Phytoecdysteroids from aerial parts of *Sileneclaviformis* and their stress-protective activity

Comment [a1]: Scientific name authencity?

Abstract

Aims: The aim of ~~this~~ the study was to investigate the phytoedysteroid of Sileneclaviformis~~(Caryophyllaceae)~~ and the biological activity of sum of phytoecdysteroids.

Place and Duration of Study: Institute of the Chemistry of Plant Substances AS RUz, Tashkent, Uzbekistan; Laboratory of the Chemistry of glycosides and department of the Farmacological and toxicology, between August 2019 and June 2020.

Methodology: The phytoecdysteroids were isolated from S.claviformis using chromatographic methods. Their structures were confirmed by NMR and IR spectroscopy. Sum of phytoecdysteroids was administered at a dose of 10 mg/kg orally. The data obtained during the experiments were processedand analyzed by the method of variation statistics using the Student t-criterion.

Results: Column chromatography (CC) investigation of S.claviformis plant allowed the identification, and the compounds 2 and 6 are reported for the first time from this genus. The adrenal gland mass increased in relation to the adrenal gland mass of intact animals by 42.6%, they showed a significant decrease in the content of ascorbic acid and cholesterol by 56.5 and 49.1%, respectively. A significant decrease in glycogen content (by 30.1%) and a noticeable activation of lipid peroxidation processes were noted in the liver, as indicated by an increase of 69.2% in the content of MDA in the organ.

Conclusion: This is the first study reporting an orally biological investigation for S.claviformis. The sum of phytoecdysteroids showed potent stress-protective activity. The stress-protective effect of phytoecdysteroids was more pronounced in compare to the eleutherococcus extract.

Keywords -phytoecdysteroids, *Sileneclaviformis,* eleutherococcus extract, stress-protective effect

1.Introduction

Nowadays the problem of increasing the general nonspecific resistance of the organism to stressful factors is relevant problem due to the frequent work of a human in extremely adverse climatic and industrial conditions, deteriorating environmental conditions, poor nutrition, and many other destabilizing factors. Therefore, the search for substances that can increase the adaptive potential and improve life quality is one of the priority tasks of modern pharmacology [1]. Earlier it was reported that some phytoecdysteroidsmay be of noticeable interest in this regard [2], [3].

An estimated 5–6% of the terrestrial plant species accumulate detectable levels of ecdysteroids, among which *Ajuga*, *Serratula*and *Silene*spp., containing high amounts of these compounds, are good sources of ecdysteroids [4]. From the point of view of the search for ecdysteroid-containing plants among the representatives of the ~~domestic~~indigenous flora, plants of the genus *Silene* (Smolevka, family *Caryophyllaceae*) seem promising. This genus, with about 400 representatives in the world flora, is represented by 153 in the CIS and 84 species in Central Asia [5].

Comment [a2]: Abbreviation ?

Caryophyllaceae species are known for their rich content in bioactive metabolites, such as flavonoids [6],triterpenesaponins [7], phytoecdysteroids and oligosaccharides [8]. The *Silene* genus (*Caryophyllaceae*) comprises more than 700 species widely distributed in temperate zones of the world [9].

First of all, the general tonic effect of these compounds on the body was quite renounced. Thus, the improvement in the activity of the central nervous system when animals were injected with ecdysterone could be judged by the activating effect revealed by him on EEG rabbits [10].

Under the influence of phytoecdysteroids, the production of conditioned defense reflexes accelerated, the endurance of animals in relation to various physical activities increased [11], [12]. Their sexual behavior intensified [13]. An increase in the resistance of animals after the introduction of phytoecdysteroids to stressful environmental factors was noted [14], [10]. Some attention should be paid to the data on the ability to resist various helminthes infections using ecdysteroids [15].

