Content of Flavonoids in Plant Species of Genus *Myosotis* L.

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Abstract

The content of flavonoids in samples of 15 plant species of genus *Myosotis* was studied. It has been established that for the plant species under investigation this value mainly amounts to 1.0–4.0 % being characterized by a high variability for the samples harvested in various regions. The variability of flavonoid content within the range of the same coenotic population was observed to be low or medium. The species belonging to section *Alpestres* of genus *Myosotis*, in particular *M. asiatica*, are abundantly supplied with flavonoids (up to 7.4 %) being of interest for the further study. Plants harvested within mass flowering period could be used as the sources of flavonoids.

Key words: *Myosotis* L., *M. asiatica*, flavonoids, variability

INTRODUCTION

The interest in the herbal medicine considerably increased for the recent years as well as the necessity of revealing new sources of biologically active substances initiate the studies concerning the objects unclaimed before.

One of such objects is presented by plant species of genus *Myosotis* L. those do not find any application in official medicine, being at the same time used in folk medicine in order to treat epilepsy, respiratory apparatus diseases, malignant tumors of mouth and genitals [1–4].

Antibacterial action of oil extracts from leaves of plant species *Myosotis palustris*, *M. arvensis*, *M. imitata* and *M. krylovii* on the cultures of eight pathogenic and opportunistic pathogenic microbial species has been studied. It is known that all the species under investigation inhibit the development of *Shigella sonnei;* the extracts of *M. palustris* and *M. arvensis* depress the development of *Candida albicans;* the extracts of *M. palustris* suppress as well the viability of blue pus bacillus, *Pseudomonas aeruginosa*; *M. arvensis* influences all the gram-positive bacterial species under investigation (*Candida albicans*, *Staphylo coccus aureus* and *Streptococcus faecalis)*, and the extracts of *M. imitata* depress the development of *Klebsiella pneumonia* [5].

The medicinal properties of plants are determined by the presence of biologically active substances therein. According to the data of [6–11], in plant species of genus *Myosotis* there are higher fatty acids, alkaloids, saponins, anthocyanins and flavonoids revealed.

Flavonoids (quercetin, dihydroquercetin, rutin, luteoline, flavin, *etc.*) find a wide application as food antioxidants, natural dyes and medical products. It was demonstrated that many phenolic compounds such as flavones, flavanones, isoslavones, chalcones and others exhibit phytoestrogenic activity [12, 13].

At the same time data concerning the composition and content of flavonoids in plant species of genus *Myosotis* are scarce in the literature. Earlier the authors of [6, 14] investigated flavonoids from six species of genus *Myosotis* and they established that the substances revealed represent quercetin glycosides, less often these substances are rhamnetin and kaempferol glycosides. The author of [8] devoted the paper to the studies of flavonoids from Siberian plants; he have obtained data

concerning the amount of flavonoids in aerial part of plant species such as *Myosotis palustris*, *M. suaveolens*, *M. alpestris*.

MATERIALS AND METHODS

We have studied the content of flavonoids in 122 samples of 15 plant species belonging to genus *Myosotis*. For the investigation we used samples of our own harvesting carried out within June–July period of 2001–2005, as well as the samples given us by the researchers of the Laboratory of Higher Plants Systematics and Plant Genetics of the Central Siberian Botanical Garden (CSBG), SB RAS (Novosibirsk).

The samples were harvested mainly over the territory of Siberia (Novosibirsk, Irkutsk, Kemerovo, and Chita Regions, Buryatia, Krasnoyarsk Territory, Altai Territory, Khakassia, Tyva and the Mountain Altai). In addition, samples of plants were investigated harvested from the territories of Moldova (*M. sylvatica),* Sakhalin Island (*M. sachalinensis* M. Pop.), Murmansk (*M. decumbens* Host.) and Moscow Regions (*M. popovii* Dobrocz.). All the plants were harvested during the period of mass flowering.

