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THE FAT-SOLUBLE CONSTITUENTS OF *Holotrichia diomphalia* LARVAE

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The subfamily Melolonthidae, which belongs to the Scarabaeoidea family [1], consists of about 5000 species, and the literature reports about 500 species in China [2]. Four species of Melolonthidae traditionally used in China are *Holotrichia diomphalia* Bates, *H. oblila* Fald, *H. sauteri* Moser, and *H. parallela* Motschulsky [3]. *H. diomphalia* Bates is a pest to field crops but has many pharmacological uses in medical science [4], but there are few studies on its chemical composition and pharmaceutical effects.

H. diomphalia larvae have been traditionally used in folk medicine as herbal drugs in China and is one of the most widely used Korean folk medicinal preparations for the treatment of chronic liver cirrhosis, contusion, edema, furuncle, and apoplexy [5]. The immunomodulatory effect of the traditional Chinese medicine extract was also investigated [6]. The petroleum ether extract from *H. diomphalia* grub showed a significant effect on human cervical carcinoma HeLa cells [7].

Potent antibacterial proteins have been isolated from *H. diomphalia* larvae [8], and prophenoloxidase from the hemolymph of *H. diomphalia* larvae has also been purified and characterized [9]. Furthermore, crystals of prophenoloxidase activating factor-II (PPAF-II) were derived from the beetle *H. diomphalia* larvae [10].

Although the chemical composition of the petroleum ether extract of *H. diomphalia* larvae was previously studied, no research has so far been conducted concerning its supercritical fluid extraction (SFE). In the present work the composition of the petroleum ether extract and SFE of *H. diomphalia* larvae is compared for the first time. The chemical composition of the fixed oil of *H. diomphalia* larvae has been systematically reported for the first time in the present study.

The expressed oils of *H. diomphalia* larvae were obtained by petroleum ether extraction and SFE, and then they were analyzed by GC/MS to compare the chemical compositions obtained by these two methods. The chemical and class composition of the oils are presented in Tables 1 and 2, respectively. Twenty-one compounds (representing of 96.3%) in the petroleum ether extract and six compounds (representing of 99.53%) in the extract of SFE were identified (Table 1).

The identified compounds and their percentages in both oils are given in Tables 1 and 2. Regarding the tables, it is evident that the compositions are different qualitatively and quantitatively.

The expressed oils obtained by petroleum ether consisted mainly of olefinic acid (59.94%), alkane acid (33.46%), and esters (1.51%). The major compounds in the petroleum ether extract were elaidic acid (50.82%), hexadecanoic acid (25.64%), (*Z*)-11-hexadecanoic acid (8.98%), pentadecylic acid (2.70%), and stearic acid (2.65%).

In the fatty oil extracted by the SFE method, the main constituents were olefinic acid (63.8%) and alkane acid (30.07%). 9-Octadecenoic acid (59.26%), hexadecanoic acid (26.34%), and (*Z*)-11-hexadecanoic acid (4.54%), were the most abundant components in the SFE oil.

It was also observed that the percentages of the main constituent (9-octadecenoic acid) in the SFE method (59.26%) were somehow different from the major constituent (elaidic acid) obtained by the petroleum ether method (50.82%). This finding may be related to the special procedure conditions in the SFE, which prevent 9-octadecenoic acid from isomerizing to elaidic acid.

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TABLE 1. Chemical Composition of Petroleum Ether Oils and Supercritical Fluid Extraction Oils

Compound	RT (PE)	PE (SFE), %	Compound	RT (PE)	PE (SFE), %
Benzenecarboxylic acid	4.855	0.33	Hexadecanoic acid	11.797	25.64 (26.34)
Acetic acid, phenyl	5.741	0.04	Palmitic acid, ethyl ester	11.924	0.41
Hydrocinnamic acid	6.668	0.06	Heptadecanoic acid	12.15	0.77
n-Tetradecane	7.275	0.11	Elaidic acid	12.977	50.82
n-Pentadecane	8.182	0.14	Stearic acid	13.057	2.65
n-Dodecanoic acid	8.689	0.06	5,8,11-Heptadecatrien-1-ol	13.898	0.30
n-Cetane	9.029	0.14	Arachic acid	14.204	0.25
n-Heptadecane	9.823	0.18	Glycol oleate	16.092	1.10
(*E*)-9-Tetradecenoic acid	10.21	0.14	9-Octadecenoic acid		(59.26)
Myristic acid	10.29	1.39	Ethyl oleate		(4.34)
n-Octadecane	10.576	0.09	9-Octadecenoic acid,		(1.32)
Pentadecylic acid	10.77	2.70 (3.73)	(*Z*)-2-Hydroxy-1-(hydroxymethyl)ethyl ester		
(*Z*)-11-Hexadecanoic acid	11.637	8.98 (4.54)			

RT: retention time; %: calculated from GLC data.

PE: petroleum ether extraction method; SFE: supercritical fluid extraction method.

TABLE 2. Comparison of Oil Class Composition between Petroleum Ether and Supercritical Fluid Extraction

Fatty acid	Content, %		Fatty acid	Content, %	
	PE	SFE		PE	SFE
Unsaturated fatty acid	59.94	63.8	Diolefin	0.66	0
Saturated fatty acid	33.46	30.07	Benzene acid	0.43	0
Total fatty acid	93.4	93.87	Alcohol	0.3	0
Esters	1.51	5.66	Total content	96.3	99.53

PE: petroleum ether extraction method; SFE: supercritical fluid extraction method.

According to Table 2, the percentages of the unsaturated fatty acid and esters extracted by the SFE (63.8% and 5.66%, respectively) were higher than that using the petroleum ether method (59.94% and 1.51%, respectively), and the saturated fatty acid extracted was lower than that in the petroleum ether extract, but the percentages of the total fatty acid extract by the two methods are nearly identical (93.4% for the petroleum ether extract and 93.87% for the SFE). This is in complete agreement with the fact that medical insects contain ditissimus fatty acid. In addition, the ratios of diolefin (0.66%), benzene acid (0.43%), and alcohol extract (0.3%) from the petroleum ether were higher than that in SFE (0%, 0%, and 0%, respectively). This is maybe due to the procedure of liquid-liquid extraction, in which diolefin, benzene acid, and alcohol were extracted from the higher polarity fraction by petroleum ether.

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