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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE *LEVISTICUM OFFICINALE* W.D.J. KOCH ESSENTIAL OIL

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Abstract. The chemical composition of industrially obtained *Levisticum officinale* W.D.J. Koch (lovage) essential oil of Moldovan origin was analysed by means of chromatographic (GC-MS) and spectral (IR, ^{1}H and ^{13}C NMR) methods. According to gas chromatography-mass spectrometry analysis of the studied essential oil, thirty-two known and two unknown constituents were identified. The main components of *L. officinale* essential oil are monoterpenic hydrocarbons, which make up to 53.50% of the total number of components. *L. officinale* essential oil is also characterized by a high content of oxygenated monoterpenes (alcohols, cetones and esters), which reaches up to 33.60%. For the first time the presence of 6-butyl-cyclohepta-1,4-diene (0.56%) and 7-formyl-4-methyl-cumarine (0.15%) in lovage essential oil is reported. Antibacterial and antifungal activities of mentioned oil were evaluated *in vitro* on five strains of microorganisms. It was found that lovage volatile oil (*L. officinale*) exhibits high antibacterial and antifungal properties in the range of concentrations 0.015-0.030%.

Keywords: *Levisticum officinale*, essential oil, GC-MS analysis, antibacterial activity, antifungal activity.

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Introduction

Levisticum officinale W.D.J. Koch (Lovage) is a perennial, aromatic species belonging to *Apiaceae* family. It is native to Southwest Asia and Southern Europe [1,2], naturalized in many temperate regions and nowadays being cultivated throughout the world. All parts of the plant (seeds, leaves and roots) are strongly aromatic, being widely used in food, pharmaceutical, perfume and tobacco industries [3-5]. This plant has been used over the centuries as a traditional medicinal remedy that has spasmolytic, diuretic and carminative activities [6,7].

Many scientific studies showed antibacterial and antibiotic-potentiation [8,9], anti-inflammatory, antitumor, antioxidant [5,10,11], hepatoprotective [12], neuroprotective [13], spasmolytic and diuretic [14,15], nephroprotective and lytolytic [16] effects of *L. officinale*. These important therapeutic and flavouring properties are mainly attributed to the content of bioactive secondary metabolites, especially polyacetilenes, essential oil, polyphenols (flavonoids, phenolic acids), coumarins (furano- and pyranocoumarins), saponins and alkaloids [11,17-20]. The composition of the essential oil of *L. officinale* has been studied extensively and over 190 compounds were reported in its root, seed and leaf oil [6,9]. The main constituents of the essential oil are phthalides (butylidene-, dihydrobutyliden-, butyl- and propylidenephthalide; sedanonic anhydride; *cis*- and *trans*-ligustilide; senkyunolide; isosenkyunolide, validene-4,5-dihydrophthalide), terpenoids (α- and β-pinene, α- and β-phellandrenes, γ-terpinene, carvacrol, eugenol, and α-terpineol) and carboxylic acids (butyric, isovaleric, maleic, and angelic acids) [4,21-25].

The purpose of this paper is to establish the chemical composition and evaluate the antimicrobial activity of the essential oil of *L. officinale* cultivated industrially in climatic and soil conditions specific to Republic of Moldova.

Experimental

Materials

The sample of *L. officinale* essential oil was offered by the Moldovan-French company "*Molsalvia*" Pervomaysk village, Causeni district.

The essential oil (n^{20}_D= 1.4810) was obtained industrially by hydrodistillation of the aerial part of *L. officinale* collected in July of 2017.

Methods

The *GC-MS analysis* of the *L. officinale* essential oil was carried out on an Agilent Technologies 7890A system with 5975C Mass-Selective Detector (GC-MSD) equipped with split-splitless injector (split, 250°C, split ratio 1:50, 1 μL) and HP-5 ms capillary calibrated column (30 m x 0.25 mm x 0.25 μm); the carrier gas: helium 1.1 mL/min; oven: 70°C-2 min, 5°C/min-200°C-20/min-300°C/5 min; MSD in scan 30-300 amu, 15 min, 30-450 amu, solvent delay 3 min 40 s.

IR spectra were recorded on a Spectrum-100FT-IR spectrometer using the attenuated total reflection technique.

^1H and ^{13}C NMR spectra were acquired in $CDCl_3$ on a Bruker Avance DRX 400 spectrometer (400 MHz). All chemical shifts are quoted on the δ-scale in ppm and referred to residual $CHCl_3$ (δ_H at 7.26 ppm) and as $CDCl_3$ (δ_C at 77.00 ppm), respectively.

