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Analysis of Alkali Chemical Constituents from *Holotrichia diomphalia* Bates by GC-MS

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The alkali chemical constituents were obtained after 95 % alcohol extraction, condensation, acidification and extraction with chloroform from *Holotrichia diomphalia* bates. The chemical constituents were analysis by GC-MS and their relative contents were calculated by area normalization method. Seventy nine components were identified and 14 nitrogen-containing compounds were found by analysis with GC-MS. The main of nitrogen-containing compounds including 2-piperidinone, 4-methyl-5-thiazoleethanol, 2-[4-(2-hydroxy-phenyl)-thiazol-2-yl]-5-phenyl-penta-2,4-dienenitrile, 4,4'-*bis*(dimethylamino)benzhydrol, 5α-pregnane-3,20α-dio,14α,18α-[4-methyl -3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-diacetate, 5,10-diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine; 9*H*-pyrido[3,4-b]indole, 5-aminovaleric acid, Ile-ser, 4,4-dimethylazetidin-2-one and 1-methyl-2-piperidinone. The novel findings of the present study provide a scientific basis for the further development and utilization of *Holotrichia diomphalia* bates.

Keywords: *Holotrichia diomphalia* bates, Chemical constituents, Alkaloids, GC-MS.

INTRODUCTION

Holotrichia diomphalia bates is a species of melolonthidae that is indigenous in the eastern Asian areas such as China and Korea[1]. This species undergoes complete metamorphosis through four stages of life, embryo, larva, pupa and imago. The larva stage is the longest stage in the species' life cycle and widely consumed in traditional Chinese medicine (TCM) for treatment of arthrolithiasis, tetanus, erysipelas and superficial infection herbal[2,3]. Furthermore, the larva is widely used in Korean folk medicinal preparations for treatment of liver cirrhosis, contusion, edema, furuncle and apoplexy[4]. Kang *et al.*[5] reported that ethanol extract from *Holotrichia diomphalia* larvae was capable of modulating macrophage activity.

Previous studies have identified the antibacterial proteins[6], prophenoloxidase[7], prophenoloxidase activating factor-I (PPAF-II) and II[8,9] from *Holotrichia diomphalia* bates. Recently, using chromatography-mass spectrometry (GC-MS) analysis, olefinic acid, such as elaidic acid and hexadecanoic acid, alkane acid, such as pentadecylic acid and stearic acid were obtained from the fatty oils and petroleum ether extract of *Holotrichia diomphalia*1. Our preliminary studies suggest that the alkali chemical constitutions possess significant anti-tumor activities. However, there is no report available on the alkali chemical constitutions from *Holotrichia diomphalia* larvae.

Therefore, in the present study, we extracted the alkali chemical constituents from *Holotrichia diomphalia* larvae and analyzed the composition profiles using GC-MS and computer spectrum search technique. The results provide a scientific basis for further comprehensive utilization of the insects.

EXPERIMENTAL

Holotrichia diomphalia bates was collected from Hancheng city in the northwest of Shaanxi province of China, in February 2012. A voucher specimen was deposited in department of natural medicine, school of pharmacy, fourth military medical university, Xi'an, China.

Dried of *Holotrichia diomphalia* bates (5 kg) was crushed into coarse powder and extracted with 95 % ethanol (4 × 5 L) for 24 h at room temperature. Then the combined macerate was filtered and the solvent was evaporated to dryness under reduced pressure. The crude extract was acidified to pH 2 with an aqueous solution of H_2SO_4 (2 %, v/v) and extracted with EtOAc. The aqueous layer was then basified up to pH 10 with NH_3 (25 %, v/v) and the alkali chemical constituents were extracted with $CHCl_3$. Finally, the purified dried alkali chemical constituents (10 mg) were re-dissolved in 2 mL of ethanol and the solution was filtered through a 0.45 μm filter for GC-MS analysis.

TABLE-1
SELECTED PARAMETERS FOR GC-MS CONDITION

Parameter	Method 1	Method 2	Method 3	Method 4
Capillary column	GSBP-5MS (30 m × 0.32 mm. ID: 0.32 μm. film: 0.25 μm), poly(5 % dipenyl, 95 % dimethylsiloxane)			
Carrier gas	Helium			
Injection Mode	Split			
Column Flow	1.4 mL/min			
Split Ratio	84:1			
Ionization energy	70 eV			
Scan range	50-650 amu			
Injection temperature	250 °C	210 °C	210 °C	210 °C
Oven temperature Progarm	Initial temperature was 80 °C (hold time 1 min) then ramped at 8 °C/min to 280 °C (hold time 2 min)	Initial temperature was 80 °C (hold time 1 min) then ramped at 8 °C/min to 280 °C (hold time 2 min)	Initial temperature was 80 °C (hold time 1 min) then ramped at 8 °C/min to 210 °C (hold time 2 min)	Initial temperature was 100 °C (hold time 1 min) then ramped at 8 °C/min to 210 °C (hold time 2 min)
Ionization source temperature	260 °C	225 °C	250 °C	250 °C
Transmission line temperature	250 °C	285 °C	210 °C	210 °C

GC-MS condition: The compositions were analyzed by an ISQ 110953 GC-MS system (Thermo Fisher Corp, USA). The condition was shown in the Table-1. Retention times were utilized as primary criterion for the peaks identification. Compounds were identified using the MS spectral database (P/N: 274, 102, 74 Thermo Data system).