Ecdysteroids represent a large family of steroid hormones that play a crucial role in arthropods' physiology. The most abundant representative of these compounds, 20-hydroxyecdysone (20E),

regulates the reproduction, embryogenesis, diapauses and molting of arthropods [16]. Their role in plants is still to be fully understood, but it had been suggested that they have high importance in several plants as defensive agents against non-adapted herbivores [17]. Ecdysteroids secreted from plants (phytoecdysteroids) being in most cases hormones for arthropods, they are not for warm-blooded ones [18]. However, they were able to regulate many physiological processes in their bodies [19], [20]. In this case, phytoecdysteroids do not have toxic or any side effects [21]. It was found that the beneficial shifts occurring under the influence of phytoecdysteroids in the mammalian organism are accompanied by a noticeable improvement in their functional status, which is manifested by activation of the central nervous system, increased efficiency, and increased adaptive capabilities of the body to environmental stressors [22].

~~Plants of the genus *Silene* (*Caryophyllaceae*) are knownassources of ecdysteroids, a class of compounds with adaptogen and anabolic activity [23].~~

Sileneclaviformis is a perennial grassy plant (size 15-25cm) growing on the slopes of the lower belt of Tashkent and Samarkand districts mountains. It is widespread in Central Asia.

Comment [a3]: Please add elevation here.

The present ~~paper~~study deals with the isolation and structure elucidation of eight known phytoecdysteroids (Figure 1) from the buthanol extract of aerial parts of *S.claviformis*. Since several isolated compounds were described as possessing an interesting stress-protector activity, we found it pertinent to evaluate the stress-protector activity of the buthanol extract containing ecdysteroid sum.

2.Material and methods

2.1 Plant material

*Sileneclaviformis*was collected in June of 2018 from Tashkent region of mountains and the plant materials were identified by Dr. Nigmatullayev A.M. at the Institute of the Chemistry of Plant Substances (ICPS), Uzbekistan. A voucher specimen (No. 2518) has been deposited in the herbarium Department of Herbal Plants in the ICPS, Tashkent, Uzbekistan.

Sample collection methodology

Comment [a4]: Sample collection methodology adopted here?

2.2 Experimental animals

This study was performed using male micewith body weight of 19-20 g. Mice were kept in one polyacrylic cage, and all mice were quarantined for 1 week before the experiments. All animals were housed under standard controlled conditions with temperature of 24 ± 2°C, a humidity of 50% ± 5% and a 12-h light/dark cycle. Mice were given free access to food (standard commercial mice chow) andwater, received care according to European Conventionfor the Protection of Vertebrate Animalsused for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Comment [a5]: Mice age?

3 Experimental Chemical part

3.1 Extraction The freshly collected whole plant material (1kg) was air dried at 230C for 1week after collection and then ground to a powder with a Waring blender. After grinding, plant material was triturated with MeOH (3*5L) (each for 3days) at room temperature (230). The combined MeOH extract was evaporated (in a rotary vacuum evaporator at 400) and 52g of dried extract was obtained. The crude extract was suspended in water and hydrophobic compounds were removed upon partition with chloroform(CHCl3) which was discarded. Then withethyl acetate (EtOAc) for obtained less polar compounds, and then with n-butanol BuOH. The combined butanol extracts were evaporated to dryness under reduced pressure (at 460C).

Comment [a6]: Check temperature range in whole text.

3.1.1Isolation of phytoecdysteroids using column chromatography. The BuOH extract (20g) was chromatographed over a silica gel column (silica particle size: 63-100μm; Chemapol, Prague, Czech Republic). The column packed using a simple dry-pack method. The butanol extract was applied in dried form mixed with silica gel and carefully added to the top of the column. The column (750g silica size 10×60cm) was eluted with $CHCl_3$-MeOH (100:1, 90:1, 80:1, 73:1, 65:1, 60:1, 50:1, 40:1, 30:1, 20:1, 12:1, 9:1, 4:1, 2:1, 1:1), to yield four fractions. Fraction 1 (2.5g) was obtained containing a mix of less polar

ecdysteroids and was further subjected to CC, eluting with MeOH-EtOAc (50:1), to yield compounds as 2-deoxyecdysterone**(1)** (0.11g),20-hydroxyecdysone (**3**) (0.31g), ecdysterone-20,22-acetalisovaleric aldehyde**(4)** (0.006g). Repeated chromatography of Fr. 2 (3.5g) over a silica gel column (MeOH-EtOAc, 30:1, 12:1, MeOH-$CHCl_3$, 4:1) yielded pureintegristeron A (**5**) (0.07g). Fraction 3 (3.1g) was subjected to CC, eluting with MeOH-EtOAc (20:1), and MeOH-$CHCl_3$ (15:1), (12:1) to obtainecdysterone-20,22-acetalisovalerian**(7)** (0.0062g). 2-deoxy-α-ecdysone (0.22g), compounds **(8)**was obtained from Fraction 4 (4.3g), which was separated through repeated column chromatography (MeOH-EtOAc 6:1, 4:1).

