Comparative Study and GC/MS Analyses of Oleo-Gum Resins extracts obtained from *Boswellia papyrifera* (Tarak tarak)

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Abstract:

Three samples of oleogum resins of *Boswellia papyrifera* grown in some parts of the Sudan were subjected to screening with the objective of evaluating phytochemical constituents of their extracts (petroleum ether, and methanol) as well as their acid fractions. Gas chromatography/Mass spectrometry (GC/MS) analysis revealed that the main components in the petroleum ether extracts were sequentially naphtho [1, 2-b] furan-2-one (24.23%), oleic acid (22.39%) and octadecanoic acid (20.45%), whereas the principal chemical compounds in the methanolic extracts were lycopene (13.78%), oleic acid (28.60%) and benzoic acid (26.90%). The dominant molecule in the acid fractions of both Kordofan and Damazine samples was hexadecanoic acid (16.46% and 23.33%, respectively), while that from Nagawa olibanum was oleic acid (15.34%).

Keywords: *Boswellia papyrifera*; chemical composition; boswellic acid; resins.

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INTTRODUCTION

Boswellia spp of the family Burseraceae produce commercially important resins. The main African species are *B. papyrifera* (Del.) Hochst found in Sudan and Ethiopia, *B. frereana* Birdw and *B. sacra* Flückiger (syn. *B. carteri* Birdw.) occurring in Somalia (**Tucker, 1986 and Coppen, 1995**).

Oleo-gum-resin that exude from Boswellia trees composed of about 65–85% alcohol-soluble resins (diterpenes and triterpenes), 5–9% aromatic essential oil (mono and sesquiterpenes), and the remaining water-soluble gums are polysaccharides (**Tucker, 1986; Khan and Farooqi, 1991**). Triterpenes like the boswellic acids, diterpenes like incensole or isoincensole, their oxide or acetate derivatives can be considered as biomarkers of olibanum (**Hamm *et al*., 2003**).

Olibanum is used as incense and it is also used in traditional medicine in the treatment of asthma and cough, as a component of embalming fluid, a diuretic stimulant and an emmenagogue. Its essential oil and absolute oil are used as fixatives in soaps, perfumes, lotions, creams and detergents (**Abdel Wahab *et al*. 1987**). Gum resin of *B. carterii* has been used for thousands of years in traditional Chinese medicine to alleviate pain and inflammation. Scientific research showed that *B. carterii* has significant anti-arthritic and anti-inflammatory effects (**Fan *et al*., 2005**). The mechanism of this reaction was attributed to boswellic acids which are related to the immune system (**Ammon, 2006**). Boswellic acids, are important in the treatment of peritumoral oedema and chronic inflammatory diseases, thus they can be considered as alternative drugs to corticosteroids (**Weber *et al*., 2006**).

The major constituents isolated from the non-volatile fraction were cembrane and verticillane diterpenes and penta- or tetracyclic triterpenoids belonging to oleanane, ursane, lupane, dammarane and tirucallane groups (**Mathe *et al*., 2004 and Badria *et al*., 2003**). *B. papyrifera* also contains particular diterpenes: incensole, incensole acetate, incensole oxide and incensole oxide acetate. Linear carboxylic acids from pentanoic acid to lauric acid were also identified in *B. papyrifera* olibanum exclusively. Pentanoic acid and undecanoic acid were present at trace level (**Hamm *et al*., 2005**).

Büchele *et al*. (2003) identified lupeolic acid, a novel pentacyclic triterpene as a component of frankincense gum resins. *Boswellia carteri* and *B. sacra* were characterized by the presence of lupeolic acid, boswellic acids and their respective O-acetyl derivatives, whereas

3-epi-lupeol was the major compound in *B. frereana* methanolic extracts (**Mathe *et al.*, 2004**). Chemically, boswellic acids (BAs) are pentacyclic triterpenes that have been identified as the active principles of the gum resin of *Boswellia* species (**Safayhi *et al.*, 1997**). Boswellic acids have been reported to possess anti-tumor and anti-inflammatory activity, which may be due to inhibition of human leukocyte elastase (**Safayhi *et al.*, 1997**) and/or 5-lipoxygenase inhibitor activity (**Safayhi *et al.*, 1992**) and to topoisomerase inhibition (**Syrovets *et al.*, 2000**) leading to apoptosis-related tumor cell death (**Hoernlein *et al.*, 1999**). Recently, β-boswellic acid as a chemo-preventive and therapeutic agent in cancers has also been of interest (**Bhahwal *et al.*, 2009; Chevrier *et al.*, 2005**). Therefore it is important to study the acid fractions of resins of Sudanese origin to find out whether they contain boswellic acids or not.

MATERIALS AND METHODS

Material collection

Two authenticated samples of oleogum resins of *Boswellia papyrifera* were obtained from Elobied Agricultural Research Station (ARS), North Kordofan State (Kordofan sample) and Eldamazine Agricultural Research Station, Blue Nile State (Damazine sample). In addition to a commercial sample (Nagawa) kindly offered by an oleogum exporter in Khartoum. Samples were identified by experts from the Forestry Department, Ministry of Agriculture and Forestry as well as Agricultural Research Stations of Elobied and Eldamazine (Plate

1).

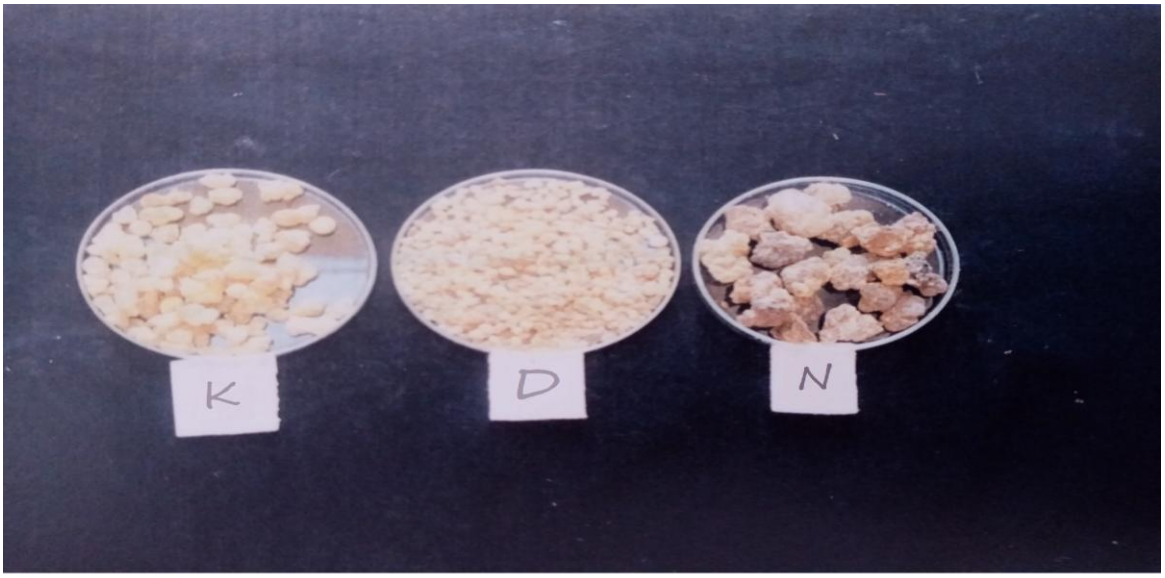


Plate 1 : Samples
K: Kordofan
D: Damazine
N: Nagawa

Preparation of extracts

Solvent extraction:

Solvent extracts were prepared according to Mothana et al. (2006). The air dried and powdered oleogum resin (50 g) was extracted under shaking at room temperature with petroleum ether (PE extract) and methanol (ME extract), for 12 hours at room temperature. The extracts were then filtered and dried using the rotary evaporator.

Isolation of the acid fractions

The acid fraction (AF) of the resin was isolated by 2% KOH extraction according to the method described by **Basar (2005).**

10g olibanum was extracted by shaking with 50 ml methanol for 12 hours. After filtration the extract was concentrated using rotary evaporator to nearly 30 ml until it becomes a thick solution. The concentrated solution was dissolved in 100 ml of 2% KOH aqueous solution and extracted five times with 30 ml ethylacetate. Every time the aqueous phase was separated from the organic phase (non acidic fraction, nAF) using separating funnel. The aqueous phase was then neutralized with 2% HCl to pH 6. The acid fraction was isolated from the aqueous phase by extraction five times with 30 ml ethylacetate. Every time the organic phase (acidic fraction) was collected separately. Finally the two fractions

were washed with distilled water, dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness.

Silylation

The silylation of the samples was achieved by the addition of 100 µl N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), Sigma Aldrich- USA, to 100 µg powdered sample. After the addition of the reagent, the sample was kept overnight at room temperature to react. The silylated samples were kept at –25°C, to avoid decomposition, for further investigations.

GC/MS conditions

GC/MS analyses were carried out using GC. MS-QP2010 (Shimadzu, Japan). Analytes were separated on a RTX-50 capillary column of 30 m × 0.25 mm with a phase thickness of 0.25 µm. The injector temperature was set to 280 °C and the temperature program was 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min. The carrier gas was helium with a column head pressure of 100 kPa. Splitless injection mode (1 min) was used. The temperatures of the transfer line and the source were 250 and 250 °C, respectively. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range *m/z* 50–680. Compounds were identified by use of the National Institute of Standards and Technology USA mass spectral library, release 98 (NIST98) as well as literature MS data and Kovatz retention indices.

