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Phytochemical research of *Galium aparine* L. Lipophilic complex and study of its antibacterial activity

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From *Galium aparine* L. herb lipophilic complex of biologically active substances had been obtained for the first time. By means of TLC and chromatography-mass-spectrometry the composition of lipophilic complex had been studied. 3 Sesquiterpenoids, squalene, 7 aromatic compounds, higher alkanes and their derivatives, fatty acids have been identified and quantified; presence of chlorophylls, carotenoids and 2 iridoids had been established. Antibacterial and antifungal activity of *Galium aparine* herb lipophilic complex and degree of these activities have been studied for the first time. Test-strains of *S. aureus*, *P. aeruginosa* and *C. albicans* were highly sensitive and *B. subtilis* is moderately sensitive to lipophilic complex of *Galium aparine* herb.

Keyword: *Galium aparine* L., chromatography-mass-spectrometry, antibacterial and antifungal activity.

1. Introduction

Cleavers or (*Galium aparine* L.) of Madder family (*Rubiaceae* Juss.) – is one of the most widely spread species of *Galium* L. genus in moderate climate zone.

Previous studies of Cleavers' biologically active substances (BAS) showed presence of hydroxycinnamic acids, coumarins, derivatives of flavones and flavonols in areal part and anthracene derivatives of alizarin type in roots and rhizomes [1, 3, 5].

Our recent research had shown presence of monoterpenoids, sesquiterpenoids, tri- and norterpenoids, aromatic compounds in *Galium aparine* herb essential oil. Presence of lipophilic compounds with potent antibacterial and antifungal activities was a background for obtaining of complexes of BAS with presumptive antibiotic properties.

The object of our research was lipophilic complex of *Galium aparine* herb.

The aim of our research was to study chemical composition, antibacterial and antifungal activity of *Galium aparine* herb lipophilic complex.

2. Materials and Methods

2.1. Plant material. *Galium aparine* L. herb was collected in flowering stage in Lugansk region, Ukraine in 2012. The plant identification was performed using voucher specimens of Herbarium of Pharmacognosy Department, the National University of Pharmacy.

Lipophilic complex was obtained by the method of exhaustive circulative extraction in Soxhlet apparatus using chloroform as extracting agent. The ratio plant material: extragent was 1: 10, an extraction time – 30 hours. After the end of extraction process the extragent was evaporated at a temperature of 35-40 ^{0}C under vacuum in a vacuum circulation apparatus till complete removal of extragent.

2.2 Study of complex's composition. By means of TLC using solvent systems ethylacetate-formic acid-water (10:2:3), hexane-acetone (6:2) and hexane-acetone (6:4) and Trimm-Hill, vanillin-sulphuric acid and phosphomolybdic acid reagents as spray reagents iridoids, chlorophylls and carotenoids have been identified.
Volatile compounds of lipophilic complex have been studied by chromatography-mass-spectrometry.

2.3 Research of antibacterial and antifungal activity. According to WHO recommendations for preliminary assessment of antibacterial and antifungal activity we used test-strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* 885-663.
In vitro study had been performed by agar well diffusion method [4]. Lipophilic complex was used as 20 g/l solution in absolute alcohol.
The microorganisms susceptibility was measured as a radius of inhibition zone in mm. The quantification of antibiotic activity and determination of minimal inhibitory concentration had been performed by serial dilutions method. Results have been processed statistically by Glantz S. [2].

3. Results and Discussion
The yield of lipophilic complex was 3,02%. It was the oleiferous mass of dark-green colour with specific herbaceous smell. The complex was soluble in chloroform, hexane and acetone, insoluble in water and water-alcohol mixtures.
By means of TLC according to the chromatographic behavior asperuloside and one unknown iridoid, chlorophyll *a*, chlorophyll *b* and two compounds belonging to carotenoids have been identified.
By means of chromatography-mass-spectrometry 36 compounds have been identified and quantified. Composition of *Galium aparine* herb lipophilic complex is represented in Table 1.

Table 1: Composition of *Galium aparine* herb lipophilic complex

S. №	Retention time, min	Compound	Content, mg/kg
1	2	3	4
1	5.63	Benzaldehyde	23.4
2	11.33	Propiophenone	69.9
3	11.70	Cinnamaldehyde	22.4
4	11.95	Methylacetophenone	71.8
5	13.30	Caprylic acid	52.5
6	14.73	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	25.9
7	15.38	Phenylacetic acid	67.5
8	16.23	2-Methoxy-4-vinylphenol	68.7
9	17.72	1,2-Dihydro-1,1,6-trimethylnaphthalene	31.4
10	17.86	1,2-Dihydro-1,6,8-trimethylnaphthalene	18.2
11	18.45	Vanillin	16.8
12	19.28	Capric acid	95.5
13	22.28	Dihydroactinidiolide	218.3
14	28.49	Loliolide	761.2
15	29.43	Myristic acid	504.0
16	30.57	Trans-neophitadiene	3485.5
17	30.84	Cis-, trans-neophitadiene	747.6
18	31.13	Cis-neophitadiene	1237.5
19	31.97	Palmitoleic acid	436.4
20	33.17	Palmitic acid	13742.2

Table 1 continued.

