ISSN : 0974 - 7508 Volume 10 Issue 7

Natural Products

An Indian Journal

Full Paper

NPAIJ, 10(7), 2014 [215-218]

Seasonal dynamics of alkaloids of *Genista tinctoria* L. growing at the Southern Ural Region

Inna P.Tsypysheva[1]*, Polina R.Petrova[2], Irina P.Baykova[1], Eugeniy G.Galkin[1], Nikolay I.Fedorov[3], Fanur Z.Galin[1,2], Marat S.Yunusov[1]

[1]Institute of Organic Chemistry, Ufa Research Center of the Russian Academy of Sciences, Ufa, prosp. Oktyabrya, 71, (RUSSIAN FEDERATION)

[2]Bashkir State University, Ufa, (RUSSIAN FEDERATION)

[3]Institute of Biology, Ufa Research Center of the Russian Academy of Sciences, Ufa, (RUSSIAN FEDERATION)

E-mail : tsipisheva@mail.ru

ABSTRACT

The seasonal dynamics of alkaloids of the aerial part of Genista tinctoria L. growing in the forest-steppe zone of the Bashkortostan Republic (Southern Ural region) was investigated by GC/MS. It was shown, that the content of quinolizidine alkaloids is maximal in the branches of the flowering period. Two alkaloids - baptifoline and (–)-cytisine are the major components of the total alkaloids of this plant.

KEYWORDS

Genista tinctoria;
Quinolizidine alkaloids;
Seasonal dynamics;
GC/MS.

INTRODUCTION

Plants of genus *Genista* (*Fabaceae*) are used by traditional medicine owing to their known biological activity - anti-inflammatory, ulceroprotective, hypoglycemic, antioxidant, phytoestrogenic and antitumor activity[1-6]. Partially these properties are caused by presence of quinolizidine alkaloids (QA) in the plants, and it is necessary to mention that some of them, first of all (-)-cytisine, is the precursor for a new drugs for treatment of neurodegenerative diseases[7], including our own researches[8-10].

It is known, that existence of accessible plant source of starting natural compounds is necessary for the achievement of such purpose. Therefore, in the course of our search for accessible sources of QA among plants of genera *Fabaceae* growing at the territory of our region[11, 12] in this work we describe the seasonal dynamics of alkaloids of *Genista tinctoria* L.

The plants of genera *Fabaceae* are one of the most popular producers of quinolizidine alkaloids which are known for their high biological activities[13-16]. And the data about alkaloids of *G. tinctoria* growing in the Eastern Europe, Turkey, and Central Asia is presented in literature[17-19]. However, the total content and the composition of the alkaloids of *G. tinctoria* growing in the Southern Ural have not yet been studied.

Genista tinctoria L. (*Fabaceae*) (a shrub, 50-150 cm in height with narrow leaves and yellow papilionaceous flowers) is wild throughout the steppe and forest-steppe zones of Western Siberia, and the Ural region[20], it is found mainly in the dry forests and their fringes, pine forests, among other shrubs, and on hill slopes primarily inhabiting limestone or sandy soils[21]. In order to identify the accessible plant sources of quinolizidine alkaloids in the territory of the

Full Paper

Bashkortostan Republic (Southern Ural) and the optimal period for collection of raw plant materials, we carried out the study of seasonal dynamics of alkaloids of the aerial parts of *G. tinctoria* collected at different phenological phases - budding, flowering, and fruiting.

EXPERIMENTAL

Plant material

The plant samples of *G. tinctoria* (the voucher specimen of *G. tinctoria* was deposited at the Herbarium of the Institute of Biology of the Ufa Scientific Center of the Russian Academy of Sciences (IB USC RAS), No. 345-2012) were collected under typical weather conditions in 2012 in the forest-steppe zone of the Bashkortostan Republic near the Bikchagul village of the Alsheevsky District. Identification of species was carried out by Professor N. I. Fedorov (IB USC RAS), Ufa. The samples have been collected at three phases: budding - buds and leaves (sample I) and branches (sample II), flowering - flowers and leaves (sample III) and branches (sample IV), fruiting - leaves and 'unripe pods' (sample V) and branches (samples VI). The plant material was dried and powdered to obtain particles 1 mm in diameter.

Extraction and isolation of total alkaloids

An air-dried and powdered sample I (100 g) was extracted with water-acetone (1:9) mixture (10 d × 3 times). After concentration under vacuum, the residue was acidified to pH 3 by 10% H_2SO_4 and extracted with Et_2O (3 × 100 ml). The aqueous fraction was cautiously adjusted to pH 10 by Na_2CO_3 and then exhaustively extracted with $CHCl_3$ (5 × 100 ml). The $CHCl_3$ fraction was dried by anhydrous Na_2SO_4 and concentrated under vacuum to yield 50 mg of total alkaloids of sample I. Extraction of total alkaloids from samples II – VI was carried out by analogy.