Antimicrobial activity assays

As test-microorganisms for the evaluation of the antimicrobial activity of lovage essential oil (*L. officinale*) were used the following: non-pathogenic Gram-positive and Gram-negative strains of *Bacillus subtilis* CNMN BB-01 and *Pseudomonas fluorescens* CNMN-PFB-01, respectively; phytopathogenic strains of *Xanthomonas campestris*, *Erwinia amylovora*, *Erwinia carotovora* and a fungus strain of *Candida utilis.*

For testing, the successive double dilution method was used. For this, at the initial stage, 1 mL of peptone broth for test bacteria and Sabouraud broth for test candida was introduced into a series of 10 tubes. Subsequently, 1 mL of the analysed preparation was dropped into the first test tube. Then, the obtained mixture was pipetted, after which 1 mL of it was transferred to the next tube, so the procedure was repeated until the tube no. 10 of the series. Thus, the concentration of the initial preparation decreased 2-fold in each subsequent tube.

At the same time, 24 hour test-microorganisms cultures were prepared.

Initially, suspensions of test microorganisms were prepared with optical densities of 2.0 for tested bacteria and 7.0 for fungus according to the McFarland index. Subsequently, 1 mL of the obtained microbial suspension was dropped in a tube containing 9 mL of sterile distilled water. The content of the tube was mixed, after which 1 mL was transferred to the tube no. 2 of the 5-tube series containing 9 mL of sterile distilled water.

From the 5-th tube of the series were taken 0.1 mL of the microbial suspension, which represent the seeded dose and added to each tube with titrated preparation. Subsequently, the tubes with titrated preparation and the seeded doses of the microorganisms were kept in the thermostat at 35°C for 24 hours. On the second day, a preliminary analysis of the results was made. The last tube from the series in which no visible growth of microorganisms has been detected is considered to be the minimal inhibitory concentration (MIC) of the preparation.

For the estimation of the minimal bactericidal and fungicidal concentrations (MBC, MFC), the contents of the test tubes with MIC and with higher concentrations are seeded on peptone and Sabouraud agar from Petri dishes with the use of the bacteriological loop. The seeded dishes are kept in the thermostat at 35°C for 24 hours. The concentration of the tested preparation that does not allow the growth of any colony of microorganisms is considered to be the minimal bactericidal and fungicidal concentrations of the preparation [26].

Results and discussion

Lovage chemical composition evaluation

According to gas chromatography-mass spectrometry analysis of studied essential oil thirty two known and two unknown constituents were identified (Figure 1).

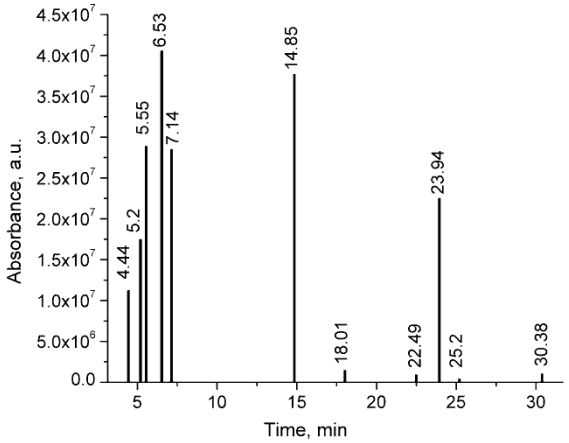


Figure 1. GC chromatogram of *L. officinale* essential oil.

It must be mentioned that the main components of *L. officinale* essential oil are monoterpenic hydrocarbons which make up to 53.50% of the total number of components (Table 1). Of these may be mentioned

β-phellandrene (22.39%), *β*-mircene (8.66%), *γ*-terpinene (6.84%), (*Z*)-*β*-ocimene (3.51%) and sabinene (3.39%) which are evidenced by a higher content.

As well, *L. officinale* essential oil is characterized by a high content of oxygenated monoterpenes (alcohols, cetones and esters) which reaches up to 33.60%. Of these, should be mentioned *α*-terpinyl acetate (30.99%), *α*-terpineol (1.11%) and geranyl acetate (0.55%). From the sesquiterpenoid series only germacrene D (0.29%) was identified.