RESULTS AND DISCUSSION

Fifty-four kinds of crude drugs derived from insects are listed in the "herbal" edited during the Chinese Song dynasty (960-1280 A.D). These drugs includes bees, wasps, flies, butterflies, moths, cockroaches, beetles, etc and are used as antiviral, antibacterial and anticancer agents[10]. Currently, more and more active compounds isolated from traditional Chinese medicine of insects show various medicinal biological activities. For example cantharidin and norcantharidin display high levels of anticancer activity[11,12]. Although *Holotrichia diomphalia* bates has a long history of the application in traditional Chinese medicine, there is little information on the bioactive compounds with great potential as pharmaceuticals in modern medicine.

In this study, to comprehensively analyze the chemical composition of *Holotrichia diomphalia* bates, we established four kinds of GC analysis methods. Total gas chromatograms of alkali chemical constituents from the *Holotrichia diomphalia* bates were shown in Fig. 1. The relative contents of chemical constituents were analysis by GC-MS and calculated by area normalization method. The results were summarized in Tables 2-5.

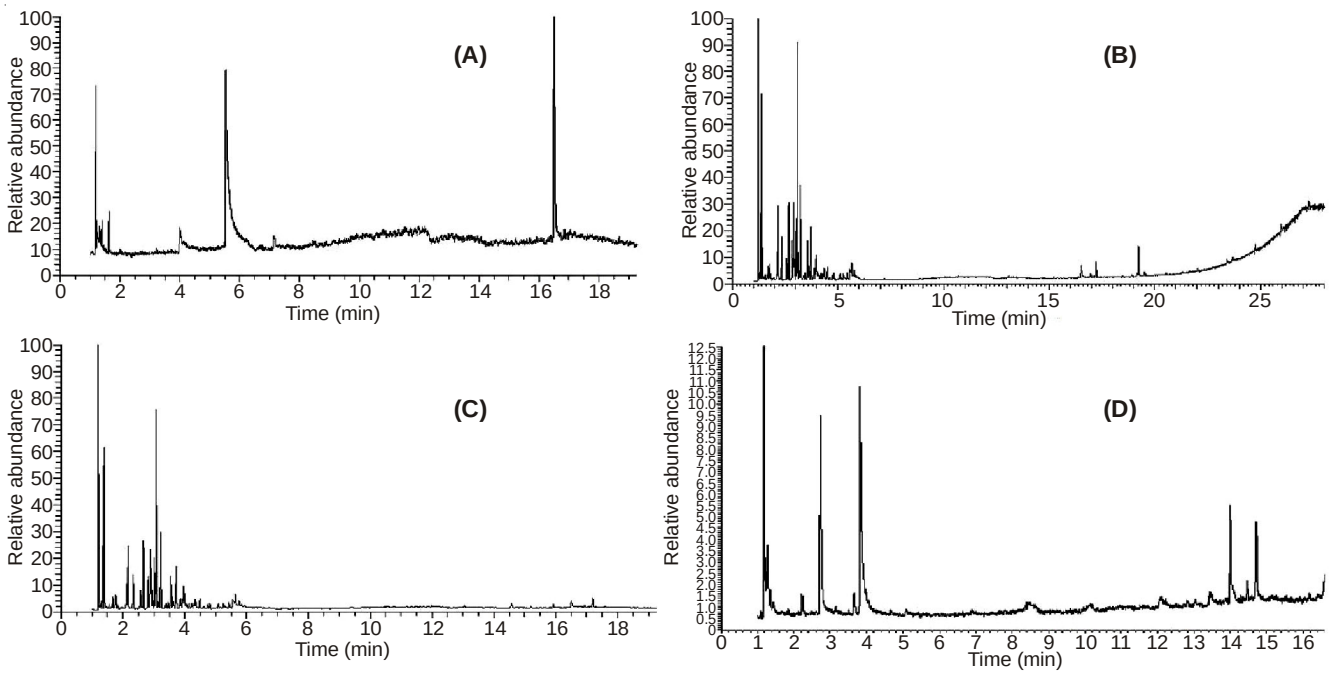
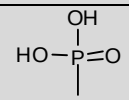
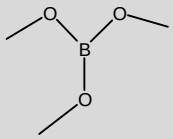
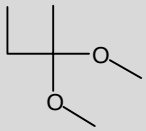
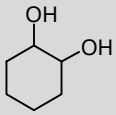
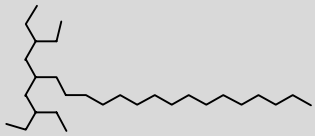
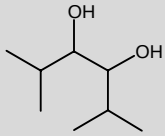
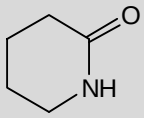
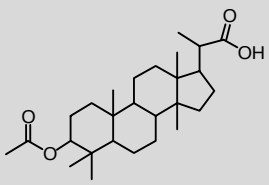
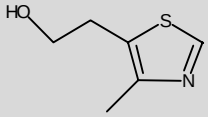
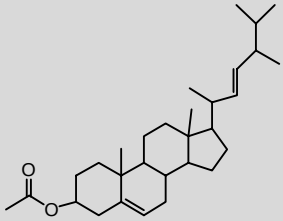
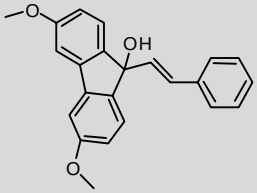
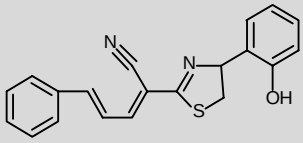
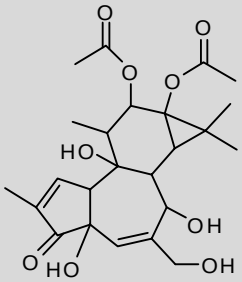
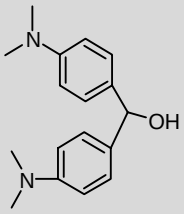
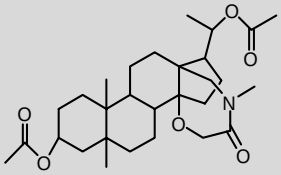
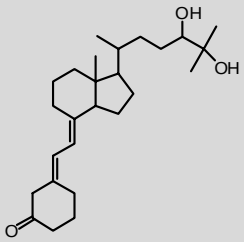
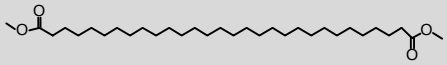
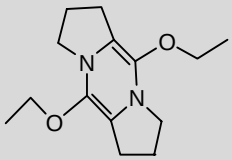
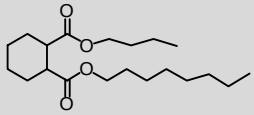
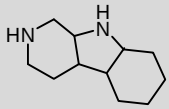
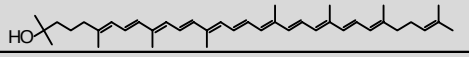


