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Fatty Acid and Volatile Oil Compositions of *Allomyrina dichotoma* Larvae

− Research Note −

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Abstract

Thirty-two different volatile oils were identified from *Allomyrina dichotoma* (*A. dichotoma*) larvae by gas chromatography/mass spectrometry (GC/MS). The major volatile components were 2,2,4-trimethyl-3-carboxyisopropyl pentanoic acid isobutyl ester (5.83%), phenol,2,6-bis(a,a-dimethyl ethyl)-4-(1-methyl-1-phenylethyl) (5.72%), heptacosane (5.49%) and phenol,2,4-bis(1-methyl-1-phenylethyl) (5.47%). The composition of the fatty acids in *A. dichotoma* larvae was also determined by gas chromatography (GC) and fourteen constituents were identified. Oleic acid (19.13%) was the most abundant fatty acid followed by palmitic acid (12.52%), palmitoleic acid (3.71%) and linoleic acid (2.08%) in 100 g of *A. dichotoma* larvae on a dry weight basis. The quantity of unsaturated fatty acids (64.00%) were higher than that of saturated ones (36.00%). The predominant fatty acids in *A. dichotoma* consist of monounsaturated fatty acid (MUFA, 57.70%) such as oleic acid, myristoleic acid and palmitoleic acid, followed by saturated fatty acids (36.00%) and polyunsaturated fatty acids (PUFA, 6.50%). In particular, the presence of essential fatty acids, such as linoleic (5.30%) and linolenic acid (0.40%) give *A. dichotoma* larvae considerable nutritional and functional value and it may be a useful source for food and/or industrial utilization.

Key words: *Allomyrina dichotoma*, fatty acids, GC, GC/MS, SDE, volatile oils

INTRODUCTION

Allomyrina dichotoma (*A. dichotoma*) is a species of rhinoceros beetle and lives most of its life subterranean (1). *A. dichotoma* is one of many precious oriental insects found in China, Japan, Taiwan and Korea and widely used in traditional medicine as treatments for many diseases, such as the liver and diabetes mellitus (2-4).

As demonstrated by several studies, *A. dichotoma* possesses various biological functions including antineoplastic, anticytotoxic and antioxidant effects (1,5,6). Lectin isolated from *A. dichotoma* has been shown to possess an immunomodulating property through cytokine production (7). Coleoptericin A and B, purified proteins derived from *A. dichotoma*, were proven to have antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (4). These two proteins also exhibited anti-inflammatory effects by inhibiting tumor necrosis factor-α (TNF-α) production (8).

Preliminary analyses suggest that *A. dichotoma* larvae contains significant amounts of unsaturated fatty acids. However, only limited studies on nutritional characteristics, profiles or functions of lipid soluble components in *A. dichotoma* have been reported. Therefore, in the present study, we have analyzed both the composition of the essential oils and fatty acids of *A. dichotoma* larvae by GC and GC/MS analysis.

MATERIALS AND METHODS

Sample preparation

The freeze-dried powder of *A. dichotoma* larvae was provided by World Way Corp. at Chungnam, Korea. Fatty acid composition was determined using gas chromatography (GC) of fatty acid methyl esters (FAME). FAME was prepared according to the method of van-Wijngaarden with minor modifications (9). Briefly, approximately 3 g of sample was weighed into a screw-capped test tube and 5 mL of tetrahydrofuran (THF) was added. The mixture was added to 30 mL of 1 N KOH in ethanol, refluxed at 85°C for 90 min. The sample was then cooled and acidified with HCl to pH 3. Distilled water and diethyl ether were added, mixed and the ether layer was discarded while the aqueous layer was acidified with concentrated HCl. The aqueous layer was backwashed with additional ether and then transferred to a new Kimax tube. The ditheyl ether was removed using a sand bath. About 40 mL of methanol and 0.5 mL of H_2SO_4 was added and the mixture was refluxed for 3 hr. After cooling, water and diethyl ether were added and the FAME extracted into the ether. The diethyl ether was removed using a stream of N_2, and the methyl esters

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were dissolved in methylene chloride for GC analysis (10).

Proximate composition

Proximate composition of *A. dichotoma* larvae was determined by AOAC methods (11). Crude protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method (6.25×N). Crude fat was determined by Soxhlet system. Ash content was determined by dry ashing in a furnace oven at 550°C for 5 hr. The moisture content was determined by drying sample in an oven at 105°C until constant weight was obtained. All analyses were done in triplicate.