The content of flavonoids was determined using a chromatography/spectrophotometry method. An accurately weighed sample (0.02–0.10 g) of air dry material was three times extracted with 50 % ethanol during 30 min with the volume ratio 1 : 20, during 20 min with the volume ratio 1 : 15, and for 10 min with the volume ratio 1 : 10. A joined extract was evaporated to obtain solid residue which was washed in an exact volume of 50 % ethanol. An aliquot taken was chromatographed on a FN 12 paper in the system of *n*-butanol–acetic acid–water (40 : 12 : 28). The chromatogram obtained was dried, investigated under UV light; visualized spots of flavonoids were cut out and placed into small glass columns (top part diameter being of 6 mm, bottom part diameter being 1 mm, h = 60–70 mm). Then we washed it with 50 % ethanol and measured the volume of eluates. The spectrophotometric measurements were performed for each eluate at the wavelength λ = 360 nm. Basing on the calibration curve plotted for routine we determined the content of flavonoids in 1 mL of the solution. The amount of flavonoids (X) was calculated according to a formula

$$X = DV_1V_2/(1000MV_3)$$

Here D is optical density (absorbance) value for the solution; V_1, V_2, V_3 are the volumes of the extract, the eluate and the extract applied onto the chromatographic layer, respectively; M is the mass of a weighed sample.

The total content of flavonoids was calculated by the summation of the quantities corresponding to individual components of the flavonoid complex present in a sample [15].

RESULTS AND DISCUSSION

The results of flavonoid content determination in leaves of 15 plant species under investigation belonging to genus *Myosotis* are presented in Table 1. One can see that this value varies within the range of 1.0–4.0 %.

The majority of samples of species belonging to section *Sylvaticae* contain ~2.0 % of flavonoids. For the species such as *M. arvensis*, *M. krylovii*, *M. austrobai-calensis* and *M. decumbens* the content of flavonoids amounts to 3.0 % and higher. The minimal content of flavonoids has been revealed in samples of *M. pseudovariabilis* (<1.0 %).

A high content of flavonoids has been revealed in species belonging to sections *Alpestres* and *Myosotis*. The minimum flavonoid percentage amounted to 1.9 % for section *Alpestres (M. baicalensis)* and 1.3 % for section *Myosotis (M. palustris)*. The maximum amount of flavonoids (7.4 %) has been revealed for one of the samples of *M. asiatica* (the Taymyr Autonomous District, the Khatanga River basin, on the outskirts of the Kresty village; sandy strips on the riverside). For the majority of samples of species belonging to these sections the content of flavonoids was higher than 2.5 % (Fig. 1).

The content of flavonoids can change depending on environmental factors, injury level and the phases of plant development, *etc.*

We have investigated the individual variations in the content of flavonoids are investigated by the example of 20 plants such as *M. arvensis* (section *Sylvaticae)* growing over the territory of the Novosibirsk Region (Novosibirsk, on the outskirts of the CSBG, a wayside)

TABLE 1

Content of flavonoids in plant species of genus *Myosotis*

Sample No.	Species	Number of samples	Flavonoids content, %
	Section *Myosotis*		
1	*M. palustris* L.	4	1.3–4.2
2	*M. caespitosa* C. F. Schultz	24	1.2–4.6
	Section *Sylvaticae*		
3	*M. sylvatica* Ehrh. ex Hoffm.	1	3.1
4	*M. sachalinensis* M. Pop.	1	1.4
5	*M. arvensis* (L.) Hill.	20	1.5–3.4
6	*M. krylovii* Serg.	14	0.9–3.3
7	*M. austrobaicalensis* O. Nikiforova	6	0.4–3.5
8	*M. sajanensis* O. Nikiforova	3	1.9–2.6
9	*M. decumbens* Host.	3	1.3–4.4
10	*M. pseudovariabilis* M. Pop.	3	0.6–0.9
	Section *Alpestres*		
11	*M. asiatica* Schischk. et Serg.	7	2.7–7.4
12	*M. austrosibirica* O. Nikiforova	4	2.1–4.0
13	*M. imitata* Serg.	21	1.4–6.5
14	*M. popovii* Dobrocz.	1	4.3
15	*M. baicalensis* O. Nikiforova	3	1.9–3.4

