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Analysis of amino acids, fatty acids and neurotoxins using gas chromatography-mass spectrometry in four scorpions species inhabiting New Valley Governorate, Egypt

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Abstract: The scorpion venom consisting of a pair of gland connected to telson has great importance for their survival, assisting in feeding and self-defense. Scorpion venoms are a complex mixture of inorganic salts, free amino acids, heterocyclic components, peptides and proteins. The present study aimed to identify telson components of four scorpion species *Leiurus quinquestriatus*, *Androctonus amoreuxi*, *Orthochirus innesi* and *Buthacus leptochelys*. Five telsons separated from four species of scorpions were preserved in 100% ethanol, homogenized in acetonitrile, centrifuged and sonicated as preparation of gas chromatography mass spectrometry (GC-MS) analysis. The present study indicated variations in the number and types of amino acids, fatty acids derivatives and neurotoxin in the four scorpion species. The four species of scorpions are included: *L. quinquestriatus* which has 14 amino acids, 15 fatty acids and 17 neurotoxic compounds; *A. amoreuxi* which has 8 amino acids, 13 fatty acids and 4 neurotoxic compounds; *O. innesi* which has 10 amino acids, 16 fatty acids and 6 neurotoxic compounds; *B. leptochelys* which has 6 amino acids, 19 fatty acids and 5 neurotoxic compounds were recorded. In conclusion, concentrations of amino and fatty acids derivatives and neurotoxic compounds showed differences among the four scorpion species. Furthermore, in all scorpion species, acetamide had the highest percentage as neurotoxin, and the highest level of neurotoxins was found in *L. quinquestriatus*, indicating this species may be the most venomous ones.

Key words: Amino acids, fatty acids, neurotoxin, telson, scorpions

1. Introduction

Scorpions belong to class Arachnida, order Scorpions. Moreover, they are one of the oldest known terrestrial animals to be used in biochemical studies (Zouari et al., 2005, 2006; Louati et al., 2011).

Telson in *Leiurus quinquestriatus* has a relatively elongated chela. Vesicle of telson has a yellow color with brown aculeus at the end. In *Androctonus amoreuxi*, aculeus at the end of telson is reddish and black in color. In *Orthochirus innesi*, the vesicle of telson is punctate, while in *Buthacus leptochelys*, telson is brown in color and with long curved aculeus (Obuid-Allah et al., 2020).

Scorpion venom contains amino acids, inorganic salts, peptides, nucleic acids, and proteins (Zlotkin, 2005). The toxic and pharmacological effect of the scorpion venom are large because it has a rich source of various small molecules and peptides. Bhavya et al. (2016) reported that scorpion venom contains variable amounts of different toxins such as neurotoxins, cardiotoxins, nephrotoxins, and hemotoxins. Venoms of scorpions belonging to family Buthidae are considered to be the most toxic and medically important species (Soleglad et al., 2003 and Bayatzadeh et al., 2020).

A variety of amino acids from the scorpion's tail and body were detected using HPLC (Lee et al., 2013). Yoshimoto et al. (2019) and Bayatzadeh et al. (2020) analyzed the venom components of the North African scorpion, (*Buthacus leptochelys*), isolated four peptides that show insect toxicity and a long chain peptide from the venom of the Iranian scorpion (*Androctonus crassicauda*), using mass spectrometric method respectively. Moreover, analyses of lipid and fatty acid composition of three species of scorpions (*Timogenes elegans*, *T. dorbignyi*, and *Brachistosternus ferrugineus*) were studied by Laino et al. (2015). El-Bitar et al. (2015) showed the enzymatic activities of the venoms from five Egyptian scorpion species (*Leiurus quinquestriatus*, *Androctonus amoreuxi*, *A. australis*, *A. bicolor* and *Scorpio maurus palmatus*) to determine the characteristics of the anti-HCV activities (Hepatitis C virus). Salama and Sharshar (2013) studied variation in venom protein composition among different scorpion species collected from different regions in Egypt. Scorpion venom has a complex mixture of about 100–700 different components, where peptides are the major once with various biological and pharmacological properties including anticancer activities. The venom of the Egyptian scorpion *Androctonus amoreuxi* has been used for treatment of human breast cancer (Salem et al., 2016).

To the best of the present authors' knowledge, there are no previous studies concerned with the analysis of the chemical components of the telsons of the following scorpion species: *L. quinquestriatus*, *A. amoreuxi*, *O. innesi* and *B. leptochelys* using gas chromatography-mass spectrometry (GC-MS). Therefore, the present study focused on determination of amino acids, fatty acids, and neurotoxins in the telsons of the four species of scorpions belonging to family Buthidae collected from the New Valley Governorate, Egypt.

2. Materials and methods

2.1. Telson organ isolation

Scorpions were collected by professional hunters, during the period from April to July, 2017, and from July to September,

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2019. A random search method was done during the survey, where the scorpions were collected under rocks, in gap of soil and leaf litter, and within vegetation. Telsons were fixed with forceps, and then were cut by scalpel to avoid other tissue contaminations. This process was carried out in the laboratory, where the place was free from dusts and other contaminants. Twenty (20) telsons were separated from adult scorpions; five from each species were used for analysis. Separated telsons were preserved in 100% ethanol, homogenized in acetonitrile as polar solvent and sonicated, then centrifuged before gas chromatography analysis.

2.2 Gas chromatography-mass spectrometer analysis

The analytical procedures using gas chromatography-mass spectrophotometer (GC-MS) analysis were done at Analytical Chemistry Unit, Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt.

GC separation was performed using GC from Agilent Technologies Model (6890N) equipped with temperature programming capability, splitless injector, capillary column, and mass quadruple spectrometry detector model 5975B. A computer data system is MSD Chemistry Station E.0201.1177 used for measuring the percent of total material. The analytical columns used were DB-5 (60 m × 250 µm × 0.25 µm), the oven temperature was set at 30 °C for 2 min, increased to 50 °C at 4 °C/min for 3 min, 150 °C at 10°C/min for 6 min then to 280 °C 15 °C/min for 2 min. The volume of the injected sample was 1 µL in split less mode. The injector temperature was set at 250 °C. Helium (99.999%, purity) was used 'as a carrier at a constant flow, 1 mLmin-1. The mass spectrometer was operated in electron impact (70 eV of ion energy), mass quadruple and mass source kept at 150 °C and 230 °C.