The Fraction 5 (2.9g) and Fr. 6 (2.7g) were subjected to CC on silica gel, eluting with $CHCl_3$-MeOH (100:1, 80:1, 60:1, 50:1, 30:1, 20:1, 15:1, 12:1, 9:1, 4:1, 1:1), to yield fractions which eluted with chloroform-methanol re-chromatographed and eluted with $CHCl_3$-MeOH increasing order of polarity.Polypodine B (**2**) (0.028g) and cyasterone**(6)** (0.075g)were obtainedfrom these fractions.

3.2Experimentalbiologicalpart

The experiments were performed on male mice weighing 19–20 g. The general stress response was caused by hanging them by the back cervical fold for 18 hours [24]. Sum of phytoecdysteroids was administered at a dose of 10 mg/kg orally (in the form of an aqueous emulsion with Arabian gum) immediately before the experiment. The reference preparation was eleutherococcus liquid extract manufactured by Dalkhimprom OJSC, administered orally at a dose of 0.2 ml/20 g body weight (previously dealcoholized), which, under appropriate conditions, was able to increase the organism's adaptive capacity for stressful effects [25]. Control animals received an adequate amount of an aqueous emulsion of Arabian gum. The effectiveness of the sum of phytoecdysteroids was assessed by the degree to which it prevented changes in the mass of the adrenal glands (the content of ascorbic acid and cholesterol in them), thymus, spleen and liver, observed under acute stress [24], [25]. Additionally, glycogen content and malondialdehyde (MDA) were determined in the liver, and the number of ulcers formed in the stomach was also calculated [26]. The sum of phytoecdysteroids and eleutherococcus extract were introduced after the first swim (water temperature 27-28^{o}C). The antitoxic and antihypoxic effect of the sum of phytoecdysteroids, also attributed toadaptogenic agents [1], was evaluated first by the survival of mice with intraperitoneal administration of a 25% ethanol solution at a dose of 9.8 g/kg, and then on the model of tissue hypoxia caused by intraperitoneal administration of sodium nitroprusside at a dose of 25 mg/kg [27], [28]. The data obtained during the experiments were processed by the method of variation statistics using the Student t-criterion.

4 Results and discussion

4.1 Chemical part

A preliminary investigation of *S.claviformis*plant has confirmed the presence of phytoecdysteroids in its composition, and allowed to isolate and identify its main ecdysteroids (e.g. see Figure 1), such as 2-deoxyecdysterone**(1)**(0.011%), polypodine B (**2**) (0.0028%), 20-hydroxyecdysone (**3**) (0.031% of dry plant's weight) [29], ecdysterone-20,22-acetalisovaleric aldehyde**(4)** (0.0006%) [30],integristeron A (**5**) (0.007%) cyasterone**(6)** (0.0075%) [31], ecdysterone-20,22-acetalisovalerian **(7)** (0.00062%) [30], 2-deoxy-α-ecdysone**(8)** (0.02%) [32].