GC analysis

The samples were analyzed on a Hewlett-Packard 6890 Series GC (Hewlett-Packard Co., Wilmington, DE, USA), equipped with a flame ionization detector (FID), along with an SP-2560 column (100 m×0.25 mm, 0.2 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The detector temperature was 260°C. The injector was set at 260°C in a split ratio of 50:1. One μL of each sample was injected. The column temperature, after an initial isothermal period of 5 min at 140°C, was increased to 240°C at a rate of 3°C/min and maintained at this temperature for 10 min.

Simultaneous distillation extraction (SDE) of volatile oils

Ten gram sample and 1 L of deionized water was placed in a 2 L round bottom flask and connected to Likens-Nickerson apparatus as described by Schultz et al. (12). Sample was extracted with 100 mL of redistilled n-pentane : diethyl ether (1:1, v/v). Extraction was carried out for 4 hr after the distilled water in the sample flask started to boil. The extract was dried over Na_2SO_4 overnight at -4°C and concentrated to 0.5 mL using a rotary vacuum evaporator (EYELA, N-1100, Tokyo, Japan).

GC/MS analysis

Analysis of samples was performed using a HP-5MS capillary column (30 m×0.25 mm, 0.25 μm, Agilent technologies Inc., Santa Clara, CA, USA) in a GC/MS (5975C, Agilent technologies Inc). Sample was injected into the column and ran using split mode (split ratio = 10:1). The helium carrier gas was programmed to maintain a constant flow rate of 1 mL/min. Oven was initially 80°C for 3 min, then finally raised to 300°C at 4°C/min.

Identification of fatty acids and volatile compounds

Fatty acids were identified by a reference standard mixture FAME (Supelco, Belle fonte, PA, USA) analyzed under the same operating conditions as those employed for FAME of the samples. Qualitative analysis of volatile compounds was carried out by identification of compounds from the mass spectrum library. The spectrum of compounds agreed with that present in the mass spectrum library of NIST 11.

Table 1. Proximate composition (%) of *Allomyrina dichotoma* larvae

Composition	%
Crude protein	38.17±0.48
Crude fat	32.72±0.76
Crude ash	4.14±0.04
Carbohydrate	22.73±0.42
Moisture	2.25±0.01

Data are expressed as mean±SD (n=3) on a dry weight basis.

RESULTS AND DISCUSSION

Proximate analysis in *A. dichotoma* larvae

The results of proximate analysis are shown in Table 1. *A. dichotoma* larvae contained 38.17±0.48% crude protein, 32.72±0.76% crude fat, 4.14±0.04% crude ash, 22.73±0.42% carbohydrates and 2.25±0.01% moisture.

Fatty acids composition of *A. dichotoma* larvae

The chromatograms shown in Fig. 1 correspond to standard fatty acid methyl ester (A) and fatty acids in *A. dichotoma* larvae (B). The peak assignation is shown in Table 2. Fourteen different fatty acids were identified; the yield of total fatty acids in *A. dichotoma* larvae was 39.87% on dry weight basis. Fatty acid data, to be useful for nutritional value, were shown as grams of each fatty acid per 100 grams of *A. dichotoma* larvae, on dry weight basis. Fatty acid composition was dominated by oleic acid (19.13 g/100 g of sample) and palmitic acid (12.52 g/100 g of sample) followed by palmitoleic acid (3.71 g/100 g of sample) and linoleic acid (2.08 g/100 g of sample). Oleic acid ($C_{18:1}$), palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$) and linoleic acid ($C_{18:2}$) combined for more than 90% of total fatty acids in *A. dichotoma* larvae. The predominant fatty acid in *A. dichotoma* was monounsaturated fatty acid (MUFA, 57.7%), such as myristoleic acid, palmitoleic acid and oleic acid, followed by saturated fatty acid (SFA, 36%) and polyunsaturated fatty acid (PUFA, 6.5%). In particular, the presence of essential fatty acids in total fatty acids such as linoleic (5.3%) and linolenic (0.4%) bestow *A. dichotoma* larvae with considerable nutritional value. Our result showed several odd-chain fatty acids such as pentadecanoic acid ($C_{15:0}$), heptadecanoic acid ($C_{17:0}$) and tricosanoic acid ($C_{23:0}$), all of which are rarely detected in analysis of insect fatty acids (13,14). The results give novel information of *A. dichotoma* larvae regarding its nutritional value and shows its possible utilization for food