2.3. Identification of components

The activity of compounds was based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of Wiley library having 775,500 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the Wiley library. The name, molecular weight and molecular formula of components of the test materials were ascertained by National Library Medicine.[1]

3. Results

3.1. Amino acid derivatives

Fourteen amino acid derivatives were recorded in the telson of *Leiurus quinquestriatus* with highest percentage of glycyl-L-valine (1.12%) (Table 1 and Figure 1), while in *Androctonus amoreuxi*, 8 amino acids were detected with highest percentage of L-Proline, N-valeryl-, dodecyl ester, (7.39%) (Table 2 and Figure 2), while in *Orthochirus innesi*, 10 amino acids were determined with highest percentage of glycyl-L-leucine (7.80%) (Table 3 and Figure 3) and in *Buthacus leptochelys*, 6 amino acids were recorded with highest percentage of glycyl-L-leucine (4.19%) (Table 4 and Figure 4).

From Tables 1–4 and Figures 1–4, there were similarities in amino acids between *L. quinquestriatus* and *A. amoreuxi*, in L-proline, N-(hexanoyl)-hexyl ester, with different concentrations. Moreover, there were coincidences between *L. quinquestriatus* and *O. innesi* in amino acid glycyl-L-leucine, and N-Acetylalanine with variable concentrations, as well as analogies were observed between *L. quinquestriatus* and *B. leptochelys*, in amino acids glycyl-L-leucine and glycyl-L-valine with distinct concentrations. The amino acid DL-alanyl-L-leucine was found in *A. amoreuxi* and *O. innesi* in variable concentrations. Furthermore, variable quantities of L-alanine, N-valeryl decyl ester were detected in *A. amoreuxi* and *B. leptochelys*. The amino acids glycyl-L-valine and glycyl-L-leucine were observed in *O. innesi* and *B. leptochelys*. Moreover, these amino acids were noted in *L. quinquestriatus, but not found in A. amoreuxi.*

3.2. Fatty acids and fatty acids esters

In *L. quinquestriatus*, 8 fatty acids and 7 fatty acid esters were identified in telsons. The fatty acids were classified as six saturated and two unsaturated ones. The highest fatty acid percentage was 9-octadecanoic acid (C18:1 n-9) and its percentage was (2.44%) (Table 1 and Figure 1). Moreover, 7 fatty acids and 6 fatty acid esters were recognized in *A. amoreuxi*, however, fatty acids were subdivided into six saturated and one unsaturated with highest percentage for 9-octadecenoic acid (C18:1n-9) (14.99%) (Table 2 and Figure 2). Furthermore, in *O. innesi*, 11 fatty acids were detected and subdivided into seven saturated and four unsaturated. In addition, 5 fatty acid esters were determined. The highest fatty acid percentage was hexadecanoic acid (palmitic acid) (C16:0) (4.82%) (Table 3 and Figure 3). On the other hand, in *B. leptochelys*, 13 fatty acids and 6 fatty acid esters were recorded. Fatty acids were categorized into 10 saturated, and 3 unsaturated ones, with highest percentage was linoleic acid (C18:2 n-6), butyl ester (3.37%) (Table 4 and Figure 4).

The components of the four scorpion species were similar in octadecanoic acid (stearic acid), 6-octadecenoic acid, 9-octadecenoic acid, 9, 12-octadecadienoic acid and hexadecanoic ethyl ester. Furthermore, *L. quinquestriatus* is similar to *B. leptochelys* in containing 9-octadecenoic acid methyl ester, and 2,6-dodecadienoic acid,10-bromo-11-hydroxy-3,7,11-trimethyl-methyl ester, and butyl 9,12-octadecadienoate with different concentrations. In addition, *L. quinquestriatus* contains 9-octadecenoic acid methyl ester such as *O. innesi* but with different concentrations. *L. quinquestriatus* is in coincidence with *B. leptochelys* in containing 11,13-dihydroxy-tetradec-5-enoic acid methyl ester. Moreover, 15-hydroxypentadecanoic acid and hexadecanoic acid (palmitic acid) were determined in *B. leptochelys* and *O. innesi* in different concentrations. Variable concentrations from 2,3-dihydroxypropyl elaidate *were identified in B. leptochelys* and *A. amoreuxi.*

[1] https://pubchem.ncbi.nlm.nih.gov/.

Table 1. List of identified compounds extract of *Leiurus quinquestriatus* telson part by GC-MS analysis.

