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CONSTITUENTS OF THE ESSENTIAL OIL IN *SOLIDAGO CANADENSIS* L. FROM EURASIA

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ABSTRACT
Hydro distilled essential oils in air-dry samples of aerial parts of *Solidago canadensis* L., (Asteraceae) from eight local invasive populations were investigated by GC-MS analysis. A comparative study on quantity and composition of the essential oils obtained from plants, growing in different ecological and climatic conditions, ontogenesis phase and different plant organs was carried out The major compounds detected in oil samples of *S. canadensis* were α-pinene (1.3 – 61.27%), limonene (0.5 – 22.5%), bornyl acetat (3.4 – 29.8%) and germacrene D (1.8 – 39.2%). Samples from inflorescences contained the maximal percentage of monoterpene hydrocarbons, while the leaves' samples showed the maximal cumulative percentage of sesquiterpene and monoterpene hydrocarbons. Data obtained from our studies confirm the availability of alien invasive species *Solidago canadensis* for medicine and many other purposes. The variability of the qualitative and quantitative composition of essential oils in different geographical locations will allow futher selection of form containing the maximum amount of active substances.

Keywords: *Solidago canadensis*; essential oil; terpenes

INTRODUCTION
Nowdays alien invasive plants spread in the secondary distribution range in mass and can displace native species especially in the antropogenically transformated sites. The resource potential of these «new species» didn't studyied in detail yet. The direct and indirect effects of occurrence of invasive plants on food production and other positive externalities of its appearence have been analysed only for a few species **(Fehér et al., 2016)**. In this regard not without interest is genus *Solidago* L. that comprises about 100 species, five of them occurring in the Old World. *Solidago virgaurea* L. is the only one native for Eurasia. The other four – *S. canadensis* L., *S. gigantea* Ait., *S. rupestris* Raf. and *S. graminifolia* (L.) Salisb. (=*Euthamia graminifolia* (L.) Nutt.) are of North American origin **(Vinogradova et al., 2010)**. *S. canadensis* and *S. gigantea* are the invasive plants, widely distributed in Russia and almost all European countries. The plant material of medicinal usage, known as *Herba Solidaginis*, includes *S. virgaurea*, *S. gigantea* and *S. canadensis* **(Kalemba and Thiem, 2004)**. This material, its extracts and derivatives serve as components in many medications, applicable while treatment of the urinary tract and prostate diseases.

S. canadensis is used in medicine for treatment of several diseases. The flowers contain analgesic, astringent, febrifuge compounds, infusion of the dried powdered herb is used as an antiseptic agent and root is applied as poultice to burns **(Moerman, 1998)**. The species has been used in European phytotherapy for over 200 years for treatment of chronic nephritis, cystitis, urolithiasis, rheumatism and as an antiphlogistic drug **(Apati et al., 2003)**. The chemical composition of the essential oil in *S. canadensis* is poorly known. The essential oils obtained from fresh green parts, dried aerial parts, inflorescences, leaves and roots of *S. canadensis* collected from Poland, India, Egypt and China were previously investigated **(Kalemba et al., 1990; Weyerstahl et al., 1993; Xia et al., 1999; Mishra et al., 2010, 2011; El-Sherei et al., 2014)**. Results of those investigations showed that inflorescences of *S. canadensis* yielded 1.7%, 0.9% (leaves), and 0.2% (steams) of the essential oil. This oil contains 85% hydrocarbons and 15% terpenoids. Earlier, α-pinene, limonene, bornyl acetate, germacrene D, myrecene and γ-cadinene had been identified.

It is known that essential oils have antibacterial, antifungal, antioxidant **(Baratta et al. 1998; Burt, 2004; Bounatirou et al., 2007)**, antimycotic **(Arras and Grella, 1992)**, antiparasitic **(Pandey et al., 2000)**, antitoxigenic **(Juglal et al., 2002)**, and insecticidal **(Konstantopoulou et al., 1992)** properties. Essential oils can be used as natural preservative in the food industry too **(Petrová et al., 2015; Foltinová et al., 2017)**. For example, essential oil as an potential sources of antimicrobial ingredients are applied for chicken thighs meat treatment **(Kačániová et al., 2016)**. Essential oils can be used as well for the control of plant pathogens such as *Aspergillus niger* and *Aspergillus tubingensis* **(Císarová et al., 2016)**.