Fig. 1. GC-MS total ion chromatograms of nitrogen-containing compounds (A) The frist method condition by analyses of GC-MS, 21 components were identified from the *Holotrichia diomphalia* bates. (B) The second method condition by analyses of GC-MS, 32 components were identified form the *Holotrichia diomphalia* bates. (C) The thirdly method condition by analyses of GC-MS, 43 components were identified from the *Holotrichia diomphalia* bates. (D) The fourthly method condition by analyses of GC-MS, 14 components were identified from the *Holotrichia diomphalia* bates

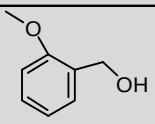
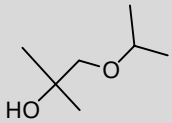
TABLE-2
MASS DATA OF 21COMPOUNDS IDENTIFIED FROM NITROGEN-CONTAINING COMPOUNDS

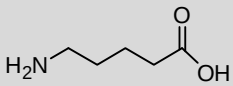
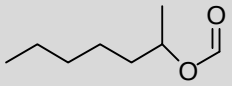
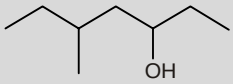
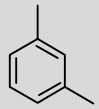
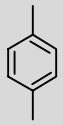
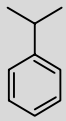
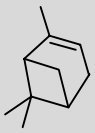
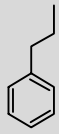
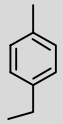
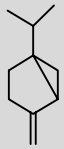
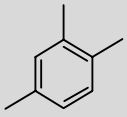
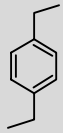
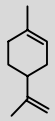
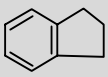
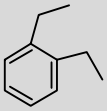
Peak No.	TR (min)	Area (%)	m.f.	Compound name	Structure
1	1.18	10.27	CH_5O_3P	Methylphosphonic acid	
2	1.31	1.08	$C_3H_9BO_3$	Trimethylborate	
3	1.63	1.80	$C_6H_{14}O_2$	2,2-Dimethoxybutane	
4	4.00	3.46	$C_6H_{12}O_2$	*trans*-1,2-Cyclohexanediol	
5	4.10	2.10	$C_{26}H_{54}$	3-Ethyl-5-(2-ethylbutyl)octadecane	
6	4.98	0.56	$C_8H_{18}O_2$	*meso*-2,5-Dimethyl-3,4-hexanediol	
7	5.53	23.71	C_5H_9NO	2-Piperidinone***	
8	6.26	0.61	$C_{27}H_{42}O_4$	2-(3-Acetoxy-4,4,14-trimethylandrost-8-en-17-yl)propanoic acid	
9	7.14	1.68	C_6H_9NOS	1-Ethyl-4-methyl-benzene	
10	8.50 8.88 10.39 10.79	0.57 0.43 0.39 0.77	$C_{30}H_{48}O_2$	(3α,22E)ergosta-5,22-dien-3-ol, acetate**	
11	11.29 11.97	0.7 0.92	$C_{23}H_{20}O_3$	3,6-Dimethoxy-9-(2-phenylethenyl)-fluoren-9-ol	
12	11.46	0.41	$C_{20}H_{14}N_2OS$	2-[4-(2-Hydroxy-phenyl)-thiazol-2-yl]-5-phenyl-penta-2,4-dienenitrile	

