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Fatty acids and their esters from *Cicuta virosa* L.

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Abstract: *n*-Hexane and chloroform fractions of aerial parts and roots of *Cicuta virosa* L. were investigated by GC-MS. As a result of the study 25 fatty acids and their esters have been identified. Two unsaturated esters such as linoleic acid ethyl ester (**IX**, 16.66%), and *n*- hexadecanoic acid ethyl ester (**VII**, 10.12%), the fatty acid *n*-hexadecanioc acid (**VI**, 8.10%) made up the bulk of the aerial parts. Four unsaturated esters such as linoleic acid ethyl ester (**IX**, 10.15%), dibutylphthalate (**XII**, 9.55%), *n*-hexadecanoic acid ethyl ester (**VII**, 8.19%) and 9, 12, 15 - octadecatrienoic acid ethyl ester (**X**, 5.9%), two fatty acids as *n*-hexadecanoic acid (**VI**, 8,15%) and 9,12-octadecadienoic acid (**VIII**, 4,5%) predominated in the roots of *Cicuta virosa* L. These known fatty acids and their esters were found for the first time in this plant species.

Keywords: Cicuta virosa L.*,* Umbelliferae, fatty acids, esters GC-MS

INTRODUCTION

Cicuta virosa L. or water hemlock is a member of the genus *Cicuta*, of the Umbelliferae family plants. Six species, native to temperate regions of North America and Asia, are belonged to the genus Cicuta [1]. Only one species *Cicuta virosa* L is widespread and found in Khentei, Khangai, Mongol Daurian, Mongol Altai, Dornot Mongol, Gobi Altai (Bayan-Tuhum-Nuur) and Transaltai Gobi (Ih tsaram) regions of Mongolia [2]. Previously some pharmacological activities such as insecticidal, antioxidative activity and antileukemic properties of *Cicuta virosa* have been investigated [3-5]. In our previous phytochemical study of *Cicuta virosa* L. growing in Mongolia resulted with identification of seven known alkaloids and eleven alcohols [6, 7]. The aim of the present study is to identify the fatty acids and esters in aerial parts and roots of *Cicuta virosa* L. subjecting the *n*-hexane and chloroform fractions by the Gas chromatography-Mass spectral analysis.

EXPERIMENTAL

Gas Chromatography-Mass spectrometry (GC-MS), well equipped with fused silica capillary column 30 m X 0.25 mm X 0.25 µm was used. Coated with HP-5 MS phase and coupled with Hewlett Packard 6890/MSD 5793 A E was used. He with 0.8 ml/min flow rate was used a carrying gas. Program of the GC-MS as follows: temperature 50-300^{o}C at 6 ^{o}/min, isotherm 0-10 min, solvent delay 2.0 min, mass range 50-750. The flame ionization detector was used at T_{inl} 260^{o}C, T_{aux}280^{o}C.

Plant material: Aerial parts of *Cicuta virosa* L. collected (275g) in July, while roots in April (280g), in July (350g) and in October (382g) at Achuutiin gol of Bulgan aimag, Central Mongolia. The plant material was identified by Prof. Ch. Sanchir, Institute of Botany Mongolian Academy of Sciences (MAS), and the voucher specimen is deposited in the Herbarium Fund of the same Institute.

Extraction and isolation: The air-dried and powdered aerial parts (275 g) and roots (280, 350 and 382 g) of *Cicuta virosa* L. were extracted with EtOH (each drug 4 x 3000 ml) at room temperature. The combined ethanol extracts were evaporated to dryness in *vacuo*. The resultant each crude extract was dissolved in distilled H_2O (400 ml) and partitioned between *n*-hexane, $CHCl_3$, ethylacetate and *n*-BuOH, respectively. The *n*-hexane and $CHCl_3$ fractions were concentrated in *vacuo* and gave 26.6 g of dry *n*-hexane fraction and 33.9, 40.0, 37.0 g of dry $CHCl_3$ fractions, respectively. These fractions were subjected to preliminary phytochemical tests.