GC/MS investigations and data analysis

Chromato-mass spectrograms were recorded on a Thermo Finnigan MAT 95 XP, electron impact ionization method (70 eV), injector temperature – 250°C, HP-5MS column, phase thickness – 30 m × 0.25 mm × 0.25 μm, initial temperature – 120 °C for 3 minutes, 250 °C isotherm for 10 minutes. The components of the alkaloid mixtures were identified from full mass spectra: the probability of similarity (Q) between the recorded spectra and the spectra found in the database (by library search[22]) is provided in TABLE 1.

Commercial available alkaloids (–)-cytisine (CAS 458-35-8), *N*-methylcytisine (CAS 486-86-2), d-lupanine (CAS 550-90-3), and also anagyrin and *N*-formylcytisine isolated from *Thermopsis lanceolata ssp. sibirica* previously[11] were used as reference substances. Relative retention index (RRT) of (–)-cytisine was 1.00. Quantitative analysis was performed using internal normalization method with respect to chromatographic peak areas without the use of correction factors. The alkaloid content is provided in % of the weight of the air-dry raw materials (ADRM). The sum of areas of peaks of the components was regarded as 100%.

RESULTS AND DISCUSSION

A comparative analysis of the content and composition of the alkaloids of the aerial parts of *Genista tinctoria L.* at the budding, flowering, and fruiting phenological phases was carried out by GS/MS method, that is the common, most convenient and fast for the analysis of extracts of quinolizidine alkaloids[23-25]. The buds and leaves (sample I), flowers and leaves (sample III), leaves and 'unripe pods' (sample V), and branches (samples II, IV, VI) of *G. tinctoria* were investigated separately. Results of the GC/MS investigation are presented in TABLE 1.

The total alkaloid content is minimal during the budding period (0.05% – sample I, 0.17% – sample II) and it increases markedly by the time the flowering (0.60% – sample III, 0.85% – sample IV) and the fruiting periods (0.59% – sample V, 0.60% – sample VI) are reached. A higher content of alkaloids was identified in the branches of the plant (the total alkaloid content was as follows: 0.17% – sample II, 0.85% – sample IV, and 0.6% – sample VI, respectively) during all phenological phases.

The analysis of composition of the total alkaloids (samples I-VI) shown that the quinolizidine alkaloids – (–)-cytisine, *N*-formylcytisine, anagyrin, baptifolin, d-lupanin, *N*-methylcytisine are the major components. It is necessary to mention, that samples I-VI differed significantly with respect to composition and content of

Full Paper

TABLE 1 : Seasonal dynamics of alkaloids of *Genista tinctoria* L.

Phenological phase				Budding		Flowering		Fruiting	
Sample				buds and leaves	branches	flowers and leaves	branches	leaves and "unripe pods"	branches
				I	II	III	IV	V	VI
Total alkaloids, %[1]				0.05	0.17	0.60	0.85	0.59	0.60
Components	RRT[2]	M+	Q[3]	Content of components, %[1]					
Ammodendrine	0.78	208	90	-	-	+[4]	-	-	-
N-methylcytisine	0.94	204	97	0.02	0.07	0.02	0.02	+	0.01
(-)-Cytisine	1.00	190	98		0.04	0.08	0.02	0.01	0.01
d-Lupanine	1.22	248	96	-	+	0.03	-	-	-
N- formylcytisine	1.51	218	94	+	-	0.05	0.16	0.06	0.10
Anagyrine	1.54	244	98	+	0.04	0.09	0.05	0.01	0.04
Baptifoline	1.91	260	89	-	-	0.10	0.22	0.05	0.22

[1]% of the weight of the air-dry raw material; [2]Injector temperature – 250°C, HP-5MS column, phase thickness – 30 m × 0.25 mm × 0.25 μm, initial temperature – 120°C for 3 minutes, 250°C isotherm for 10 minutes; [3]Probability of similarity between the recorded spectra and the spectra found in the database; [4]Traces

the individual components.

Thus, (–)-cytisine was identified in all samples but its maximal content was recorded at the flowering phase, in sample IV (0.2%). Also, the aerial part of *Genista tinctoria* contains *N*-methylcytisine and anagyrin during all phenological phases. Their relevant contents reach maximal values in the branches during the flowering period (0.07% in sample IV), and in the leaves and flowers during the flowering period (0.09% in sample III), respectively.

N-Formylcytisine presents in all samples except sample II. The content of this alkaloid in the stems during the flowering and fruiting periods is 0.16 and 0.10% (samples IV and VI, respectively). Baptifolin is a major component of total alkaloids of the branches of *Genista tinctoria*. It is accumulated in the plant during the flowering and fruiting periods; its content in samples IV and VI reached 0.22%.

Quinolizidine alkaloid d-lupanin was identified exclusively in the branches during the budding period and in the flowers and leaves during the flowering phase (samples II and III). It should be emphasized that a pyridine alkaloid ammodendrin (often accompanying quinolizidine alkaloids in plants of the genus *Genista*[26]) was identified in the flowers and leaves of the *G. tinctoria* during the flowering phase (sample III).

