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BIOCHEMICAL FEATURES OF COMMON COCKLEBUR (*XANTHIUM STRUMARIUM* L.)

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Biochemical features of wild common cocklebur (*Xanthium strumarium* L.) were studied in order to assess its possible cultivation. Analyses showed high contents of fatty oil in seeds (up to 40%) and fruit (up to 12%) that consisted of unsaturated (palmitic, stearic) and more valuable polyunsaturated (linoleic, linolenic) acids. Oils extracted from seeds and whole fruit had practically identical chemical compositions. The results indicated that free iodide ion was absent in the plant and that the contents of organically bound iodine in oils from the fruit (385 mg/L) and fruit pulp after pressing (215 mg/kg) were elevated.

Keywords: common cocklebur, *Xanthium strumarium* L., fatty oil, saturated and unsaturated fatty acids, organic iodine compounds.

The study and introduction into cultivation of new and endangered medicinal plants is an important endeavor. Therefore, much research at VILAR is currently focused on the introduction of little studied promising medicinal and aromatic plants, including species from the genus *Xanthium* L. (cockleburs), in particular, *X. strumarium* L. (common cocklebur) (Asteraceae/Compositae) [1 – 4].

The common names are cocklebur, burr, sheep burr, etc. [1 – 4]. The plant is an annual 30 – 120 cm in height and is a short-day plant that flowers in July-August. Each cocklebur bur contains two seeds. The seeds are covered by a hard green husk with hooked spines.

Cocklebur grows in the southern and middle bands of European Russia, the Caucasus, southern Siberia, and Central Asia. The plant inhabits moist sandy soil along banks of rivers and ditches and neglected sites [1 – 5].

Leaves, stems, fruit, and roots are used medicinally. Leaves and stems are harvested in July-August; fruit, September-October; roots, in late autumn.

According to the literature, the aerial part contains 17 classes of various organic compounds including mono- and sesquiterpene lactones, steroids, carotinoids, phenols, lignans, flavonoids, anthraquinones, N-containing compounds, halide-containing compounds, organic acids, higher fatty acids, fatty oil, etc. [1 – 14]. Seeds contain up to 40% oil consisting of saturated acids (8.2%), oleic acid (27.1%), linoleic acid (63.4%), and extractable substances (26.1%). Furthermore, seeds contain xanthostrumarin glycosides (up to 1.27%); fruit, the sesquiterpene lactones xanthatin and xanthinosin [11]; the herb, alkaloids. It is noteworthy that elevated iodine contents in all plant organs [3, 4] and the presence of chlorine compounds [2, 7] were reported. However, the chemical composition of the plant and its possible medicinal applications are insufficiently studied.

The structures of eight isolated phenolic acids, six of which were hydroxycinnamic acids, were recently elucidated by Chinese researchers using modern research methods (NMR, GC, HPLC) [11, 12]. In addition to common cocklebur (*X. strumarium*), research is also being conducted globally on the chemical composition and pharmacological activity of *X. sibiricum* Patrin ex Widder [11], *X. spinosum* L. [1 – 4], and *X. italicum* Moretti [10].

Increased attention has been paid to cocklebur since it was recognized as a possible valuable source of medicinal preparations containing iodine compounds, fatty oil, higher fatty acids, phenolic compounds, and other biologically active compounds [2 – 17].

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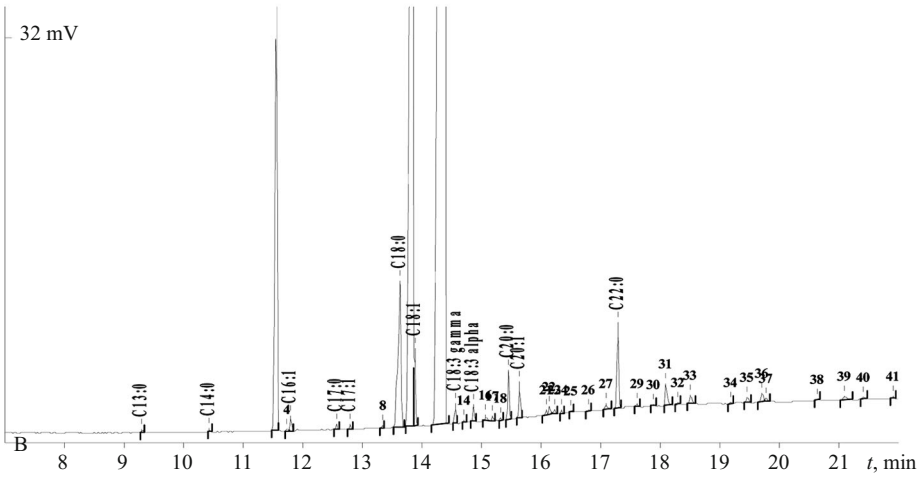

Fig. 1. HPLC chromatogram of methyl esters of fatty-acids from cocklebur seeds (cold pressing) after alkaline hydrolysis and methylation by diazomethane.