Statistical Analysis

Data were statistically analyzed by the general linear model (GLM) procedure of SAS (SAS Institute, 2003). Duncan's multiple range test (Steel andTorrie, 1980) was used to separate the treatments means with significant difference. Standard error of means (SEM) was calculated by dividing standard deviation by the square root of the sample size.

RESULTS AND DISCUSSION:

Yield (%) of the extracts

As shown in Table 1, Nagawa sample yielded the highest PE (69.89%), which was significantly ($p \leq 0.05$) higher than that of Damazine sample (64.17%). However, frankincense from Kordofan gave 68.90% PE, which was comparable to that of both Nagawa and Damazine.

On the other hand, the three samples gave comparable quantities of methanolic extracts (54.30, 62.54 and 56.78% for Kordofan, Damazine and Nagawa respectively).

Nagawa oleogum resin exhibited high yield of AF (19.78%), which was analogous to that of Damazine (16.10%). Kordofan frankincense showed significantly ($p \leq 0.05$) lower value of 5.97%.

Formerly, Mothana *et al*. (2009) reported that *Boswellia dioscorides*, *Boswellia socotrana* and *Commiphora ornifolia* gave sequentially 40.45, 33.77 and 19.50% methanolic extracts.

Table (1): Yield (%) of extracts

Sample	PE	ME	AF
Kordofan	$68.90^{ab} \pm 0.569$	$54.30^{a} \pm 8.047$	$5.97^{b} \pm 2.446$
Damazine	$64.17^{b} \pm 4.569$	$62.54^{a} \pm 4.474$	$16.10^{a} \pm 2.143$
Nagawa	$69.89^{a} \pm 0.456$	$56.78^{a} \pm 0.795$	$19.78^{a} \pm 0.514$
SEM	1.54	3.09	1.10

PE: petroleum ether extract; ME: methanolic extract; AF: acid fraction;
SEM: standard error of the mean.

Each value is an average of three experimental samples.
Values are (means) ± standard deviation.
Means not sharing a common superscript small letter in a column are significantly different at ($P \leq 0.05$).

Extracts and Acid fractions

The major compound in Kordofan petroleum ether extract (KPE) was Nophtho [1, 2b] furan-2-one, 2, 3, 3a, 4, 5, 5a, 6, 7, 9a, ab-deca-hydro-3, 5a, 9-trimethyl-7, 9a-peroxy (24.23%). Ergost-25-ene-6, 12-dione, 3, 5-dihydro-, (3-β, 5-α.) and Hexadecanoic acid,

trimethylester were also detected in reasonably high concentrations (9.51% and 9.11%, respectively) as shown in Table 2. GC chromatogram of KPE is shown in Fig. 1. In addition, varying amounts of the following pentacyclic triterpenes were found; Ursa-9 (11), 12-dien-28-oic acid, 3-(-acetyl (0xy), methyl ester, (3-β), β -Amyrin methyl ester, β-Amyrenone, β-Amyrene, β-Amyrenol (0.31%) and Urs-11-en-28-oic acid, 2, 13-dihydroxy-3, 23-[(-methyl ethylidene) bis (oxy)]-, γ-lactone, (2-α-, 3-β,4-α). The main components in Kordofan methanol extract (KME) were Lycopene, 1, 1, 2, 2, -tetrahydro-1, 1-dimethoxy-, all-trans (13.78%) and Naphthalene, decahydro-1, 4-dimethoxy-, (1-α., 4.α. 4a.α., 8a.β.) (10.16%). GC chromatogram of KME is shown in Fig. 2. Also, chemical investigation showed that KME contain the pentacyclic triterpenes; β-Amyrine methyl ether (4.25%), β-Amyrene (3.15%), β-Amyrenol (2.84%), Urs-12-ene, 1, 2, 11-triol,3-(acetyloxy)-, (1.β., 2.α., 3.β., 11. α) (1.43%), Urs-11en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methyl ethylidene) bis (oxy)]-, γ-lactone, (2.α., 3. β., 4.α) (3.20%) and β-Amyrenone (3.61%). The results in Table 2 and Fig. 3, demonstrated acid fraction of Kordofan sample (KAF) composed mainly Hexadecanoic acid, trimethysilylester (16.46%) and Tricosane-1, 15-diol, bis (O-trimethylsilyly) (13.53%).

In addition, the following pentacyclic triterpenes were distinguished: β-Amyrene (7.07%), β-Amyrenol (4.64%), β amyrin methyl ether (3.86%), Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]-, γ-lactone, (2.α., 3. β., 4.α) (1.42%) and β-Amyrenone (8.40%),

The principal chemical compound of Damazine petroleum ether extract (DPE) was found to be Oleic acid, trimethylsilylester (22.39%) as shown in Table 2. Moderately higher concentrations of 3-Oxatricyclo [20, 8-O-O-(7, 16) triaconta-1(22), 7(16), 9, 13, 23, 29, hexaene (9.07%) and Hexadecanoic acid, trimethylsilylester (7.90%) were also documented. However, traces of Pentacyclic triterpenes, β-Amyrene (2.36%) as well as β-Amyrenone (2.31%) and Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]- , γ-lactone, (2.α., 3.β., 4.α) (0.18%) were presented in DPE. GC chromatogram of DPE is shown in Fig. 4.

In Table 2, the two dominant molecules in the methanol extract of Damazine sample (DME) were Oleic acid, trimethylsilylester (28.60%) and Hexadecanoic acid, trimethylsilylester (21.71%); along with little quantities of the following pentacyclic triterpenes; β- Amyrenol (6.87%), β-Amyrenone (4.32%), Lupeol acetate (0.41%) and

Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]-, γ-lactone, (2.α, 3.β, 4.α) (1.69%). GC chromatogram of DME is shown in Fig. 5.

The acid fraction of Damazine sample (DAF) was found to contain Hexadecanoic acid, trimethylsilylester as the chief component (23.33%). Evidently elevated concentrations of pentacyclic triterpenes β-Amyrenol (18.64%), and β-Amyrenone (12.16%) were observed (Table 2). Additionally, minor amounts of Olean-13 (18) ene (0.86%) and Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]-, γ-lactone, (2.α.,3. β., 4.α) (1.06%) were detected. GC chromatogram of DAF is shown in Fig. 6. As shown in Table 2, Nagawa petroleum ether extract (NPE) major compound was Octadecanoic acid, trimethylsilylester (20.45%), followed by Hexadecanoic acid, trimethylsilylester (12.41%). Further, the following pentacyclic triterpenes were identified: Lupeol (3.46%), Jasminol (2.61%), α-Amyrin (1.11%), β- Amyrene (1.03%), β-Amyrin methylether (0.51%), α-amyrenol (0.75%), β-Amyrenone (0.35%) and Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]-, γ-lactone, (2.α., 3. β., 4.α) (0.19%). GC chromatogram of NPE is shown in Fig. 7.

The methanolic extract of Nagawa sample (NME) was found to contain three major compounds, Benzoic acid, 2-[(trimethylsilyl) oxy]-, trimethylsilylester (26.90%), Hexadecanoic acid, trimethylsilylester (26.23%) and Octadecanoic acid, trimethlsilylester (22.93%). Beside that it contained trivial quantities of pentacyclic triterpenes such as Jasminol (1.28%), β-Amyrene (2.44%), β-Amyrenol (1.92%), α-Amyrin acetate (0.56%), Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]-, γ-lactone, (2.α., 3.β., 4.α) (0.39%) and Oleanane 3, 16.diol, 13, 28-epoxy, diacetate (3.β., 16.α) (0.23%) (Table 2 and Fig. 8).

The Nagawa acid fraction (NAF) was found to contain substantial amounts of Oleic acid, trimethylsilylester (15.34%) and Hexadecanoic acid trimethylsilylester (13.76%) as well as the following pentacyclic triterpenes; β Amyrenone methylether (12.16%), β-Amyrene (12.34%), Oleana-11, 13, 18-diene (7.92%) and Urs-11-en-28 oic acid, 2, 13-dihydroxy-3, 23-[(1-methylethylidene) bis (oxy)]- , γ-lactone, (2.α., 3. β., 4.α) (5.24%). GC chromatogram of NAF is shown in Fig. 9.

As displayed in Table 2, Hexadecanoic acid trimethylsilyl ester was found in petroleum extracts of Kordofan, Damazine, and Nagawa samples as well as in their methanolic extracts and acid fractions. The compound seemed to be considerably higher in NPE

and NME compared to NAF. Also, it was noticeably higher in DME and DAF than those of Kordofan sample, but it was slightly higher in KPE compared to that of Damazine sample.

Oleic acid trimethylsilyl ester was found in petroleum extracts of Kordofan and Damazine samples and in their methanol extracts (1.47% and 28.6 %, respectively). It is also noticed in the acid fractions of the three samples. The compound showed markedly higher concentrations in DPE and DME than that in the corresponding extracts of Kordofan sample. While it was not detected in the petroleum and methanolic extracts of Nagawa sample. However it was detected in higher amount in the acid fraction of Nagawa sample compared to that of Kordofan and Damazine samples (Table 2).