Scientific hypothesis

The major goal of this work was the determination chemical composition of the essential oils and the characterization essential oil chemical polymorphism of *S.canadensis* invasive populations from Eurasia.

MATERIAL AND METHODOLOGY

Locating plants and data collection

The specimens of *S. canadensis* (Figure 1) were collected in eight local invasive populations from Austria, Ukraine, Kazakhstan and Russia (Moscow, Penza, Tver and Tula regions) geographically distant from each other (Table 1). The specimens were collected both at vegetative and flowering buds' formation phases (June – August 2015). For the purpose of phytochemical analysis, one to three specimens (all the aerial shoots) were sampled within 0.2 m^2 – patches in all the examined local populations, simultaneously with collection of the herbarium specimens. The herbarium specimens are kept in the herbarium of Main Botanical Garden RAS (MHA).

Chemicals

Various reference chemicals used in this study were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used in this study were purchased from Merck (Darmstadt, Germany).

Methods of the Essential Oil Composition Study

Essential oil was obtained from an average sample of aerial parts (leaves, inflorescences and a mixture of inflorescences and leaves) of plants. The oil was extracted from chopped air-dried material by hydrodistillation according to **Ginzberg (1932)**. Qualitative composition of the oil was determined by gas chromatography at the Center for Collective Use of the Federal Research Center “Fundamentals of Biotechnology”, Russian Academy of Sciences, Moscow (RFMEFI62114X0002), using a Shimadzu GS 2010 gas chromatograph with a GCMS-QP 2010 mass detector and SPB-1 nonpolar column (solid-phase-bound methyl silicone) (Supelco, Sigma-Aldrich Corporation) with the length of 30 m and diameter of 0.25 mm. The samples were dissolved in benzene at a ratio of 1 : 150 and chromatographed with the temperature-programming mode under the following conditions: injector temperature, 180 °C; interface, 205 °C; detector, 200 °C. The carrier gas was helium. The flow through the column was 1 cm^3/min and flow division was 1 : 10. Mass detector settings: logging mode, TIC; mass range, 30 – 400 m/z. The temperature regime for the substances with the retention index of 1300 consisted of the thermostat at 60 °C for 3 min; then, 2 °C.min^{-1} until 230 °C; and isotherm for 2 min. The temperature regime for the substances with a retention index higher than 1300

Figure 1 *Solidago canadensis* L. in the Moscow district, photo by Yu.Vinogradova, September 2016.

Table 1 Examined invasive populations of *Solidago canadensis* L.

Specimen	Locality	Content of essential oil, %
M-1	Moscow, Main Botanical Garden RAS, phase of vegetation (leaves)	0.7
M-2	Moscow, Main Botanical Garden RAS, phase of blooming (leaves)	0.1
M-3	Moscow, Main Botanical Garden RAS, phase of blooming (inflorescens)	0.4
Ms	Moscow region, Krasnogorsk district (N 55º48'; E 37º18')	0.3
As	Austria, Urban district of Vienne (N 48º11'; E 16º18')	0.4
Tv	Tver’ region, Kimru district (N 56º51'; E 35º54')	0.1
Pz	Penza region, Zemetrino district (N 53º50'; E 42º62')	0.4
Kz	Kazakhstan, Urban district of Alma-Ata (N 43º15'; E 76º54')	0.2
Bs	Altaj region, Bijsk district (N 51º38'; E 84º30')	0.3
Sh	Sakhalin region, Urban district Yuzhno-Sakhalinsk (N 46º57'; E 142º44')	0.3
Uk	Ukraine, Kiev region, Mironovka district (N 49º38'; E 30º57')	0.2
Tl	Tula region, Venevo district (N 54º51'; E 38º35')	0.4

consisted of the thermostat at 60 °C for 1 min; then, 4 °C.min^{-1} until 230 °C; and isotherm for 2 min. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors. The identity of the components was assigned by comparison of their retention indices, relative to a C9 – C17 hydrocarbon standard mixture.

