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Investigation of liposoluble constituents from the root of Ligularia narynensis

Abstract. In this work, chemical constituents of the liposoluble portion from the root part of medicinal plant Ligularia narynensis have been determined for the first time. The constituents extracted from the root part of L. narynensis by chloroform were analyzed by GC-MS method. Total fifty nine compounds were separated and their relative contents were determined by area normalization in which the major constituents were n-Hexadecanoic acid (13.44%), 9,12-Octadecadienoic acid (Z,Z)- (11.79%), (3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-b]furan-2(3H)-one (6.90%), Octadecanoic acid (6.17%), .gamma.-Sitosterol (5.50%), 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (4.05%), respectively.

Key words: Ligularia narynensis, chloroform extract, liposoluble constituents, GC–MS.

Introduction

Ligularia is the genus of perennial herbs of the family Compositae, containing about 180 Eurasian species, 17 species growing in mountains of Kazakhstan [1]. Some species in this genus have been used for a long time as folk remedies for their antibiotic, antiphlogistic, and antitumor activities [2-5]. More than 27 Ligularia species have been used as traditional Kazakh and Chinese medicinal herbs for the treatment of fever, pain, inflammation, and intoxication, and to invigorate blood circulation [6-9]. Previous studies confirmed the presence of sesquiterpenes, triterpenes, sinapyl alcohol derivatives, lignans, alkaloids, and steroids in Ligularia [10]. Eremophilane sesquiterpenes are considered as the major secondary metabolites and taxonomic markers of Ligularia genus. More than 500 eremophilane sesquiterpenes have been reported from this genus [11; 12]. Additionally, oplopane sesquiterpenes have been reported from L. narynensis [13].

We have previously reported the chemical investigation results on total bioactive components from root part of L. narynensis such as organic acids (0.64 %), flavonoids (0.52 %), moisture content (5.14 %), total ash (13.24 %), and extractives content (27.7 %). Together with eleven macro-micro elements from the ash of plant (main contents: K (2214.13 µg/mL), Ca (391.31 µg/mL), and Fe (311.73 µg/mL) were determined by using method of multi-element atomic emission spectral analysis. And same time, twenty amino and eight fatty acids were analyzed from this plant. The results showed that major contents of amino acids were glutamate (2452 mg/100g), aspartate (1238 mg/100g) and alanine (748 mg/100g), as well as in fatty acids were oleic (33.5 %) and linoleic (41.2 %) acids, respectively [14].

In our continuously study of the plant, fifty nine liposoluble constituents in chloroform extract from medicinal plant, L. narynensis have been identified by GC-MS methods which grown in Almaty region of Kazakhstan for the first time.

Materials and methods

Plant material. The root part of plant L. narynensis was collected in September 2017 from Butakovsky gorge of the Zailiysky Alatau Mountains of Almaty region and identified by Dr. Alibek Ydyrys. Specimens (1217-BH-17) were deposited in the Herbarium of Laboratory Plant Biomorphology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. The air dried roots of L. narynensis were cut into small pieces and stored at room temperature.

Extraction and isolation. The air-dried roots of L. narynensis (100 g) were pulverised and extracted with 70% ethyl alcohol (1:1) three times (seven days each time) at room temperature. After evaporation of the solvent under reduced pressure, the residues were
mixed and suspended in water and then successively partitioned with hexane, chloroform, EtOAc, and n-BuOH to afford the corresponding extracts. The obtained chloroform extract (173 mg) was analyzed by GC-MS method.

**Experimental part.** To determine the liposoluble constituents’ composition was made erenow of the raw material used GC/MS device. The root part of *L. narynensis* were analyzed by Electron Impact Ionization (EI) method on Agilent 7890A-5975C GC-MS (Gas Chromatograph coupled to Mass Spectrometer) fused silica capillary column (30m x 0.25mm; 0.25 μm film thickness), coated with HP-5MS were utilized. The carrier gas was helium (99.999 %). The column temperature was programmed from 50°C (held for 10 min), with 10°C/min rate program to increase temperature to 300°C.

The latter temperature maintained for 40 min (Acquisition parameters full scan; scan range 30-1000 amu). The injector temperature was 310°C. Injection: with a 1 μl. Detector ion source (EI-70eV). Samples were injected by splitting with the split ratio 5:1.