No.	RT	Name of the compound	Molecular formulae*	Molecular weight*	Value %	Biological activity**
Amino acids						
1	20.851	Glycyl-L-valine (Essential)	$C_7H_{14}N_2O_3$	174.2	1.12%	Anticancer of liver and lung, antitumor of breast, lung, prostate, and liver due to inhibition of the production of tumor necrosis factor and increase natural killer cell activity and antioxidants.
2	19.772	L-Proline,N-(hexanoyl)-, hexyl ester (Nonessential)	$C_{17}H_{31}NO_3$	297.4	0.57%	
3	22.36	L-Proline,N-valeryl-, heptadecyl ester (Non-essential)	$C_{26}H_{49}NO_3$	423.7	0.44%	
4	22.477	L-Proline,N isobutoxycarbonyl-, heptadecyl ester (Nonessential)	$C_{27}H_{51}NO_4$	453.7	0.39%	
5	21.439	L-Valine, N allyloxycarbonyl-,propyl ester (Essential	$C_{12}H_{21}NO_4$	243.3	0.38%	
6	32.764	5-Oxo-L-prolyl- L-Tyrosinamide (Nonessential)	$C_{14}H_{17}N_3O_4$	291.3	0.07%	Anticancer of liver and lung, antitumor of breast, lung and prostate, increase T-lymphocyte proliferation, Antioxidant.
7	11.233	DL-Leucine (Essential)	$C_6H_{13}NO_2$	131.17	0.06%	Increase aromatic amino acid decarboxylase activity inhibit production of uric acid.
8	11.344	N-(2-Amino-4-methylpentanoyl) leucine (Essential)	$C_{12}H_{24}N_2O_3$	244.33	0.06%	Antitumor of nasopharynx, increase natural killer (NK) cell activity, neuroinhibitor, tumor necrosis factor-inhibitor.
9	11.624	Cycloserine (Nonessential)	$C_3H_6N_2O_2$	102.09	0.05%	Anticancer, inhibit destruction of glycosaminoglycans.
10	12.044	N-Acetylalanine (Nonessential)	$C_5H_9NO_3$	131.13	0.01%	Antitumor of nasopharynx, increase natural killer (NK) cell activity, inhibit production of tumor-necrosis-factor, neurotoxic.
11	21.008	Norvaline, 2-propyl-, ethyl ester (Essential)	$C_{10}H_{21}NO_2$	187.28	0.63%	Increase aromatic amino acid decarboxylase activity.
12	28.084	Glycyl-L-leucine (Essential)	$C_8H_{16}N_2O_3$	188.22	0.02%	Anticancer of liver and lung, antitumor of lung, liver and prostate, antioxidant.
13	20.157	2,5-dioxo-3-isopropyl-6-methylpiperazine (Essential)	$C_8H_{14}N_2O_2$	170.21	0.67%	No activity reported.
14	11.402	DL-Leucine ethyl ester (Essential)	$C_8H_{17}NO_2$	159.23	0.09%	
Fatty acids						
1	25.671	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284.5	1.40%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity.
2	25.671	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	2.44%	
3	20.874	2,6-Dodecadienoic acid, 10-bromo-11-hydroxy-3,7,11-trimethyl-methyl ester	$C_{16}H_{27}BrO_3$	347.29	0.58%	
4	25.088	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	0.28%	
5	22.815	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.13%	
6	26.137	Hexadecanoic acid ethyl ester	$C_{18}H36O2$	284.5	0.12%	
7	30.066	3-Cyclopentylpropionic acid, 4-cyanophenyl ester	$C_{11}H_9NO_2$	187.19	0.10%	
8	41.915	Butyric acid, 4-methoxy, trimethylsilyl ester	$C_8H_{18}O_3Si$	190.31	0.01%	
9	32.415	11,13-Dihydroxy-tetradec-5-enoic acid methyl ester	$C_{15}H_{28}O_4$	272.38	0.02%	
10	12.947	2-Butenedioic acid	$C_4H_4O_4$	116.07	0.02%	
11	31.366	Oleic Acid	$C_{18}H_{34}O_2$	282.5	0.01%	
12	32.543	3-phenyl-5-hexynoic acid	$C_{12}H_{12}O_2$	188.22	0.01%	

Table 1. (continued)

Amino acids						
No.	**RT**	**Name of the compound**	**Molecular formulae***	**Molecular weight***	**Value %**	**Biological activity****
13	32.345	9-Octadecenoic acid - methyl ester	$C_{19}H_{36}O_2$	296.49	0.01%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity, provide zinc.
14	32.286	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879	0.03%	
15	35.434	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336.6	0.05%	No activity reported.
Neurotoxins						
1	8.348	Acetamide	C_2H_5NO	59.07	84.29%	No activity reported.
2	8.908	3-Hydroxybutanal	$C_4H_8O_2$	88.11	0.32%	
3	9.91	[1]benzothieno[2,3-c]quinolin-6(5H)-thione	$C_{15}H_9$	267.37	0.04%	
4	11.286	2-(Butylamino)-4'-chloroacetanilide	$C_{12}H_{17}ClN_2O$	240.73	0.03%	
5	11.84	3,5-Dimethylbenzaldehyde	$C_9H_{10}O$	134.17	0.17%	
6	25.799	1,4,7,10,13,16,19-Heptaoxacyclohenicosane	$C_{14}H_{28}O_7$	308.37	0.28%	
7	41.7	Dimethyl (methylthio) acetamide	$C_7H_{15}NOS$	161.27	0.14%	
8	11.537	Benzoic acid	$C_7H_6O_2$	122.13	0.24%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity.
9	12.714	N,N-Dimethyl acrylamide	C_5H_9NO	99.13	0.01%	Antitumor of nasopharynx, increase natural killer (NK) cell activity, inhibit production of tumor necrosis factor.
10	14.41	N,N-Dimethylacetoacetamide	$C_6H_{11}NO_2$	129.16	0.01%	Antitumor of nasopharynx, increase natural killer (NK) cell activity, inhibit production of tumor necrosis factor.
11	15.022	Oxaluric acid	$C_3H_4N_2O_4$	132.08	0.01%	Increase aromatic amino acid decarboxylase activity, inhibit production of uric acid.
12	15.051	2-Aminoacetamide	$C_4H_{10}N_2O_3$	134.13	0.10%	No activity reported.
13	16.054	butylated hydroxy toluene	$C_{15}H_{24}O$	220.35	0.01%	Testosterone-hydroxylase-inducer
14	16.159	N-(Alpha.-methylphenethyl)-Acetamide,	$C_{11}H_{15}NO$	177.24	0.02%	Increase natural killer (NK) cell activity, inhibit production of tumor-necrosis-factor, antitumor of nasopharynx.
15	26.907	2,5-Piperazinedione, 3-(phenylmethyl)	$C_{11}H_{12}N_2O_2$	204.22	0.04%	No activity reported.
16	27.274	2-Methyl-6-nitro-4-quinolinol	$C_{10}H_8N_2O_3$	204.18	0.02%	
17	32.957	2-Methyl-1-phenyl-1-pentanol	$C_{12}H_{18}O$	178.27	0.21%	Catechol-O-Methyl-transferase-inhibitor.

** Source: Dr. Duke's Phytochemical and Ethnobotanical Databases[2].
* Source: Molecular formulae and molecular weight[3].
RT (min): Retention time in minutes.

3.3. Neurotoxins found in telsons

The current study revealed presence of 17 compounds with neurotoxic activity in *L. quinquestriatus* with a highest percentage for acetamide (84.29%) (Table 1 and Figure 1). Furthermore, in *A. amoreuxi*, 4 neurotoxins were recognized with a highest percentage for acetamide (25.21%) (Table 2 and Figure 2). A total number of 6 neurotoxins were identified in *O. innesi*, with highest neurotoxin percentage for acetamide (65.15%) (Table 3 and Figure 3). In *B. leptochelys*, 5 neurotoxins were detected, and the highest percentage was for acetamide (79.52%) (Table 4 and Figure 4). Furthermore, acetamide was the most common neurotoxin with variable concentrations in the four scorpion species investigated in the present study. Benzene acetaldehyde was found in *A. amoreuxi* and *B. leptochelys*, but in different concentrations. Moreover, dimethyl benzaldehyde was recognized in the four species of scorpions. In addition, N (aminocarbonyl)-acetamide was found in *O. innesi* and *Buthacus leptochelys* in variable amounts.