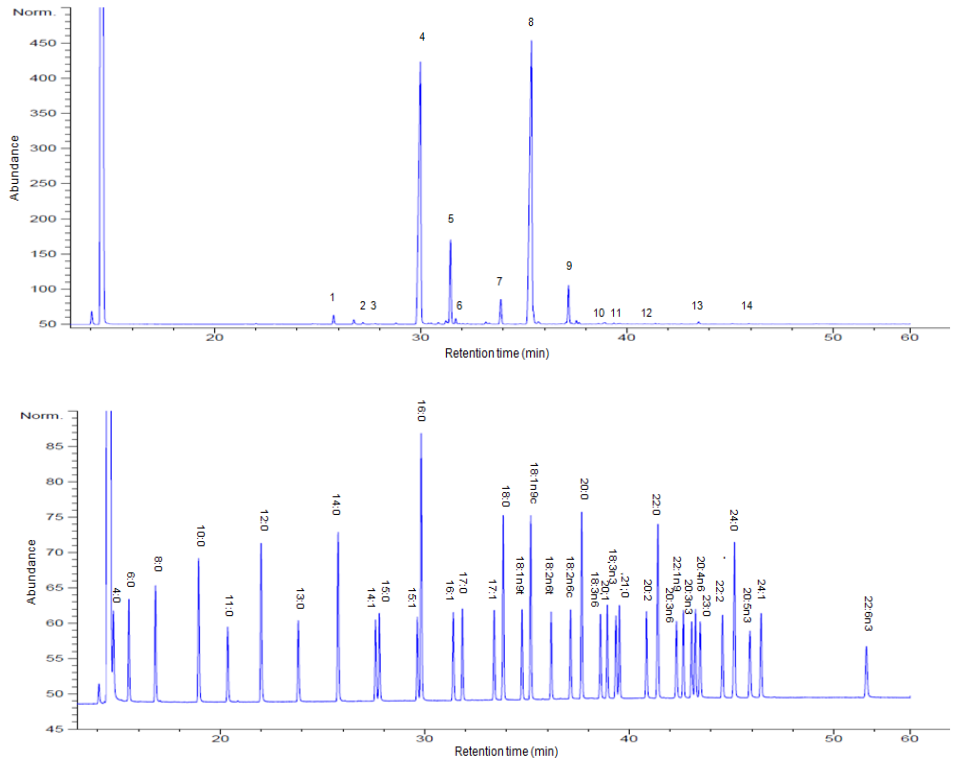

Fig. 1. Gas chromatogram of standard fatty acids (A) and fatty acids of *Allomyrina dichotoma* larvae (B). Four major compounds are indicated as 4, palmitic acid; 5, palmitoleic acid; 8, oleic acid; 9, linoleic acid. For peaks numbers, see Table 2.

Table 2. Fatty acid composition of *Allomyrina dichotoma* larvae

No.	Fatty acid	Mol. formula	Retention time	Amount (g/100 g of sample)
1	Myristic acid	C14:0	25.78	0.45
2	Myristoleic acid	C14:1	27.61	0.13
3	Pentadecanoic acid	C15:0	27.80	0.13
4	Palmitic acid	C16:0	29.99	12.52
5	Palmitoleic acid	C16:1	31.45	3.71
6	Heptadecanoic acid	C17:0	31.87	0.13
7	Stearic acid	C18:0	33.89	0.91
8	Oleic acid	C18:1	35.37	19.13
9	Linoleic acid	C18:2	37.17	2.08
10	γ-Linolenic acid	C18:3	38.62	0.12
11	α-Linolenic acid	C18:3	39.37	0.15
12	cis-11,14-Eicosadienoic acid	C20:2	40.85	0.11
13	Tricosanoic acid	C23:0	43.48	0.20
14	cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5	45.91	0.10

and/or industrial applications.

Volatile constituents of *A. dichotoma* larvae

The results of volatile compounds in *A. dichotoma* larvae analyzed by GC/MS were exhibited in Fig. 2. Thirty two volatile compounds belonging to chemical classes of acids (2), alcohols (5), esters (1), hydrocarbons (16), terpenes (2) and others (6) were tentatively determined (Table 3, 4). Hydrocarbons were detected as the dominant group due to the highest proportion (50.46%). The major compounds belonging to hydrocarbons were heptacosane (5.49%), hexacosane (5.43%), tetracosane (5.20 %), octacosane (4.88%), pentacosane (4.87%) and heneicosane (4.41%).