Identification of the compounds: Identification of compounds was done by comparing the NIST and Wiley library data of the peaks and mass spectra of the peaks with those reported in literature. Percentage composition was computed from GC peak areas on HP-5MS column without applying correction factors.

**Figure 1 – Total ionization chromatogram of chloroform extract from the root part of *L. narynensis***
Results and discussion

The liposoluble constituents extracted from the root part of *L. narynensis* by chloroform were analyzed by GC-MS method. The yield from whole herbs of *L. narynensis* was found to be 0.173%. Total fifty-nine compounds were separated and their relative contents were determined by area normalization in which the major constituents were n-Hexadecanoic acid (13.44 %), 9,12-Octadecadienoic acid (Z,Z)-(11.79 %), (3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4a,5,6,7,9,9a-octahydroazuleno[6,5-b]furan-2(3H)-one (6.90 %), Octadecanoic acid (6.17 %), gamma.-Sitosterol (5.50 %), 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) - (4.05 %), 3,6,6-Trimethylundecane-2,5,10-trione (3.94 %), Achillicin (3.86 %), 3-Pyrazolidinone, 1-phenyl- (3.44 %), 2-Pentadecen-4-yne, (Z)- (2.93 %), Linoelaidic acid (2.70 %), Alloaromadendrene (2.34 %), 1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butanedienyl)- (1.99 %). GC-MS chromatogram of the liposoluble constituents from root part of *L. narynensis* was presented in Figure 1. Table 1 report the composition of the liposoluble constituents of *L. narynensis*.

According to the report the n-Hexadecanoic acid (13.44 %) might function as an anti-inflammatory agent [15]. Furthermore, this acid has an inhibitory activity. These findings further confirm the medicinal value of plant and its anticancer cytotoxic potential [16; 17]. And second major liposoluble constituent 9,12-Octadecadienoic acid (Z,Z)-(11.79 %) have been reported to have antimicrobial activity [18]. (3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-b]furan-2(3H)-one (6.90 %) also called as columellarin has a termitecidal activity [19].

