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NEUTRAL LIPIDS AND BIOLOGICAL ACTIVITY OF THE $CHCl_3$ EXTRACT OF THE AERIAL PART OF *Silene guntensis*

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Investigations of the plants from the genus *Silene* showed that they are sources of ecdysteroids [1] and that many of them contain significant quantities of biologically active compounds.

We studied lipids occurring in the $CHCl_3$ extract of *S. guntensis* B. Fedtsch. during extraction from it of ecdysteroids in order to utilize the plant more completely and safely and to establish the effect of the lipid components on the effectiveness of the antibacterial and cytotoxic activity.

Neutral lipids were isolated by separating the extract over a column of silica gel into hydrocarbons (3.34%), esters of fatty acids (FAE) and alcohols (7.83), triacylglycerides (TAG) (2.48), free fatty acids (FFA) (3.12), aliphatic and polyprene alcohols (5.0), and compounds eluted by Et_2O (19.58), $CHCl_3$ (3.38), and MeOH (55.26).

It can be seen that FAE, FFA, and TAG made up 13.43% of the total.

Fatty acids were analyzed as the methyl esters using GLC (Table 1) [2].

Table 1 shows that the FAE and FFA were more enriched in saturated acids than TAG and that the TAG have more unsaturated acids. This is typical for these classes of neutral lipids. The principal saturated acid was palmitic (16:0). Moreover, the FAE fraction included a significant quantity of 24:0 and 26:0 acids (21.3% total) whereas the FFA had 10.3% of the 26:0 acid. The dominant unsaturated acids in these lipid classes were oleic (18:1), linoleic (18:2), and linolenic (18:3).

Preliminary screening of the $CHCl_3$ part of the extract from the aerial part of *S. guntensis* found that it exhibited antibacterial and cytotoxic activities.

Cell Cultivation. Antitumor activity of the tested samples was evaluated using *HeLa* cells that were cultivated at 37°C under an atmosphere with 90% humidity and 5% CO_2 in 96-well plates (2×10^4 cells/well) in DMEM culture medium (pH 7.4) containing inactivated fetal calf serum (10%), penicillin/streptomycin (100 U/mL), sodium pyruvate (1 mM), and non-essential amino acids (10 mM). After this, the $CHCl_3$ extract at a concentration of 0.97–500 μg/mL was added to the *HeLa* cells, which were placed in an incubator for 24 h at 37°C. The control was *HeLa* cells (2×10^4 cells/well) without the extract. The results were calculated in percent of the control. All experiments were conducted in triplicate.

MTT-test. Cytotoxic activity was evaluated using the MTT test [3]. Medium in each well was replaced 4 h before the end of cultivation by fresh medium (DMEM, 100 μL) containing MTT (0.5 mg/mL). The plates were placed in the incubator for 3 h. The crystals of formazane that were present at the end of the experiment were dissolved in DMSO. Absorption was measured in an apparatus for reading the plates. Cytotoxic activity of the extracts and compounds was determined from the percent reduction in the controls of the color at 595 nm.

The preliminary *in vitro* investigations showed that the $CHCl_3$ extract of *S. guntensis* suppressed the growth of *HeLa* cells. The inhibiting concentration of the $CHCl_3$ extract according to the MTT tests was a dose of 26.58 μg/mL. At that concentration 50% of the cells died compared with the control. This indicated that the extract had antitumor activity.

Antibacterial Activity. Cultures of *Staphylococcus aureus* MRSA16, *Micrococcus luteus*, *Escherichia coli* NCTC9001, *Pseudomonas aureginosa* NCTC6749, *Acinetobacter* sp. T16, and *Staphylococcus saprophyticus* T415 were used for the tests.

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TABLE 1. Fatty-Acid Composition of Acyl-Containing Classes, GLC, mass%

Acid	FAE	TAG	FFA	Acid	FAE	TAG	FFA
10:0	0.2	0.8	0.3	18:3	12.4	20.6	19.0
12:0	0.4	1.3	1.2	20:0	0.7	1.3	0.3
14:0	1.5	4.6	1.8	21:0	1.9	–	1.6
15:0	0.5	Tr.	1.2	22:0	7.3	3.4	4.9
16:0	17.4	19.4	26.5	23:0	2.7	–	2.2
16:1	0.5	1.3	1.3	24:0	11.2	Tr.	5.1
17:0	0.4	1.6	0.4	25:0	4.1	–	1.4
18:0	2.9	3.4	3.1	26:0	10.1	–	10.3
18:1	9.1	18.2	8.3	$\Sigma_{sat.}$	61.3	35.8	60.3
18:2	16.6	24.1	11.1	$\Sigma_{unsat.}$	38.6	64.2	39.7

Antibacterial activity of the $CHCl_3$ extract dissolved in DMSO was studied using the disk diffusion test [4]. Microorganisms were grown on agar dishes at 30°C overnight in Mueller–Hinton medium (Oxoid). The suspension (100 μL) contained bacteria (10^8 CFU/mL). Sterile filter disks (5-mm diameter) were impregnated with extract solution (20 μL, 5 mg/mL) and placed on the surface of the innoculated Petri dishes, which were incubated at 37°C for 24 h. Antibacterial activity was estimated by measuring inhibition zones formed around the disks. Five disks in each Petri dish were used. Each test was conducted in triplicate.

Activity against *E. coli*, *P. aureginosa*, and *Acinetobacter* was found. This may have been related to the presence of slightly polar lipids. The bacteria *S. aureus*, *M. luteus*, and *S. saprophyticus* were resistant to the extract.

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