Statistical analyses

Results are expressed as mean of triplicate trials. Data were analyzed by one-way analysis of variance (ANOVA) on the means of values (p <0.05). The essential oils composition was used to determine the relationship between the different samples by cluster analysis using PAST 2.17.

RESULTS AND DISCUSSION

Essential oil contents of *S. canadensis* varied between 0.07 and 0.70%. Accumulation of essential oil of this species depends on the ecological and climatic conditions of growth, ontogenesis phase and testing plant's organs.

The maximum content of oil was found in the leaves collected at vegetative phase (0.7%). Inflorescences collected at flowering buds' formation phase yielded more oil than the leaves (Table 1).

The trace amount of essential oil (0.1%) was recorded in the plants at the northernmost collection point- Tver region, Kimry district; the maximal quantity (0.4%) – in the plants from three local invasive populations (As, Pz and Tl).

Qualitative and quantitative variations in the oils' composition in leaves, inflorescences and aerial parts for all the specimens collected were observed (Table 2, 3).

In the composition of essential oil 63 components were identified. Mono- and sesquiterpene hydrocarbons were dominant in all the oil samples. Among the monoterpenes dominated α- and β-pinene, β-myrcene, limonene and bornyl acetate; sesquiterpenes – germacrene D, α- and β-caryophyllene, β-elemene. The remaining components of these classes were encountered in small amounts.

Composition of the essential oil significantly varies within different phases of plants ontogenesis (Table 2). During the vegetative period the monoterpene and the sesquiterpenes contents in leaves decrease from 80 to 20% after the flowering stage; oxygenated monoterpenes increases from 7% to 30%, respectively. Within a vegetatitive phase (before flowering) the major components of essential oil of leaves are α-pinene – 28.1%, germacrene D – 39.2%, bornyl acetate – 7.3%, limonene – 7.0%, β-myrcene – 7.3%. At the flowering buds' formation phase amount of essential oil in the leaves decreased to 0.10% and the main components' proportion changed (α-pinene – 1.3%, germacrene D – 16.6%, bornyl acetate 29.8%, limonene – 0.5%, β-myrcene – 0.2%). The inflorescences contain 0.4% essential oil and the major component is α-pinene – 61.2%, limonene – 13.7% and bornyl acetate – 8.5%.

The composition of essential oil significantly varies in aerial parts of *S. canadensis*, depending on the geographical locality of the invasive population, too. The monoterpene hydrocarbons were significantly higher in all the samples compared to sesquiterpenes.

Mostly quantitative rather than qualitative variation was observed in all the essential oils analyzed. The percentage of monoterpene hydrocarbons was higher in samples Pz, Ms, As, Tv, Uk and Tl (64 – 85%) compared to those of Kz, Bs and Sh (46 – 54%). α-Pinene (12.6 – 55.4%), β-pinene (0.7 – 5.4%), β-myrecene (1.8 – 6.9%) and limonene (6.4 – 22.5%) were the major monoterpenes in all the samples. The sesquiterpenes: germacrene D (3.0 –

Table 2 Essential oil composition (%) of the leaves and inflorescens *Solidgo canadensis* L.within different phases of plant's ontogenesis.