Gas-Chromatography - Mass Spectrometry analysis: Two µl of *n*-hexane and chloroform fractions of aerial parts and roots from *Cicuta virosa* L. were employed for GC-MS analysis. The molecular weight and structure of compounds of test materials were ascertained by interpretation on mass spectrum of GC -MS using the database of National Institute Standards and Technology (NIST).

RESULTS AND DISCUSSION

The fatty acids and esters composition of aerial parts of *Cicuta virosa* L. isolated from the *n*-hexane and chloroform fractions were summarized in table 1.

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Table 1. Fatty acids and esters of aerial parts of *Cicuta virosa* L.

N	Name of compounds and molecular formula	Content in the aerial parts, %	Retention time, min.	M^+, characteristic ions
1	Ethyl hydrogen succinate, $C_6H_{10}O_4$ (I)	0.28	10.987	146(2%), 101(100%)
2	Benzene acetic acid, $C_8H_8O_2$ (II)	0.18	12.806	136(34%), 91(100%)
3	Tetradecanoic acid, $C_{14}H_{28}O_2$ (III)	0.30	23.141	228(24%), 73(100%)
4	Tetradecanoic acid, ethyl ester, $C_{16}H_{32}O_2$ (IV)	0.36	23.753	256(8%), 88(100%)
5	Pentadecanoic acid ethyl ester, $C_{17}H_{34}O_2$ (V)	0.20	25.462	270(8%), 88(100%)
6	n-Hexadecanoic acid, $C_{16}H_{32}O_2$ (VI)	8.10	26.623	256(40%), 73(100%)
7	n-Hexadecanoic acid ethyl ester, $C_{18}H_{36}O_2$ (VII)	8.12	27.109	284(7.5%), 88(100%)
8	9,12-Octadecadienoic acid (Z,Z)- $C_{17}H_{32}O_2$ (VIII)	4.5	29.305	280(7.5%), 67(100%)
9	Linoleic acid ethyl ester, $C_{20}H_{36}O_2$ (IX)	16.66	29.697	308(1.4%), 81(100%)
10	9,12,15-Octadecatrie-noic acid ethyl ester, $C_{20}H_{34}O_2$ (X)	5.9	29.791	306(2%), 79(100%)
11	n-Hexadecanoic acid butyl ester, $C_{20}H_{40}O_2$ (XI)	2.26	30.026	312(1.4%), 56(100%)
12	Eicosanoic acid ethyl ester, $C_{22}H_{44}O_2$ (XII)	0.39	32.944	340(20%), 88(100%)
13	9,12-Octadecadienoic(Z,Z)-2-hydroxy-1-(hydroxymethyl) ester, $C_{20}H_6O_4$ (XIII)	3.93	36.739	310(5%), 67(100%)

As a result of GC-MS analysis of the *n*-hexane fraction of *Cicuta virosa* 13 known compounds have been identified, as 4 fatty acids and 9 their esters. Of these substances linoleic acid ethyl ester (IX, 16.66%), hexadecanoic acid ethyl ester (VII, 8.12%) and *n*-hexadecanioc acid (VI, 8.10%) have detected as a major one. The fatty acids and their esters compositions of chloroform fractions from roots *Cicuta virosa* L are summarized in Table 2.

Table 2. Fatty acids and their esters of the chloroform fraction from roots of *Cicuta virosa* L.