The β-Amyrenone was detected in the petroleum extracts of the three samples as well as the methanolic extracts and acid fractions (except for NME). The compound was present in higher quantity in the petroleum extract of Kordofan than that of Damazine and Nagawa. On the other hand it was higher in the methanol extract and acid fraction of Damazine sample compared to Kordofan sample. However, DAF and NAF showed identical β-Amyrenone levels. β-amyrene (olean -12-ene) was found in the petroleum extracts of kordofan, Damazine and Nagawa as well as in methanolic extracts and acid fractions of Kordofan and Nagawa samples, but it was not detected in methanol extract and acid fraction of Damazine sample. It is clear that β-amyrene was higher in methanol extract of Kordofan than that of Nagawa, while the opposite was true in the case of the acid fraction (Table 2). β-amyrin methyl ether (olean-12-ene, 3 methoxy, (3.β)) was detected in KPE, NPE, KME and KAF. It was not present in all methanolic extracts excluding that from Kordofan sample. Also, it was absent in the three extracts obtained from Damazine sample. β-amyrin methyl ether was higher in KPE than that for NPE. β-Amyrenol was found in considerable amounts in methanolic extracts of the three samples. But it was noticed in KPE in trace amount of 0.31%. It was also detected in acid fraction of Kordofan and that of Dmazene. It was markedly higher in the later than in the former one. For Nagawa sample the compound was only detected in the methanol extract. β-terpineol was found in KPE, KME and DME. The compound was not detected in acid fractions of all samples as well as DPE. It is clear from Table 4, that Nagawa sample was lacking this compound. Urs-11-en-28-oic acid, 2, 13-dihydroxy-3, 23-[(-methyl ethylidene) bis (oxy)]-, γ-lactone, (2-α-, 3-β,4-α) was observed in all examined extracts prepared from Kordofan, Damazine and Nagawa samples. The compound was slightly higher in petroleum and methanol extracts

of Kordofan sample than that of the related extracts of the other samples, although it was higher in the acid fraction of Nagawa sample compared to the fractions of the rest samples. Ursa-9 (11), 12-dien-28-oic acid, 3-(acetyloxy)-, methylester, (3.β) as well as 9, 12-Octadecadienoic acid, 18-(trimethylsiloxy),-methylester and Cholestone-3(2-methoxy ethoxy) (3.β., 5.α) were found only in KPE in percentage of 7.16%, 4.19% and 5.00%, respectively. The three compounds were absent in the other examined extracts, except for KME which contained 1.39% Ursa-9 (11), 12-dien-28-oic acid, 3-(acetyloxy)-, methylester, (3.β). Undecanoic acid-11-bromo-trimethyl ester was noticed in petroleum extract (3.11%) and acid fraction (4.84%) of Kordofan sample. On the other hand, petroleum extract and acid fraction of Damazine sample as well as the acid fraction of Nagawa contained Undecanoic acid-11-fluoro-trimethyl ester as 2.55%, 1.41% and 3.68%, respectively. Interestingly, methanol extract of Nagawa sample contained Undecanoic acid-11-iodo-trimethyl ester (1.93%). However, Undecanoic acid derivatives were not detected in the methanolic extracts of Kordofan and Damazine sample (Table 2). It was observed that petroleum extract of Kordofan sample contained 11-trans-Octadecenoic acid, trimethylsilyl (2.62%). Although this compound was absent in DPE and NPE. On the other hand methanol extract of Kordofan sample contained 11-cis-Octadecenoic acid, trimethylsilyl (1.32%), while DME and NME were free of this compound. The acid fractions of Kordofan, Damazine and Nagawa were found to contain nearly the same amount of 11-cis-Octadecenoic acid, trimethylsilyle (5.09%, 5.69% and 4.59%, respectively). Additionally, trans-9-Octadecenoic acid was found only in methanol extract (2.84%) and acid fraction (11.38%) of Kordofan, but it was absent in the extracts and acid fractions of the other samples. Octadecanoic acid, 12 (trimethylsiloxy)-, trimethylsilylester was found in petroleum extract of Damazine and Nagawa, whereas Octadecanoic acid, trimethylsilylester occurred in large amounts of 20.45% and 22.93% sequentially in petroleum ether and methanolic extracts of Nagawa. However, the above mentioned two compounds were not detected in the other tested extracts.

For Damazine sample, Octanoic acid, 7-oxo, trimethylsilylester was recognized in the petroleum and methanol extract as well as the acid fraction (1.58%, 0.83% and 2.71%, respectively), while for Nagawa sample, the compound was just found in both petroleum extract and acid fraction (3.31% and 6.17%, respectively). In the case of Kordofan sample, the same compound was detected only in the petroleum extract (0.57%). However, NAF showed the highest concentration of Octanoic acid, 7-oxo, trimethylsilylester (Table 2).

Ample quantity of Triconsane-1, 15, diol, bis (trimethylsilyl) (13.53%) was detected in acid fraction of Kordofan sample, while Damazine acid fraction contained much lesser amount of Oxirane, 2, 2-dimethyl-3-[3, 7-dimethyl-9-(phenylthio)-3,7. nonadienyl] (2.43%). On the other hand acid fraction of Nagawa sample showed traces of Lycopene, 1, 1, 2, 2, -tetrahydro-1, 1-dimethoxy-, all-trans (0.21%). The rest of the scanned extracts were missing the above mentioned compounds excluding KPE and NME (containing 2.40% and 0.60% Oxirane, respectively); and KME (having 13.78% Lycopene, 1, 1, 2, 2, -tetrahydro-1, 1-dimethoxy-, all-trans). In addition, the acid fractions of the three samples contained 0.23%, 0.42% and 0.44%, Stigmasta-4, 7, 22-trien-3-β.-ol, respectively. The later compound was not identified in the other extracts except KPE (0.24%). Cyclopentadecanone, oxime and Linoleic acid, trimethylsilylester were found in petroleum extracts of Damazine (5.78% and 2.92%, respectively) and Nagawa (7.32% and 3.31%, correspondingly). The two compounds were not detected in the other extracts except DME which contain 2.27% Cyclopentadecanone, oxime and NAF which had 2.21% Linoleic acid, trimethylsilylester (Table 2). Dodecanoic acid methyl ester was found only in petroleum extract and acid fraction of Kordofan sample (3.50% and 5.48%, respectively), while, petroleum extract and acid fraction of Damazine sample contained sequentially 3.98% and 1.16% Tetracosanoic acid, trimethylsilylester. In contrast, the other extracts were lacking these compounds.

Nophtho [1, 2b] furan-2-one, 2, 3, 3a, 4, 5, 5a, 6, 7, 9a, ab-deca-hydro-3, 5a, 9-trimethyl-7, 9a-peroxy (St.st. Peroxide), the major component of KPE, was trace in DPE (0.89%) and absent in NPE. It was also detected in DME (3.62%) and DAF (2.43%), but missing in the other two samples (Table 2).

Oleana-11, 13, 18-diene as well as 7-trimethylsilyloxy Octanoic acid, trimethylsilylester and Cholan-24-oic acid, 3, 7-bis [(trimethylsilyl) oxy] trimethylsilylester (3.α., 5.β., 7.α) were detected only in Nagawa acid fraction with values of 7.92%, 5.29% and 5.93%, respectively.

Crinan-3-one and Benzoic acid, 2-[(trimethylsilyl) oxy]-, trimethylsilylester were only found in the methanol extract of Nagawa sample (3.61% and 20.90%, respectively). In contrast, similar extract of Kordofan sample contained Cedran-diol, 8s, 13 as well as 1.2-Pentanediol, 5-(6-bromodecahydroxy-2, 5, 5a, 8a-tetramethyl-1-naphthalenyl)-3-methylene and Furan, 2-[(ethoxy-3, 4-dimethyl-2-cyclohexen-1-ylidene) methyl]. However, the other scanned extracts were free of these compounds. Furthermore, the methanol extracts of the

three samples contained 1.06%, 2.04% and 3.44%, Tetradecanoic acid, trimethylsilylester, correspondingly. The later compound was not detected in the rest materials except DAF (5.43%). Additionally, the methanol extracts of Damazine and Nagawa showed consecutively 2.35% and 1.21% 3, 5-Cylo-28, 33-dinorgorgostan-24-one, 6-methoxy-, (3.β, 5.α, 6.β), which was not detected in the other extracts except DPE (1.12%).

Verticiol and Naphthalene, decahydro-1, 4-dimethoxy-, (1.α, 4.α, 8a.β) were detected in petroleum extract of Damazine (1.05% and 4.25%, respectively) as well as the methanolic extract of Kordofan. The two compounds were not detected in the other extracts with the exception of DME which contained the later compound.

Small quantities of 3-Oxatricyclo [20.8.O.O] (7, 16)] triaconta-1 (22), 7, (16), 9, 13, 23, 29-hexaene (9.07%), 2-Propanoic acid-1-methylundecyl ester (5.78%), Cyclohexanol. 5-methyl-2 (1-methel ethyl), (1.α, 2.β, 5.β) (4.69%) and Iodquinol (4.25%) were detected only in the petroleum extract of Damazine sample (Table 2).

Oleo-gum-resin of *Boswellia serrata* composed of higher terpenoids (25-35 %), boswellic acid and several other triterpenoids include α-amyrins,11-keto-a-boswellic acid,

3’ hydroxyl urs-9,11-dien-24-oic acid, 3’-acetoxy urs-9, 11-dien-24-oic- acid (**Aman and Balu, 2009**).