It is significant the presence of some phtalides like (*Z*)-3-buthylidene phtalide **29** (RT= 22.428, 0.23%), (*Z*)-ligustillide **31** (RT= 23.942, 11.19%) and (*E*)-ligustillide **32** (RT= 25.201, 0.20%) (Figure 2).

For the first time, the presence of 6-butyl-cyclohepta-1,4-diene **18** (0.56%) and 7-formyl-4-methyl-cumarine **30** (0.15%) in lovage essential oil is reported (Figure 3).

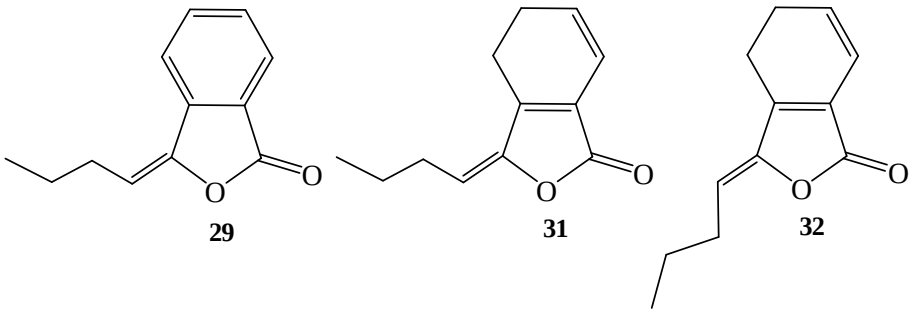
The molecular mass of all identified compounds was confirmed by mass-spectrometry analysis.

Table 1

Phytochemical composition of *L. officinale* essential oil of Moldovan origin.

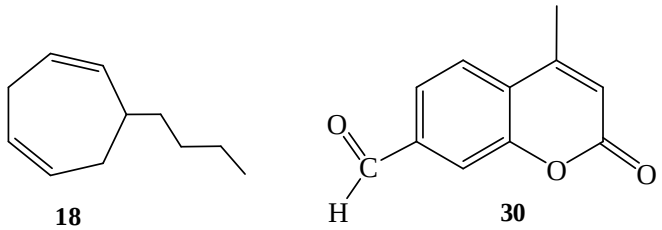
No.	*RT* (min)*	*Component*	*%*	***No.***	*RT* (min)*	*Component*	*%*
1	4.294	*α*-Thujene	0.578	***18***	9.641	6-Butyl-cyclohepta-1,4-diene	0.557
2	4.442	*α*-Pinene	1.998	***19***	10.187	Terpinen-4-ol	0.278
3	4.739	Camfene	0.240	***20***	10.460	Cryptone	0.130
4	5.209	Sabinene	3.396	***21***	10.533	*α*-Terpineol	1.111
5	5.298	*β*-Pinene	0.588	***22***	12.248	Linalyl acetate	0.107
6	5.550	*β*-Mircene	8.657	***23***	13.040	Bornyl acetate	0.085
7	5.866	*α*-Phellandrene	2.693	***24***	13.985	(*E*)-Sabynil acetate	0.044
8	6.001	*δ*-3-Carene	0.043	***25***	14.859	*α*-Terpinyl acetate	30.992
9	6.141	*α*-Terpinene	0.204	***26***	15.085	Perillyl alcohol	0.147
10	6.339	*p*-Cymene	1.455	***27***	15.520	Geranyl acetate	0.545
11	6.536	*β*-Phellandrene	22.393	***28***	18.010	Germacrene D	0.292
12	6.608	(*Z*)-*β*-Ocimene	3.506	***29***	22.428	(*Z*)-3-Buthylidene phtalide	0.232
13	6.836	(*E*)-*β*-Ocimene	0.204	***30***	23.484	7-Formyl-4-methyl-cumarine	0.146
14	7.149	*γ*-Terpinene	6.841	***31***	23.942	(*Z*)-Ligustillide	11.188
15	7.858	(+)-4-Carene	0.699	***32***	25.201	(*E*)-Ligustillide	0.202
16	8.134	Linalool	0.107	***33***	30.385	$[M]^+$ 258 m/z	0.142
17	8.780	(*E*)-4-Thujanol	0.050	***34***	30.471	[M]+ 286 m/z	0.084

**RT - retention time.*



29 **31** **32**

Figure 2. The structure of (*Z*)-3-buthylidene phtalide *29*, (*Z*)-ligustillide *31* and (*E*)-ligustillide *32*.