4. Discussion

The present study reveals the chemical analysis of telsons in four scorpion species. According to the authors' knowledge,

[2] https://phytochem.nal.usda.gov/
[3] https://pubchem.ncbi.nlm.nih.gov/

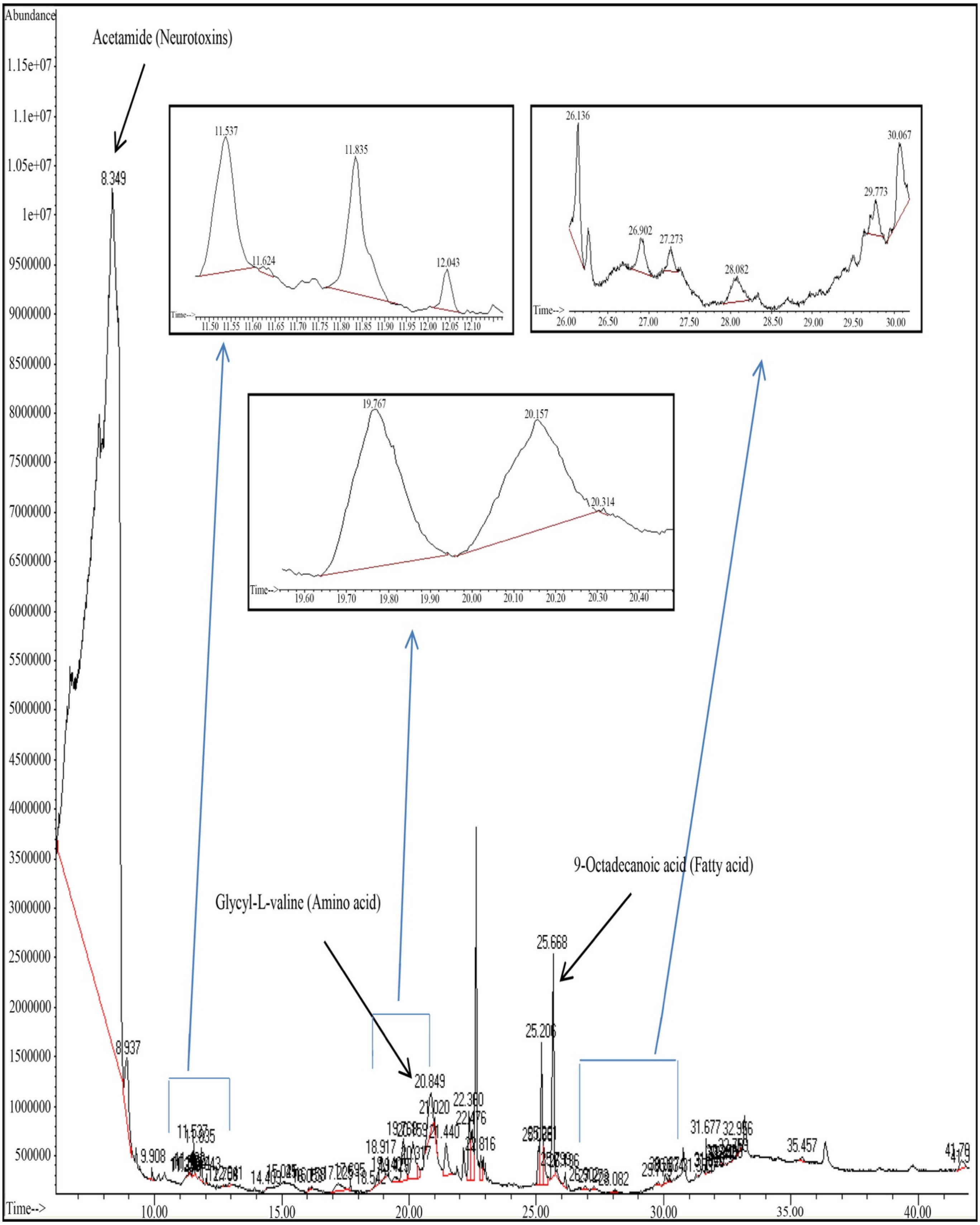

Figure 1. GC-MS chromatogram of *Leiurus quinquestriatus.*

no previous studies compared the percentages of amino acids, fatty acids, and neurotoxins in *Leiurus quinquestriatus*, *Androctonus amoreuxi*, *Orthochirus innesi* and *Buthacus leptochelys*.

Table 2. List of identified compounds extract of *Androctonus amoreuxi* telson part by GC-MS analysis.