Furthermore, **Büchele *et al*. (2003)** identified lupeolic acid, as a constituent of frankincense gum resins.

According to **Dekebo *et al*. (2002)** triterpenes from the resin of *Boswellia negelecta* were found to be canaric acid, α -amyrin, β -amyrone and *epi*-amyrin. Recently, α- and β-amyrin and their derivatives were found as constituents of *B. neglecta* and *B. rivae* while their derivatives were detected in *B. serrate*. (**Basar, 2005**).

Culioli *et al*. (2003) stated that a new lupane-type triterpene, 3-alpha-hydroxy-lup-20(29)-en-24-oic acid, was isolated from the methanolic extract of "Eritrean-type" resin of commercial frankincense together with 3-alpha-hydroxy-olean-12-en-24-oic acid (alpha-boswellic acid) and 3-alpha-hydroxy-urs-12-en-24-oic acid (beta-boswellic acid). Also, **Mathe *et al*. (2004)** reported that the resin of the Eritrean-type’’ olibanum contained alpha-boswellic acid, 3-O-acetyl-a-boswellic acid, beta-boswellic acid, 3-O-acetyl-b-boswellic acid, α-amyrin, β-amyrin, lupeol, 3-epi-α-amyrin, 3-epi-β-amyrin, 3epi-lupeol, α-amyrenone, β-amyrenone, lupenone, lupeolic acid and 3-O-acetyl-lupeolic acid). However, lupeol and *epi*-lupeol were found in the acid fraction of *B. frereana* (**Basar, 2005**).

Ursa-9(11), 12- dien-3-ol and oelana-9(11):12-dien-3-ol were isolated from *Protium Heptaphyllum* of the family Burseraceae (**Rüdigera *et al*. (2007**). α -myrin and β -amyrin, together with lupeol are constituents of the resin from *Protium species*, as well as their corresponding ketones α-amyrone, β-amyrone and lupenone (**Oliveira *et al*. (2004**) and **Vieira-Junior *et al*. (2005).**

Unexpectedly, in all three samples analyzed in this study α- and β-boswellic acids were not detected. However, **Basar (2005)** studied five *Boswellia* species. The investigations on their acid fractions revealed that *B. carterii* and *B. serrata* are the only species that have boswellic acids. On the contrary, α- and β-boswellic acids were detected at low concentrations in *B. rivae*, *B. frereana* and *B. neglecta*.

Table (2): Chemical composition (%) of extracts and acid fractions

Compound	Ext	K	D	N
Nophtho [1, 2b] furan-2-one, 2, 3, 3a, 4, 5, 5a, 6, 7, 9a, ab-deca-hydro-3, 5a, 9-trimethyl-7, 9a-peroxy	**PE**	24.23	0.89	-
	ME	-	3.62	-
	AF	-	2.43	-
Ergost-25-ene-6, 12-dione, 3, 5-dihydro-, (3-β, 5-α.)	**PE**	9.51	-	-
	ME	-	-	-
	AF	-	-	-
Hexadecanoic acid, trimethylsilylester	**PE**	9.11	7.90	12.41
	ME	4.86	21.71	26.23
	AF	16.46	23.33	13.76
B-Terpineol	**PE**	7.49	-	-
	ME	8.84	4.49	-
	AF	-	-	-
Ursa-9 (11), 12-dien-28-oic acid, 3-(-acetyl (0xy)-, methyl ester, (3-β)	**PE**	7.16	-	-
	ME	-	-	-
	AF	-	-	-
Oleic acid, trimethylsilyl ester	**PE**	6.38	22.39	-
	ME	1.47	28.60	-
	AF	10.70	14.52	15.34
Cholestone-3 (2-methoxyethoxy), (3-β, 5-α)	**PE**	5.00	-	-
	ME	-	-	-
	AF	-	-	-
β -Amyrin methyl ether	**PE**	4.47	-	0.51
	ME	4.25	-	-
	AF	3.86	-	-
β-Amyrenone	**PE**	4.24	2.31	0.35
	ME	3.61	4.32	-
	AF	8.40	12.16	12.16
β-Amyrene	**PE**	0.92	2,36	1.03
	ME	3.15	-	2.44
	AF	7.07	-	12.34
Urs-11-en-28-oic acid, 2, 13-dihydroxy-3, 23-[(-methyl ethylidene) bis (oxy)]-, γ-lactone, (2-α-, 3-β,4-α)	**PE**	2.98	0.18	0,19
	ME	3.20	1.69	0.39
	AF	1.42	1.06	5.24
Lycopene, 1, 1, 2, 2, -tetrahydro-1, 1-dimethoxy-, all-trans	**PE**	-	-	-
	ME	13.78	-	-
	AF	-	-	0.21
Naphthalene, decahydro-1, 4-dimethoxy-, (1-α., 4.α. 4a.α., 8a.β.)	**PE**	-	4.25	-
	ME	10.16	6.97	-
	AF	-	-	-
Cedran-diol, 8s, 13-	**PE**	-	-	-
	ME	7.14	-	-
	AF	-	-	-
Verticiol	**PE**	-	1.05	-
	ME	6,04	-	-
	AF	-	-	-
1, 2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2, 5, 5a, 8a-tetramethy-1-naphthalenyl)-3-methylene	**PE**	-	-	-
	ME	8.13	-	-
	AF	-	-	-
Furan, 2-[(ethoxy-3, 4-dimethyl-2-cyclohexen-1-ylidene) methyl]	**PE**	-	-	-
	ME	7.82	-	-
	AF	-	-	-
β-Amyrenol	**PE**	0.31	-	-
	ME	2.84	6.87	1.92
	AF	4.64	18.64	-

Table (2): Continue 1

Compound	Ext	K	D	N
Urs-12-ene, 1, 2, 11-triol,3-(acetyloxy)-, (1.β., 2.α., 3.β., 11. α)	**PE**	-	-	-
	ME	1.43	-	-
	AF	-	-	-
Tricosane-1, 15-diol, bis (O-trimethylsilyly)	**PE**	-	-	-
	ME	-	-	-
	AF	13,53	-	-
Trans-9-octadecenoic acid, trimethylsilylester	**PE**	-	-	-
	ME	2.84	-	-
	AF	11.38	-	-
3-Oxatricyclo [20, 8-O-O] (7, 16) triaconta-1(22), 7(16), 9, 13, 23, 29, hexaene	**PE**	-	9.07	-
	ME	-	-	-
	AF	-	-	-
2-Propanoic acid-1-methylundecyl ester	**PE**	-	5.78	-
	ME	-	-	-
	AF	-	-	-
Cyclopentadecanone, oxime,	**PE**	-	5.78	7.32
	ME	-	2.27	-
	AF	-	-	-
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.α., 2.β., 5.β)	**PE**	-	4.69	-
	ME	-	-	-
	AF	-	-	-
Octadecanoic acid, 12-(trimethylsiloxy)-trimethylsilylester	**PE**	-	4.35	5.28
	ME	-	-	-
	AF	-	-	-
Lupeol acetate	**PE**	-	-	-
	ME	-	0.41	-
	AF	-	-	-
11-Cis-Octadecenoic acid, trimetylsilylester	**PE**	-	-	-
	ME	1.32	-	-
	AF	5.09	5.69	4.59
Tetradecanoic acid trimethylsilyl ester	**PE**	-	-	-
	ME	1.06	2.04	3.44
	AF	-	5.34	-
Olean-13 (18) ene	**PE**	-	-	-
	ME	-	-	-
	AF	-	0.86	-
Octadecanoic acid, trimethylsilylester	**PE**	-	-	20.45
	ME	-	-	22.93
	AF	-	-	-
Cholestan-3-ol, 5-chloro-6-nitro-, (3.β, 5.α, 6.β)	**PE**	-	-	6.18
	ME	-	-	-
	AF	-	-	-
Lupeol	**PE**	-	-	3.46
	ME	-	-	-
	AF	-	-	-
Jasminol	**PE**	-	-	2.61
	ME	-	-	1.28
	AF	-	-	-
α-Amyrin	**PE**	-	-	1.11
	ME	-	-	-
	AF	-	-	-
α-amyrenol	**PE**	-	-	0.75
	ME	-	-	-
	AF	-	-	-