S. №	Retention time, min	Compound	Content, mg/kg
21	33.72	Heptadecanoic acid	220.2
22	34.20	Linolenic acid	692.9
23	35.30	Linoleic acid	18937.3
24	36.31	Tricosane	229.1
25	36.60	4,8,12,16-Tetramethylheptadecane-4-olide	120.6
26	37.32	Tetracosane	65.1
27	38.31	Pentacosane	221.2
28	40.20	Octacosane	788.5
29	41.05	Heptacosane	300.4
30	41.14	Squalene	264.0
31	42.06	Nonacosane	3315.9
32	42.71	Triacontane	248.5
33	43.31	Stigmasta-3,5-diene	127.0
34	43.50	Vitamin E	154.0
35	43.52	Untriacontane	297.9
36	45.08	γ-Sitosterol	265.4

Content of volatile compounds in *Galium aparine* herb lipophilic complex was 4.8% of which terpenoids (0.12%) – sesquiterpenoids dihydroactinidiolide and loliolide, triterpenoid squalene; aromatic compounds (0.03%) – benzaldehyde, propiophenone, cinnamaldehyde, methylacetophenone, phenylacetic acid, 2-methoxy-4-vinylphenol and vanillin; steroids (0.04%) – stigmasta-3,5-diene and γ-sitosterol; higher alkanes and their derivatives (1.1%) and fatty acids (3.5%). Also vitamin E had been identified and quantified (0.01%).

Chromatographic profile of *Galium aparine* herb lipophilic complex is shown on Fig. 1.

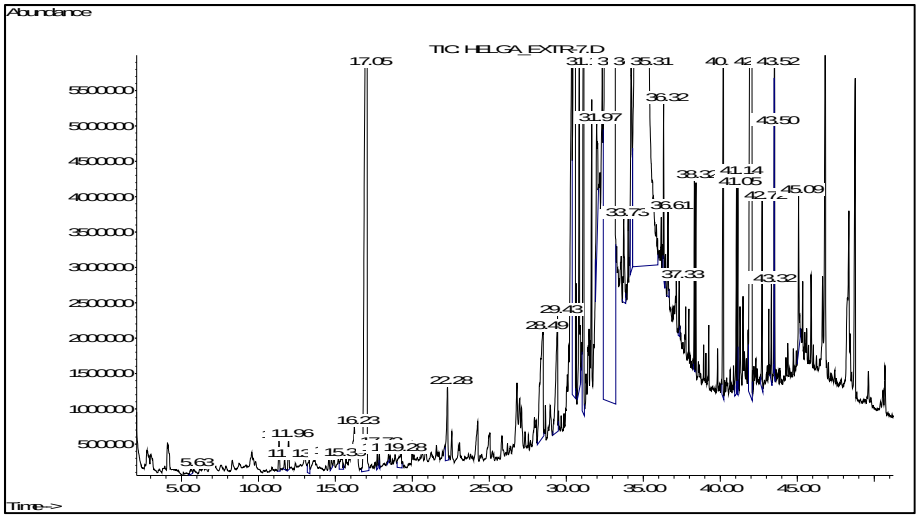

Fig 1: Chromatographic profile of *Galium aparine* herb lipophilic complex

Results of research of antibacterial and antifungal activity are shown in Table 2.

Table 2: Antibacterial and antifungal activity of *Galium aparine* herb lipophilic complex

Test-strains					
S. aureus 25923	*E. coli* 25922	*P. aeruginosa* 27853	*B. subtilis* 6633	*P. vulgaris* 4636	*C. albicans* 885-663
Zone of growth inhibition, mm, (M±m)					
35,4±0,1	10,0±0,1	26,0±0,4	23,1±0,3	14,2±0,1	33,1±0,2
Minimum inhibitory concentration, µg/ml					
31,25	250,00	62,50	125,00	250,00	31,25
Minimum bactericidal concentration, µg/ml					
62,50	500,00	125,00	250,00	500,00	62,50

Obtained results show that test-strains of *S. aureus*, *P. aeruginosa* and *C. albicans* are highly sensitive to lipophilic complex of *Galium aparine* herb; *B. subtilis* has moderate sensitivity; *E. coli* and *P. vulgaris* are non-sensitive to *Galium aparine* herb lipophilic complex or chosen concentration of complex.

4. Conclusions

1. From *Galium aparine* L. herb lipophilic complex of biologically active substances had been obtained for the first time.
2. By means of TLC and chromatography-mass-spectrometry the composition of lipophilic complex had been studied. 3 Sesquiterpenoids, squalene, 7 aromatic compounds, higher alkanes and their derivatives, fatty acids have been identified and quantified; presence chlorophylls, carotenoids and 2 iridoids had been established.
3. Antibacterial and antifungal activity of *Galium aparine* herb lipophilic complex and degree of these activities have been studied for the first time. It was established that test-strains of *S. aureus*, *P. aeruginosa* and *C. albicans* are highly sensitive and *B. subtilis* is moderately sensitive to lipophilic complex of *Galium aparine* herb.
4. Obtained results indicate the prospect of further in-depth research of antibiotic properties of *Galium aparine* herb lipophilic complex using clinical strains of microorganisms.

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