The alcohol group (17.04%) was characterized as the second major chemical group. The major constituents of

Table 3. Relative content of functional groups of volatile oils in *Allomyrina dichotoma* larvae

Functional groups	Relative peak area percentage (%)	Number of compounds
Acids	6.95	2
Alcohols	17.04	5
Esters	2.43	1
Hydrocarbons	50.46	16
Terpenes	1.78	2
Miscellaneous (including unknowns)	21.34	6
Total	100	32

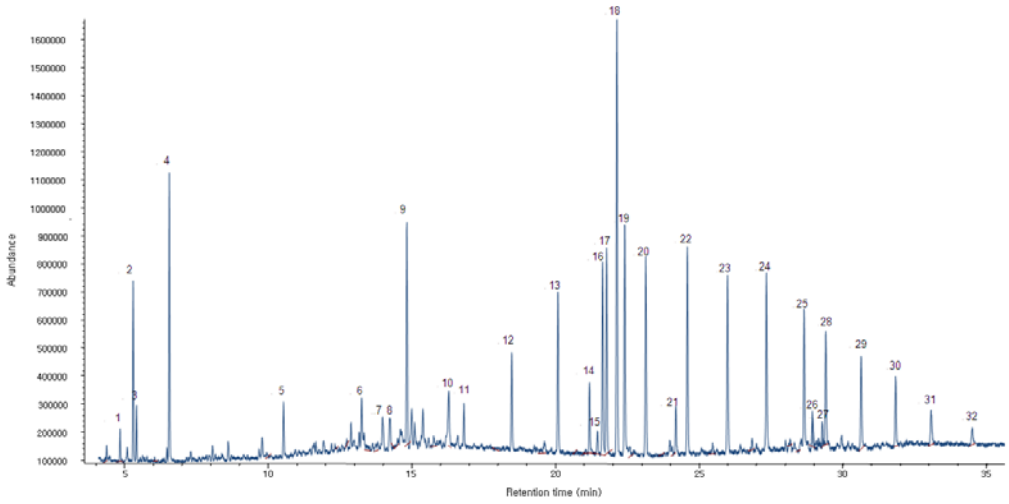

Fig. 2. GC/MS Chromatogram of volatile compounds in *Allomyrina dichotoma* larvae.

Table 4. Volatile compounds of *Allomyrina dichotoma* larvae

No.	Compound name	Retention time	Relative peak area%
1	4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one	4.34	0.66
2	Phenol,2,5-bis(1,1-dimethylethyl)	4.80	3.37
3	Butylated hydroxytoluene	4.91	1.03
4	Pentanoic acid,2,2,4-trimethyl-3-carboxy isopropyl, isobutyl ester	6.06	5.83
5	2,4-Diphenyl-4-methyl-2(E)-pentene	10.03	1.14
6	Hexadecanoic acid, ethyl ester	12.75	1.12
7	Phenol,2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	13.48	1.35
8	10,18-Bisnorabieta-8,11,13-triene	13.73	1.31
9	Phenol,2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	14.33	5.72
10	Ethyl oleate	15.79	2.43
11	Heptadecane	16.32	1.04
12	Eicosane	17.98	2.46
13	Heneicosane	19.59	4.41
14	1,21-Docosadiene	20.69	1.61
15	2,4-Diphenyl-4-methyl-1-pentene	20.96	0.64
16	Pentacosane	21.14	4.87
17	2,4-bis(1-methyl-1-phenylethyl)-phenol	21.28	5.47
18	Unknown	21.64	11.74
19	Unknown	21.91	5.92
20	Hexacosane	22.64	5.43
21	1,13-Tetradecadiene	23.69	1.34
22	Heptacosane	24.09	5.49
23	Tetracosane	25.49	5.20
24	Octacosane	26.85	4.88
25	Triacontane	28.16	3.69
26	Phenol,2,4,6-tris(1-methyl-1-phenylethyl)-	28.46	1.13
27	1-Allyl-5-bromo-6-hydroxypyridazin-6-one	29.30	0.68
28	Tetratriacontane	29.43	3.33
29	Hexadecane, 1-iodo-	30.66	2.79
30	Tetratetracontane	31.85	1.98
31	Octadecane	33.09	1.32
32	Eicosane, 2-methyl-	34.52	0.69

alcohols were phenol,2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) (5.72%), phenol,2,4-bis(1-methyl-phenylethyl) (5.47%), phenol,2,5-bis(1,1-dimethyletyl) (3.37%) and phenol,2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) (1.35%). Acids (6.95%) include pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl (5.83%) and hexadecanoic acid, ethyl ester (1.12%). The main compounds of esters (2.43%), and terpenes (1.78%) were ethyl oleate (2.43%) and 2,4-diphenyl-4-methyl-2(E)-pentene (1.14%), respectively.

In this study, we determined the profile of fatty acids and volatile constituents of *A. dichotoma* larvae. The idea of eating insects is not well received due to their appearance. However, many insects are rich sources of proteins, lipids, minerals and vitamins (15). The present study clearly suggests, for the first time, the composi-

tions of fatty acids and volatile oils in *A. dichotoma* larvae. The overall result gives novel information on the property of *A. dichotoma* larvae as a good source of oil and suggests a potential source of oil for nutritional and medicinal purposes.

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