Table (2): Continue 2

Compound	Ext	K	D	N
6a, 14a-methano-picen, perhydro-, 1, 2, 4a, 6b, 9, 9, 12a-heptamethyl-10-hdroxy	**PE**	-	-	2.68
	ME	-	-	-
	AF	-	-	-
Benzoic acid, 2-[(trimethylsilyl) oxy]-,trimethylsilylester	**PE**	-	-	-
	ME	-	-	26.90
	AF	-	-	-
α-Amyrin acetate	**PE**	-	-	-
	ME	-	-	0.56
	AF	-	-	-
Oleanane 3, 16.diol, 13, 28-epoxy, diacetate (3.β., 16.α)	**PE**	-	-	-
	ME	-	-	0.23
	AF	-	-	-
Oleana-11, 13, 18-diene	**PE**	-	-	-
	ME	-	-	-
	AF	-	-	7.92
Ursa-9 (11), 12-dien-28-oic acid, 3-(acetyloxy)-, methyl ester, (3.β)	**PE**	7.16	-	-
	ME	1.39	-	-
	AF	-	-	-
9, 12-Octadecadienoic acid, 18-(trimethylsiloxy),-methylester	**PE**	4.19	-	-
	ME	-	-	-
	AF	-	-	-
Undecanoic acid-11-bromo-trimethyl ester	**PE**	3.11	-	-
	ME	-	-	-
	AF	4.84	-	-
Undecanoic acid-11-flouro-trimethyl ester	**PE**	-	2.55	-
	ME	-	-	-
	AF	-	1.41	3.68
Undecanoic acid-11-iodo-trimethyl ester	**PE**	-	-	-
	ME	-	-	1.93
	AF	-	-	-
11-trans-Octadecenoic acid, trimethylsilyl	**PE**	2.62	-	-
	ME	-	-	-
	AF	-	-	-
Octanoic acid, 7-oxo, trimethylsilylester	**PE**	0.57	1.58	3.31
	ME	-	0.83	-
	AF	-	2.71	0.17
Oxirane, 2, 2-dimethyl-3-[3, 7-dimethyl-9-(phenylthio)-3, 7. nonadienyl]	**PE**	2.04	-	-
	ME	-	-	0.60
	AF	-	2.43	-
Stigmasta-4, 7, 22-trien-3-β.-ol	**PE**	0.24	-	-
	ME	-	-	-
	AF	0.23	0.42	0.44
Linoleic acid, trimethylsilylester	**PE**	-	2.92	3.31
	ME	-	-	-
	AF	-	-	2.21
Dodecanoic acid methyl ester	**PE**	3.50	-	-
	ME	-	-	-
	AF	5.48	-	-
Tetracosanoic acid, trimethylsilylester	**PE**	-	3.98	-
	ME	-	-	-
	AF	-	1.16	-
7-trimethylsilyloxy Octanoicacid, trimethylsilyl ester	**PE**	-	-	-
	ME	-	-	-
	AF	-	-	5.29

Table (2): Continue 3

Compound	Ext	K	D	N
Cholan-24-oic acid, 3, 7-bis [(trimethylsilyl) oxy] trimethylsilylester (3.α., 5.β., 7.α)	**PE**	-	-	-
	ME	-	-	-
	AF	-	-	5.93
Crinan-3-one	**PE**	-	-	-
	ME	-	-	3.61
	AF	-	-	-
3, 5-Cylo-28, 33-dinorgorgostan-24-one, 6-methoxy-, (3.β, 5.α, 6.β)	**PE**	-	1.12	-
	ME	-	2.35	1.21
	AF	-	-	-
Iodquinol	**PE**	-	4.25	-
	ME	-	-	-
	AF	-	-	-

K: Kordofan D: Damazine N: Nagawa
PE: petroleum extract ME: Methanol extract AF: Acid fraction

Conclusion:

The major compound in Kordofan petroleum ether extract was Naphtho [1, 2-b] furan-2-one, 2, 3, 3a, 4, 5, 5a, 6, 7, 9a, 9b-decahydro-3, 5a, 9-trimethyl-7, 9a-peroxy- (24.23%). Ergost-25-ene-6, 12-dione, 3, 5-dihydro-, (3-β, 5-α.) and Hexadecanoic acid, trimethylester were also detected in reasonably high concentrations (9.51% and 9.11%, respectively). Whereas the main components of the methanolic extract were Lycopene, 1, 1, 2, 2, -tetrahydro-1, 1-methoxy-, all-trans (13.78%) and Naphthalene, decahydro-1, 4-dimethoxy-, (1-α., 4.α. 4a.α., 8a.β.) (10.16%). However, the acid fraction composed mainly of Hexadecanoic acid, trimethysilylester (16.46%) and Tricosane-1, 15-diol, bis (O-trimethylsilyly) (13.53%).

The principal chemical compound of Damazine petroleum ether extract was Oleic acid, trimethylsilylester (22.39%). Moderately higher concentrations of 3-Oxatricyclo [20, 8-O-O-(7, 16) triaconta-1(22), 7(16), 9, 13, 23, 29, hexaene (9.07%) and Hexadecanoic acid, trimethylsilylester (7.90%) were also detected. On the other hand, the two dominant molecules in the methanolic extract were Oleic acid, trimethylsilylester (28.60%) and Hexadecanoic acid, trimethylsilylester (21.71%). While the acid fraction was found to contain Hexadecanoic acid, trimethylsilylester (23.33%) and β-Amyrenol (18.64%) as the chief components.

Nagawa petroleum ether extract major compound was Octadecanoic acid, trimethylsilylester (20.45%), followed by Hexadecanoic acid, trimethylsilylester (12.41%). The methanolic extract contained three major compounds,

Benzoic acid, 2-[(trimethylsilyl) oxy]-,trimethylsilylester (26.90%), Hexadecanoic acid, trimethylsilylester (26.23%) and Octadecanoic acid, trimethlsilylester (22.93%). The acid fraction was found to contain substantial amounts of Oleic acid, trimethylsilylester (15.34%), Hexadecanoic acid trimethylsilylester (13.76%), β Amyrenone methylether (12.16%) and β-Amyrene (12.34%).

In accordance with the previous studies, the pentacyclic triterpenes Lupeol and Lupeol acetate were identified in Nagawa and Damazine samples, respectively. Unexpectedly, in all three samples analyzed in this study α- and β-boswellic acids were not detected. However, Sudanese olibanum was characterized by the presence of the following pentacyclic triterpenes: Urs-11-en-28-oic acid, 2, 13-dihydroxy-3, 23-[(-methyl ethylidene) bis (oxy)]-, γ-lactone, (2-α-, 3-β, 4-α) which was detected in all materials prepared from the three samples; Ursa-9 (11), 12-dien-28-oic acid, 3-(acetyloxy)-, methylester, (3.β) and Urs-12-ene, 1, 2, 11-triol,3-(acetyloxy)-, (1.β., 2.α., 3.β., 11. α) which were found in Kordofan sample; Olean-13 (18) ene (in Damazine sample); Oleana-11, 13, 18-diene and Lup-20 (29)-en-28-ol (Jasminol) which were existed in Nagawa sample.

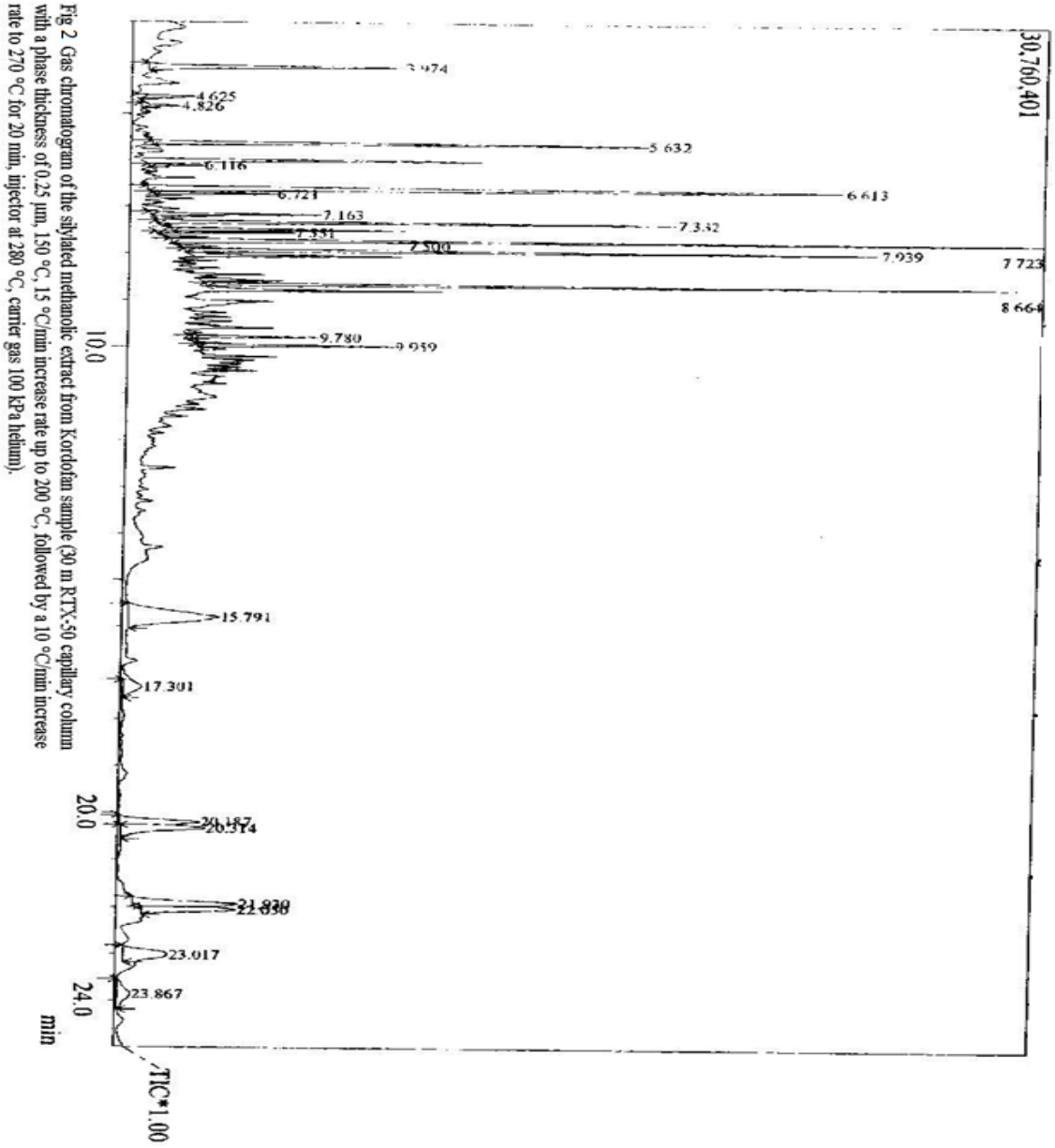


Fig 2 Gas chromatogram of the silylated methanolic extract from Kordofan sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

