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CHEMICAL STUDY OF *Panzerina lanata*

D. N. Olennikov* **and L. M. Tankhaeva**

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Panzerina lanata (L.) Sojak. is a plant species of the family Lamiaceae. It is widely distributed over the territory of Buryatia. *P. lanata* is used in Tibetian medicine under the name *gang ga′ chung* as a substitute for *Gentiana urnula* Smith. (Gentianaceae) to treat stomach, intestinal, and gynecological diseases [1]. A chemical study of this plant found ballonigrine, 13-hydroxyballonigrine [2], stachydrin [3], and phenolic compounds [4]. The goal of our work was to study the chemical composition of the aerial part of *P. lanata* growing in Buryatia.

The aerial part of *P. lanata* was collected in the vicinity of Naryn-Atsagat (Republic of Buryatia, Russia, June 17, 2009; 52°01′65″ N, 108°27′95″ E). The species was determined by Doctor of Pharmaceutical Sciences T. A. Aseeva (IGEB, SB, RAS). A sample of the raw material was preserved in the herbarium of the Department of Biologically Active Compounds, IGEB, SB, RAS (No. L/L2111-09).

Ground raw material of *P. lanata* (340 g) was extracted with EtOH (70%) on a boiling-water bath (5×, 1:15 ratio). The combined alcohol extract was concentrated to an aqueous residue that was extracted successively with C_6H_{14}, $CHCl_3$, and EtOAc to produce hexane (11.295 g, 3.32% of air-dried mass), $CHCl_3$ (6.514 g, 1.92%), and EtOAc (6.601 g, 1.94%) fractions and an aqueous residue (92.303 g, 27.15%).

The hexane fraction (9 g) was chromatographed over a column of SiO_2 (2 × 40 cm) using C_6H_{14}:EtOAc (100:0→75:15) with subsequent rechromatography on TLC (solvent system 1, detector 1). This isolated seven compounds were identified as *β*-carotene (3 mg, **1**), lutein (6 mg, **2**), zeaxanthin (4 mg, **3**), violaxanthin (4 mg, **4**), neoxanthin (2 mg, **5**) [5], *β*-sitosterol (12 mg, **6**), and ursolic acid (25 mg, **7**) [6]. The composition of free fatty acids in the hexane fraction was determined by GC–MS after methylation [7]. A total of 12 compounds was found (% of total mass of fatty acids): 15:0 (0.36), 16:0 (30.31), 16:1 (n-9) (0.38), 17:0 (0.78), 18:0 (10.81), 18:1 (n-9) (10.92), 18:1 (n-7) (2.08), 18:2 (n-6) (12.00), 19:0 (9.41), 18:3 (n-3) (2.04), 20:0 (11.19), 22:0 (9.71).

The $CHCl_3$ (6 g) and EtOAc (6.5 g) fractions were separated using column chromatography over SiO_2 (1.5 × 30 and 2 × 40 cm, respectively) and a $CHCl_3$:EtOH gradient (100:0→65:35) with subsequent rechromatography of subfractions over Sephadex LH-20 (EtOAc:EtOH, 100:0→0:100) and TLC (solvent system 2, detector 2). The $CHCl_3$ fraction afforded **7** (10 mg), quercetin (2 mg, **8**) [8], and kaempferol (9 mg, **9**) [9]; the EtOAc fraction, caffeic acid (22 mg, **10**) [10], acteoside (6 mg, **11**) [11], 2-*O*-caffeylmalic acid (phaselic acid, 11 mg, **12**) [12], 3-*O*-caffeylquinic acid (chlorogenic acid, 12 mg, **13**), 5-*O*-caffeylquinic acid (neochlorogenic acid, 14 mg, **14**) [13], quercetin-3-*O*-rutinoside (rutin, 28 mg, **15**) [14], quercetin-3-*O*-rhamnoside (quercitrin, 11 mg, **16**) [8], and luteolin-7-*O*-glucoside (cinaroside, 9 mg, **17**) [14].