Compounds	RI	Leaves, phase of vegetation (M-1)	Leaves, phase of blooming (M-2)	Inflorescens, phase of blooming (M-3)
		Monoterpene		
α-Pinene	928	28.1	1.25	61.2
Camphene	940	0.99	0.13	1.40
Sabinen	960	0.53	–	0.91
β-Pinene	965	2.75	0.17	3.25
β-Myrcene	976	7.28	0.20	2.28
α-Phellandrene	989	1.47	–	–
Limonene	1016	6.98	0.52	13.7
α-Campholenal	1105	0.33	1.60	1.06
Bornyl acetate	1270	7.34	29.8	8.49
		Sesquiterpene		
β-Elemene	1477	1.05	0.71	–
β-Caryophyllene	1483	0.46	0.45	–
α-Caryophyllene	1492	0.30	0.39	–
Germacrene D	1497	39.2	16.6	1.81
β-Eudesmene	1499	0.18	1.13	0.23
Aromadendrene oxide	1557	–	1.74	0.15
Caryophyllene oxide	1571	–	2.50	–
Spathulenol	1603	0.17	2.97	–

Legend: RI – retention index relative to C_9-C_{17} n-alkanes on SPB-1 column (Supelco); M-1, M-2, M-3 – Moscow, Main Botanical Garden RAS; «–» – component is not present.

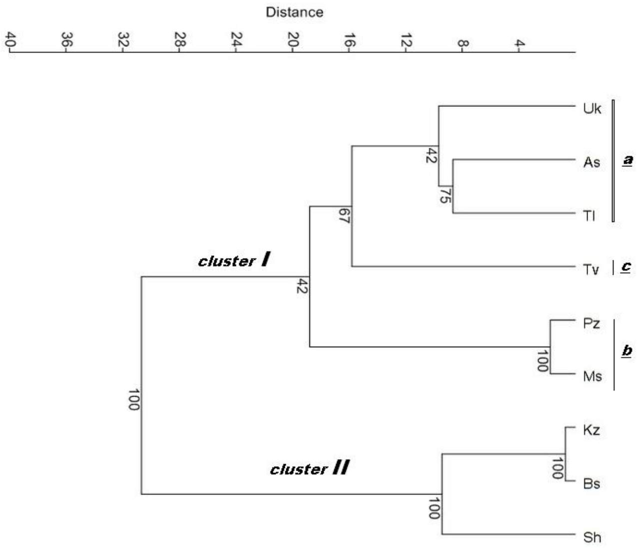

Figure 2 Dendrogram obtained by cluster analysis of the percentage composition of essential oils from the *Solidgo canadensis* L. samples examined. For abbreviations, see Table 1 and the text.

Table 3 Essential oil composition of *Solidgo canadensis* L. (%).

Compounds	RI	Ms	As	Tv	Pz	Kz	Uk	Tl	Bs	Sh
				Monoterpene						
α-Pinene	928	41.6	43.6	25.1	52.4	12.6	30.0	24.3	14.1	13.8
Camphene	940	1.28	1.70	0.97	0.75	0.94	1.07	0.97	1.22	0.20
Sabinen	960	0.62	0.78	0.42	0.96	1.23	0.46	0.32	0.39	0.28
β-Pinene	965	3.52	5.36	2.48	4.73	3.91	4.20	2.42	3.62	0.71
β-Myrcene	976	6.38	1.82	4.10	1.12	4.88	6.90	2.60	4.88	2.29
Limonene	1016	22.5	10.7	6.42	20.3	19.7	15.4	17.3	18.1	17.5
α-Campholenal	1105	0.39	0.68	1.37	0.31	0.19	0.21	0.83	0.49	0.32
Bornyl acetate	1270	8.34	7.87	26.3	3.35	10.9	8.71	15.2	9.40	11.8
				Sesquiterpene						
β-Elemene	1477	0.60	0.50	0.82	0.21	0.67	–	0.83	0.20	–
β-Caryophyllene	1483	0.56	1.09	0.66	0.61	0.93	1.41	0.77	018	0.64
α-Caryophyllene	1492	0.44	0.28	0.39	0.21	0.56	0.51	0.43	0.24	0.55
Germacrene D	1497	8.67	14.9	2.98	10.3	31.8	24.0	15.9	32.4	36.2
β-Eudesmene	1499	0.47	0.35	0.36	–	0.24	0.20	0.59	0.12	–
Aromadendrene oxide	1557	0.31	0.20	1.45	0.34	–	0.40	1.78	–	0.67
Caryophyllene oxide	1571	0.51	0.88	4.17	–	1.23	0.46	2.34	0.32	0.11
Spathulenol	1603	–	–	2.33	0.28	0.46	0.11	1.39	0.83	1.31

Note: RI – retention index relative to C_9-C_{17} n-alkanes on SPB-1 column (Supelco); «–» – component is not present.