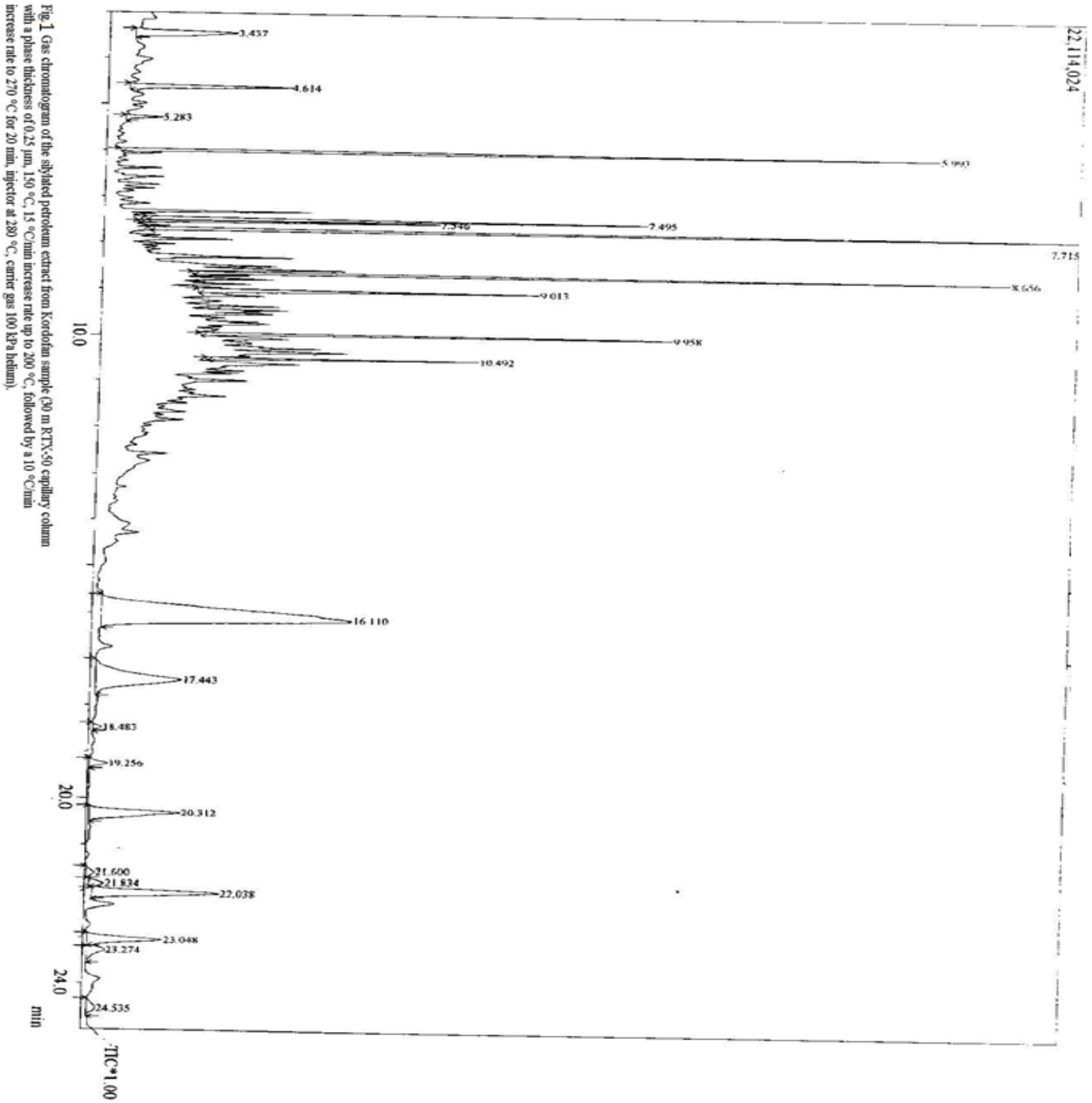


Fig. 1 Gas chromatogram of the silylated petroleum extract from Kordofan sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

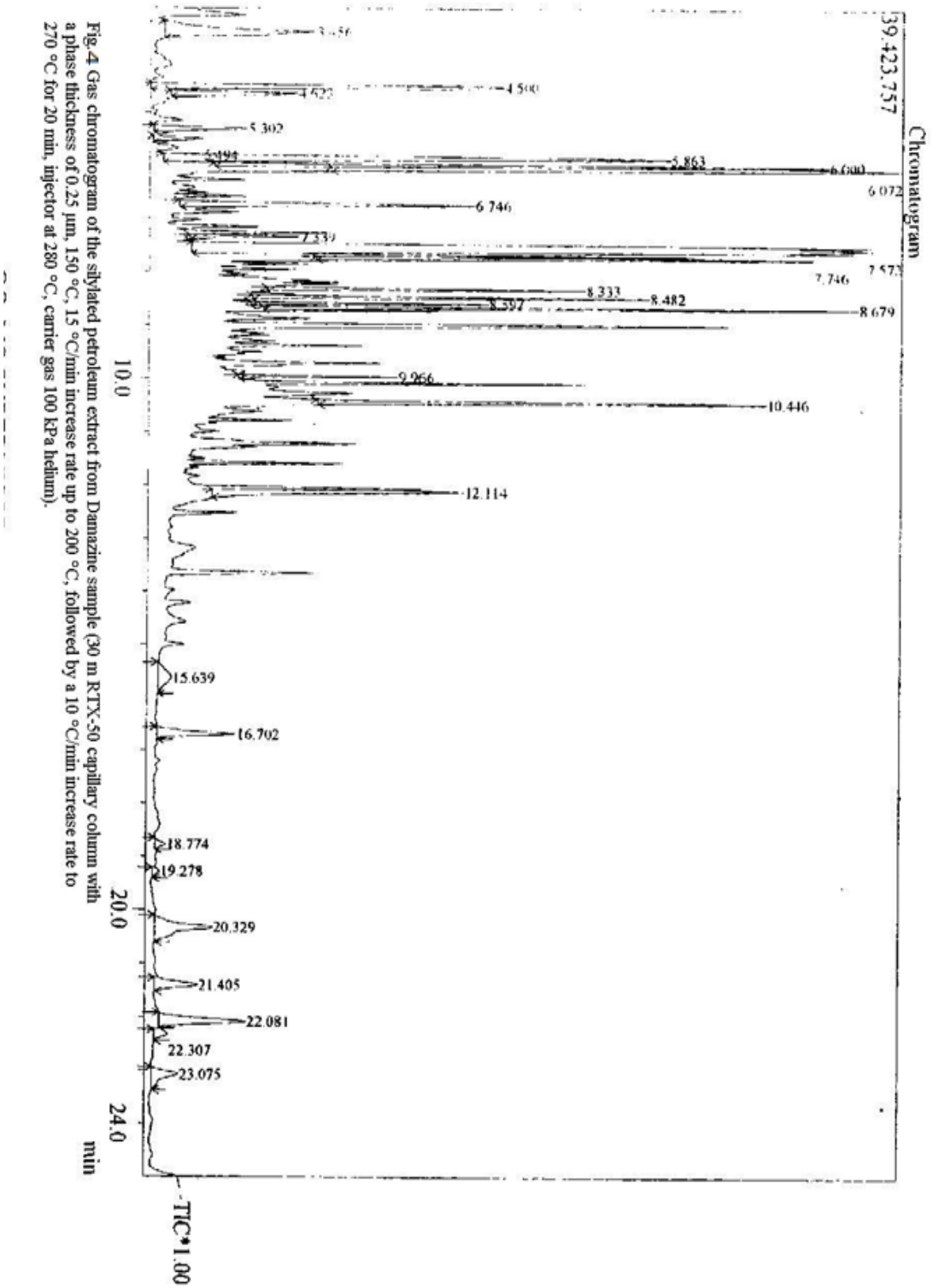


Fig.4 Gas chromatogram of the silylated petroleum extract from Damazine sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