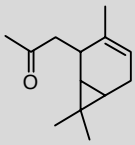
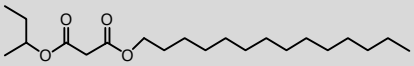
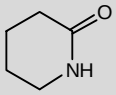
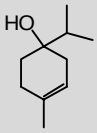
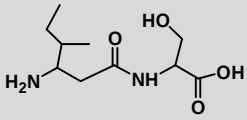
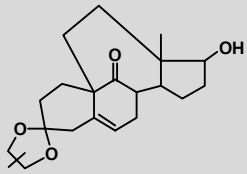
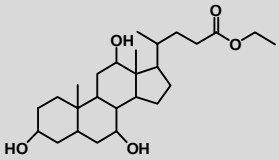
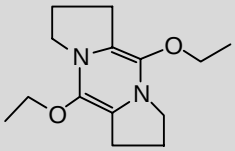
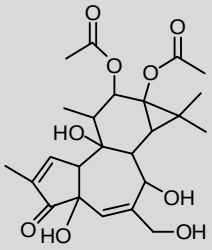
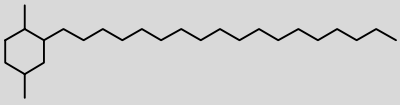
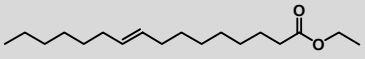
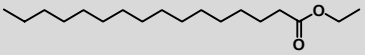
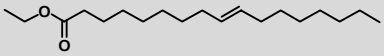
13	11.52 12.19 12.71 13.23 13.61 13.79 14.06	0.96 3.67 0.85 0.66 0.62 0.45 0.80	$C_{24}H_{32}O_9$	[1aR-(1aα,1bα,2α,4aα,7aα,7bα,8α,9α,9aα)]-5*H*-cyclopropa[3,4]bcnz[1,2-e]azulen-5-one,9,9a-*bis*(acetyloxy)1,1a,1b,2,4a,7a,7b,8,9,9adecahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl****	
14	11.83	0.90	$C_{17}H_{22}N_2O$	4,4'-*bis*(Dimethylamino)benzhydrol	
15	12.57 12.85	0.45 0.53	$C_{28}H_{43}NO_6$	5α-Pregnane-3,20α-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]diacetate	
16	14.56	0.47	$C_{27}H_{44}O_3$	(3α,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol***	
17	16.38	0.40	$C_{32}H_{62}O_4$	Triacontanedioic acid dimethyl ester	
18	16.50	22.19	$C_{14}H_{22}N_2O_2$	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine****	
19	16.84	0.73	$C_{20}H_{30}O_4$	1,2-Benzenedicarboxylic acid, butyl octyl ester	
20	17.04	0.90	$C_{11}H_8N_2$	9*H*-Pyrido[3,4-b]indole	
21	18.67	0.39	$C_{40}H_{58}O$	Rhodopin	

****This component had appeared in four methods. ***This component had appeared in three methods. **This component had appeared in two methods.

TABLE-3
MASS DATA OF 32 COMPOUNDS IDENTIFIED FROM NITROGEN-CONTAINING COMPOUNDS

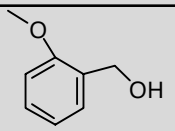
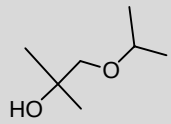
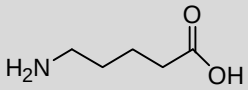
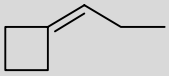
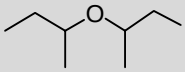
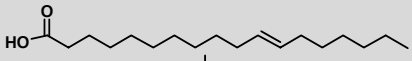
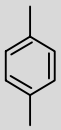
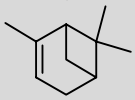
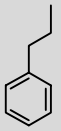
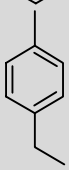
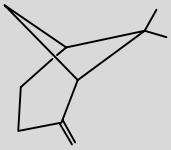
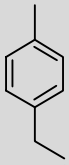
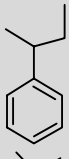
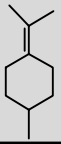
Peak No.	TR (min)	Area (%)	m.f.	Compound Name	Structure
1	1.20	10.39	$C_8H_{10}O_2$	2-Methoxybenzylalcohol***	
2	1.27	0.62	$C_7H_{16}O_2$	2-Propanol,1-isopropoxy-2-methyl**	