36.2%), α- and β-caryophyllene (0.4 – 1.9%), β- and γ-elemene (0.2 – 1.8%) were present in all the oil samples. Oxygenated compounds, especially bornyl acetate (3.4 – 26.3%) showed their highest percentage in all the samples.

Cluster analysis (Figure 2), confirmed a high chemical correlation among all populations (S_{corr} >0.80%). Two clusters were defined in the bases of the amount of monoterpenes and germacrene D. In cluster 1, which included 6 out of the 9 samples, germacrene D ranged from 3 to 24%, whereas the three sample of cluster 2 showed a higher percentage (32 – 36%). Correlation was detected between the clusters and the geographical collection site. The most part of samples the essential oils studied belonged to the "European" chemotype. And only the three samples (Kz, Bs, Sh) belonged to the "Asian" chemotype. Sub-cluster a has a relative amount between 15.9 and 24.0%, sub-cluster b between 8.7 and 10.3% and sub-cluster c (Tv) has the lower relative amount (3%). No significant correlation was detected between the clusters and the geographical collection site (p = - 0.35, r= 0.49).

The presence of germacrene D as the major component in overwhelming majority oil samples is in agreement with the previous reports on its occurrence as a major constituent in the oils obtained from the fresh green parts (23.8%), leaves (28.4 and 64.06%) and fresh flowers (29.5%) of *S. canadensis* collected from Poland **(Kalemba et al., 1990; Weyerstahl et al., 1993; Kalemba and Thiem, 2004),** China **(Xia et al., 1999)**, India **(Mishra et al., 2010, 2011)** and Egypt **(El-Sherei et al., 2014)**, respectively. On the contrary, γ-cadinene previously identified as main constituents (27.1 and 12.7 – 20.4%) in the aerial parts and fresh flowers of *S. canadensis* collected Poland and Egypt during the flowering stage **(Kalemba et al., 1990; Weyerstahl et al., 1993; Kalemba and Thiem, 2004; El-Sherei et al., 2014)** was not detected in our oil samples. Moreover, all the samples collected in Poland, India and Egypt lacked bornyl acetate while the latter was identified in considerable amounts (3.4 – 26.3%) in the oil of aerial parts of the plants, collected in Russia, Austria, Ukraine and Kazakhstan.

CONCLUSION

There is a great potential in finding new antimicrobial drugs from the wild. Natural crude drug extracts and biologically active compounds obtained from plant species used in traditional medicine can be prolific resources for new drugs. Moreover, according to literature, some monoterpenes and sesquiterpenes as α-pinene, myrcene, limonene and germacrene D have cytotoxic effects against different cell lines. We can support usage of such natural products as potent preservative agents, as well as possible candidates for new medical preparations. Data from our studies confirm the availability of *Solidago canadensis*: its aerial part containes from 0.1 to 0.7% of essential oil in the leaves and from 0.1 to 0.4% of essential oil in the inflorescens. The major compounds detected in oil samples of *S. canadensis* were α-pinene (1.3 – 61.27%), limonene (0.5 – 22.5%), bornyl acetat (3.4 – 29.8%) and germacrene D (1.8 – 39.2%). Samples from inflorescences contained the maximal percentage of monoterpene hydrocarbons, while the leaves' samples showed the maximal cumulative percentage of sesquiterpene and monoterpene hydrocarbons. The variability of the qualitative and quantitative composition of essential oils in different geographical locations will allow futher selection of form containing the maximum amount of active substances.

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