The effect of cocklebur on animals has been studied. Tests showed that total alkaloids from this plant at doses exceeding the maximum tolerated ones caused cardiac insufficiency and hypotension. Noticeable effects on the cardiovascular system were not observed at non-toxic doses. Therapeutic doses strengthened and slowed breathing and relaxed intestinal smooth muscle. Based on these results, alkaloids from common cocklebur could be recommended for clinical trials as an agent for stimulating breathing and relaxing intestinal smooth muscle. The plant assisted thyroid shrinkage with goiter and possessed anticonvulsive activity with colic.

Cocklebur herb is used in folk medicine for thyroid diseases, diarrhea, gastrointestinal diseases, and cholera. Fresh herb juice is given to hives patients. Cocklebur is an effective agent for treating various skin diseases. It is used internally and externally for eczema, impetigo, and fungal skin and nail infections. Cocklebur is a poisonous plant that requires caution if used internally [1 – 5].

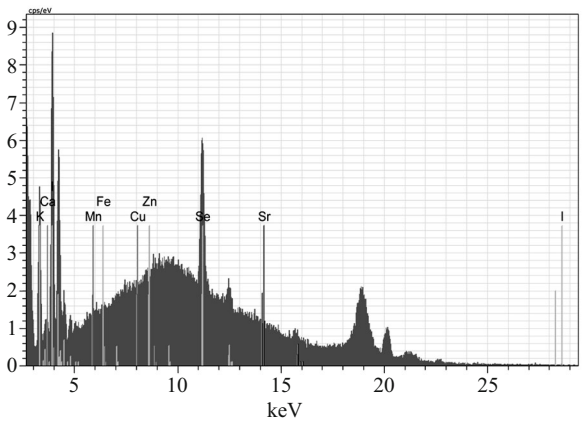

Fig. 2. Typical XRF spectrum of cocklebur seed pulp.

Cocklebur is not used in official medicine of the RF. The plant is used in classical homeopathy [4] and is officially recognized in China (cocklebur fruit is included in the Chinese Pharmacopoeia) and several other countries. The preparation Adenostop is manufactured from cocklebur in Romania and is used to treat prostate adenoma. High anticancer activity of cocklebur (for breast, lung, stomach, and colon cancer) was recently reported [13 – 15]. It possesses pronounced antiprotozoal activity. Therefore, modern highly effective domestic medicinal preparations may be created after a thorough study of the biochemical features of the plant and its biological activity.

EXPERIMENTAL PART

The studies were carried out in 2013 – 2014. Fruit and seeds isolated from *X. strumarium* fruit collected during rip-

TABLE 1. Oil Yield from Cocklebur and Oxidation Index (2013 Harvest)

Sample No.	Part	Moisture, % of abs. dry mass	Oil content, % of abs. dry mass	Oxidation index	
				Cold pressing	Soxhlet
1	Fruit with husk	5.5	12.1	7.7	7.7
2	Fruit with husk	5.3	13.3	7.0	10.0
3	Greenish seeds	5.2	34.7	5.7	5.9
4	Green seeds	5.6	37.0	6.8	6.2
5	Greenish-brown seeds	4.0	37.9	4.9	5.6
6	Brown seeds	5.3	36.8	7.4	6.2
7	Yellowish-green seeds	6.0	38.8	2.9	–

TABLE 2. Principal Fatty Acid Contents in Cocklebur Oil from Fruit and Seeds Extracted by *n*-Hexane in a Soxhlet Apparatus

Average	Acid					
	palmitic C16:0	stearic C18:0	oleic C18:1	linoleic C18:2	α-linolenic C18:3	γ-linolenic C18:3
From fruit (for 3 tests)	5.4 ± 0.2	2.27 ± 0.1	19.9 ± 0.4	67.43 ± 0.39	0.26 ± 0.02	0.2 ± 0.02
From seeds (for 3 tests)	5.59 ± 0.3	2.14 ± 0.15	20.07 ± 0.44	68.06 ± 1.15	0.29 ± 0.05	0.18 ± 0.07

ening in August 2013 in Eletskii District, Lipetsk Region, on the banks of Bystraya Sosna River near Cherkassy were studied.