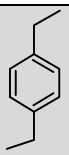
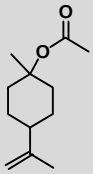
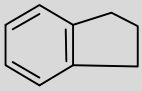
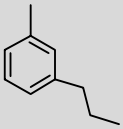
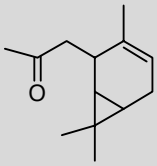
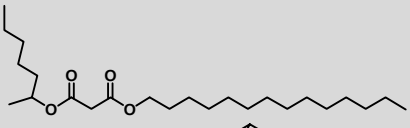
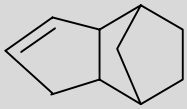
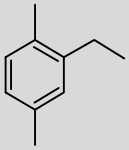
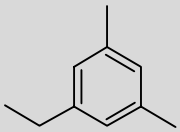
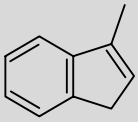
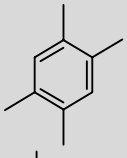
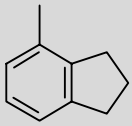
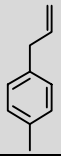
3	1.38	12.73	$C_5H_{11}NO_2$	5-Aminovaleric acid**	
4	1.54	0.20	$C_8H_{16}O_2$	Formic acid, hept-2-yl ester	
5	1.76	1.28	$C_8H_{18}O$	5-Methyl-3-heptanol	
6	2.17	5.48	C_8H_{10}	1,3-Dimethyl-benzene	
7	2.33	2.66	C_8H_{10}	*p*-xylene**	
8	2.57	1.70	C_9H_{12}	(1-Methylethyl)-benzene	
9	2.66	4.02	$C_{10}H_{16}$	α-Pinene	
10	2.82	2.24	C_9H_{12}	*n*-Propylbenzene	
11	2.89	6.12	C_9H_{12}	1-Ethyl-4-methyl-benzene**	
12	3.02	2.95	$C_{10}H_{16}$	4-Methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane**	
13	3.07 3.15	12.46 0.39	$C_{10}H_{16}$	(1S)-Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-**	
14	3.22	5.10	C_9H_{12}	1,2,4-Trimethyl-benzene**	
15	3.55	2.24	$C_{10}H_{14}$	1,4-Diethyl-benzene**	
16	3.62	1.06	$C_{10}H_{16}$	Limonene	
17	3.72	2.81	C_9H_{10}	Indane	
18	3.86	1.35	$C_{10}H_{14}$	*n*-Propylbenzene	

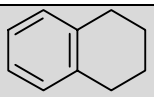
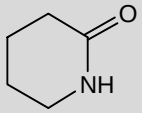
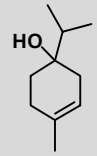
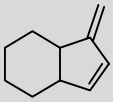
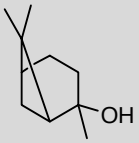
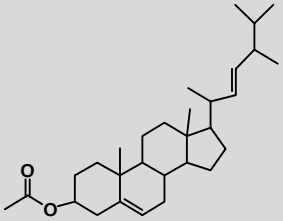
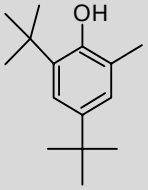
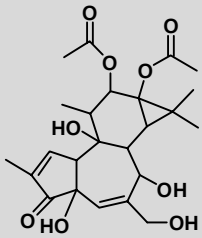
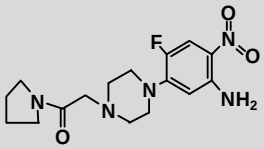
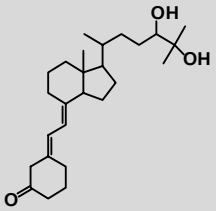
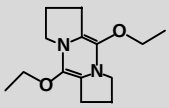
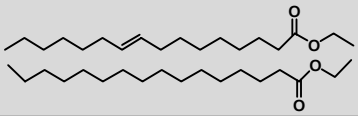
19	3.97	3.15	$C_{13}H_{20}O$	(+)-3-Carene,2-(acetylmethyl)-**	
20	4.07	0.35	$C_{21}H_{40}O_4$	Malonic acid,2-butyltetradecyl ester	
21	4.49	0.79	$C_{10}H_{16}$	Octahydro-4,7-methano-1*H*-indene**	
22	5.56	1.31	C_5H_9NO	2-Piperidinone****	
23	5.65	1.07	$C_{10}H_{18}O$	Terpinen-4-ol**	
24	5.77	0.43	$C_9H_{18}N_2O_4$	Ile-ser	
25	5.94	0.16	$C_{20}H_{28}O_4$	Tetracyclo[7.7.1.0(1,12),0(4,8)] heptadec-11-en-5-ol-17-one, 14,14-ethylenedioxy	
26	10.09	0.16	$C_{26}H_{44}O_5$	Ethyl iso-allocholate	
27	16.52	1.55	$C_{14}H_{22}N_2O_2$	5,10-Diethoxy-2,3,7,8-tetrahydro-1*H*, 6*H*-dipyrrolo[1,2-a:1',2'-d] pyrazine****	
28	12.22 15.99	0.16	$C_{24}H_{32}O_9$	[1aR-(1aα,1bα,2α,4aα,7aα,7bα,8α,9α,9aα)]-5*H*-cyclopropa[3,4]bcnz[1,2-e]azulen-5-one,9,9a-*bis*(acetyloxy)1,1a,1b,2,4a,7a,7b,8,9,9adecahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-****	
29	16.07	0.15	$C_{26}H_{52}$	Cyclohexane,1,4-dimethyl -2-octadecyl	
30	16.96	0.30	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate***	
31	17.22	1.21	$C_{18}H_{36}O_2$	Hexadecanoic acid ethyl ester***	
32	19.22	2.64	$C_{20}H_{38}O_2$	Ethyl oleate	

****This component had appeared in four methods. ***This component had appeared in three methods. **This component had appeared in two methods.