CONCLUSIONS

Thus, it was shown, that the content of quinolizidine alkaloids in the aerial parts of *Genista tinctoria* growing in the forest-steppe zone of the Bashkortostan Republic (Southern Ural) is maximal in the branches of the flowering period; the baptifoline and (-)-cytisine are the major components of the total alkaloids of this plant. Resulting data confirms that *G. tinctoria* can be used as an accessible source of the two mentioned above quinolizidine alkaloids.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research under Grant No. 14-03-31503-mol-a; Foundation of the RF President for Leading Scientific Schools under Grant NSh-1700.2014.3.

REFERENCES

[1] I.llarionov, L.Rainova, N.Nakov; Farmatsiya, **29**, 39 **(1979)**.
[2] L.Rainova, N.Nakov, S.Bogdanova, E.Minkov, D.Staneva-Stoicheva; Phytother.Res., **2**, 137 **(1988)**.
[3] A.P.Rauter, A.Martins, C.Borges, J.Ferreira, J.Justino, M.R.Bronze, A.V.Coelho, Y.H.Choi, R.Verpoorte; J.Chromatogr.A, **1089**, 59 **(2005)**.

Full Paper

[4] S.Raja, H.N.Ahamed, V.Kumar, K.Mukherjee, A.Bandyopadhyay, P.K.Mukherjee; Iran J.Pharmacol.Therapeutics, **6**, 15 **(2007)**.

[5] M.Luczkiewicz, D.Glod; Plant Sci., **165**, 1101 **(2003)**.

[6] S.Banerjee, Y.Li, Z.Wang, F.H.Sarkar; Cancer Lett., **269**, 226 **(2008)**.

[7] J.Rouden, M.C.Lasne, J.Blanchet, J.Baudoux; Chemical Reviews, **114**, 712 **(2013)**.

[8] I.P.Tsypysheva, A.V.Kovalskaya, N.S.Makara, A.N.Lobov, I.A.Petrenko, E.G.Galkin, T.A.Sapozhnikova, F.S.Zarudii, M.S.Yunusov; Chemistry of Natural Compounds, **48**, 629 **(2012)**.

[9] P.Tsypysheva, A.V.Kovalskaya, A.N.Lobov, M.Kh.Salimgareeva, U.Sh.Fatkullina, P.R.Petrova, S.F.Gabdrakhmanova, N.S.Makara, K.Yu.Suponitskii, Yu.V.Vakhitova, F.S.Zarudii, M.S.Yunusov; Chemistry of Natural Compounds, **49**, 707 **(2013)**.

[10] Y.V.Vakhitova, I.P.Tsypysheva, M.K.Salimgareeva, A.V.Koval'skaya; Chemistry of natural compounds, **50**, 498 **(2014)**.

[11] P.Tsypysheva, E.G.Galkin, A.S.Erastov, O.A.Karimova, I.P.Baikova, A.V.Kovalskaya, I.U.Khalilova, L.M.Abramova, M.S.Yunusov; Khimiya rastitelnogo syrja, **4**, 181 **(2012)**.

[12] P.Tsypysheva, E.G.Galkin, A.S.Erastov, O.A.Karimova, I.P.Baikova, A.V.Kovalskaya, I.U.Khalilova, L.M.Abramova, M.S.Yunusov; Khimiya rastitelnogo syrja, **4**, 55 **(2013)**.

[13] J.Buckingham, K.H.Baggaley, A.D.Roberts, L.F.Szlabo; 'Dictionary of Alkaloids', 2nd Edition, CRC **(2010)**.

[14] M.Wink; Planta medica, 509 **(1987)**.

[15] J.P.Michael; Nat.Prod.Rep., **21**, 625 **(2004)**.

[16] M.R.Roberts, M.Wink; 'Alkaloids. Biochemistry, ecology and medical applications', Plenum; New York **(1998)**.

[17] V.Cristov, L.Evstatieva; Dokladi na Bulgarskata Akademiya na Naukite, **53**, 23 **(2000)**.

[18] D.Knoefel, H.R.Schuette; Journal fuer Praktische Chemie (Leipzig), **312**, 887 **(1970)**.

[19] F.Tosun, M.Tanker, A.Tosun, T.Ozden; Pharmacia (Ankara), **31**, 5 **(1991)**.

[20] B.K.Shishkin; 'Flora of the U.S.S.R., Genista L.', **11**, **(1945)**.

[21] N.I.Fedorov, O.I.Mikhailenko, A.A.Muldashev, M.R.Lugmanova; Basic grounds of biological resources management, KMK: Scientific Press Ltd; 2005. Part 2, Screening for alkaloids in plants of Southern Ural, 203-211 **(2005)**.

[22] National Institute of Standards and Thecnology (http://www.nist.gov/).

[23] A.Martins, M.Wink, A.Tei, M.Brum-Bousquet, F.Tillequin, A.P.Rauter; Phytochemical Analysis **(2005)**.

[24] N.Erdemoglu, S.Ozkan, F.Tosun; Phytocemical Rev., **6**, 197 **(2007)**.

[25] Y.Li, Z.Hu, L.He; Pharmaceutical and biomedical analysis, **43**, 1667 **(2007)**.

[26] Hagers Handbuch der Pharmazeutischen Praxis, Folgeband 2: Drogen A-K, Springer **(2012)**.