sources of organic matter in environmental ecosystem. However, triterpenol esters may be useful for assessing early diagenesis (hydrolysis) of terrestrial higher plant detritus during river transport (Basyuni et al; 2007, Jaffe et al; 2006). The triterpenoids identified (taraxerol and amyrins) are of higher plant source and is indicative of mangrove-derived inputs (Koch et al; 2011).

The n-alkan-2-ones ranging from C17 to C35 have been reported in many sedimentary environments, including marine and lacustrine sediments (Wang et al; 2003). The Cmax at 25, 27 and 29 for high tide and 25 and 35 for low tide samples in the distribution pattern of the n-alkan-2-ones when compared to those of the n-alkanes showed that direct microbial oxidation of n-alkanes did not occur in the SPM samples from GRK at both tidal phases. This however, could be traceable to the direct incorporation from epicuticular plant waxes or vascular plant inputs. The slight significant variation in the total concentrations of alkan-2-ones in the samples at different tidal phases, suggesting the direct microbial oxidation of the n-alkanes identified as the primary source of alkan-2-one in the samples occur at high tide than low tide.

Table 1. *Concentration of PTMEs in SPM collected at High and Low Tide from* **GKR**

Samples Stations/Concentrations (mg/kg)

			High Tide					**Low Tide**				
Compound Name	**MW**	**MF**	**SH1**	**SH2**	**SH3**	**SH4**	**SD**	**SL1**	**SL2**	**SL3**	**SL4**	**SD**
Taraxer-12-en-3-one	424	$C_{30}H_{48}O$	Nd	Nd	nd	0.12	0.06	0.1	nd	0.47	nd	nd
Taraxer-14-en-3-one	424	$C_{30}H_{48}O$	Nd	Nd	nd	0.1	0.05	0.07	nd	0.43	nd	0.21
Taraxerone	424	$C_{30}H_{48}O$	Nd	Nd	nd	nd	nd	0.07	nd	0.61	nd	0.29
Taraxerol	426	$C_{30}H_{48}O$	Nd	Nd	nd	0.12	0.06	0.08	nd	0.19	nd	0.23
Miliacin	440	$C_{31}H_{52}O$	Nd	Nd	nd	0.12	0.06	0.06	nd	0.38	nd	0.18
β-Amyrin Methylether	440	$C_{31}H_{52}O$	Nd	Nd	nd	0.13	0.06	0.07	nd	0.53	nd	0.25
α –Amyrin methyl ether	440	$C_{31}H_{52}O$	Nd	Nd	nd	0.12	0.06	0.05	nd	0.64	nd	0.31
β – Amyrenyl acetate	468	$C_{32}H_{52}O$	Nd	Nd	nd	nd	nd	0.05	nd	0.59	nd	0.29
α- Amyrenyl acetate	468	$C_{32}H_{52}O$	Nd	Nd	nd	nd	nd	0.07	nd	0.44	nd	0.21
Friedelin	426	$C_{30}H_{52}O$	Nd	Nd	nd	nd	nd	0.09	nd	0.51	nd	0.24

nd= not detected

Table 2. *Concentration of n-alkanones in SPM collected at High and Low Tide from GKR*

Samples Stations (mg/kg)

			High Tide						**Low Tide**					
Compound Name	**MW**	**MF**	**SH1**	**SH2**	**SH3**	**SH4**	**Mean**	**SD**	**SL1**	**SL2**	**SL3**	**SL4**	**Mean**	**SD**
6,10,14,-trimethyl Pentadecan-2-one	268	$C_{18}H_{36}O$	Nd	nd	nd	nd	nd	nd	0.05	nd	nd	Nd	0.01	0.02
Pentacosan-2-one	366	$C_{25}H_{50}O$	0.14	0.34	0.21	nd	0.17	0.14	0.06	nd	0.43	0.34	0.21	0.21
Heptacosan-2-one	394	$C_{27}H_{54}O$	0.1	0.40	0.21	0.11	0.11	0.14	0.04	nd	0.21	0.31	0.14	0.14
Octacosa-2-one	408	$C_{28}H_{50}O$	0.09	0.18	0.17	nd	0.19	0.09	nd	nd	0.34	Nd	0.05	0.17
Nonacosan-2-one	422	$C_{29}H_{56}O$	Nd	0.29	0.29	0.18	0.22	0.14	0.06	nd	0.33	Nd	0.09	0.16
Triacontan-2-one	436	$C_{30}H_{60}O$	0.11	0.34	0.27	0.14	0.03	0.11	nd	nd	nd	Nd	nd	nd
Hentriacostan-2-one	450	$C_{31}H_{62}O$	nd	nd	nd	0.13	0.3	0.06	0.07	nd	nd	Nd	0.02	0.04
Dotriacontan-2-one	464	$C_{32}H_{64}O$	Nd	nd	nd	0.12	nd	0.06	0.07	nd	nd	Nd	0.02	0.04
Tritriacontan-2-one	478	$C_{33}H_{66}O$	Nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	