TABLE-4
MASS DATA OF 42 COMPOUNDS IDENTIFIED FROM NITROGEN-CONTAINING COMPOUNDS

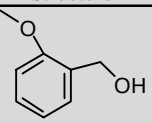
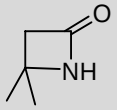
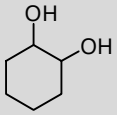
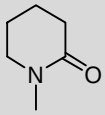
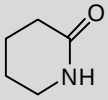
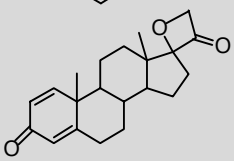
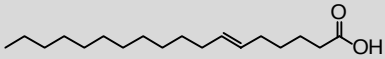
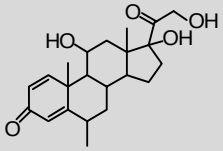
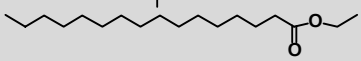
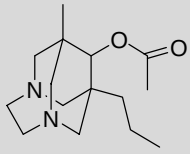
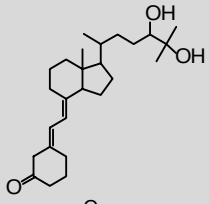
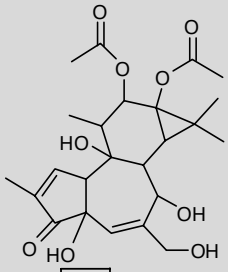
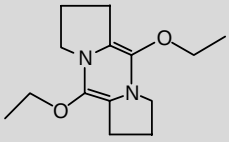
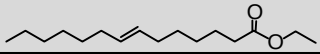
Peak No.	TR (min)	Area (%)	m.f.	Compound name	Structure
1	1.20	7.29	$C_8H_{10}O_2$	2-Methoxybenzylalcohol***	
2	1.27	0.63	$C_7H_{16}O_2$	1-Isopropoxy-2-methyl-2-propanol**	
3	1.38	12.73	$C_5H_{11}NO_2$	5-Aminovaleric acid**	
4	1.67	0.96	C_7H_8	2-Propenylidene-cyclobutene	
5	1.76	1.65	$C_8H_{18}O$	Di-*sec*-butyl ether	
6	1.98	0.25	$C_{18}H_{34}O_2$	*cis*-Vaccenic acid	
7	2.17 2.33	5.52 2.72	C_8H_{10}	*p*-Xylene**	
8	2.66	4.05	$C_{10}H_{16}$	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	
9	2.82	2.43	C_9H_{12}	Propyl-benzene	
10	2.89	6.50	C_9H_{12}	1-Ethyl-4-methyl-benzene**	
11	3.02	3.13	$C_{10}H_{16}$	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)- **	
12	3.08 3.15	12.93 0.47	$C_{10}H_{16}$	(1S)-Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene **	
13	3.22	5.38	C_9H_{12}	1,2,4-Trimethyl-benzene**	
14	3.41	0.54	$C_{10}H_{14}$	(1-Methylpropyl)benzene	
15	3.47	0.34	$C_{10}H_{16}$	1-Methyl-4-(1-methylethylidene)-cyclohexene	

16	3.55	2.45	$C_{10}H_{14}$	1,4-Diethyl-benzene**	
17	3.62	1.18	$C_{12}H_{20}O_2$	1-Methyl-4-(1-methylethenyl)acetate cyclohexanol	
18	3.72	3.07	C_9H_{10}	Indane	
19	3.86	1.37	$C_{10}H_{14}$	1-Methyl-3-propyl-benzene	
20	3.97	3.48	$C_{13}H_{20}O$	2-(acetylmethyl)-(+)-3-Carene,**	
21	4.08	0.35	$C_{24}H_{46}O_4$	Malonic acid 2-heptyltetradecyl ester	
22	4.15	0.24	$C_{10}H_{14}$	Endo-4,7-methanoin-dene,3a,4,5,6,7,7a-hexahydro-	
23	4.22	0.62	$C_{10}H_{14}$	2-Ethyl-1,4-dimethyl-benzene	
24	4.32	0.91	$C_{10}H_{14}$	1-Ethyl-3,5-dimethyl-benzene	
25	4.36	0.58	$C_{10}H_{12}$	1-Methyl-indan	
26	4.49	0.87	$C_{10}H_{16}$	Octahydro-4,7-methano-1*H*-indene**	
27	4.75 4.81	0.38 0.50	$C_{10}H_{14}$	1,2,4,5-Tetramethyl-benzene	
28	5.10	0.66	$C_{10}H_{12}$	2,3-Dihydro-4-methyl-1*H*-indene	
29	5.26	0.76	$C_{10}H_{12}$	1-Methyl-4-(2-propenyl)benzene	