2-Caffeylmalic Acid. UV spectrum (λ_{max}, nm): 221, 245, 327. MS (*m*/*z*): 295. ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm): malate: 175.4 (C-1), 71.9 (C-2), 37.9 (C-3), 174.0 (C-4); caffeyl: 126.9 (C-1′), 115.2 (C-2′), 146.4 (C-3′), 148.2 (C-4′), 116.2 (C-5′), 124.4 (C-6′), 147.2 (C-7′), 114.8 (C-8′), 167.8 (C-9′).

HPLC (conditions 1) was used to establish the content of several phenolic compounds in morphological groups of *P. lanata* (Table 1). It was found that flowers typically accumulated alkaloids including quercetin (**8**) up to 0.03%, phaselic acid (**12**) up to 0.1%, rutin (**15**) up to 1.4%, and quercitrin (**16**) up to 1.5%. Leaves had high concentrations of caffeic acid (**10**) up to 0.15% and neochlorogenic acid (**14**) up to 1.5%. The dominant compounds of the aerial part of *P. lanata* were flavonoids such as rutin (up to 0.8%) and quercitrin (up to 0.75%) and phenylpropanoids such as chlorogenic (up to 0.52%) and neochlorogenic acids (0.57%).

Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, 670047, Ulan-Ude, fax: (3012) 43 30 34, e-mail: oldaniil@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 5, September–October, 2011, pp. 703–705. Original article submitted February 22, 2011.

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TABLE 1. Content of Phenolic Compounds in *P. lanata*

Compound, group of compounds	Morphological group				
	leaves	flowers	perianths	stems	aerial part
8, μg/g	23.76	257.50	Tr.	–	77.34
9, μg/g	Tr.	11.87	Tr.	–	Tr.
10, μg/g	1502.45	128.08	189.89	Tr.	524.63
12, μg/g	563.64	1121.03	853.64	Tr.	675.12
13, mg/g	2.48	2.48	1.24	Tr.	5.27
14, mg/g	15.43	0.24	0.15	2.36	5.74
15, mg/g	9.45	14.17	2.99	1.40	8.01
16, mg/g	6.48	14.49	6.52	0.62	7.52
17, mg/g	1.58	–	–	–	0.43
Flavonoids, mg/g	17.53	28.93	9.51	2.02	16.04
Phenylpropanoids, mg/g	19.98	3.97	2.43	2.36	12.21

Tr: traces; –, not observed.

Chromatographic analysis of the aqueous fraction of *P. lanata* (HPTLC, solvent system 3, detector 3) showed the presence of four compounds. These were isolated by dissolving the fraction (10 g) in H_2O (200 mL), adding NH_3 solution (25%) until the pH was 10, and extracting with $CHCl_3$ until a negative reaction with Dragendorff's solution was obtained. The combined organic extracts were filtered through anhydrous Na_2SO_4 and concentrated to dryness. The solid was dissolved in MeOH (20 mL) and chromatographed under preparative HPTLC conditions. The separation isolated two compounds that were identified as choline [6 mg, **18**, mp 182°C, MS (*m*/*z*): 104] and prolinebetaine {stachydrin, 8 mg, **19**, mp 280°C, MS (*m*/*z*): 143 $[M + H]^+$} [15]. Liquid chromatography (conditions 2) detected in this fraction proline (**20**) and 4-hydroxyproline (**21**).

Chromatography of the aqueous fraction of *P. lanata* detected the free carbohydrates (% of aqueous fraction mass/air-dried raw material) glucose (**22**, 5.63/1.55), arabinose (**23**, 5.12/1.47), saccharose (**24**, 0.88/0.24), fructose (**25**, 0.40/0.12) (HPLC, conditions 3) and the organic acids succinic (**26**, 4.97/1.41), citric (**27**, 2.05/0.56), malic (**28**, 1.22/0.37), tartaric (**29**, 0.63/0.21), and fumaric (**30**, 0.42/0.12) (HPLC, conditions 4).