N	Name of compounds and molecular formula	Content in the roots, %			Retention time, min.	M^+, characteristic ions
		April	July	October		
1	Tetradecanoic acid, $C_{14}H_{28}O_2$ (III)	0.74			23.141	228(24%), 73(100%)
2	Tetradecanoic acid ethyl ester, $C_{16}H_{32}O_2$ (IV)		0.39		23.753	256(8%), 88(100%)
3	Pentadecanoic acid ethyl ester, $C_{17}H_{34}O_2$ (V)		0.67	0.67	25.462	270(8%), 88(100%)
4	n-Hexadecanoic acid, $C_{16}H_{32}O_2$ (VI)	3.60	8.15		26.623	256(40%), 73(100%)
5	n-Hexadecanoic acid ethyl ester, $C_{18}H_{36}O_2$ (VII)		8.19	0.37	27.109	284(7.5%), 88(100%)
6	9,12-Octadecadienoic acid, $C_{17}H_{32}O_2$ (VIII)		4.5	0.27	29.321	280(7.5%), 67(100%)
7	Linoleic acid ethyl ester, $C_{20}H_{36}O_2$ (IX)	4.77	10.15	4.18	29.697	308(1.4%), 81(100%)
8	9,12,15-Octadecatrienoic acid ethyl ester, $C_{20}H_{34}O_2$ (X)	5.90	4.7		29.791	306(2%), 79(100%)
9	Eicosanoic acid ethyl ester, $C_{22}H_{44}O_2$ (XII)	1.65	1.65		32.944	340(1.4%), 88(100%)
10	9,12-Octadecadienoic-2-hydroxy-1-(hydroxymethyl) ester, $C_{20}H_6O_4$ XIII)			2.58	36.739	310(20%), 67(100%)
11	Malic acid, $C_4H_{36}O_5$ (XIV)	2.81			13.543	146(5%), 89(100%)
12	Dibutyl phthalate, $C_{16}H_{22}O_4$ (XV)	9.55			26.654	278(2%), 149(100%)
13	Pentanoic acid, phenyl- methyl ester, $C_{12}H_{16}O_2$ (XVI)		0.31		16.101	278(2%), 91(100%)
14	Phthalic acid, isobutyl-undecyl ester, $C_{12}H_{16}O_2$ (XVII)		0.52		25.119	278(2%), 149(100%)
15	n-Heptadecanoic acid ethyl ester, $C_{19}H_{38}O_2$ (XVIII)		0.33	0.37	28.679	298(14%), 88(100%)
16	Octadecanoic acid ethyl ester, $C_{20}H_{40}O_2$ (XIX)		1.71		30.279	312(20%), 88(100%)
17	Ethyl tetracosanoate, $C_{26}H_{52}O_2$ (XX)		0.34		37.979	396(42%), 88(100%)
18	Octanoic acid, $C_{26}H_{52}O_2$ (XXI)		0.16		11.021	396(2%), 60(100%)
19	Benzene acetic acid, 4-hydroxy -3-methoxy-, $C_{26}H_{52}O_2$ (XXII)			0.19	17.576	396(2%), 137(100%)
20	Ethyl(2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate, $C_9H_{10}O_4$ (XXIII)		1.18		17.623	222(100%), 177 (70%)
21	Ethyl oleate, $C_{20}H_{38}O_2$ (XXIV)		1.18		21.419	312(2%), 55(100%)
22	n-Hexanedioic acid, bis-(2-ethyl hexyl) ester, $C_{22}H_{42}O_4$ (XXV)		0.66		24.681	380(4%), 129(100%)

As a result of GC-MS investigation 22 compounds have been identified including 4 esters and 3 fatty acids, 14 esters and 3 fatty acids, 5 esters and 2 fatty acids in the roots *Cicuta virosa* L. collected in April, July and October respectively. Analysis resulted that in the root harvested in April linoleic acid ethyl ester (4.77%), 9,12,15-octadecatrienoic acid ethyl ester (5.90%) and dibutyl phthalate (9.55%) have dominated. Whereas, in the roots collected in July *n*-hexadecanoic acid (8.15%), *n*-hexadecanoic acid ethyl ester (8.19%), linoleic acid ethyl ester (10.15%) have dominated. Finally, in the roots harvested in October linoleic acid ethyl ester (4.18%) has dominated too. Thus lenoleic acid ethyl ester **IX** prevails in roots collected all vegetation periods.