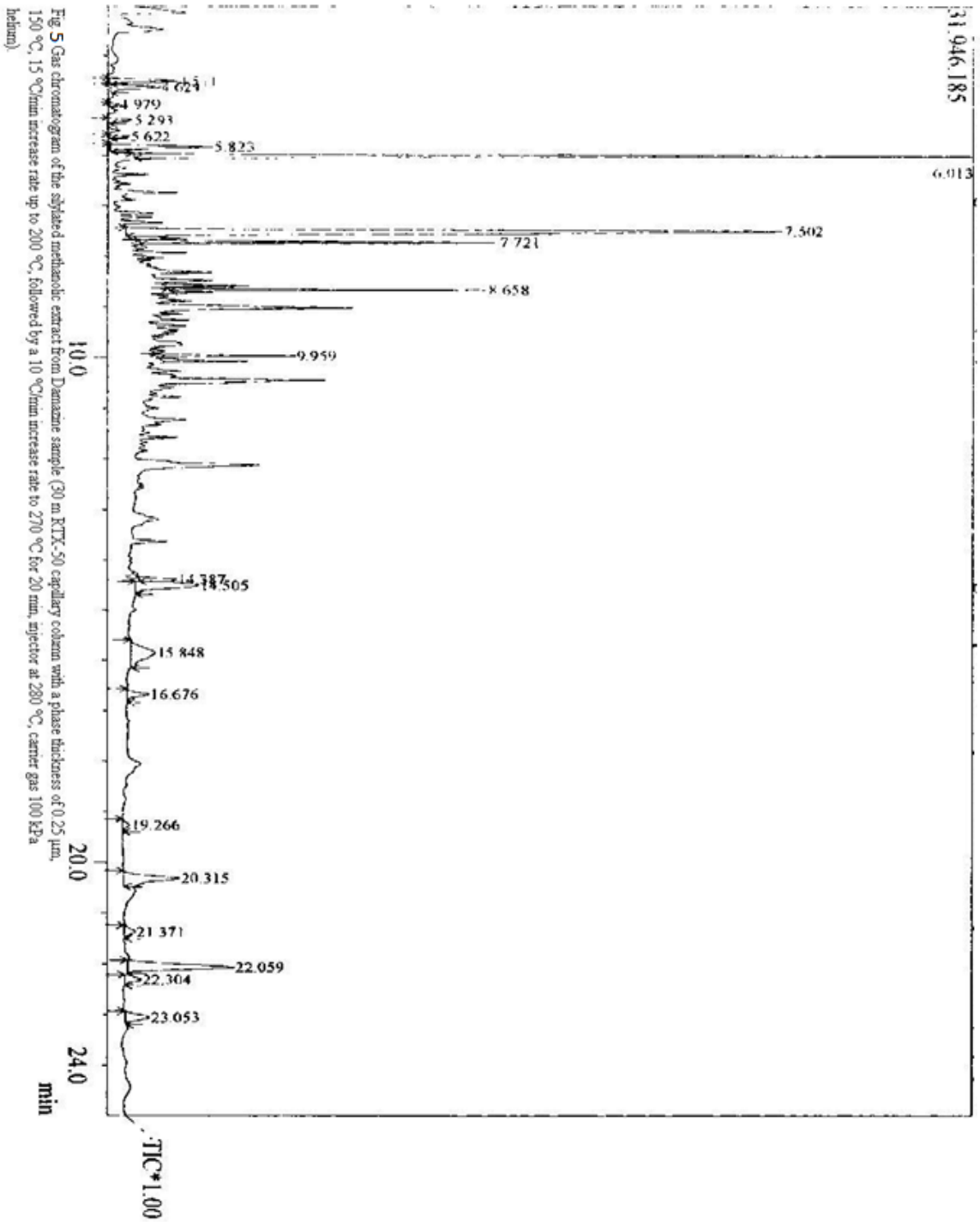


Fig. 5 Gas chromatogram of the silylated methanolic extract from Damazane sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

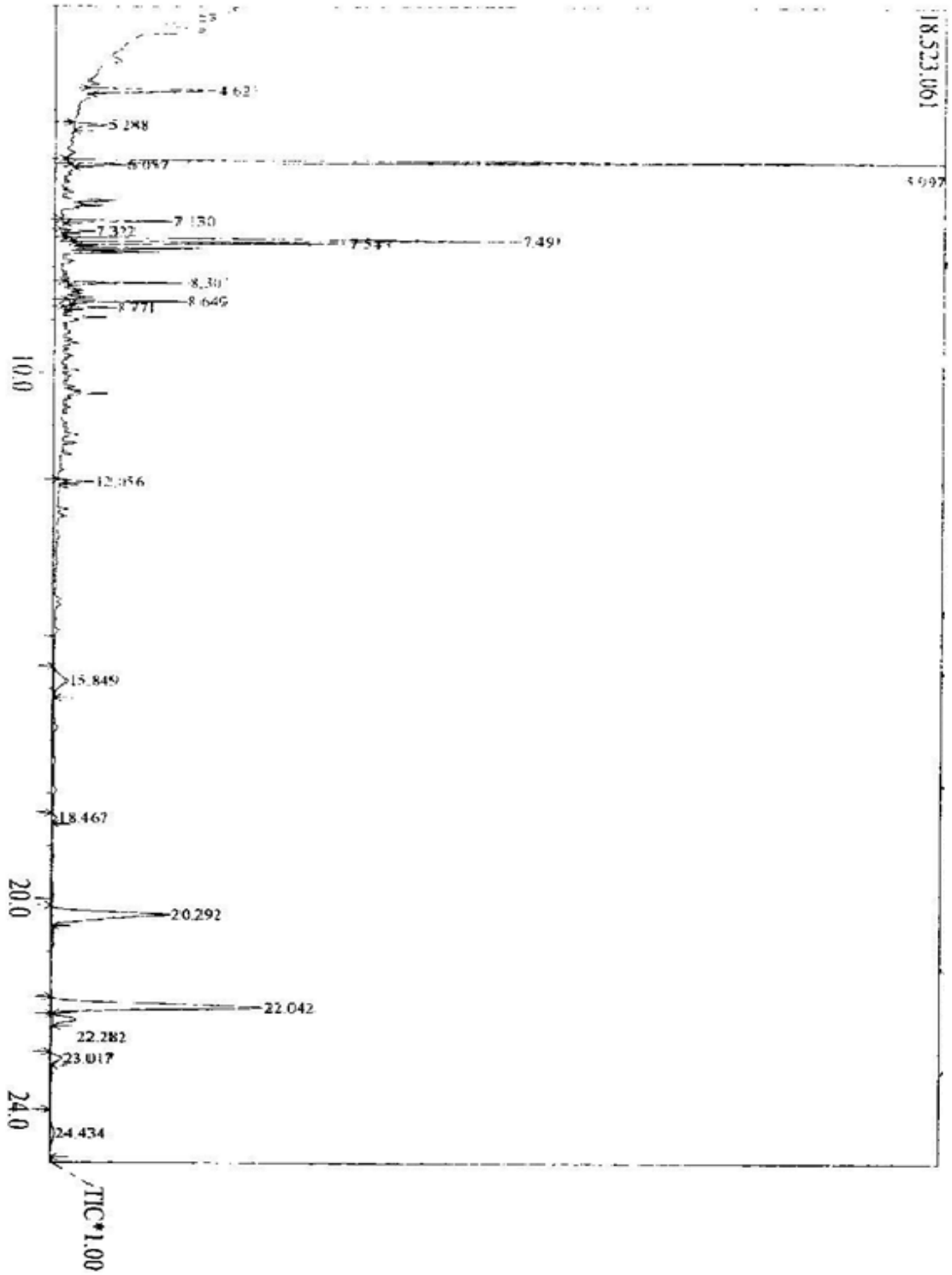
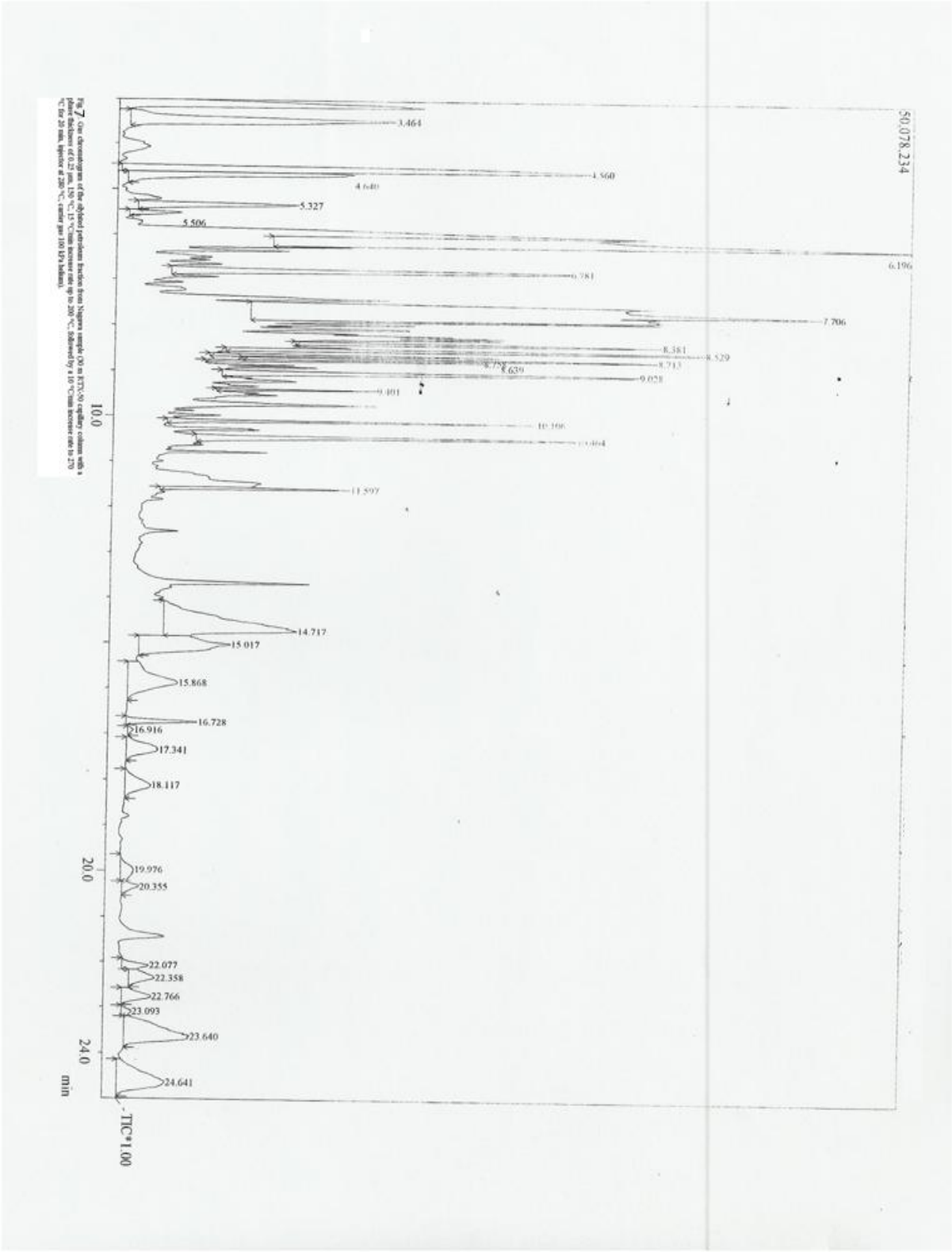