nd= not detected

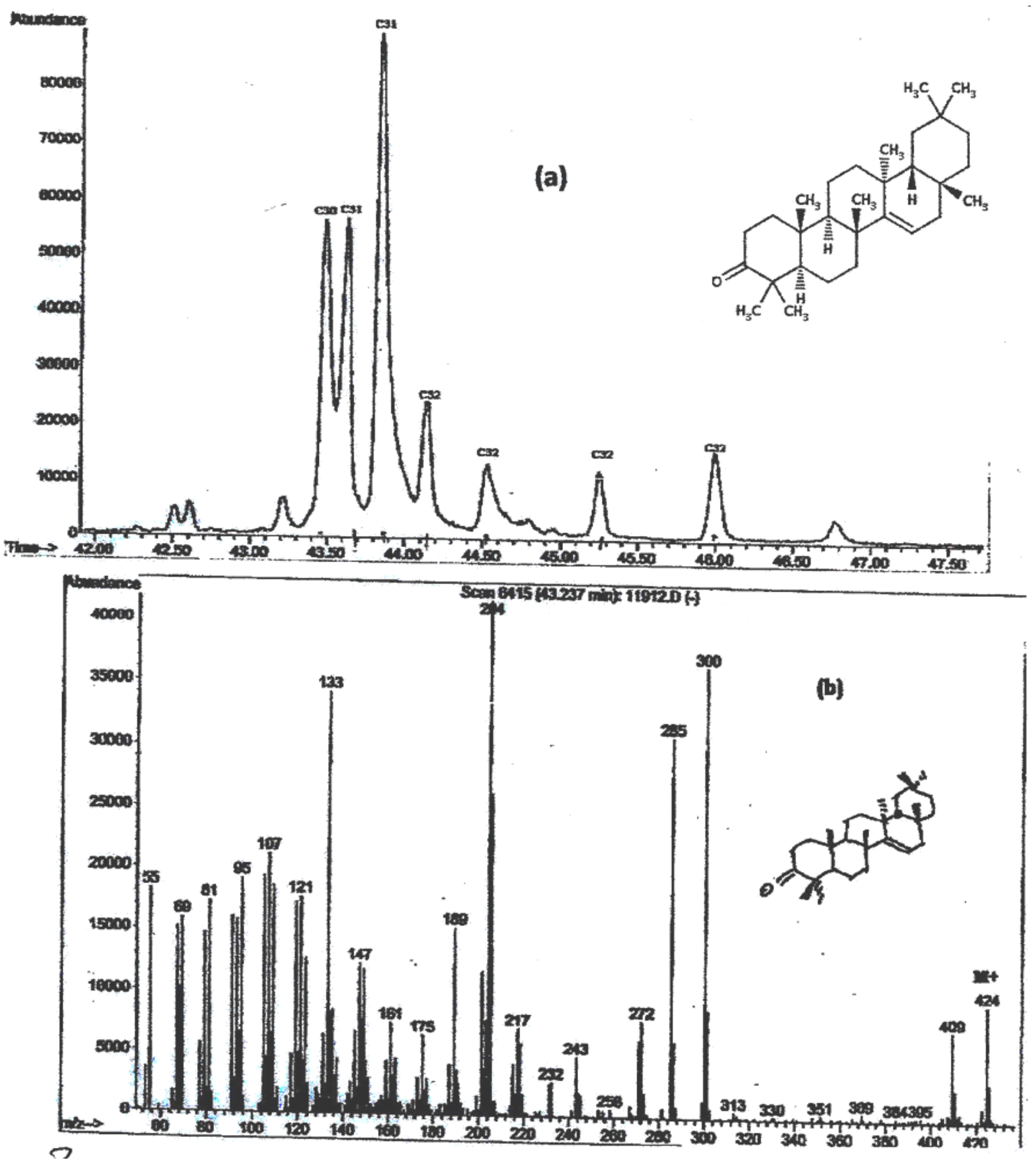

Figure 2. (a) *Representative gas chromatogram showing the occurrence of PTMEs and (b) Mass spectrum of taraxerone in SPM from Great Kwa River*

