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MEDICINAL PLANTS

CHEMICAL COMPOSITION OF *ALLIUM SCHOENOPRASUM* LEAVES AND INHIBITORY EFFECT OF THEIR EXTRACT ON TUMOR GROWTH IN MICE

T. I. Shirshova,[1] I. V. Beshlei,[1] V. P. Deryagina,[2] N. I. Ryzhova,[2] and N. V. Matistov[1]

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A comprehensive chemical study of the cultivated plant *Allium schoenoprasum* L. from the collection of the Botanical Garden of the Institute of Biology (Komi Scientific Center, UB, RAS) showed that its leaves contained a wide range of biologically active substances and trace elements involved in the antioxidant and anticarcinogenic protection system of humans. An experiment for the evaluation of the antitumor potential of these substances showed that the aqueous and aqueous-alcohol extracts of onion leaves tended to inhibit the growth of subcutaneously grafted Ehrlich carcinoma in male BDF mice at the stage of intense tumor development.

Keywords: *A. schoenoprasum* L., extract, anticarcinogenic effect

Various onion species have been used since antiquity as food and medicinal plants [1]. Drugs created from extracts of onion *Allium cepa* L. and garlic *A. sativum* L. that affect movements of the gastro-intestinal tract and cardiovascular system or exhibit bactericidal properties are included among the tools of modern medicine [2, 3]. Experimental and epidemiological evidence that several biologically active compounds (BACs) found in onion can suppress proliferation of various types of tumor cells and reduce the risk of developing neoplasms of several organs including the colon, stomach, reproductive organs, etc. were obtained [4 – 7]. Several methods for preparing drugs with antitumor activity that contained essential oils of garlic and onion were developed [1]. The broad spectrum of activity of onion is due to the richness of its chemical composition, which includes vitamins, phytoncides, flavonoids, sugars, alkaloids, steroidal glycosides, and other valuable BACs and useful trace elements. High antioxidant properties of perennial onions due to the presence of specific chemical selenium species and high concentrations of flavonoids and vitamins E and C were reported several times in the literature [8].

The genus *Allium* is represented in Komi Republic by the three species *A. angulosum* L., *A. schoenoprasum* L., and *A. strictum* Schrad. Among these, *A. schoenoprasum* (chive) has the broadest distribution. It grows over practically the whole territory of the republic [9], has been cultivated, and is widely used by the population as a food and medicinal plant. A comprehensive study of the chemical composition of this onion species enabled us to compose for the first time the most complete list of the quantitative content and constituent composition of the most important BAC classes that play an important role as natural food components with pronounced physiological and pharmacological effects on the body and its principal regulatory and metabolic processes. Drugs obtained from this onion species were expected to exhibit various physiological activities because of the high content of ascorbic acid, selenium, and chromium in addition to steroidal glycosides involved in antioxidant protection of the

[1] Institute of Biology, Komi Scientific Center, Ural Branch, Russian Academy of Sciences, Syktyvkar, Russia.
[2] Research Institute of Carcinogenesis, Blokhin Russian Oncological Research Center, Russian Academy of Medical Sciences, Moscow, 115478 Russia.

body. They could be used to treat and prevent several diseases.

EXPERIMENTAL PART

We studied *A. schoenoprasum* from the collection of the Botanical Garden, Institute of Biology, Komi Scientific Center, Ural Branch, RAS. Onion leaves were ground and dried at room temperature with constant ventilation. Table 1 describes the isolation of neutral lipids (NL) and the analysis of their fatty-acid (FA) composition, the extraction of steroidal glycosides, and the isolation and identification of pure compounds. All analyses were performed in the eco-analytical laboratory Ekoanalit of the Inst. Biol., Komi Sci. Cent., Ural Br., RAS. The analysis for Se content was carried out in the Food Research Institute, RAMS (Moscow).

The antitumor potential of the tested substance was studied in 40 male BDF mice (29 – 30 g) from the Stolbovaya nursery. Dried and ground onion leaves were suspended in distilled H_2O and in aqueous EtOH (8%) in a 1:19 ratio, stored in a thermostat for 2 h at 37°C, and filtered through a metallic sieve. The aqueous onion extract was also stirred before peroral administration to the mice in volumes of 0.8 and 0.6 mL; the aqueous EtOH extract, 0.8 mL.