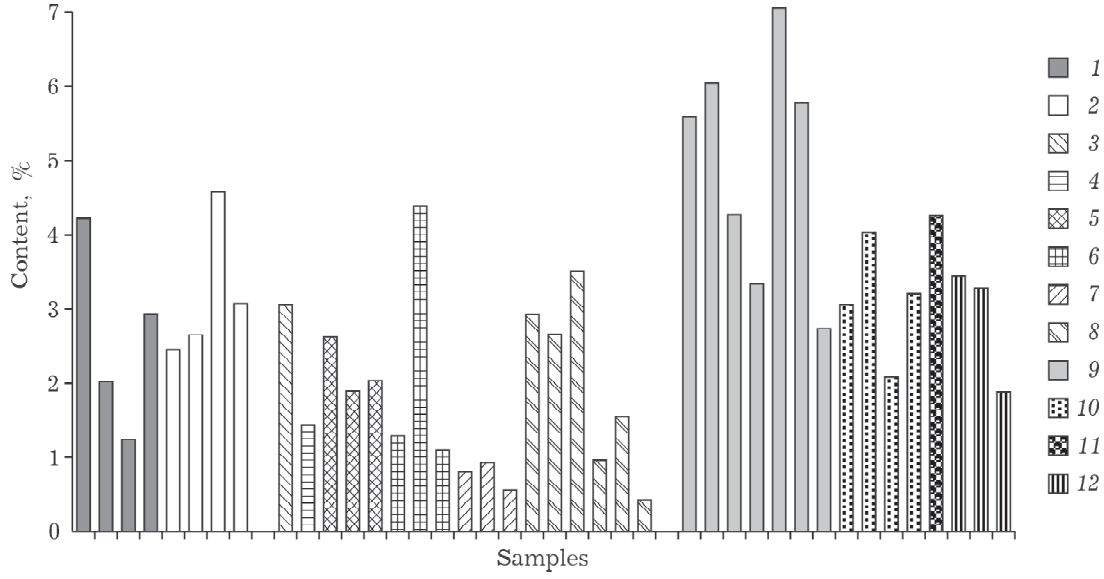

Fig. 1. Content of flavonoids in leaves of plant species belonging to genus *Myosotis*. On the abscissa the following plant species of genus *Myosotis* are plotted: *1* – *M. palustris*, *2* – *M. caespitosa*, *3* – *M. sylvaticae*, *4* – *M. sachalinensis*, *5* – *M. sajanensis*, *6* – *M. decumbens*, *7* – *M. pseudovariabilis*, *8* – *M. austrobaicalensis*, *9* – *M. asiataca*, *10* – *M. austrosibirica*, *11* – *M. popovii*, *12* – *M. baicalensis*.

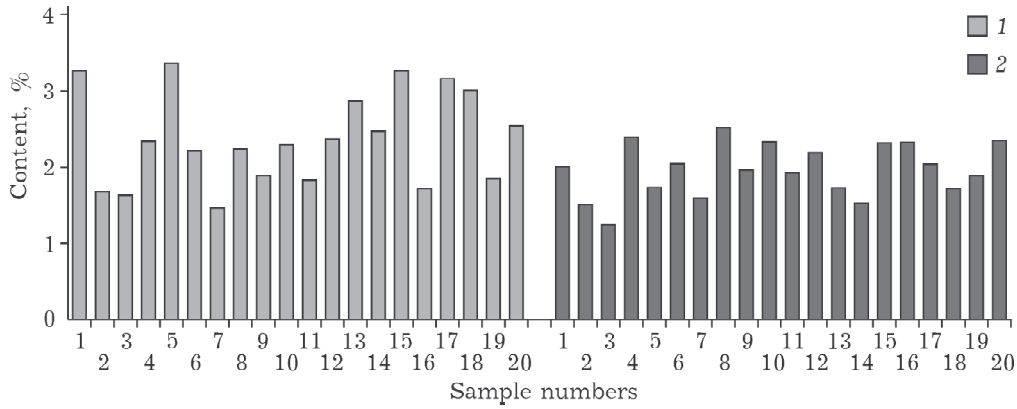

Fig. 2. Flavonoid content variation in individual plants by the example of *Myosotis arvensis* (*1*) and *M. caespitosa* (*2*).

and 20 plants belonging to *M. Caespitosa* species (section *Myosotis)* harvested over the territory of the Kemerovo Region (Chebula District, on the outskirts of the Kurakovo District, a macereed swamp) (Fig. 2).

Basing on the data obtained we have determined the variation coefficient (*V*). The evaluation of variation coefficient was carried out according to the scale presented in [16].