Air-dried material was ground in a manual grinder to a particle size passing through a 0.2-mm sieve. Moisture of ground fruit and seeds was determined by the usual gravimetric method. Total lipids were extracted by *n*-hexane using a modified Soxhlet method. The hexane extracts were filtered through a layer of anhydrous Na_2SO_4, evaporated in vacuo in a rotary evaporator at <50°C until the odor of the solvent disappeared, and dried to constant mass in a desiccator over anhydrous $CaCl_2$. The masses of the obtained fatty-acid fractions were determined gravimetrically. The degree of oxidation of the fat extracted by cold pressing or using the Soxhlet apparatus was estimated from the oxidation index that was measured using the optical density in the UV spectrum at 232 nm of a solution with an accurately known concentration of the fat in *n*-hexane [15]. The fatty-acid composition of the oils was studied using an HRGC 5300 mega series high-performance capillary GC (Carlo Erba) with an OmegaWax quartz capillary column (30 m × 0.32 mm, stationary phase 0.25 μm, Supelco), temperature programmed linearly by a column thermostat from 100 to 250°C at 10°C/min, an FID, detector temperature 260°C, injector temperature 250°C, and flow division (Split, He 6.0) 25:1. Chromatograms were recorded (FID) and processed using Ampersand Multichrom v1.7 software. Figure 1 shows a typical chromatogram of a mixture of fatty acids (as methyl esters, FAME) from cocklebur fatty oil after alkaline hydrolysis by KOH solution (1 N) in anhydrous EtOH followed by methylation using diazomethane in ethoxypropane with a MeOH catalyst.

Separated chromatographic fractions of the analyzed mixtures were analyzed qualitatively and identified using a standard mixture of 37 FAMEs (Supelco). The mixture of FAME from cocklebur fatty oil was analyzed quantitatively using an internal normalization method.

Organic iodine compounds in pulp and oil were analyzed quantitatively using optical-emission spectroscopy on a Vista-PRO simultaneous CCD ICP-AES spectrometer (Varian, Switzerland) with a SOLIS-500 laser ablation system [17] in combination with XRF using a S2 Picofox spectrometer (Bruker) (Fig. 2).

RESULTS AND DISCUSSION

The fatty-oil samples obtained by us were white to pale-green transparent oily liquids with a sharp specific aroma depending on the starting material, i.e., fruit or seeds, and its ripeness. Considering the difficulty of separating the seeds from the fruit, the oil could be obtained directly from the pulverized fruit. Table 1 presents the yield of oil with respect to the dry mass of all studied samples. Our results agreed with those published earlier [5, 6].

Fruit of cocklebur averaged 12.7% fatty oil; seeds, 37%. The oil consisted of saturated palmitic (5.53%) and stearic acids (2.12%); monounsaturated oleic acid (20.0%); and the more valuable polyunsaturated linoleic (67.8%), α-linolenic (0.28%), and γ-linolenic acids (0.20%) (Table 2).

Table 2 shows that oil obtained from cocklebur fruit had a fatty-acid composition close to that of its seed oil. This simplified the oil production technology. Thus, cocklebur fatty-acid oil had a characteristic high content of easily oxidized linoleic acid and, in contrast with evening-primrose

TABLE 3. Principal Fatty Acid Contents in Cocklebur Oil (2013 Harvest) as a Function of Extraction Method

Sample No.	Fatty acid	% of total content in oil	
		cold pressing	Soxhlet apparatus
1	Palmitic C16:0	5.06	4.98
2	Stearic C18:0	2.31	1.98
3	Oleic C18:1	20.42	19.43
4	Linoleic C18:2	69.84	70.64
5	γ-Linolenic C18:3	0.15	0.31
6	α-Linolenic C18:3	0.13	0.14

TABLE 4. Organically Bound Iodine Content in Cocklebur Organs

Sample No.	Sample type	Organically bound iodine content	
		mg/L	mg/kg
1	Oil from clean seeds	114.6	-
2	Oil from fruit with seeds	340.2	-
3	Pulp from clean seeds	-	76.7
4	Pulp from fruit husk	-	242.5
5	Pulp from fruit with seeds	-	217.2

(*Oenothera biennis* L.) oil [18], an insignificant amount of the more biologically active linolenic acids.

Clinical observations established a 1:5 ratio of polyunsaturated fatty acids omega-3 and omega-6 was most favorable for treating cardiovascular diseases. Therefore, medicinal preparations could be formulated by fractionating the fatty acids and enriching the preparations with a source of one or the other of these fatty acids [19].