Tumor strain Ehrlich carcinoma (EC) was obtained from the stores of Blokhin Russian Oncological Scientific Center, RAMS. We used 3 – 4 passages of the tumor *in vivo*. Spontaneous breast cancer provided the initial tumor for the solid EC strain. Mice were housed in a vivarium on solid feed pellets with free access to water. They were divided into four groups of 10 animals each. The first group included mice with grafted EC as a control; the second group, mice that received the aqueous extract of onion leaves (AEL) at a dose of 1.3 g/kg of animal body mass (abm) via peroral administration five times per week for 2.5 weeks before EC grafting and during the course of the whole experiment afterward; the third group, mice that received AEL according to the above schedule at a dose of 1.0 g/kg abm; the fourth group, mice that received the aqueous-EtOH (8%) extract of onion leaves (AAEL) at a dose of 1.3 g/kg abm according to the above schedule. Each mouse of the second group during the whole experiment received 1360 mg; of the third group, 1020 mg; and of the fourth group, 1360 mg of substance. The EC tumor cells were grafted s.c. by inoculation into the right inguinal area. Each mouse was injected with 10^6 EC cells in

TABLE 1. Content of Biologically Active Compounds and Trace Elements in *A. schoenoprasum* Leaves

Compound class	Component composition	Quantitative content	Study methods	Reference
Neutral lipids (% of dry substance)	Sterols (sitosterol, stigmasterol, campesterol, cholesterol) and their esters, free fatty acids and their esters, mono-, di-, and triacylglycerins	0.75 – 1.94	TLC, HPLC, C—MS	[10]
Including higher fatty acids (% of their total amount)	linoleic (C 18:2)	22.8 – 32.3	GC	[10]
	linolenic (C 18:3)	34.4 – 38.7		
	palmitic (C 16:0)	24.5 – 25.9		
Steroidal glycosides (% of air-dried mass)	Spirostanols (deltonin, saponin A)	1.42 – 2.49	HPLC, C—MS	Results partially published in [11]
	Furostanols (deltoside, protodioscin)	1.33 – 2.14		
Vitamins (mg/100 g of moist raw matl.)	Ascorbic acid	81 ± 2	Titration with basic solution of 2,6-dichlorophenolindophenol	Results not published
Macroelements (% of dry substance)	K	1.40 – 4.00	Metal content in acid-soluble form determined on Spectro ICP-AES	Results partially published in [12]
	Na	0.011 – 0.070		
	Mg	0.12 – 0.18		
	Ca	0.55 – 1.49		
	P	0.24 – 0.67		
	S	0.4 – 1.44	GC	
Microelements (mg/kg of dry substance)	Fe	15 – 160	Spectro ICP-AES	
	Mn	14 – 220		
	Cr	1.5 – 10.0		
	Cu	2 – 7		
	Zn	10 – 38		
	Ni	1.2 – 7.7		
	Al	36 – 140		
	Se	0.063 – 0.174	Fluorometric method	[13]

Hanks solution (0.5 mL). The inhibiting effect of AAEL was estimated from tumor growth inhibition (TGI) determined from the volume and mass of tumors in animals of the test groups compared with the control (group 1, EC).

$$TGI = [(V_t (V_c)/V_c]\cdot 100\%,$$

where V_c is the average tumor volume in the control group and V_t, the average tumor volume in the test group (mm^3).

Keeping in mind that fact that the tumor did not have a perfectly spherical shape but was more reminiscent of an ellipsoid, the tumor volume was determined using the formula for calculating the volume of an ellipsoid:

$$V = A \cdot B \cdot C \cdot \pi/6,$$

where A, B, and C are the lengths of the large, medium, and small axes [14].

Mice were euthanized under ether anesthesia at the end of the experiment (on the 52nd day). Tumors were excised. Their masses were determined.

RESULTS AND DISCUSSION

The study of the chemical composition of *A. schoenoprasum* showed that it produced a whole series of BACs and trace elements (Table 1). Leaves of *A. schoenoprasum* contained from 0.75 to 1.94% neutral lipids, the principal constituents of which were sterols ($R_f = 0.81$) and their esters ($R_f = 0.8$); free FA ($R_f = 0.36$) and their esters ($R_f = 0.48$); and mono-, di-, and triacylglycerins ($R_f = 0.57$). Our investigations showed that the sterols made up a substantial part of the NLs from *A. schoenoprasum* leaves. According to HPLC data, the principal component of the sterols was sitosterol, the structure of which was confirmed by chromatography-mass spectrometry (C–MS). Insignificant quantities of other sterols such as stigmasterol, campesterol, and cholesterol were detected by this same method. Sitosterol and stigmasterol are the sterols encountered most frequently in plants. Phytosterols, which occur in significant quantities in vegetables and fruit, exhibit a protective effect from atherosclerosis and colon cancer [15]. The FA composition of the NLs included acids with C_{16}-C_{20} chains and an even number of C atoms. The principal FAs by content were the unsaturated higher FAs linoleic and linolenic (Table 1).