30	5.44	0.59	$C_{10}H_{12}$	1,2,3,4-Tetrahydro-naphthalene	
31	5.55	2.05	C_5H_9NO	2-Piperidinone****	
32	5.65	1.26	$C_{10}H_{18}O$	Terpinen-4-ol**	
33	5.77	0.44	$C_{10}H_8$	1-Methylene-1*H*-indene	
34	5.84	0.16	$C_{10}H_{18}O$	*trans*-2-Pinanol	
35	10.19 11.94	0.13 0.21	$C_{30}H_{48}O_2$	(3α,22E)-Ergosta-5,22-dien-3-ol,acetate**	
36	10.82	0.18	$C_{15}H_{24}O$	4,6-Di(1,1-dimethylethyl)-2-methyl phenol	
37	13.85	0.13	$C_{24}H_{32}O_9$	[1aR-(1aα,1bα,2α,4aα,7aα,7bα,8α,9α,9aα)]-5*H* cyclopropa-[3,4]bcnz-[1,2-e]azulen-5-one,9,9a-*bis*(acetyloxy)1,1a,1b,2,4a,7a,7b,8,9,9adecahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-****	
38	14.43	0.58	$C_{16}H_{22}FN_5O_3$	5-[4-(Pyrrolidin-1-yl)carbonylmethylpiperazin-1-yl]-4-fluoro-2-nitroaniline	
39	15.36	0.15	$C_{27}H_{44}O_3$	(3α,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol***	
40	16.52	1.79	$C_{14}H_{22}N_2O_2$	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine****	
41	16.96	0.36	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate***	
42	17.22	1.47	$C_{18}H_{36}O_2$	Hexadecanoic acid ethyl ester***	

****This component had appeared in four methods. ***This component had appeared in three methods. **This component had appeared in two methods.

TABLE-5
MASS DATA OF 14 COMPOUNDS IDENTIFIED FROM NITROGEN-CONTAINING COMPOUNDS

Peak No.	TR (min)	Area (%)	m.f.	Compound name	Structure
1	1.18	48.65	$C_8H_{10}O_2$	2-Methoxybenzylalcohol***	
2	1.28	2.68	C_5H_9NO	4,4-Dimethylazetidin-2-one	
3	2.73	8.01	$C_6H_{12}O_2$	*cis*-1,2-Cyclohexanediol	
4	3.65	0.94	$C_6H_{11}NO$	1-Methyl-2-piperidinone	
5	3.82	12.81	C_5H_9NO	2-Piperidinone****	
6	5.20	0.23	$C_{21}H_{26}O_3$	3-Oxo-androsta-1,4-dien-17α-spiro-2'-3'-oxo-oxetane	
7	8.43 8.64	0.93 0.22	$C_{18}H_{34}O_2$	6-Octadecenoic acid	
8	10.14	0.96	$C_{22}H_{30}O_5$	Methylprednisolone	
9	12.22 14.72	0.60 3.41	$C_{18}H_{36}O_2$	Hexadecanoic acid ethyl ester****	
10	12.48	0.24	$C_{15}H_{26}N_2O_2$	9-Acetoxy-1-methyl-8-propyl-3,6-diazahomoadamantane	
11	12.58 13.58 13.88	0.15 0.18 0.16	$C_{27}H_{44}O_3$	(3α,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol***	
12	13.45	1.14	$C_{24}H_{32}O_9$	[1aR-(1aα,1bα,2α,4aα,7aα,7bα,8α,9α,9aα)]-5H-cyclopropa[3,4]bcnz[1,2-e]azulen-5-one,9,9a-*bis*(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9adecahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-****	
13	14.01	4.83	$C_{14}H_{22}N_2O_2$	5,10-Diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine****	
14	14.46	0.68	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate***	

****This component had appeared in four methods. ***This component had appeared in three methods. **This component had appeared in two methods.

Using the first GC-MS analysis method, 30 peaks were obtained and 21 components were identified from the *Holotrichia diomphalia* bates, accounting for 84.43 % of total alkali chemical constituents (Fig. 1a). According to the percentage content of comparison, the main constituents were 2-piperidinone (23.71 %); 5,10-diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine (22.19 %); methyl-phosphonic acid (10.27 %); [1aR-(1aα,1bα,2α,4aα,-7aα,7bα,8α,9α,9aα)]-5*H*-cyclopropa[3,4]bcnz[1,2-e]azulen-5-one,9,9a-*bis*(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9adecahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl (8.01 %) (Table-2).

Using the second GC-MS analysis method, 40 peaks were obtained and 32 components were identified from the *Holotrichia diomphalia* bates, accounting for 89.23 % of total alkali chemical constituents (Fig. 1B). According to the percentage content of comparison, the main constituents were (1S)-bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene (12.85 %); 5-aminovaleric acid (12.73 %); 2-methoxybenzyl alcohol (10.39 %); 1-ethyl-4-methyl-benzene (6.12 %); 1,3-dimethyl-benzene (5.48 %); 1,2,4-trimethyl-benzene (5.10 %); α-pinene (4.02 %) (Table-3).

In the third GC-MS analysis condition, 50 peaks were obtained and 43 components were identified from *Holotrichia diomphalia* bates, accounting for 94.44 % of total alkali chemical constituents (Fig. 1C). According to the percentage content of comparison, the main constituents were (1S)-bicyclo-[3.1.1]heptane,6,6-dimethyl-2-methylene; 5-aminovaleric acid (12.73 %); *p*-xylene (8.24 %); 2-methoxybenzyl alcohol (7.29 %); 1-ethyl-4-methyl-benzene (6.50 %) (Table-4).

Using the fourth GC-MS analysis method, 20 peaks were obtained and 14 components were identified from the *Holotrichia diomphalia* bates, accounting for 86.82 % of total alkali chemical constituents (Fig. 1D). According to the percentage content of comparison, the main constituents were 2-methoxybenzylalcohol (48.65 %); 2-piperidinone (12.81 %); *cis*-1,2-cyclohexanediol (8.01 %); 5,10-diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine (4.83 %); hexadecanoic acid ethyl ester (4.01 %) (Table-5).