Air-dried raw material of *P. lanata* (300 g) was treated by steam distillation in a Clevenger apparatus in order to study the essential oil (EO). Liquid extraction by hexane was used to isolate the EO because of its good solubility in water. EO of *P. lanata* was a grayish-brown thick liquid with a specific aroma, yield 0.64% of the air-dried raw material mass. The EO contained according to GC–MS 21 compounds (89.7% of the EO mass). The predominant components were monoterpenes (54.3%) including camphor (12.4%), α-pinene (10.3%), and linalool (9.1%).

The chemical composition of *P. lanata* EO was as follows:

Compound	*Content of essential oil mass, %*	*Compound*	*Content of essential oil mass, %*
α-Pinene	10.3	Terpinen-4-ol	6.4
β-Pinene	1.5	Carvone	0.8
β-Myrcene	1.9	Geraniol	0.9
Limonene	0.8	β-Burbonene	1.4
1,8-Cineol	2.4	β-Caryophyllene	8.3
Terpinolene	2.1	α-Humulene	1.1
Linalool	9.1	Germacrene B	1.0
2-Methylbenzofuran	0.3	Caryophyllene oxide	4.2
Camphor	12.4	Palmitic acid	14.3
p-Mentha-3-en-8-ol	3.3	Phytol	4.8
Borneol	2.4	Unidentified	89.7.

Sesquiternene derivatives made up 16% of the EO and were dominated by β-caryophyllene (8.3%) and caryophyllene oxide (4.2%). The EO of *P. lanata* had a high (14.3%) content of palmitic acid, which was the dominant compound, and phytol (4.8%).

The study found 30 compounds in the aerial part of *P. lanata*. Compounds **1–9**, **11**, **12**, **16–18**, and **20–30** were observed in this plant species for the first time. Information on the free fatty acids and EO of *P. lanata* is also reported for the first time.

Column chromatography was performed over silica gel (Chemapol) and by gel permeation over Sephadex LH-20 (Pharmacia); TLC and HPTLC, on Sorbfil PTSKh-AF-A and Sorbfil PTSKh-AF-B (Imid Ltd.) silica-gel plates. The solvent systems were C_6H_{14}:Me_2CO (1, 2:1), MeC_6H_5:EtOAc:HCOOH (2, 5:4:1), CH_2Cl_2:MeOH:H_2O (3, 5:4:1); the detectors, $KMnO_4$ (5%) (1), 2-aminoethyldiphenylborinate in MeOH (1%) (2), and Dragendorff's solution/$NaNO_2$ (3). Isolated compounds were identified by melting point, specific rotation, chemical transformations, and UV, IR, and ^{13}C NMR spectroscopy. Spectrophotometric studies were performed on an SF-2000 spectrophotometer (LOMO); optical rotation, on an SM-3 polarimeter (Zagorsk Optical-Mechanical Plant); IR spectra, on an FT-801 IR-Fourier spectrometer (Simex); ^{13}C NMR spectra, on a VXR 500S NMR spectrometer (Varian, operating frequency 125.7 MHz, 1% solutions in DMSO-d_6). HPLC condition 1 used a Summit liquid chromatograph (Dionex), Prodigy ODS 3 column (Phenomenex, 250 × 4.6 mm, 5 μm), H_2O:MeOH:AcOH (14:6:1) mobile phase at flow rate 1 mL/min, 20°C, UVD 170S UV-detector at λ 330 nm; condition 2, an AAA-339 automated amino-acid analyzer (Microtechna); condition 3, a Milikhrom A-02 liquid chromatograph (Econova), a Separon 5-NH_2 (Tessek Ltd., 80 × 2 mm, 5 μm) column, mobile phase MeCN:H_2O (3:1) at flow rate 0.1 mL/min, 22°C, and λ 190 nm; condition 4, a Milikhrom A-02 chromatograph (Ekonova), ProntoSil-120-5-C18 AQ column (Metrohm AG, 75 × 2 mm, 5μm), mobile phase A (4 M $LiClO_4$–0.2 M H_3PO_4):H_2O (1:19) and B (MeCN), A:B 99:1, flow rate 0.05 mL/min, 22°C, and λ 210 nm. The chromatographic mobility, spectrum in the stopped solvent flow, and spectral ratios (if necessary) were determined during the course of the analysis. Experiments with added standards were also performed. GC–MS was performed on a 6890N GC–MS combined with a 5973N mass-quadrupole detector (Agilent Technologies; electron-impact ionization, 70-eV ionization energy, total ion-current detection, scan range 41–450 amu) using HP-Innowax capillary columns (Agilent Technologies; 30 m × 0.25 mm × 0.50 μm; stationary phase polyethyleneglycol; fatty acids) and HP-5MS (Agilent Technologies; 30 m × 0.25 mm × 0.50 μm; stationary phase diphenyl- and dimethylpolysiloxane 5:95; EO). The mobile phase (He) flow rate was 1 mL/min. The sample volume (1% solution in hexane) was 0.2 μL; flow division, 20:1. The column temperature was 150–250°C (heating rate 2°C/min), vaporizer 250°C, ion source 230°C, detector 150°C, the line joining the chromatograph to the mass spectrometer 280°C. A mass-spectra library (NIST 05, Wiley) was used for identification. Retention times were also compared with those of standard samples.