The variability level of flavonoid content for individual plants *M. arvensis* is $V = 16.1$ %, with the variation range equal to 1.5–3.4 %, $\bar{m} = 2.4$ %. For plants *M. caespitosa* $V = 7.2$ %, the variation range is equal to 1.2–2.5 %, $\bar{m} = 2.0$ %.

The variability level of is considered to be very low in case of $V \le 7$ %, low at $V = 8$–12 %, medium at $V = 13$–20 %, high at $V = 21$–40 % and very high at $V > 40$ %.

We have established that the variability level of flavonoid content for individual plants of *Myosotis caespitosa* species within same coenotic population is low, whereas for samples of *M. arvensis* species this value is medium.

The comparative analysis of variability levels for the content of flavonoids in *M. arvensis* and *M. caespitosa* based on Fisher criterion [17] has demonstrated that the species are significantly differing in this characteristic: $F' = 3.17$ whereas $F'_{\text{tabl}} = 2.12$.

The seasonal and age variation factors exert a considerable influence upon the composition and content of chemical compounds [8, 18]. So, the authors of [19, 20] demonstrated that for the majority of plants the content of flavonoids increases during the vegetation period reaching a maximum value at the budding or flowering stage.

The seasonal dynamics for the accumulation of the sum of flavonoids we investigated in vegetative organs of *Myosotis krylovii* (Novosibirsk, on the outskirts of the CSBG, cultivated plants). We studied plants at seven development stages such as vegetation stage, budding stage, budding–flowering beginning stage, mass flowering stage, flowering–fructification beginning stage, flowering end–fructification stage, fructification stage. In order to minimize the influence of weather conditions upon the

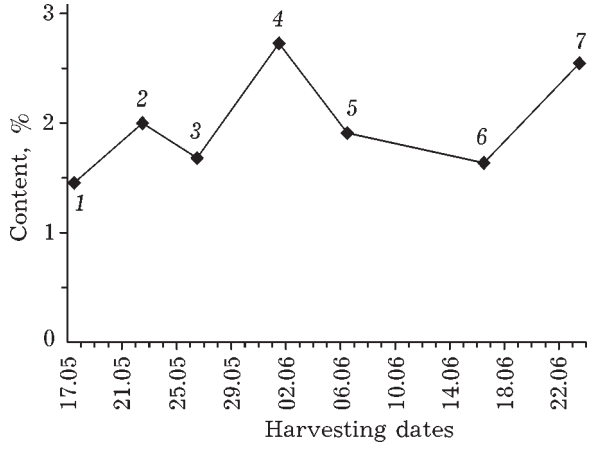

Fig. 3. Seasonal dynamics for flavonoid content in vegetative organs of *Myosotis krylovii*. On the ordinate the content of flavonoids is plotted; on the abscissa the development stages of plants are plotted: *1* – vegetation stage, *2* – budding stage, *3* – budding–flowering beginning stage, *4* – mass flowering stage, *5* – flowering–fructification beginning stage, *6* – flowering end–fructification stage, *7* – fructification stage.

content of flavonoids, the plant material was harvested within fine sunny days at the temperature of 25–28 °C, weather setting fair not later than 1 day before harvesting (Fig. 3).

The minimal content of flavonoids (1.45 %) has been revealed for plants in the vegetation stage. The first maximum value falls within the stage of mass flowering and is equal to 2.7 %. At the beginning of fructification stage the content of flavonoids decreases down to 1.9 %, and then down to 1.6 %. The second maximum of the content of flavonoids (2.5 %) was revealed at the fruitification stage.

The variation coefficient for the data obtained amounted to 11.4 %, *i.e.* the seasonal variability of flavonoid content is low. This fact allows one to perform a comparative analysis for the content of flavonoids in plant samples harvested within the periods of different development stages.

The mass of plant species belonging to genus *Myosotis* is maximal during the flowering stage and it is usually reduced towards the fruitification stage at the expense of radical leaves die-off and an increase in the portion of injured tissues. In order to elucidate the value of flavonoid-containing raw material of *Myosotis* species it would be appropriate to determine the content of flavonoids in the samples harvested during the mass flowering stage.

The differences in the chemical composition of plants one often connect with so-called geographical factor, *i.e.* with the sum of ecological and phytocoenotic factors determining the zoning of the vegetation cover [21]. The presence of chemical heterogeneity within the natural habitat could be of a great practical importance, since the content and qualitative composition of biologically active substances determine the characteristics and value of a raw material [22, 23].