Alkaloids are a group of naturally occurring chemical compounds, which contain mostly basic nitrogen atoms and have diverse and important physiological effects on humans and other animals. Terpenoid indole alkaloids have been used as anticancer, antimalarial, antihypertensive and hypoglycemic agents for more than 40 years[13]. Monoterpene indole alkaloids constitute a large group of specialised metabolites with many potent pharmaceutical properties, including the antitumoral vinblastine and hypotensive ajmalicine[14]. Moreover, diterpenoid alkaloids showed significant cytotoxic effects against human tumor cell lines, such as A172, A549, HeLa and Raji[15]. The C18-diterpenoid alkaloids demonstrated antiarrhythmic activities[16]. In the present study, we identified 14 nitrogen containing compounds from *Holotrichia diomphalia* Bates, which were detected by GC-MS for the first time. The activities of these nitrogen containing compounds need further study.

Conclusion

In summary, in this study, a total of 79 components were identified from the alkali extract of *Holotrichia diomphalia* bates using four GC-MS analysis methods. 14 nitrogen containing compounds were found for the first time by GC-MS analysis with different methods, including 2-piperidinone; 1-ethyl-4-methyl-benzene; 2-[4-(2-hydroxy-phenyl)-thiazol-2-yl]-5-phenyl-penta-2,4-dienenitrile; 4,4'-*bis*(dimethylamino) benzhydrol; 5α-pregnane-3,20α-dio,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-diacetate; 5,10-diethoxy-2,3,7,8-tetrahydro-1*H*,6*H*-dipyrrolo[1,2-a:1',2'-d]pyrazine; 9*H*-pyrido[3,4-b]indole; 5-aminovaleric acid; Ile-ser; 5-[4-(pyrrolidin-1-yl)carbonylmethylpiperazin-1-yl]-4-fluoro-2-nitroaniline; 4,4-dimethylazetidin-2-one; 1-methyl-2-piperidinone; 9-acetoxy-1-methyl-8-propyl-3,6-diazahomoadama ntane. The study on the alkali chemical constituents provided the test basis for the further development and utilization of *Holotrichia diomphalia* bates resources.

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REFERENCES

1. Q.F. Dong, J.L. Wang, S.F. Zhang, Z. Wang, C.-X. Zhang, H. Gao, H.-M. Zhang and L. Zhang, *Bioresour. Technol*., **99**, 8521 (2008).
2. K. Pei, W. Cao and Q.Q. Guo, *Zhong Yao Cai*, **35**, 357 (2012).
3. Q.-F. Dong, J.-L. Wang, S.-F. Zhang, Z. Wang, C.-X. Zhang, H. Gao, H.-M. Zhang and L. Zhang, *Bioresour. Technol*., **99**, 8521 (2008).
4. N.-S. Kang, S.-Y. Park, K.-R. Lee, S.-M. Lee, B.-G. Lee, D.-H. Shin and S. Pyo, *J. Ethnopharmacol*., **79**, 89 (2002).
5. N.K. Kang, S.Y. Park, K.R. Lee, S.-M. Lee, B.-G. Lee, D.-H. Shin and S. Pyo, *J. Ethnopharmacol*., **79**, 89 (2002).
6. S.Y. Lee, H.J. Moon, S. Kurata, T. Kurama, S. Natori and B.L. Lee, *J. Biochem*., **115**, 82 (1994).
7. T.H. Kwon, S.Y. Lee and J.H. Lee, *Mol. Cells*, **7**, 90 (1997).
8. S. Piao, D. Kim, J. Won Park, B. Leul Lee and N.-C. Ha, *Biochim. Biophys. Acta*, **1752**, 103 (2005).
9. S.Y. Lee, M.Y. Cho, J.H. Hyun, K.M. Lee, K.-I. Homma, S. Natori, S.-I. Kawabata, S. Iwanaga and B.L. Lee, *Eur. J. Biochem*., **257**, 615 (1998).
10. N.A. Ratcliffe, C.B. Mello, E.S. Garcia, T.M. Butt and P. Azambuja, *Insect Biochem. Mol. Biol*., **41**, 747 (2011).
11. G.S. Wang, *J. Ethnopharmacol.*, **26**, 147 (1989).
12. M.T. Goldfarb, A.K. Gupta and W.S. Sawchuk, *Dermatol. Clin*., **9**, 287 (1991).
13. G.D. Goldhaber-Pasillas, Y.H. Choi and R. Verpoorte, *Adv. Bot. Res*., **68**, 233 (2013).
14. B. St-Pierre, S. Besseau, M. Clastre, V. Courdavault, M. Courtois, J. Crèche, E. Ducos, T.D. de Bernonville, C. Dutilleul, G. Glévarec, N. Imbault, A. Lanoue, A. Oudin, N. Papon, O. Pichon and N. Giglioli-Guivarc'h, *Adv. Bot. Res*., **68**, 73 (2013).
15. K. Wada, *Stud. Nat. Prod. Chem*., **38**, 191 (2012).
16. F.P. Wang, Q.H. Chen and X.T. Liang, *Chem. Biol*., **67**, 1 (2009).