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REFERENCES

1. *Tibetian Medicine in Buryatia* [in Russian], RAS, Novosibirsk, 2008.
2. G. Savona, F. Piozzi, and J. R. Hanson, *Phytochemistry*, **17**, 2132 (1978).
3. T. A. Khokhrina and V. A. Peshkova, *Chem. Nat. Comp.*, **10**, 284 (1975).
4. *Plant Resources of the USSR. Families Hippuridaceae-Lobeliaceae* [in Russian], Nauka, Leningrad, 1991.
5. F. H. Foppen, *Chromatogr. Rev.*, **14**, 133 (1971).
6. T. Kurihara, M. Kikuchi, S. Suzuki, and E. Toyoda, *Yakugaku Zasshi*, **96**, 1407 (1976).
7. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York, 1972.
8. J. S. Choi, H. S. Young, J. C. Park, J.-H. Choi, and W. S. Woo, *Arch. Pharm. Res.*, **9**, 233 (1986).
9. M.-H. Yang and L.-Y. Kong, *Chem. Nat. Comp.*, **44**, 98 (2008).
10. E.-R. Woo and M. S. Piao, *Arch. Pharm. Res.*, **27**, 173 (2004).
11. G. M. Pettit, A. Numata, T. Takemura, R. H. Ore, A. S. Narula, J. M. Schmidt, G. M. Cragg, and C. P. Pase, *J. Nat. Prod.*, **53**, 456 (1990); H. Ravn, S. Nishibe, M. Sasahara, and L. Xuebo, *Phytochemistry*, **29**, 3627 (1990).
12. Y.-S. Liang, H. K. Kim, A. W. M. Lefeber, C. Erkelens, Y. H. Choi, and R. Verpoorte, *J. Chromatogr. A*, **1112**, 148 (2006); H. Tazaki, M. Ito, M. Miyoshi, J. Kawabata, E. Fukushi, T. Fujita, M. Motouri, T. Furuki, and K. Nabeta, *Biosci. Biotechnol. Biochem.*, **66**, 255 (2002).
13. H. A. Jung, J. C. Park, H. Y. Chung, J. Kim, and J. S. Choi, *Arch. Pharm. Res.*, **22**, 213 (1999).
14. V. M. Malikov and M. P. Yuldashev, *Chem. Nat. Comp.*, **38**, 358, 473 (2002).
15. T. Ohsugi, R. Nishida, and H. Fukami, *Appl. Entomol. Zool.*, **26**, 29 (1991); I. Uriarte-Pueyo, M. Goicoechea, A. G. Gil, A. L. de Cerain, A. L. de Munain, and M. I. Calvo, *J. Agric. Food Chem.*, **57**, 11055 (2009).