Fig.6 Gas chromatogram of the silylated acid fraction determined by 2% KOH from Damazine sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).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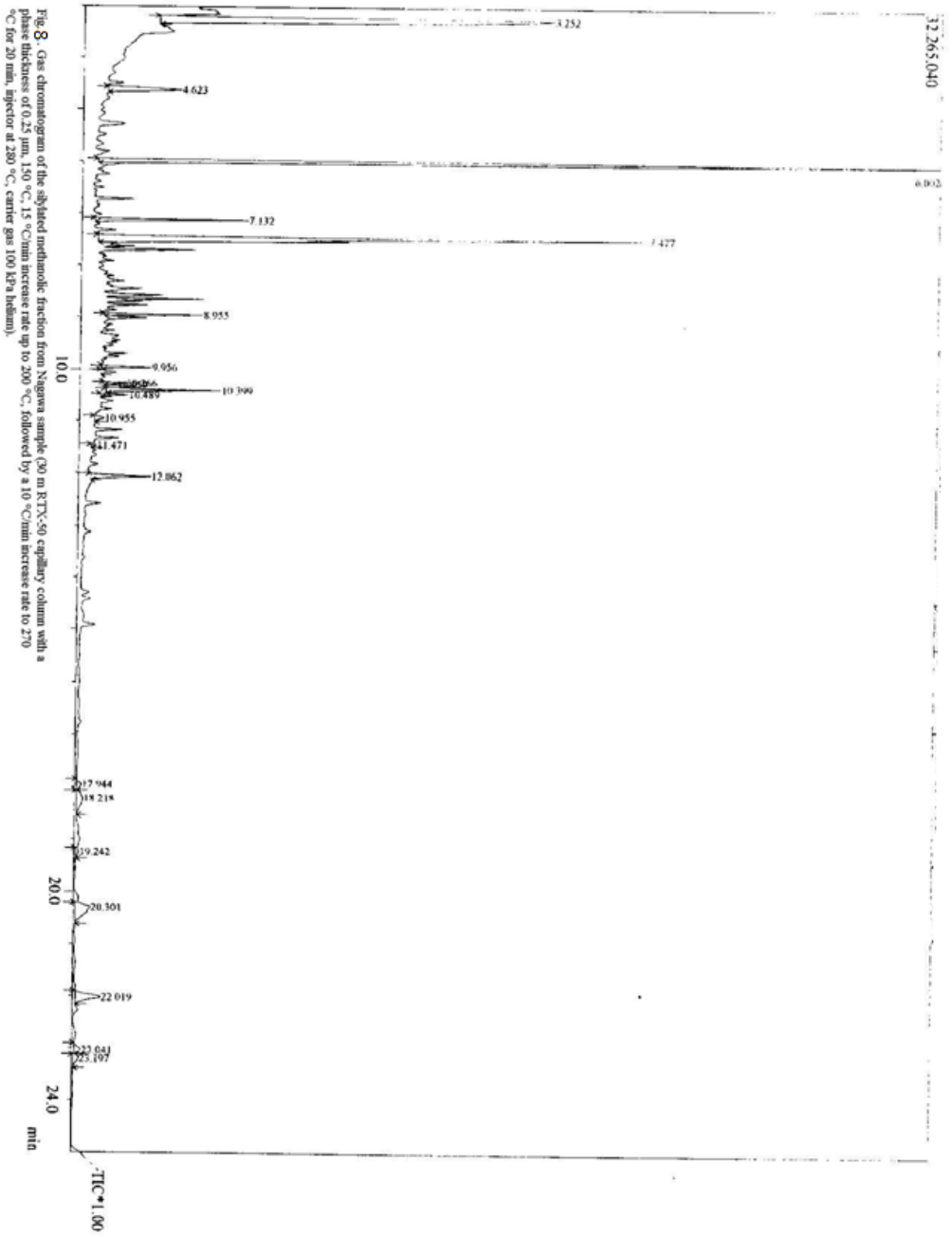

Fig. 8: Gas chromatogram of the silylated methanolic fraction from Nagawa sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 °C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

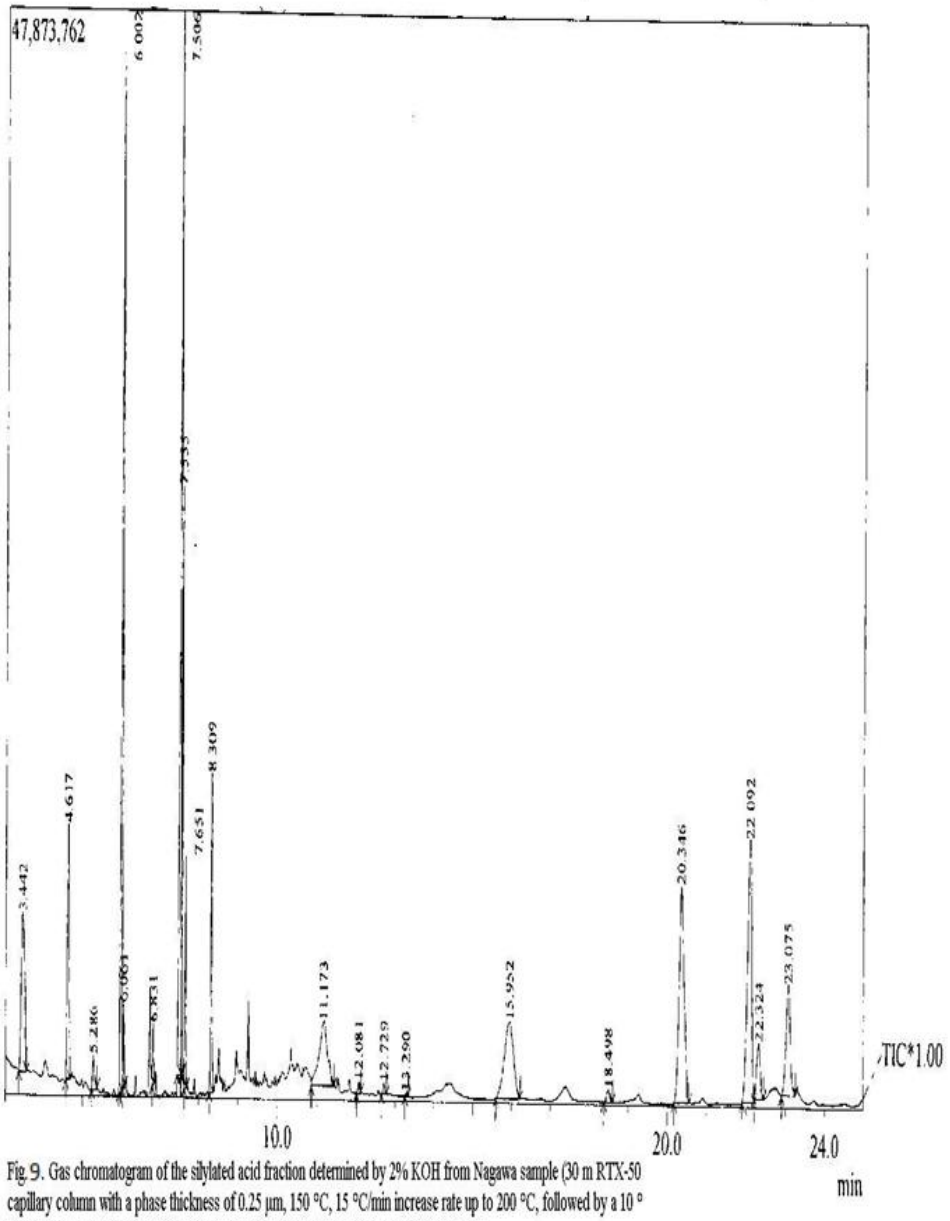

Fig. 9. Gas chromatogram of the silylated acid fraction determined by 2% KOH from Nagawa sample (30 m RTX-50 capillary column with a phase thickness of 0.25 µm, 150 °C, 15 °C/min increase rate up to 200 °C, followed by a 10 ° C/min increase rate to 270 °C for 20 min, injector at 280 °C, carrier gas 100 kPa helium).

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