Table 1 – The liposoluble constituents from the root part of *L. narynensis*

| Peak No. | Constituents                        | t<sub>R</sub> (min) | Molecular Formula | Structure | MW  | Content (%) |
|---------|-------------------------------------|---------------------|------------------|----------|-----|-------------|
| 1       | t-C<sub>9</sub>H<sub>17</sub>COCH<sub>2</sub>CH<sub>3</sub> | 4.17                | C<sub>10</sub>H<sub>16</sub>O<sub>2</sub> | ![Structure](image1) | 144 | 0.54        |
| 2       | Ethane, hexachloro-                 | 6.63                | C<sub>6</sub>Cl<sub>6</sub>         | ![Structure](image2) | 234 | 0.09        |
| 3       | 2-Methoxy-4-vinylphenol             | 9.84                | C<sub>9</sub>H<sub>12</sub>O<sub>2</sub> | ![Structure](image3) | 150 | 0.34        |
| 4       | Phenol, 2,6-dimethoxy-              | 10.22               | C<sub>8</sub>H<sub>10</sub>O<sub>3</sub> | ![Structure](image4) | 154 | 0.32        |
| 5       | Phenol, 4-(ethoxymethyl)-           | 10.55               | C<sub>10</sub>H<sub>12</sub>O<sub>2</sub> | ![Structure](image5) | 152 | 0.19        |
| 6       | Vanillin                            | 10.82               | C<sub>9</sub>H<sub>8</sub>O<sub>3</sub>  | ![Structure](image6) | 152 | 0.24        |
| 7       | Diphenyl ether                      | 11.02               | C<sub>10</sub>H<sub>10</sub>O         | ![Structure](image7) | 170 | 0.74        |
|Peak No.| Constituents                                                                 | $t_r$ (min) | Molecular Formula | Structure | MW  | Content (%) |
|--------|-----------------------------------------------------------------------------|-------------|-------------------|-----------|-----|-------------|
|8       | 2H-1-Benzopyran-2-one, 8-hydroxy-                                           | 12.81       | C$_{7}$H$_{6}$O$_{3}$ | ![structure](image) | 162 | 0.18        |
|9       | 2,3,5,6-Tetrafluoroanisole                                                  | 12.91       | C$_{7}$H$_{4}$F$_{4}$O | ![structure](image) | 180 | 0.95        |
|10      | (1,4-Dimethylpent-2-ethyl)benzene                                            | 13.40       | C$_{15}$H$_{34}$  | ![structure](image) | 174 | 0.36        |
|11      | Benzaldehyde, 4-hydroxy-3,5-dimethoxy-                                      | 13.89       | C$_{7}$H$_{10}$O$_{4}$ | ![structure](image) | 182 | 0.60        |
|12      | 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-buteryl)-1,5,5-trimethyl-     | 14.22       | C$_{15}$H$_{22}$O$_{3}$ | ![structure](image) | 226 | 0.62        |
|13      | (4R,4aR)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,7-hexahydronaphthalene | 14.38       | C$_{15}$H$_{22}$  | ![structure](image) | 202 | 0.78        |
|14      | (E)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol                                  | 14.49       | C$_{7}$H$_{10}$O$_{3}$ | ![structure](image) | 194 | 0.24        |
|15      | beta-Vatirenene                                                             | 14.59       | C$_{15}$H$_{22}$  | ![structure](image) | 202 | 0.19        |
|16      | Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-                                 | 14.74       | C$_{11}$H$_{12}$O$_{3}$ | ![structure](image) | 196 | 0.36        |
|17      | Coniferyl aldehyde                                                          | 14.81       | C$_{15}$H$_{16}$O$_{3}$ | ![structure](image) | 178 | 0.42        |
| Peak No. | Constituents | \( t_r \) (min) | Molecular Formula | Structure | MW | Content (%) |
|---------|--------------|-----------------|------------------|----------|----|-------------|
| 18      | 3-Pyrazolidinone, 1-phenyl- | 14.93 | C_{9}H_{10}N_{2}O | ![Structure](image) | 162 | 3.44 |
| 19      | 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-buteryl)- | 15.35 | C_{16}H_{12}O_2 | ![Structure](image) | 222 | 0.62 |
| 20      | (S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-ene | 15.47 | C_{16}H_{13}O | ![Structure](image) | 222 | 0.35 |
| 21      | Benzene, 1,4-diethyl-2-methyl- | 16.26 | C_{25}H_{16} | ![Structure](image) | 148 | 0.41 |
| 22      | 2H-Benz[e]inden-3-ol, 3,3a,4,5-tetrahydro-3a-methyl-, (3S-cis)- | 16.57 | C_{24}H_{20}O | ![Structure](image) | 200 | 0.21 |
| 23      | Isopulegol | 16.81 | C_{10}H_{18}O | ![Structure](image) | 154 | 0.17 |
| 24      | Hexadecanoic acid, methyl ester | 17.06 | C_{17}H_{34}O_2 | ![Structure](image) | 270 | 0.16 |
| 25      | trans-Sinapaldehyde | 17.28 | C_{11}H_{12}O_4 | ![Structure](image) | 208 | 0.26 |
| 26      | n-Hexadecanoic acid | 17.52 | C_{13}H_{26}O_2 | ![Structure](image) | 256 | 13.44 |
| 27      | Hexadecanoic acid, ethyl ester | 17.74 | C_{16}H_{34}O_2 | ![Structure](image) | 284 | 1.68 |
| 28      | 1H-Indene, 2-butyl-5-hexyloctahydro- | 18.48 | C_{15}H_{36} | ![Structure](image) | 264 | 0.30 |
| 29      | Pallensin | 18.68 | C_{13}H_{26}O_4 | ![Structure](image) | 264 | 1.43 |
### Table 1: Liposoluble Constituents from the Root of *Ligularia narynensis*