Polyunsaturated fatty acids are known to be thermally unstable and unstable to storage at positive temperatures. Therefore, a determination of the oxidation index and the fatty-acid composition as a function of the oil isolation method (cold pressing or Soxhlet apparatus) were of great interest (Table 3).

The results indicated that the tested isolation methods had an insignificant influence on the content and quality of the fatty-oil composition.

Considering the conflicting reports about the quantitative content of iodine in all plant organs, we studied isolated total lipids from seeds and pulp from oil separation for their iodine contents. Studies using the known procedure [20] showed that free iodide ion was absent in them. Apparently, the iodine in cocklebur was only bonded to other organic compounds. In order to check this hypothesis, organic iodine compounds from the plant were studied in more detail using optical-emission spectroscopy in combination with XRF. Table 4 presents the results for the contents of organically bound iodine in the cocklebur samples.

Thus, the iodine content was elevated in oil isolated from fruit with seeds. The relatively low content of organically bound iodine in pulp from clean cocklebur seeds was noteworthy. The iodine content was elevated in pulp from fruit husks after separating oil. XRF analysis of seed pulp detected iodine in addition to the trace elements Se, Mn, Zn, and Cu, which are valuable for humans.

REFERENCES

1. *Flora of the USSR* [in Russian], Vol. 25, Izd. Akad. Nauk SSSR, Moscow, Leningrad (1959), pp. 521 – 522.
2. *Plant Resources of Russia. Wild Flowering Plants, Their Quantitative Composition and Biological Activity* [in Russian], Vol. 5, Part 2, St. Petersburg, Moscow (2013), pp. 139 – 141.
3. V. P. Makhlayuk, *Medicinal Plants in Folk Medicine* [in Russian], Povolzhskoe Kn. Izd., Saratov (1991), pp. 131 – 132.
4. E. A. Ladynina, *Wisdom of Herbs. Herbal Cures and Homeopathy* [in Russian], AIF Print, Moscow (2003), pp. 216 – 217.
5. K. S. Shrivastava, R. S. Krisnamurthy, and C. N. Haksar, *J. Sci. Ind. Res., Sect. B*, **16**, 427 – 428 (1957).
6. H. Handtl, *Phyton*, **15**, Fasc. 1 – 2, 2 – 25 (1973).
7. N. P. S. Bisht and R. Singh, *J. Indian Chem. Soc.*, **54**(8), 797 – 798 (1977).
8. T. Han, H. L. Li, Q. Y. Zang, et al., *Chem. Nat. Compd.*, **42**(5), 567 – 570 (2006).
9. T. Han, H. L. Li, Q. Y. Zang, et al., *Chem. Nat. Compd.*, **44**(6), 814 – 816 (2008).
10. A. Kovacs, A. Vasas, P. Forgo, et al., *Z. Naturforsch.*, **64**(5 – 6), 343 – 349 (2009).
11. T. Han, H. L. Li, Y. Hu, et al., *J. Chin. Integr. Med.*, **4**(2), 194 – 198 (2006).
12. T. Han, Q. Y. Zhang, H. Zhang, et al., *Anal. Chim. Acta*, **634**(2), 272 – 278 (2009).
13. L. Tao, F. Fan, Y. Liu, et al., *PLoS One*, **8**(11), e81945 (2013).
14. L. Zhang, L. Tao, J. Ruan, et al., *Planta Med.*, **78**(09), 890 – 895 (2012).
15. I. Ramirez-Eroza, Y. Huang, R. A. Hickie, et al., *Can. J. Physiol. Pharmacol.*, **85**(11), 1160 – 1172 (2007).
16. T. A. Sokol'skaya, A. I. Malakhov, V. P. Golubev, and V. V. Semikin, *Vedom. Nauchn. Tsentra Eksp. Gos. Kontrolya Lek. Sredstv Minzdrava RF*, **2**(3), 77 – 78 (2000).
17. P. A. Yarvig, M. V. Kakhetelidze, and L. I. Churkadze, *Issled. Obl. Estestv. Nauk*, **1**(25) (2014); http: // science.snauka.ru / 2014 / 01 / 6585.
18. G. I. Klimakhin, V. S. Fonin, V. V. Semikin, et al., in: *Proceedings of the International Scientific-Methodical Conference…VNIISSOK* [in Russian], Izd. RUDN, Moscow (2011), pp. 178 – 182.
19. S. N. Kulakova, *Masla Zhiry*, No. 1, 10 – 12 (2011).
20. V. G. Belikov (ed.), *Laboratory Techniques in Pharmaceutical Chemistry* [in Russian], Vysshaya Shkola, Moscow (1989).