The variation of flavonoid content in plant species of genus *Myosotis* is considered depending on geographical factors by the example of widespread species such as *M. imitata* (21 samples) and *M. krylovii* (14 samples) (Fig. 4).

The content of flavonoids in samples of species *M. krylovii,* harvested from various regions is characterized by a medium variability level (V = 16.0 %, the variation range being of 0.9–3.3 %), whereas for samples of *M. imitata* species this value is very high (V = 45.2 %, the variation ranging within 1.4–6.5 %). The average $\overline{m}$ values amounted to 1.9 and 3.3 % for *M. krylovii* and *M. imitate,* respectively.

The analysis of the results obtained allows one to assume that higher flavonoid content values for *M. imitata* as compared to those of *M. krylovii* could be caused by ecological attaching the species. As it is known, one of the basic functions of flavonoids in a plant consists in the protection against excess dozes of UV light [8]. Plant species *M. imitate* represents heliophytes those prefer open well-exposed habitats, whereas plant species *M. krylovii* represents sciophytes and grow mainly in forests [24].

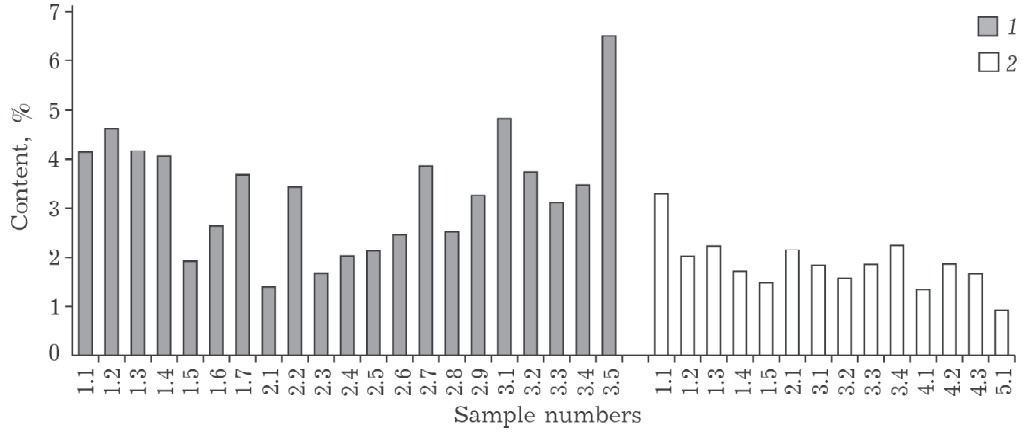

Fig. 4. Flavonoid content variation depending on plant habitat by the example of *Myosotis imitata* and *M. krylovii: 1* – *M. imitata*, *2* – *M. krylovii.* The second figure of sample number corresponds to trial number; the first figure corresponds to the region wherein the plants were harvested: *1* – Novosibirsk Region, *2* – Mountain Altai, *3* – Irkutsk Region and Buryatia, *4* – Khakassia, *5* – Tyva.

The maximal content of flavonoids (6.5 %) is revealed in a sample of *M. imitata* from Buryatia (Eravnoye District, the Bolshoe Eravnoye Lake; northern lakeside, steppe meadow). To a smaller extent flavonoids are contained in plants of this species harvested in the territory of Republic Mountain Altai (Kosh-Agach District, the Kuray Depression; larch forest – 4.8 %) and the Novosibirsk Region (Toguchin District, on the outskirts of the Gorny settlement, Bugotak hills; northern slope, grass-stone bramble birch forest – 4.6 %; Kargat District, on the outskirts of the Kargat River; riverside, meadow – 4.2 %; Suzun District, on the outskirts of the Rozdestvenskoye village, the Karasuk River valley; cereal-forb meadow – 4.1 %).

The comparative analysis of the values of flavonoid content in samples of *M. krylovii* and *M. imitata* based on the Fisher criterion has demonstrated that the variability level of flavonoid content in *M. imitata* is sufficiently differs from that in *M. krylovii:* $F' = 5$ whereas $F'_{\text{tabl}} = 2.11$.