Steroidal glycosides (SG) exhibiting antisclerotic, antioxidant, and antitumor properties were especially interesting [16].

The onion contained rather high concentrations of trace elements such as zinc, copper, and chromium (Table 1) [17 – 20].

Our investigations showed that onion leaves contained from 111 to 121 μg/kg of Se (Table 1). The biological accumulation coefficients for Se were 0.9 – 1.1. This was indicative of the accumulating properties of *A. schoenoprasum* for this trace element. These results suggest that the onion may contribute to the anticancer protection of the human body.

Table 2 presents results from the study of the antitumor potential of AEL and AAEL against grafted EC in BDF mice from 5 to 33 days after tumor development resulting from s.c. inoculation of tumor cells. The TGI index was from 10.2 to 38.4% for peroral administration to mice. However, it was not statistically significant. With respect to the group of ani-

TABLE 2. Effect of Aqueous and Aqueous-Alcohol Extracts of *A. schoenoprasum* Leaves on Growth of Subcutaneously Grafted Ehrlich Carcinoma (EC) in Male BDF Mice

Group, active substance (dose, g/kg abm)	Tumor volume, mm^3, $M \pm SD$ (volume change, %)								Tumor mass, g, 35 d
	Days after tumor grafting								
	5	8	12	15	19	22	28	33	
1 (EC)	41.6 ± 18.1 $n = 10$	195.5 ± 88.7 $n = 10$	844.5 ± 419.4 $n = 10$	960.7 ± 538.7 $n = 10$	1189.6 ± 824.9 $n = 10$	1946.7 ± 1286.4 $n = 9$	2624.3 ± 937.2 $n = 8$	4258.8 ± 1405 $n = 8$	4.21 ± 1.81 $n = 8$
2 EC + AEL (1.3)	43.4 ± 21.2 $n = 10$ (– 4.3)	201.1 ± 172.6 $n = 10$ (+ 2.9)	577.7 ± 463.3 $n = 10$ (– 31.6)	592.2 ± 429.8 $n = 10$ (– 38.4)	878.9 ± 638.1 $n = 10$ (– 26.1)	1283.3 ± 957.8 $n = 10$ (– 34)	2230.8 ± 1048.3 $n = 10$ (– 15.0)	3869.6 ± 1391.9 $n = 10$ (– 9.1)	4.19 ± 1.40 $n = 10$
3 EC + AEL (1.0)	38.6 ± 13.6 $n = 10$ (– 7.2)	265.9 ± 301.2 $n = 10$ (+ 36.0)	964.4 ± 473.0 $n = 10$ (+ 14.2)	1161.1 ± 619.8 $n = 10$ (+ 20.9)	1284.9 ± 873.2 $n = 10$ (+ 8.0)	1683.6 ± 1103 $n = 10$ (– 13.5)	2284.6 ± 870.7 $n = 10$ (– 13.0)	3470.2 ± 969.5 $n = 10$ (– 18.5)	4.07 ± 1.15 $n = 10$ (– 3.3)
4 EC + AAEL (1.3)	30.4 ± 11.6 $n = 10$ (– 26.9)	282.2 ± 183.9 $n = 10$ (+ 44.4)	584.7 ± 166.6 $n = 9$ (– 30.8)	592.2 ± 429.9 $n = 9$ (– 38.4)	1068 ± 455.5 $n = 9$ (– 10.2)	1332.9 ± 658.3 $n = 9$ (– 31.5)	1892.2 ± 727.8 $n = 9$ (– 27.9)	3203.8 ± 1431.9 $n = 9$ (– 24.8)	3.85 ± 1.13 $n = 9$ (– 8.6)

Note. Results expressed as mean arithmetic values ± standard deviations (SD). Values in % potentiation (+) or inhibition (() of tumor growth given in parentheses.

mals that received AEL at a dose of 1.0 g/kg abm, AEL at this dose did not have a potentiating effect on EC growth according to the TGI indices at all controlled times and the uncertainties in the measurement of the tumor volume. Tumors from mice were weighed 35 d after inoculation of tumor cells. The results showed that this parameter was not reduced (group 2) or decreased insignificantly (3.3 and 8.6% for groups 3 and 4, respectively) compared with the control (Table 2).