No.	RT	Name of the compound	Molecular formulae*	Molecular weight*	Value %	Biological activity**
Amino acids						
1	22.296	L-Proline, N-valeryl-, dodecyl ester (Nonessential)	$C_{22}H_{41}NO_3$	367.6	7.39%	Antitumor and anticancer of liver and lung, antitumor of breast, lung, prostate, and liver, antioxidant, anticarcinomic of lung, inhibit production of tumor necrosis factor, increase natural killer (NK) cell activity.
2	19.65	L-Proline, N-(hexanoyl)-, hexyl ester (Nonessential)	$C_{17}H_{31}NO_3$	297.4	3.60%	
3	22.086	L-Proline, N-valeryl-, hexadecyl ester (Nonessential)	$C_{26}H_{49}NO_3$	423.7	1.68%	
4	21.323	N-Acetoacetylvaline methyl ester (Essential)	$C_8H_{15}NO_3$	173.21	2.39%	Antitumor of nasopharynx, inhibit production of tumor-necrosis-factor, increase natural killer (NK) cell activity, neurotoxic, stimulate sympathetic nervous system.
5	18.677	L-Alanine, N-valeryl-, hexyl ester (Nonessential)	$C_{14}H_{27}NO_3$	257.2	0.91%	
6	18.735	L-Alanine, N-valeryl-, decyl ester (Nonessential)	$C_{18}H_{35}NO_3$	313.26	1.171%	
7	20.14	N-BOC-trans-3-methyl-l-proline (Non-essential)	$C_{11}H_{19}NO_4$	229.13	0.46%	
8	20.023	DL-Alanyl-L-leucine (Essential)	$C_9H_{18}N_2O_3$	202.25	1.94%	Anticancer of liver and lung, antitumor of breast, lung, prostate and liver, antioxidant.
Fatty acids						
1	25.17	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	14.99%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity.
2	25.607	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	284.5	6.97%	
3	31.552	2Hydroxy1Hexadecanoicacidhy Droxy methyl) ethyl ester	$C_{19}H_{38}O_4$	330.50	3.61%	
4	33.999	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	2.26%	
5	25.065	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	1.60%	
6	26.137	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.5	1.52%	
7	22.914	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.5	1.74%	
8	19.289	Butanedioic acid, cyclic hydrazide	$C_4H_6N_2O_2$	19.289	0.34%	
9	25.496	Ethyl linoleate	$C_{20}H_{36}O_2$	308.5	0.94%	No activity reported.
10	20.213	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}$	356.54	0.79%	
11	33.324	Benzyl mandelate	$C_{15}H_{14}O_3$	242.27	0.39%	
12	33.073	Butyl 9-tetradecenoate	$C_{18}H_{34}O_2$	282.5	0.13%	
13	32.753	Methyl oleate	$C_{19}H_{36}O_2$	296.5	0.40%	Methyl-Donor, catechol-O-Methyl-transfers-inhibitor.
Neurotoxins						
1	6.093	Acetamide	C_2H_5NO	59.07	25.21%	No activity reported.
2	9.275	Benzeneacetaldehyde	C_8H_8O	120.14	0.83%	
3	11.84	2,3-dimethyl- Benzaldehyde	$C_9H_{10}O$	134.17	0.73%	
4	9.87	Nonadecylamine	$C_{19}H_{41}N$	283.5	0.22%	

** Source: Dr. Duke's Phytochemical and Ethnobotanical Databases[4].

* Source: Molecular formulae and molecular weight[5].

RT (min): Retention time in minutes.

[4] https://phytochem.nal.usda.gov/

[5] https://pubchem.ncbi.nlm.nih.gov/

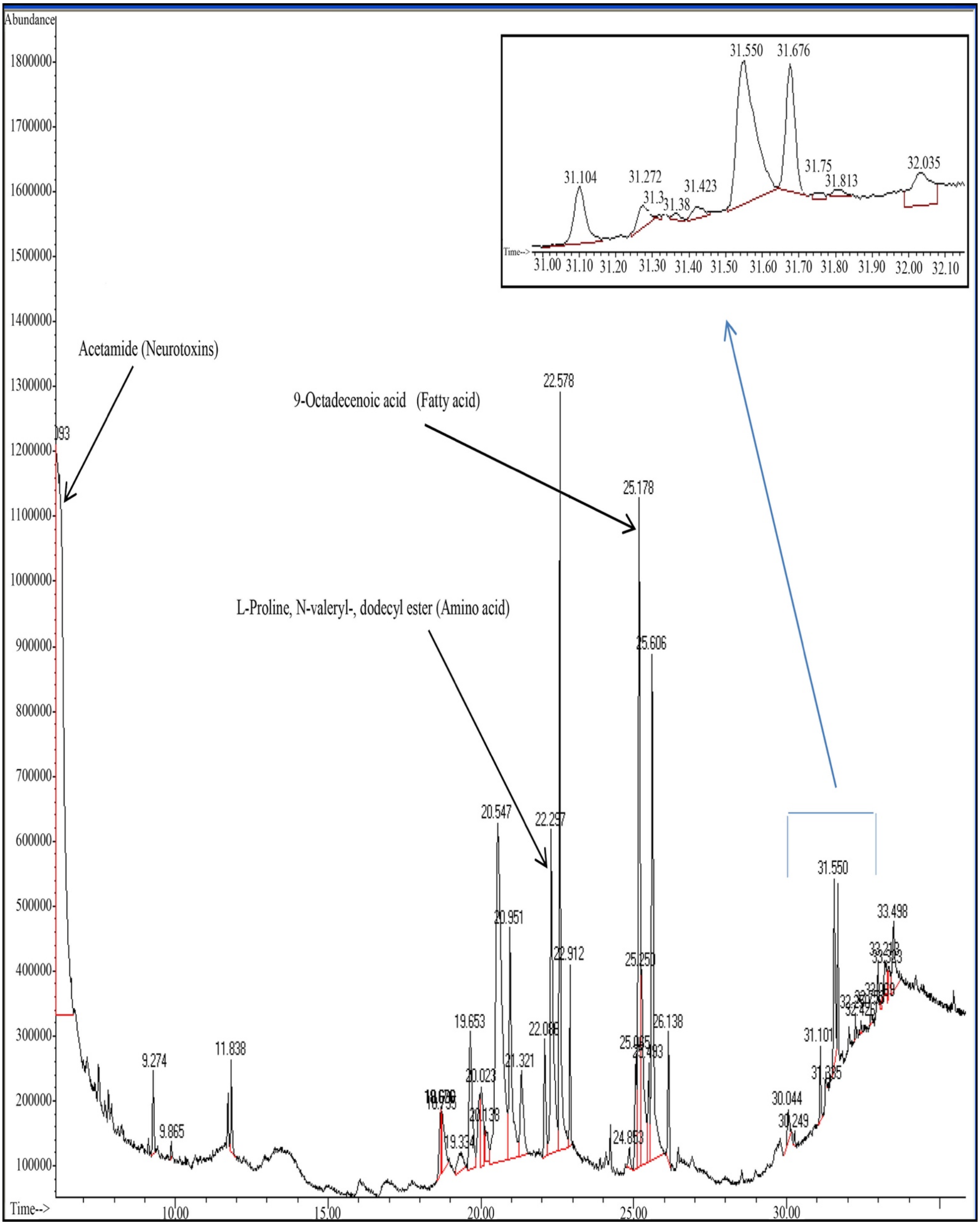

Figure 2. GC-MS chromatogram of *Androctonus amoreuxi*

Table 3. List of identified compounds extract of *Orthochirus innesi* telson part by GC-MS analysis.