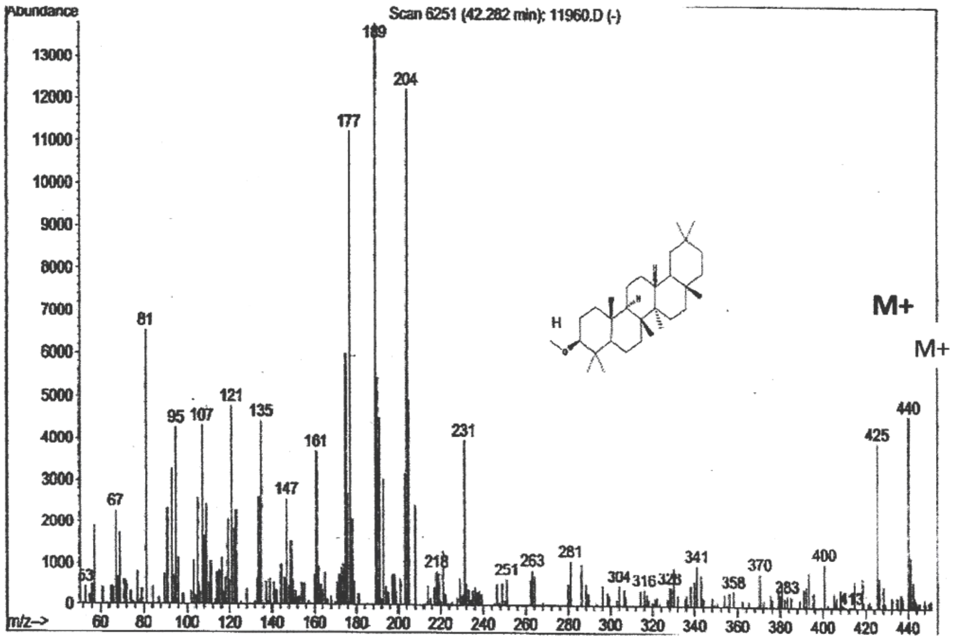

Figure 3. Representative mass spectrum of milliacin in SPM from Great Kwa River

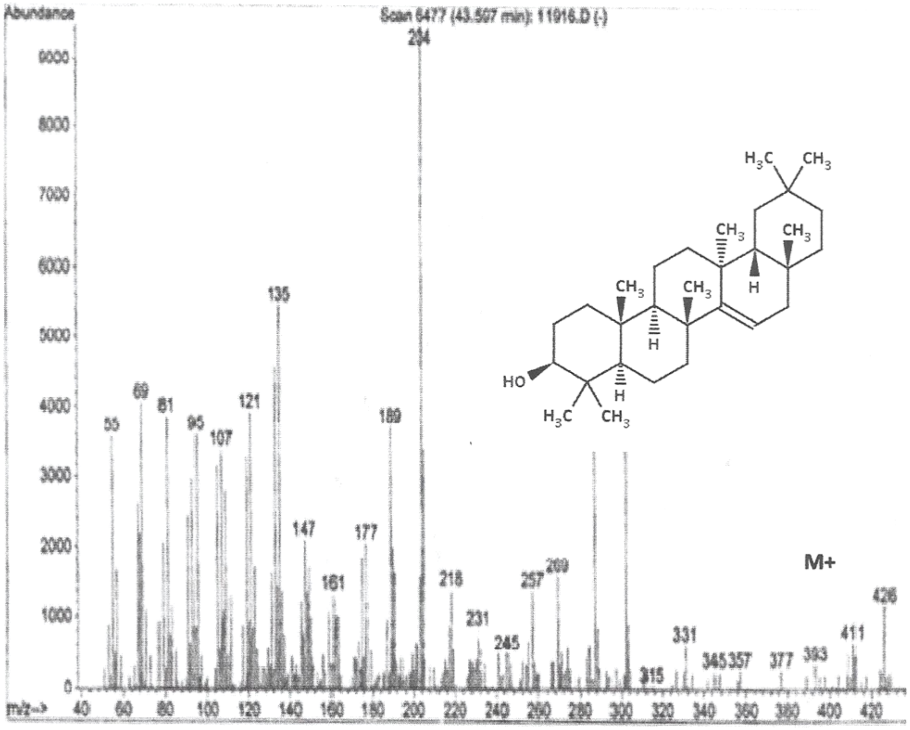

Figure 4. Mass spectrum of taraxerol in SPM from Great Kwa River

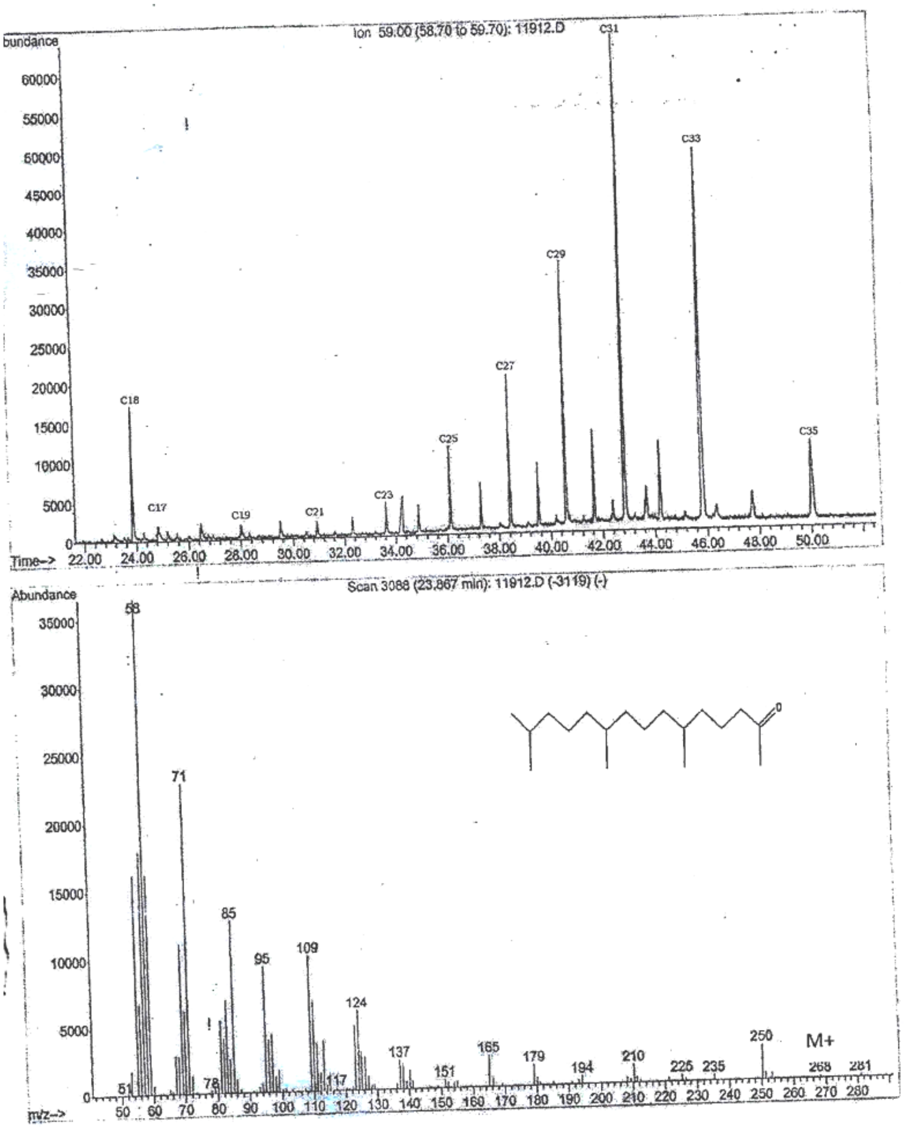

Figure 5. *Representative gas chromatogram showing the occurence of alkanones in aromatic fraction of SPM and mass spectrum of 6, 10, 14 – trimethylpentadecan-2-one from Great Kwa River*

CONCLUSION

The present study brought new insight on how pentacyclic triterpenol derivatives, as the methyl ethers (PTMEs) and alkanones were characterized in the suspended particulate matter of the Great Kwa River. The PTMEs that were characterized included taraxer-12-en-2-one, taraxer-14-en-2-one, taraxerone, taraxerol, miliacine, β_ amyrin methyl ether, ∝-amyrin methyl ether, β-amyrenyl acetate, ∝-amyrenyl acetate and friedelin, while the alkanones were mainly 6, 10, 14 – trimethyl pentadeean-2-one, pentacosan-2-one, heptacosan-2-one, octacosa-2-one, nonacosan-2-one, triacotan-2-one, hentriacostan-2-one and dotriacontan-2-one. These distinct biomarkers are readily extractable from SPM using solvent extraction techniques and are identifiable with GC-MS analysis by their characteristic mass spectrometric fragmentation pattern. PTMEs are biomarkers of specific higher plants subspecies, while the triterpenol esters are indicators of early diagenetic alteration of higher plants detritus. The presence of taraxerol and its ketone counterpart taraxerone in the sample is an indication of dominant inputs from the study area vegetation and also reflect the oxidation, preservation of the precursor in these river although direct biological inputs for these compounds are possible. The presence of n-alkan-2-ones compounds in the samples could be due to the direct microbial oxidation of the n-alkanes derived from epicuticular vascular plant wax. PTMEs accumulation was high during low tide than high tide. This could be traceable for organic matter sedimentation and oxidative process.

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