It must be noted that no mice died in the groups of animals that received AEL whereas one animal that received AAEL (group 4) died and two mice in the control group (group 1) died.

It was interesting that a healing effect was observed after ulceration of tumors caused by the development of necrotic processes in mice that received AEL or AAEL of onion.

Considering existing requirements for substances of natural origin during evaluation of their antitumor activity, the TGI value as affected by the tested substance should be >50.0% ($p < 0.05$) [14]. In our instance, AEL or AAEL of onion exhibited weaker inhibition for EC that was not confirmed statistically. However, the arithmetic mean of tumor volume from 12 to 33 d of EC growth in mice that received the maximum dose of onion extract was consistently lower.

Thus, AEL and AAEL of onion exhibited a tendency to inhibit the growth of s.c. grafted EC in male BDF mice during its vigorous development. We propose that performing the experiment using onion extracts enriched with SG will enable their antitumor protection effect to be enhanced and prolonged.

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REFERENCES

1. G. I. Deineko, *Rastit. Resur.*, **21**(2), 221 – 229 (1985).
2. E. Lyutomski, *Novosti Farm. Med.*, No. 4, 193 – 202 (1980).
3. M. D. Mashkovskii, *Drugs* [in Russian], Vol. 2, Meditsina, Moscow (1984).
4. C. Galeone, C. Pelucchi, L. Dal Maso, et al., *Public Health Nutr.*, **12**(9), 1576 – 1579 (2009).
5. K. Lazarevic, A. Nagorni, N. Rancic, et al., *J. BUON.*, **15**(1), 89 – 93 (2010).
6. S. Tache, A. Ladam, and D. E. Corpet, *Eur. J. Cancer*, **43**(2), 454 – 458 (2007).
7. E. Viry, A. Anwar, G. Kirsch, et al., *Int. J. Oncol.*, **38**(4), 1103 – 1111 (2011).
8. D. Stajner, N. Milic, J. Canadanovic-Brunet, et al., *Phytother. Res.*, **20**(7), 581 – 584 (2006).
9. A. I. Tolmachev (ed.), *Flora of Northeast European USSR* [in Russian], Vol. 2, Nauka, Leningrad (1976), pp. 112 – 114.
10. T. I. Shirshova, I. V. Beshlei, and I. V. Gruzdev, *Rastit. Resur.*, **44**(1), 75 – 81 (2008).
11. T. I. Shirshova and G. A. Volkova, *Rastit. Resur.*, **42**(3), 59 – 66 (2006).
12. T. I. Shirshova and I. V. Beshlei, *Rastit. Resur.*, **45**(2), 97 – 105 (2009).
13. T. I. Shirshova, I. V. Beshlei, N. V. Matistov, et al., *Rastit. Resur.*, **47**(1), 112 – 118 (2011).
14. E. M. Treshchalina, O. S. Zhukova, G. K. Gerasimova, et al., *Handbook of Experimental (Preclinical) Study of New Drugs* [in Russian], Meditsina, Moscow (2005), pp. 637 – 651.
15. N. A. Golubkina, S. M. Sirota, V. F. Pivovarov, et al., *Biologically Active Compounds from Vegetables* [in Russian], VNIISSOK, Moscow (2010).
16. I. S. Vasil'eva and V. A. Paseshnichenko, *Usp. Biol. Khim.*, **40**, 153 – 204 (2000).
17. A. V. Shabrov, V. A. Dadali, and V. G. Makarov, *Biochemical Principles of Action of Food Microcomponents* [in Russian], Avvalon, Moscow (2003), pp. 99 – 135.
18. B. P. Yu, *Physiol. Rev.*, **74**, 139 – 162 (1994).
19. C. Ip, *J. Nutr.*, **128**(11), 1845 – 1854 (1998).
20. P. R. Harrison, J. Lanfear, L. Wu, et al., *Proceedings of the 6th Int. Symposium on Selenium in Biology and Medicine*, Van Nostrand Reinhold, New York (1996), pp. 74 – 82.