| Peak No. | Constituents                                                                 | $t_\text{R}$ (min) | Molecular Formula | Structure                  | MW  | Content (%) |
|----------|------------------------------------------------------------------------------|--------------------|-------------------|----------------------------|-----|-------------|
| 30       | (3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazulen| 18.87              | C$_{15}$H$_{20}$O$_2$ | ![Structure](image)         | 250 | 6.90        |
| 31       | Achillicin                                                                   | 18.99              | C$_{15}$H$_{22}$O   | ![Structure](image)        | 246 | 3.86        |
| 32       | 9,12-Octadecadienoic acid (Z,Z)-                                             | 19.12              | C$_{16}$H$_{26}$O$_2$ | ![Structure](image)        | 280 | 11.79       |
| 33       | Octadecanoic acid                                                            | 19.34              | C$_{18}$H$_{32}$O$_2$ | ![Structure](image)        | 284 | 6.17        |
| 34       | Linoelaidic acid                                                            | 19.42              | C$_{18}$H$_{32}$O$_2$ | ![Structure](image)        | 280 | 2.70        |
| 35       | Alloaromadendrene                                                            | 19.68              | C$_{16}$H$_{26}$     | ![Structure](image)        | 204 | 2.34        |
| 36       | 1H-Indene-1,3(2H)-dione, 2-(2-methylbutylidene)-                             | 19.81              | C$_{16}$H$_{26}$O$_2$ | ![Structure](image)        | 214 | 1.98        |
| 37       | Benzo[f]quinoline, 2-methyl-                                                | 20.11              | C$_{15}$H$_{11}$N    | ![Structure](image)        | 193 | 1.64        |
| 38       | 1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-              | 20.30              | C$_{16}$H$_{26}$O    | ![Structure](image)        | 206 | 1.99        |
| 39       | Longifolenaldehyde                                                          | 20.41              | C$_{15}$H$_{26}$O    | ![Structure](image)        | 220 | 0.88        |
| 40       | 11,13-Dimethyl-12-tetradecen-1-ol acetate                                   | 20.48              | C$_{16}$H$_{34}$O$_2$ | ![Structure](image)        | 282 | 0.97        |

Continuation of table 1

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| Peak No. | Constituents                                                                 | $t_r$ (min) | Molecular Formula | Structure | MW  | Content (%) |
|---------|------------------------------------------------------------------------------|-------------|-------------------|-----------|-----|-------------|
| 41      | 3,6,6-Trimethylundecane-2,5,10-trione                                        | 20.56       | C$_{14}$H$_{24}$O$_3$ | ![Structure](image1) | 240 | 3.94        |
| 42      | (-)-Isolongifolol, methyl ether                                              | 20.64       | C$_{16}$H$_{28}$O   | ![Structure](image2) | 236 | 0.96        |
| 43      | 2-Pentadecen-4-yne, (Z)-                                                     | 20.81       | C$_{15}$H$_{26}$    | ![Structure](image3) | 206 | 2.93        |
| 44      | Cedrol                                                                       | 20.95       | C$_{15}$H$_{26}$O   | ![Structure](image4) | 222 | 0.79        |
| 45      | 2-Heptyne, 7-bromo-                                                          | 21.05       | C$_{17}$H$_{36}$Br  | ![Structure](image5) | 174 | 1.15        |
| 46      | 2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7. beta.,8a.beta.)- | 21.23       | C$_{14}$H$_{26}$O   | ![Structure](image6) | 208 | 0.85        |
| 47      | 1,4-Dioxaspiro[4.5]decan-8-one, 7-(hydroxymethyl)-                           | 21.70       | C$_{15}$H$_{24}$O$_4$ | ![Structure](image7) | 186 | 0.26        |
| 48      | Bis(2-ethylhexyl) phthalate                                                  | 22.31       | C$_{24}$H$_{38}$O$_4$ | ![Structure](image8) | 390 | 1.05        |
| 49      | Pyridine-3-carboxamide, oxime, N-(2-trifluoromethyl)phenyl)-                 | 25.03       | C$_{13}$H$_{10}$F$_3$N$_2$O | ![Structure](image9) | 281 | 0.11        |
| 50      | Campesterol                                                                  | 27.75       | C$_{28}$H$_{48}$O   | ![Structure](image10) | 400 | 0.61        |
| 51      | Stigmasterol                                                                 | 28.08       | C$_{29}$H$_{48}$O   | ![Structure](image11) | 412 | 1.26        |
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**Conclusion**

In summary, the investigation of the liposoluble constituents from roots of *L. narynensis* of Kazakhstan have been made for the first time. As the results of this study fifty nine liposoluble compounds were quantified from medicinal plant in which the major constituents were n-Hexadecanoic acid (13.44%), 9,12-Octadecadienoic acid (Z,Z)- (11.79%), (3aR,4aS,5R,9aS)-5,8-Dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-b]furan-2(3H)-one (6.90%), Octadecanoic acid (6.17%), .gamma.-Sitosterol (5.50%), 2(1H)Naphthalenone, 3,5,6,7,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (4.05%), respectively. Presence of these bioactive constituents, may indicate that the plant extract has anti-inflammatory, antimicrobial and anticancer activities. From the results we can estimate that *L. narynensis* extracts poetically useful in medicine. Further and comprehensive investigation is scheduled to be implemented in the next research stage.

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