Chemistry of Natural Compounds, Vol. 33, No. 6, 1997

LIPIDS OF *Ziziphora pedicellata*

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UDC 547.915:665.3

The amounts and compositions of the lipids, pigments, and fatty acids in the inflorescences, leaves, and stems and the total air-dry biomass of Ziziphora pedicellata *Pazij. et Vved. have been established. The essential oil content of the biomass has been determined. A similarity of the qualitative composition of the lipids and pigments, an increase in the amount of unsaponifiable substances, and a substantial fall in the level of chlorophylls on the natural drying of the biomass have been found.*

The essential-oil plant *Ziziphora pedicellata* Pazij. et Vved. (Lamiaceae) is an endemic species for Central Asia [1]. In folk medicine, a tincture and a decoction of the epigeal part are used as hypotensive, diuretic, and wound-healing agents. Thanks to its peculiar attractive odor and bacterial properties, the essential oil has found use in the food and perfumery industries as a perfume for tooth powders and pastes and for soap [2]. The seeds of *Z. pedicellata* contain more than 17% of reserve lipids with an attractive odor and a high level of the 18:3 polyunsaturated acid and carotenoids [3]. The lipids of the epigeal part have scarcely been studied.

We have made a comparative investigation of the lipids and pigments of the fresh epigeal part of *Z. pedicellata* gathered in the flowering period, and of an air-dry sample of it. The amounts of essential oil in the fresh and the air-dry biomasses were determined by steam distillation and it was then used as a standard sample of total terpenoids in the analysis of the lipids by TLC.

To estimate the amounts of lipids in individual organs, the fresh biomass was separated into leaves, inflorescences, and stems, while the air-dry plant was not differentiated. The lipids were extracted from comminuted samples by Folch's method. The yields of volatile and extractive substances are given in Table 1.

The leaves of the fresh biomass were the richest in lipids. The level of lipids in the air-dry epigeal part differed little from the mean value of this index for the fresh biomass. In the natural drying of the plant, more than 20% of the essential oil (calculated on the a.d.w.) was lost.

The amounts of lipophilic pigments in extracts were determined spectrophotometrically. After alkaline hydrolysis of the extracts and isolation of the products, we evaluated the ratio of unsaponifiable substances and total fatty acids (FAs). The results are given in Table 2.

Carotenoids were present in all parts of the fresh biomass, but their level in the leaves was somewhat higher. The amounts of chlorophyll b in the inflorescences and leaves were 2.0-1.9 times, and in the stems 1.7 times, more than those of chlorophyll *a*. As was to be expected, the unsaponifiable substances were concentrated in the stems, and the FAs equally in the inflorescences and the leaves.

In the air-dry biomass, as compared with the fresh biomass (taking the proportion of each organ into account), the level of chlorophyll *a* had fallen 3.5-fold and that of chlorophyll b 2-fold, while the levels of carotenoids and total FAs were almost the same, and that of unsaponifiable substances had doubled.

The qualitative sets of lipids and lipophilic substances of all the extracts were similar and, according to TLC, consisted of paraffins, carotenoids, esters of FAs with triterpenols, with phytosterols and with fatty and low-molecular-mass alcohols, tocopherols (leaves, inflorescences), triacylglycerols (leaves, inflorescences), free FAs, triterpenols, phytosterols, and fatty alcohols. Chromatograms of the neutral lipids of all the extracts showed spots of chlorophylls and their more polar derivatives.

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0009-3130/97/3306-0633$18.00

TABLE 1

Sample	Weight of the organ, %	Content, %		Yield of extractive substances	
		moisture and volatile substances	essential oil	% of the crude biomass	mg/g a.d.w.
Fresh biomass	–	–	2.02	–	–
inflorescences	27.5	30.2	–	3.4	48.7
leaves	28.2	36.1	–	5.3	82.9
stems	44.3	7.6	–	1.4	15.2
Air-dry biomass	–	9.5	1.6	3.0	33.0

TABLE 2

Sample	Pigments, mg/g a.d.w.			Unsaponifiable substances	Total fatty acids
	chlorophyll		sum of carotenoids	% of the weight of the lipids	
	a	b			
Fresh biomass					
inflorescences	0.329	0.164	0.039	12.6	46.3
leaves	0.562	0.287	0.049	7.0	44.9
stems	0.107	0.063	0.011	15.6	35.8
Air-dry biomass	0.084	0.075	0.026	23.8	37.9