No.	RT	Name of the compound	Molecular formulae*	Molecular weight*	Value %	Biological activity**
Amino acids						
1	20.588	Glycyl-L-leucine (Essential)	$C8H16N2O3$	188.22	7.80%	Anticancer of liver and lung, antitumor of breast, lung, prostate and Lung, antioxidant.
2	20.956	Glycyl-L-valine (Essential)	$C_7H_{14}N_2O_3$	174.2	1.13%	
3	19.656	L-Proline (Nonessential)	$C_5H_9NO_2$	115.13	1.00%	
4	20.145	N-Acetylalanine (Nonessential)	$C_5H_9NO_3$	131.13	0.47%	Antitumor of nasopharynx, increase natural killer (NK) cell activity, inhibit production of tumor-necrosis-factor.
5	21.329	N-(Aminoacetyl) leucine (Essential)	$C_8H_{15}NO_3$	173.21	0.44%	
6	22.086	L-Leucine,N-cyclopropylcarbonyl,methyl ester (Essential)	$C_{11}H_{19}NO_3$	213.27	0.39%	
7	19.947	DL-Alanyl-L-leucine (Essential)	$C_9H_{18}N_2O_3$	202.25	0.33%	Anticancer of liver and lung, antitumor of breast, lung, prostate and liver, antioxidant.
8	32.321	Cyclo-(l-alanyl-tyrosyl) (Essential)	$C_{12}H_{14}N_2O_3$	234.25	0.08%	
9	13.466	N-(Glycyl) alanine (Non-essential)	$C_5H_{10}N_2O_3$	146.14	0.06%	
10	18.717	2,5-dioxo-3-isopropyl-6-methylpiperazine (Essential)	$C_8H1_4N_2O_2$	170.21	0.94%	No activity reported.
Fatty acids						
1	22.588	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.42	4.82%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity.
2	25.607	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284.5	3.26%	
3	25.164	Oleic Acid	$C_{18}H_{34}O_2$	282.5	2.13%	
4	31.558	15-Hydroxypentadecanoic acid	$C15H30O3$	258.4	0.80%	
5	33.213	Oleyl alcohol, heptafluorobutyrate	$C_{22}H_{35}F_7O_2$	464.5	0.46%	
6	33.505	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	0.41%	
7	35.51	2- Chloropropionic acid, octadecyl ester	$C_{21}H_{41}ClO_2$	361	0.40%	
8	25.053	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	0.32%	
9	24.855	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.26%	
10	26.137	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.5	0.23%	
11	22.914	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.5	0.18%	
12	32.432	10-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.5	0.14%	
13	33.662	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.41	0.08%	
14	22.22	3-Hexenoic acid (Caproic acid)	$C_6H_{10}O_2$	114.14	0.07%	
15	31.75	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.03%	
16	32.257	2-Hydroxy-(Z) 9-pentadecenyl propanoate	$C_{18}H_{34}O_3$	298.5	0.09%	Increase zinc bioavailability, provide zinc, testosterone-hydroxylase-inducer.
Neurotoxins						
1	6.687	Acetamide	C_2H_5NO	59.07	65.15%	No activity reported.
2	10.639	N-(aminocarbonyl)- (Acetamide	$C_3H_6N_2O_3$	118.09	0.08%	
3	11.723	3,4-Dimethylbenzaldehyde	$C_9H_{10}O$	134.17	0.72%	
4	20.029	2-Methylpiperazine	$C_5H_{12}N_2$	100.16	0.36%	
5	30.147	4N-methylamino-2 (1H)-pyrimidinone	$C_6H_9N_3O$	139.16	0.04%	Histamine-inhibitor, hormone-balancing, increase natural killer (NK) cell activity, tumor necrosis factor-inhibitor, stimulate norepinephrine production.
6	18.921	3-Methyl-6-(1 methylethyl)-2,5 Piperazinedione	$C_8H_{14}N_2O_2$	170.20	0.04%	Catechol-O-Methyl-transferase-inhibitor, catechol-O-Methyl-transferase-inhibitor.

** Source: Dr. Duke's Phytochemical and Ethnobotanical Databases[6].

* Source: Molecular formulae and molecular weight[7].

RT (min): Retention time in minutes.