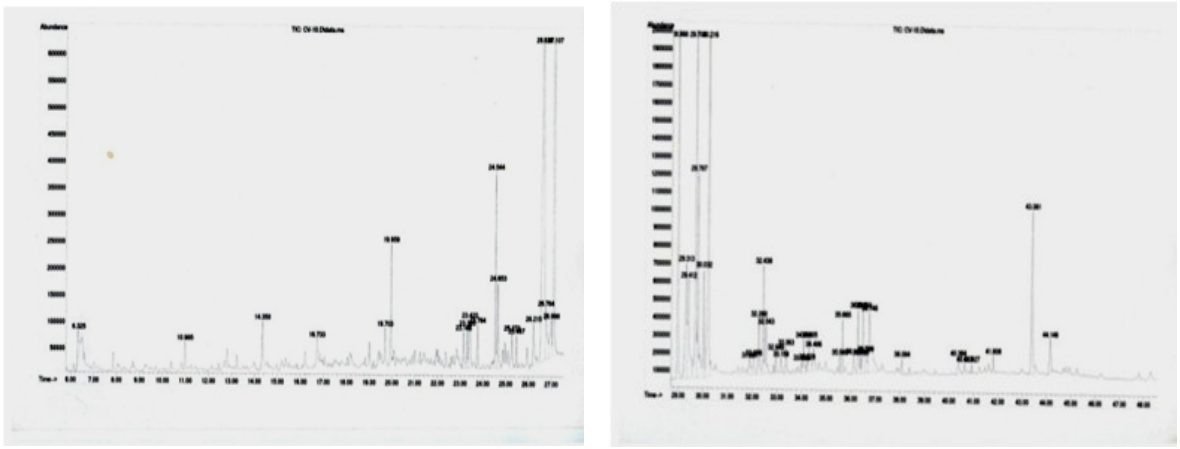

Fig. 1. GC-MS Chromatograms of the chloroform fraction of the roots (July) of *Cicuta virosa* L.

The GC-MS chromatograms of the chloroform fraction of the roots (July) of *Cicuta virosa* L are shown in figure 1. The GC-MS spectral studies revealed the presence of 49 volatile compounds. Amongst them we have been identified thirteen esters (IV, V, VII, IX, XII, XVI, XVII, XVIII, XIX, XX, XXII, XXIV and XXV) and two fatty acids (VI, XXI). Linoleic acid ethyl ester (IX, 10.15%), *n*-hexadecanoic acid ethyl ester (VII, 8.19%), *n*-hexadecanoic acid (VI, 8.15%), 9,12,15-octadecatrienoic acid ethyl ester (X, 5.9%) and 9,12-octadecadienoic acid (VIII, 4.5%) were in the highest quantity among constituents identified in the roots collected in July.