TABLE 3

Acid	Amount of the FA in the extracts (%, GLC)			
	fresh biomass			air-dry biomass
	inflorescences	leaves	stems	
10:0	3.4	2.4	1.9	3.3
12:0	9.9	8.8	12.5	14.2
13:0	6.8	3.1	2.7	2.3
14:0	2.2	2.0	1.5	1.8
15:0	Tr.	Tr.	0.6	Tr.
16:0	17.2	14.2	26.2	17.2
16:1	6.9	4.2	7.6	2.2
17:0	Tr.	Tr.	Tr.	Tr.
18:0	10.0	12.3	10.1	3.2
18:1	7.4	8.1	4.7	5.1
18:2	9.8	6.7	14.6	16.5
18:3	22.9	32.0	12.2	29.5
20:0	3 .	6.2	5.2	4.7
Σ_{sat}	53.0	49.0	60.9	46.7
Σ_{unsat}	47.0	51.0	39.1	53.3

Of the polar lipids, in all the organs we identified steryl glycosides and their esters, monogalactosyldiacylglycerols (MGDGs) and digalactosyldiacylglycerols, N-acylphosphatidylethanolamines, phosphatidylglycerols, phosphatidylethanolamines, phosphatidylcholines (PhCs), and phosphatidylinositols (PhIs).

As well as lipids, in extracts of the inflorescences and, to a smaller degree, of the leaves, by comparison with the chromatographic mobilities of the components of the essential oil of *Z. pedicellata*, we found three spots of terpene compounds. They had R_f values coinciding with those of free FAs, triacylglycerols, and hydrocarbons, and were readily stained by iodine vapor. On chromatograms of hydrolyzed extracts and the products of their methylation, the terpene spots retained their R_f values. It is known that the main compound in the essential oil of *Z. pedicellata* is pulegone, while menthone, isomenthone, and γ-pinene are present in appreciable amounts [4].

When aliquot samples were subjected to TLC we observed a predominance of (apart from chlorophyll) the spots of wax esters, MGDGs, PhCs, and PhIs in the stems, and of terpenes (inflorescences) FFAs, MGDGs, and PhCs in extracts of the leaves and inflorescences.

The individual classes of lipids were isolated from extracts of the air-dry biomass by CC. The compositions of the main components of these classes were determined by mass spectrometry. We identified paraffins of the C20:0–C31:0 series with a predominance of C29 (M^+ 408), C31 (M^+ 436), and C33 (M^+ 464); esters of the 16:0, 18:0, 18:1, and 18:2 FAs with a 4,4-dimethylsterol, with amyrin (M^+ 464, 492, 490, 488), with β-sitosterol (M^+ 652, 680, 678) and with stigmasterol (M^+ 650, 678, 676), and esters of the 16:0, 18:0, and 18:1 acids with the C22:0, C24:0, and C26:0 alkan-1-ols (M^+ 564-648), α-tocopherol (M^+ 430, 165), amyrin (M^+ 426, *m/z* 218), β-sitosterol (M^+ 414), stigmasterol (M^+ 412), and campesterol (M^+ 400). The fatty acid composition of the extracts according to GLC is given in Table 3. In addition to the FAs listed in Table

3, by mass spectrometry we found in the total methyl esters from the fresh and the air-dry biomass the series of saturated acids from 22:0 to 28:0, with the odd homologs as minor components.

As can be seen from Table 3, in the inflorescences and leaves of the fresh biomass, the 18:3 species of unsaturated acids predominated, while in the stems the saturated FAs, particularly 12:0 and 16:0, and, of unsaturated acid the 18:2 type were concentrated. The proportion of medium-molecular-mass FAs, 10:0-13:0, was higher in the inflorescences (20.1%). During the natural drying of the plant the component composition and the level of the main unsaturated FAs and the total amount of medium-molecular-mass FAs did not change.

We have shown previously that it is possible to obtain concentrates of biologically active lipids and lipophilic substances [5] forming valuable products for the cosmetic and pharmaceutical industry from the processing wastes of essential-oil-bearing species of the same family, *Lavandula vera* D.C. and *Salvia sclarea* L., after the isolation of the essential oil.

The results of the present work show that both the fresh and the dry biomass of *Ziziphora pedicellata* and the wastes from their steam distillation can be used to obtain lipophilic extracts containing biologically active components.

EXPERIMENTAL

General Observations. The conditions for isolating the total lipids have been described in [6] and those for the separation of the neutral and polar lipids by CC and TLC and for their identification, and the recording conditions for UVS and mass spectra, in [7, 8]. The amounts of chlorophyll and carotenoids were calculated from a formula in [9].

The essential oil was isolated by Clevenger's method [10]. Moisture and volatile substances were determined, the extracts were subjected to alkaline hydrolysis, and the unsaponifiable substances were isolated as in [11].

The fresh plants were gathered in the period of mass flowering in the environs of Bel'dersaya (Tashkent oblast) in June, 1995. The air-dry plants were obtained after their natural drying in the air without access of light.

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