[6] https://phytochem.nal.usda.gov/

[7] https://pubchem.ncbi.nlm.nih.gov/

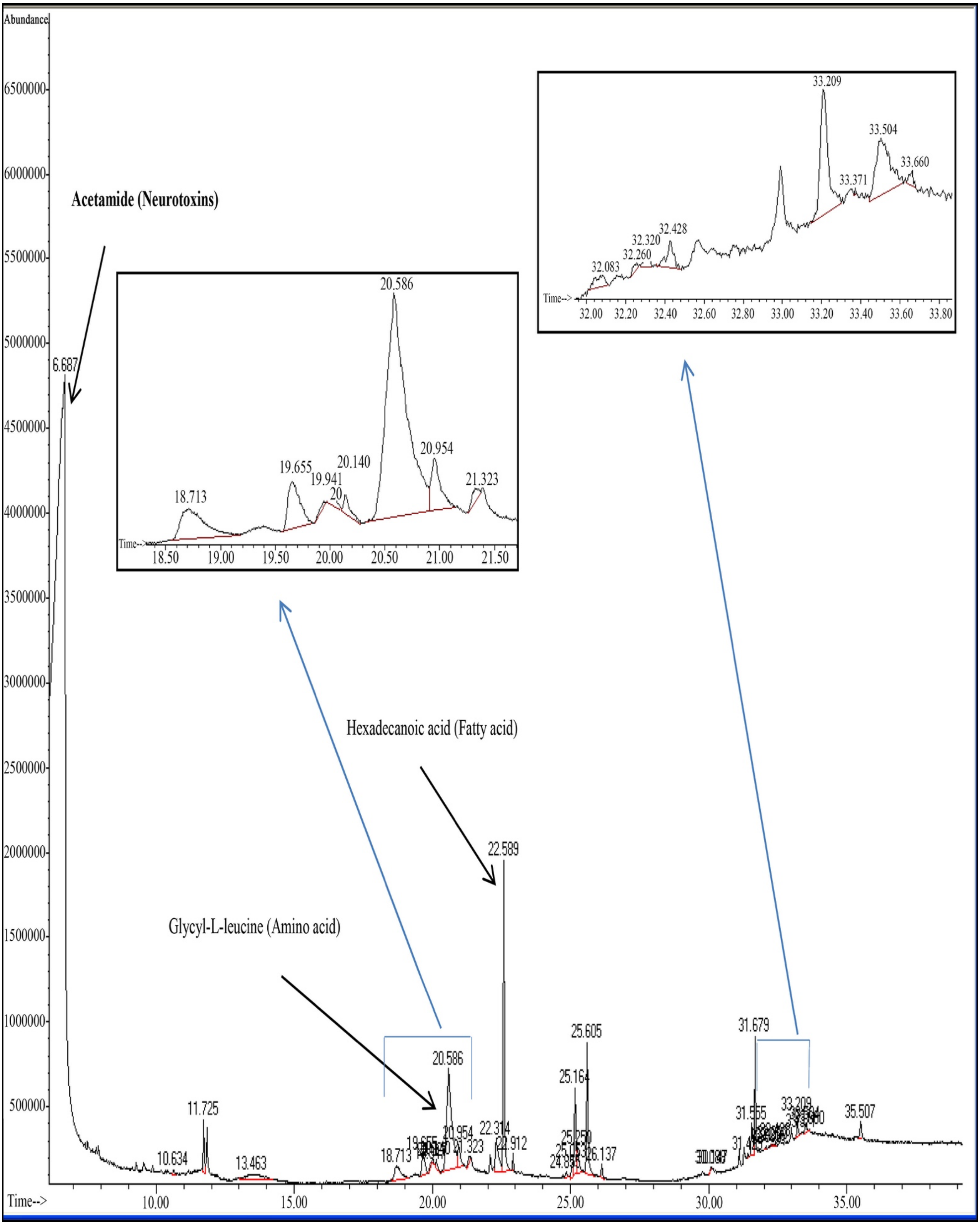

Figure 3. GC-MS chromatogram of *Orthochirus innesi*.

Table 4. List of identified compounds extract of *Buthacus leptochelys* telson part by GC-MS analysis.

No.	RT	Name of the compound	Molecular formulae*	Molecular weight*	Value %	Biological activity**
Amino acids						
1	20.023	Cyclo (glycyl-L-leucyl) (Nonessential)	$C_8H_{14}N_2O_2$	170.21	0.23%	Anticancer of liver and lung, antitumor of lung, liver, and prostate, antioxidant.
2	22.605	Glycyl-L-leucine (Essential)	$C_8H_{16}N_2O_3$	188.22	4.19%	
3	22.941	Glycyl-L-valine (Essential)	$C_7H_{14}N_2O_3$	174.2	0.41%	
4	39.864	N-Formylleucine methyl ester (Essential)	$C_8H_{15}NO_3$	173.21%	0.01%	Antitumor of nasopharynx, inhibit production of tumor-necrosis-factor, increase natural killer (NK) cell activity, stimulate sympathetic nervous system.
5	18.723	N-(2Aminopropanoyl) leucine (Essential)	$C_9H_{18}N_2O_3$	202.25	0.14%	
6	21.329	L-Alanine, N-valeryl-, decyl ester (Nonessential)	$C_{18}H_{35}NO_3$	313.26	0.182%	
Fatty acids						
1	25.17	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	1.53%	Inhibit production of uric acid, increase aromatic amino acid decarboxylase activity.
2	20.606	2,6-Dodecadienoic acid, 10-bromo-11-hydroxy-3,7,11-trimethyl- methyl ester	$C_{16}H_{27}BrO_3$	347.29	1.20%	
3	33.213	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.5	0.75%	
4	31.552	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258.4	0.52%	
5	33.505	Propanoic acid, 3-hydroxy-2-methyl-3-phenyl-, tert-butyl ester	$C_{14}H_{20}O_3$	236.31	0.41%	
6	25.053	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	0.26%	
7	32.555	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	0.17%	
8	33.376	Oleic acid, propyl ester	$C_{21}H_{40}O_2$	324.5	0.13%	
9	35.306	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	0.12%	
10	31.75	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.10%	
11	32.152	3-Hydroxy-2,6,6-trimethyl-hept-4-enoic acid	$C_{10}H_{18}O_3$	186.25	0.09%	
12	11.274	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.5	0.09%	
13	33.662	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	0.08%	
14	29.623	14-Pentadecenoic acid	$C_{15}H_{28}O_2$	240.38	0.04%	
15	32.648	11,13-Dihydroxy-tetradec-5-enoic acid, methylester	$C_{15}H_{28}O_4$	272.38	0.02%	
16	33.347	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	0.01%	
17	33.499	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}$	356.54	0.13%	
18	25.624	Linoleic acid, butyl ester	$C_{24}H_{44}O_2Si$	392.7	3.37%	
19	35.475	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336.6	0.38%	No activity reported.
Neurotoxins						
1	7.124	Acetamide	C_2H_5NO	59.07	79.52%	No activity reported.
2	9.881	N-(aminocarbonyl)- Acetamide	$C_3H_6N_2O_3$	118.09	0.08%	
3	9.287	Benzeneacetaldehyde	C_8H_8O	120.14	0.09%	
4	20.956	3,4-Dimethylbenzaldehyde	$C_9H_{10}O$	134.17	0.8%	
5	26.143	N-(n-Propyl) Acetamide	$C_5H_{11}NO$	101.15	0.40%	Increase natural killer (NK) cell activity, inhibit production of tumor necrosis factor, stimulate sympathetic nervous system, tumor necrosis factor-inhibitor.

**Source: Dr. Duke's Phytochemical and ethnobotanical Databases[8].

* Source: Molecular formulae and molecular weight[9].

RT (min): Retention time in minutes.

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[8] https://phytochem.nal.usda.gov/