CONCLUSION

The content of flavonoids in 122 samples of 15 plant species belonging to genus *Myosotis* has been studied. The seasonal dynamics of flavonoid accumulation in vegetative organs of *Myosotis krylovii* is characterized by the presence of the two maxima corresponding to the stage of mass flowering (2.7 %) and to the fruiting stage (2.5 %). The seasonal variability of the flavonoid content was observed to be low. The content of flavonoids in samples of species belonging to genus *Myosotis* from various regions is characterized by a sufficiently high variability. The variability of flavonoid content within the same one coenotic population was observed to be low or medium. Plant species from section *Alpestres,* in particular *M. asiatica,* are of interest for the further studying due to a high content of flavonoids (up to 7.4 %). Plants harvested within mass flowering period could be used as the sources of flavonoids.

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REFERENCES

1 M. N. Varlakov, Izbrannye Trudy, Medgiz, Moscow, 1963.
2 S. A. Vardanyan, *Rast. Res.,* 15, 3 (1979) 460.
3 A. A. Makarov, Lekarstvennye Rasteniya Yakutii i Perspektivy Ikh Osvoyeniya, Nauka, Novosibirsk, 2002.
4 J. L. Hartwell, *Lloydia*, 34, 2 (1971) 204.
5 Yu. V. Shinkarenko, Yu. L. Yakimova, V Mezhdunar. Nauch.-Prakt. Konf. "Problemy Botaniki Yuzhnoy Sibiri i Mongolii" (Proceedings), Barnaul, 2006, p. 321.
6 V. A. Bandyukova, S. F. Dzhumyrko, N. V. Sergeeva, A. L. Shinkarenko, Pervy Vsesoyuz. S'yezd Farmatsevtov (Treatises), Moscow, 1970.
7 M. D. Alaniya, E. V. Kereselidze, G. E. Dekanosidze *et al.*, Biologicheski Aktivnye Veshchestva Flory Gruzii, Metsniereba, Tbilisi, 1976, p. 64.
8 V. G. Mionaeva, Flavonoidy v Ontogeneze Rasteniy i Ikh Prakticheskoye Ispolzovaniye, Nauka, Novosibirsk, 1978.
9 G. R. Jamieson, E. H. Reid, *Phytochem.*, 8, 8 (1969) 1489.
10 N. Ishikura, *Kimamoto J. Sci. Biol.*, 12, 1 (1974) 17.
11 J. F. Resch, D. F. Rosberger, J. Meinwaid, *Lloydia*, 45, 3 (1982) 358.
12 M. N. Zaprometov, Osnovy Biokhimii Fenolnykh Soyedineniy, Vysshaya Shkola, Moscow, 1974.
13 A. I. Nikitin, *Probl. Reproduktsii*, 3 (2000) 16.
14 V. A. Bandyukova,E. T. Avanesov, *Rast. Res.,* 7, 3 (1971) 321.
15 G. I. Vysochina, I*bid.*, 4 (1998) 47.
16 S. A. Mamaev, Formy Vnutrividovoy Izmenchivosti Drevesnykh Rasteniy, Nauka, Moscow, 1973.
17 G. N. Zaytsev, Matematicheskiy Analiz Biologicheskikh Dannykh, Nauka, Moscow, 1991.
18 M. G. Pimenov, L. F. Borisova, *Itogi Nauki i Tekhniki. Botanika*, 6 (1987) 7.
19 G. A. Syrtanova, Konf. "Introduktsiya i Akklimatizatsiya Poleznykh Rasteniy v Kazakhstane" (Thesises), Alma-Ata, 1972, p. 105.
20 A. D. Bobrova, Konf. "Prirodnaya Flora Ukrainy i Moldavii i Obogashcheniye Yeye Putem Introduktsii" (Thesises), Kiev, 1972, p. 12.
21 I. L. Krylova, Sezonnaya Ritmika i Produktivnost' Dikorastushchikh Lekarstvennykh Rasteniy, Nauka, Moscow, 1988, p. 3.
22 S. Melibaev, U. Rakhmankulov, A. Saidkhodzhaev, *Rast. Res.*, 16, 3 (1980) 431.
23 N. K. Shokhina, G. I. Vysochina, A. P. Golgikh, *Sib. Ekol. Zh.*, 3 (1999) 257.
24 Yu. V. Shinkarenko, *Ibid.*, 10, 1 (2003) 79.