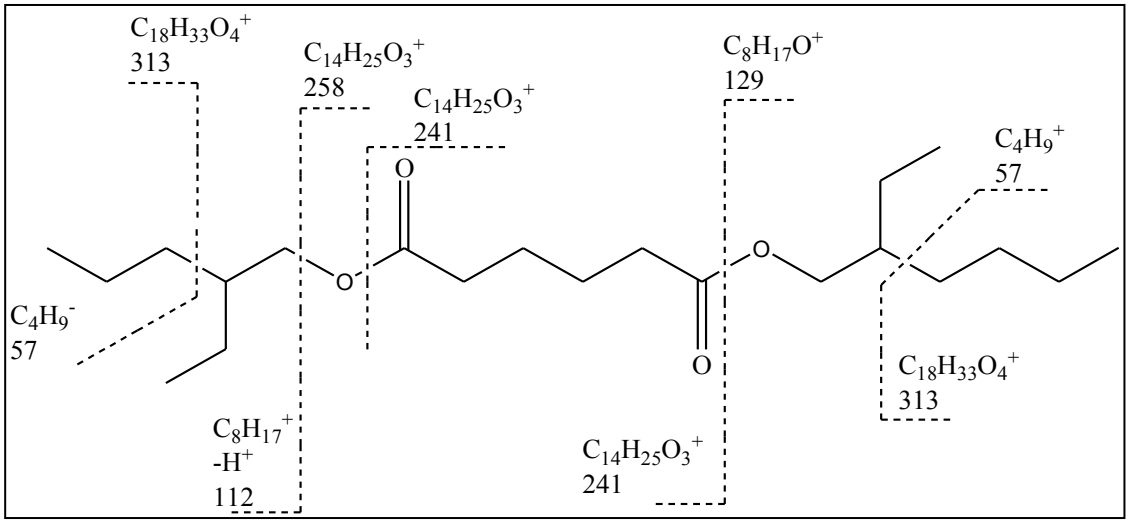

Fig. 2. Schematic representation of mass fragmentation pattern for spectral data of *n*-hexanedioic acid, bis-(2-ethylhexyl) ester (XXV)

Mass fragmentation of *n*-hexanedioic acid, bis-(2-ethylhexyl) ester (XXV) is shown in Figure 2. Molecular formula $C_{22}H_{42}O_4$, m/z 370M^+, 371$(M^+{+}1)^+$ (92.4%), 259(52%), 241(28.4%) 147(44.6%), 129(100%), 113 (15.4%), 71(11.4%).

Unsaturated fatty acids and esters are crucial to every cell in the body for normal growth. Especially, they provide lubricating quality for blood vessels and nerves to keep skin and other tissues young [8].

Table 3. Therapeutic activity of some fatty acids and their esters identified from the *n*-hexane and chloroform fractions of aerial parts and roots of *Cicuta virosa* L.

N	Name of the compounds	Therapeutic activity [9]
1	9,12-Octadecadienoic acid	Hypocholesterolemic, antieczemic, antihistaminic, antiandrogenic, antiarthritic, nematicide, hepatoprotective, anti-inflammatory, cancer preventive, antiacne, 5-alpha reductase inhibitor, anticoronary, antiplasmodial activities
2	Hexadecanoic acid ethyl ester	Lubricant, antiandrogenic, hypocholesterolemic, antioxidant, 5-α-reductase inhibitor, cancer preventive, nematicide activity
3	Benzeneacetic acid	Quinone reductase(QR) induction activity
4	Tetradecanoic acid	Antifungal activity
5	Tetradecanoic acid ethyl ester	Analgesic, ulcerogenic and anti-inflammatory activity
6	Linoleic acid ethyl ester	Hypocholesterolemic, antiarthritic, antihistaminic, antiandrogenic, antieczemic, cancer preventive, hepatoprotective, nematicide, antiacne, 5-alpha reductase inhibitor, anticoronary, antiplasmodial activity
7	9,12,15-Octadecatrienoic acid ethyl ester	Anti-oxidative activity
8	Malic acid	Antiplatelet, antimicrobial, antibacterial activity
9	Dibutyl phthalate	Antagonistic, thyroid receptor (TR) activity
10	Ethyl Oleate	Anti-oxidative activity

Plants and other natural products with medicinal properties can be a source of antioxidant, anti-inflammatory, anti-cancer agents to continue to be relevant in the research areas and leads to the development of traditional drugs.

CONCLUSIONS

In the present study 25 fatty acids and esters have been identified from the *n*-hexane and chloroform fractions from aerial parts and roots of *Cicuta virosa* L. by GC-MS analysis. Two unsaturated esters such as linoleic acid ethyl ester (**IX,** 16.66%) and *n*-hexadecanoic acid ethyl esters (**VII**, 10.12%), one fatty acid *n*-hexadecanioc acid (**VI**, 8.10 are constituted the bulk of the aerial parts, while four unsaturated esters such as linoleic acid ethyl ester (**IX,** 10.15%), dibutylphthalate (**XII,** 9.55 *n*-hexadecanoic acid ethyl ester (**VII**, 8.19%) and 9,12,15-octadecatrienoic acid ethyl ester (**X,** 5.9%), two fatty acids *n*-hexadecanoic acid (**VI**, 8.15%) and 9,12-octadecadienoic acid (**VIII**, 4.5%) were dominated in the roots of *Cicuta virosa* L. These known fatty acids and their esters have been found for the first time in this species.

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