[9] https://pubchem.ncbi.nlm.nih.gov/

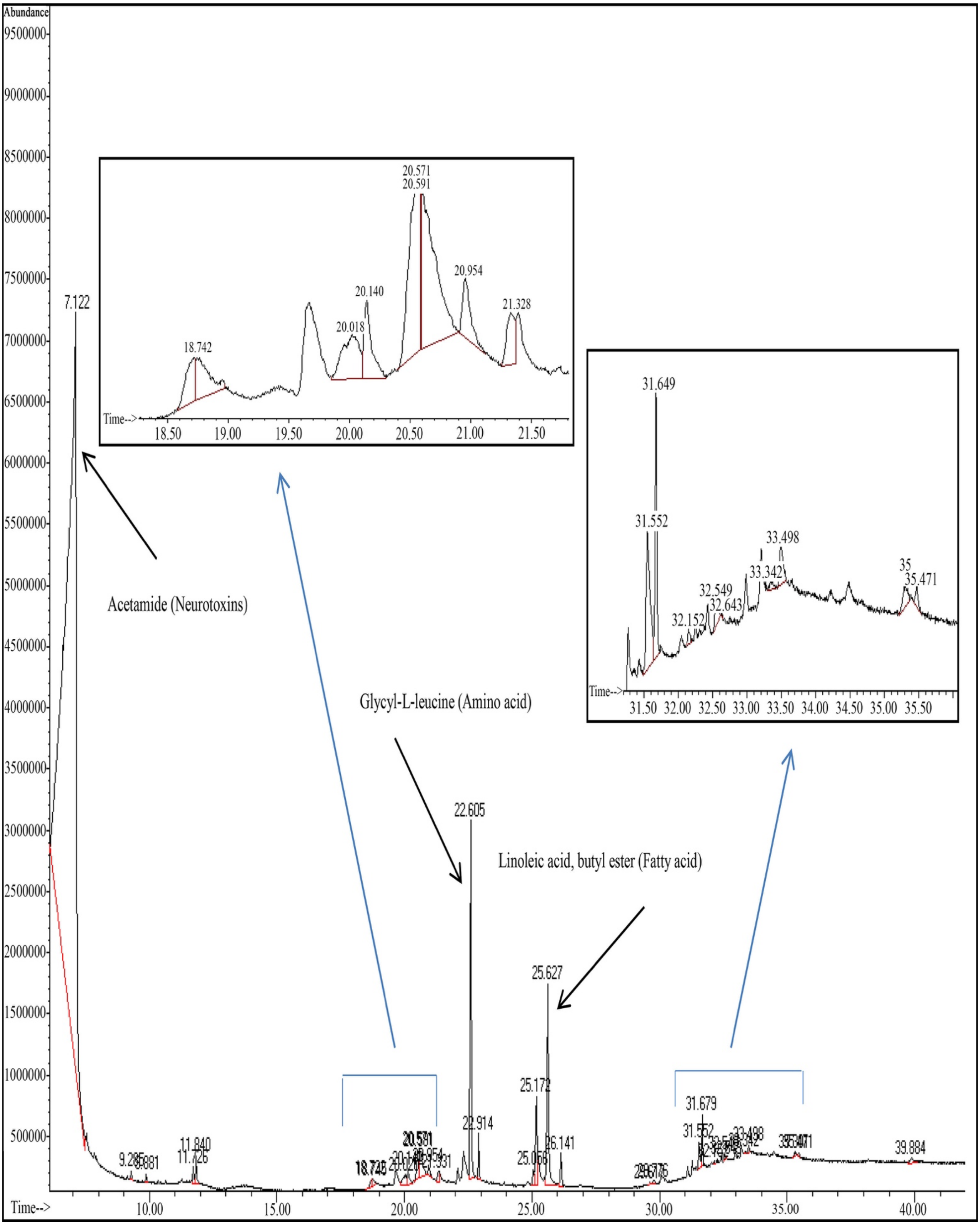

Figure 4. GC-MS chromatogram of *Buthacus leptochelys*.

In the present study, 14 amino acid derivatives were determined in *L. quinquestriatus.* In contrast, Emteris et al. (2006) found 20 amino-acids. Such difference could be attributed to a wide variety of amino acid peptides (Abdel-Rahman et al., 2013). In this work, 6 amino acid derivatives were detected in *B. leptochelys*. However, Lee et al. (2013) reported 17 amino acids in the scorpion telsons of *Buthus martensii Karsch*. This variation may be due to the difference between scorpion species. In the current research, 8 and 10 amino acid derivatives were recorded in *A. amoreuxi* and *O. innesi*, respectively. Unfortunately, based to the author's knowledge, no literatures were available to compare the present finding with *A. amoreuxi* and *O. innesi*. Referring to the importance of amino acids in scorpions, they have antitumor and anticancer activities (Salem et al., 2016), inhibit the proliferation of cancer cells (Hyun et al., 2006). Moreover, some amino acids of scorpions such as Aspartic acid prevent body fatigue, protect the immune system by producing immunoglobulin, and remove excess ammonia and toxins from the bloodstream, preventing damage to the liver and the central nervous system (Alam et al., 2007).

In the present study, fatty acid numbers of the species investigated were found between 15 (*L. quinquestriatus*), 13 (*A. amoreuxi*), 16 (*O. innesi*) and 19 (*B. leptochelys*). Laino et al. (2015) detected fatty acids in telsons of three scorpion species namely: *Timogenes elegans* with percentage 36%, *T. dorbignyi* with percentage 48% and *Brachistosternus ferrugineus* with percentage between 32% and 40% wet weight. Such differences could be explained by variations of scorpion species.

In the present research, the highest amount of neurotoxins was detected in *Leiurus quinquestriatus*, indicating this scorpion has higher levels of the neurotoxic compounds when compared to other species investigated. As it was mentioned in a previous study (Salama, 2013), *Leiurus quinquestriatus* secretes one of the most noxious venoms among buthidae scorpions. Moreover, the amount of neurotoxins in scorpions is variable, even within the same species and from place to place, it also varies with the age, size, nutrition of the scorpion and climatic conditions (Gueron et al., 1980). In addition, it was reported that the venom of *Buthacus leptochelys* has a significant toxicity against insects and crickets (*Acheta domesticus*) (Gurevitz et al., 2007; Yoshimoto et al., 2019). Venomous animals including scorpions have a wide variety of natural peptide toxins which are used for predation and defense (Lewis et al., 2003). Furthermore, scorpion neurotoxins are usually used to increase natural killer cell activity; inhibit the production of tumor necrosis factor and work as antitumor in vitro and in vivo (Loret and Hammock, 2001; Ding et al., 2014; Salem et al., 2016; Bhavya et al., 2016; Béchohra et al,. 2016). Moreover, scorpion neurotoxic components are also used to make scaffolds for the development of drugs (Costa et al., 2015).

5. Conclusion

The amount and percentages of amino acids, fatty acids, and neurotoxins varied among the four studied scorpion species. The highest amount of neurotoxins was found in *Leiurus quinquestriatus*, indicating this species may be the most venomous scorpion. Furthermore, variations in the concentrations of these analytes among the different scorpion species indicate the presence of potential patterns of telson components that could be used in medicinal purposes, however, further researches are necessary to approve such use, but the present study is a reasonable starting point.

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