18 **30**

Figure 3. The structure of 6-butyl-cyclohepta-1,4-diene *18* and 7-formyl-4-methyl-cumarine *30*.

The presence of constituents mentioned above is confirmed by spectral analysis. Thereof, in IR spectra of *L. officinale* essential oil there are absorption peaks of exocyclic and trisubstituted double bonds from identified terpenic molecules at 3011, 1670, 1636, 1366 and 876 cm^{-1}. Peaks representing ester groups (acetates) are localized at 1730 and 1256 cm^{-1} and that from 1772 cm^{-1} confirm the presence of unsaturated lactones.

The ^{1}H NMR spectrum can be divided in 2 zones. The first one includes singlet signals of methyl groups localized in strong field: *gem*-dimethyls at 0.86-0.99 ppm, methyl groups attached to hydroxylated carbon atoms at 1.38-1.41 ppm. Singlets of the methyl groups adjacent to double bonds are visible at 1.58-1.69 ppm, and that of methyl groups from acetates are localized at 1.93 ppm. Protons of exocyclic methylene groups and those adjacent to double bonds are visible in the weaker field as doublets or broad singlets from 4.69 ppm to 6.12 ppm.

The ^{13}C NMR spectra are in accordance with proton spectra. The signal of primary carbon atoms ($-CH_3$) are localized from 19.41 ppm to 42.54 ppm, of tertiary hidroxylated carbon atoms (≥C-OH) at 84.69 ppm, of secondary exocyclic carbons ($=CH_2$) at 109.83 ppm, of tertiary unsaturated carbon atoms (-CH=CH- or >C=CH) at 120.3 ppm. The signal of quaternary carbon atoms (>C=O and lactonic) are visible at 170.28 ppm.

Antimicrobial activity evaluation

Lovage volatile oil (*L. officinale*) exhibits high antibacterial and antifungal properties in the range of concentrations 0.015-0.030% (Table 2). It can be mentioned that the antimicrobial properties of the lovage extract are due to the high content of *β*-phellandrene (RT= 6.536, 22.39%), *α*-terpinyl acetate (RT= 14.859, 30.99%) and (*Z*)–ligustillide (RT= 23.942, 11.19%). The above-mentioned compounds exhibit pronounced antimicrobial properties through mechanisms that include: breaking of the cell wall and cytoplasmic membrane, reduction of the cytoplasm around the nucleus, disturbance of the lipid fraction of the plasma membrane resulting in the alteration of its permeability and the leakage of the intracellular content [27-29].

Table 2

The antimicrobial activity *(MBC, MFC) of the oil extracted from the *Levisticum officinale* plants.**

Test-microorganisms	*Concentration (%)*							
	0.25	*0.12*	*0.06*	*0.03*	*0.015*	*0.007*	*0.0035*	*0.0017*
Bacillus subtilis CNMN BB-01 (4.8×10^8 CFU/mL)	-	-	-	-	+	+	+	+
Pseudomonas fluorescens CNMN-PFB-01 (4.8×10^8 CFU /mL)	-	-	-	-	+	+	+	+
Xanthomonas campestris (4.8×10^8 CFU /mL)	-	-	-	-	-	+	+	+
Erwinia amylovora (4.8×10^8 CFU /mL)	-	-	-	-	+	+	+	+
Erwinia carotovora (4.8×10^8 CFU /mL)	-	-	-	-	-	+	+	+
Candida utilis (3.0×10^7 CFU /mL)	-	-	-	-	+	+	+	+

* *MBC- minimal bactericidal concentration;*
MFC- minimal fungicidal concentration.

Conclusions

The qualitative (IR, ^{1}H and ^{13}C NMR) and quantitative (GC-MS) analyses of industrially obtained *Levisticum offcinale* essential oil of Moldovan origin were performed for the first time. As a result, thirty-two constituents, most of them, belonging to monoterpens, their derivatives and sesquiterpenoids with the total content of 87.30% were identified, together with some specific for the mentioned species butyl phtalides (11.62%). The *in vitro* tests have shown that the minimal bactericidal and fungicidal concentrations of oil extracted from *L. officinale* against *B. subtilis*, *P. fluorescens*, *X. campestris*, *E. amylovora*, *E. carotovora* and *C. utilis* are quite low 0.015-0.03%, which denotes its high antibacterial and antifungal activity.

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