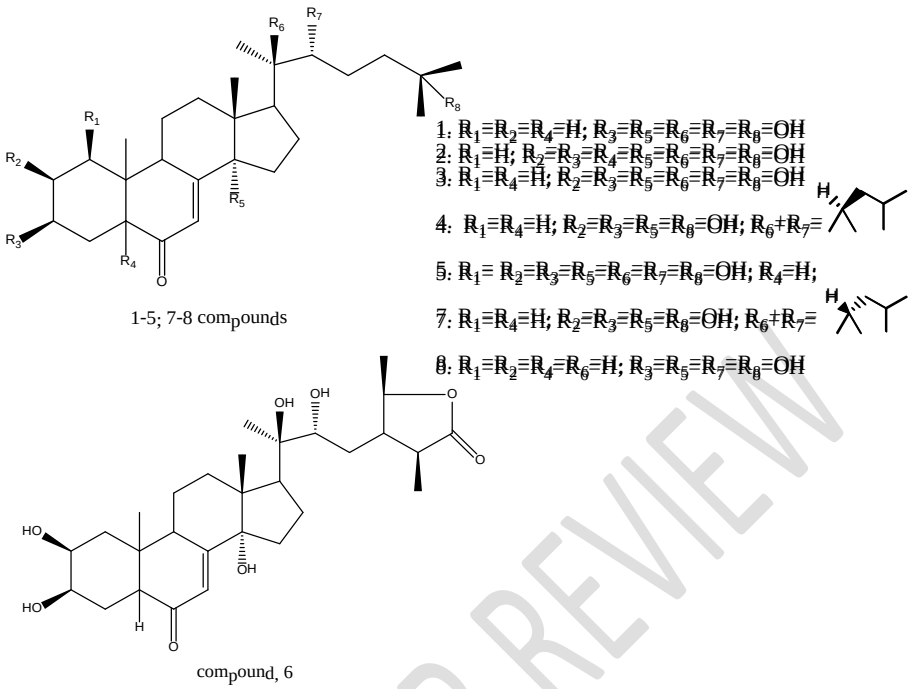

Fig. 1. Structure of compounds 1-8 from *S.claviformis*

The isolated individual ecdysteroids have been identified on the basis of the IR spectroscopy, and ^{1}H NMR spectroscopy,R_f and melting point on the A.KrussOptronic Germany, M 5000; 90-264 VIAC, as well as by comparison with reference compounds. Table 1 provides the physicochemical data for the individual substances and ecdysteroids yield from *S.claviformis*plant. SilicagelKSK 100/160 μmhave been used for column chromatography. Thin-layerchromatographymadeon SilufolUV-254 andMerck plates, FlukaAnalytical 254 nm, Germany, by spraying with alcohol solution of vanillin and heating for 1-2 minutes for 90-100^{0}. The NMR ^{1}H and ^{13}C spectra were recorded by VN MRS-400 (Varian) NMR spectrometer with an operating frequency of 400 MHz.

Table 1. Physical and chemical properties of phytoecdysteroids isolated from *Sileneclaviformis*

Compound No.	Compound name	Composition	T.,°C	Yield,% of plant mass
1	2-Deoxy-20-hydroxyecdysone	$C_{27}H_{44}O_6$	254-255	0.011%
2	Polypodine B	$C_{27}H_{44}O_8$	253-254	0.028%
3	20-Hydroxyecdysone	$C_{27}H_{44}O_7$	242-243	0.031%
4	Hydroxyecdysone-20,22-acetylizovalericaldehide	$C_{32}H_{52}O_7$	-	0.0006%
5	Integristerone A	$C_{27}H_{44}O_8$	247-248	0,007%
6	Cyasterone	$C_{29}H_{44}O_8$	163-164	0.0075%
7	Hydroxyecdysone-20,22-acetylizovalericaldehide	$C_{32}H_{52}O_7$	-	0.00062%
8	2-Deoxy-α-ecdysone	$C_{27}H_{44}O_5$	233-234	0.02%

Comment [a7]: Maintain uniformity after decimal like as either three or four.

2-deoxyecdysterone(1). White powder, mp 240–241°C, $[\alpha]_D$ + 81.2 ± 2° (*c* 0.50; metanol).^{13}C NMR spectrum (100 MHz, C_5D_5N-d6, δ, ppm): 30.01 (C-1), 28.05 (C-2), 65.28 (C-3), 34.03 (C-4), 55.70 (C-5),

202.16 (C-6), 120.12 (C-7), 165.94 (C-8), 34.98 (C-9), 38.54 (C-10), 21.47 (C-11), 31.67 (C-12), 47.51 (C-13), 83.87 (C-14), 31.76 (C-15), 21.77 (C-16), 49.88 (C-17), 17.69 (C-18), 23.29 (C-19), 76.87 (C-20), 21.65 (C-21), 76.86 (C-22), 28.77 (C-23), 42.58 (C-24), 68.89 (C-25), 30.21 (C-26), 30.44 (C-27).

Polypodine B (2). White powder, mp 250–251°C, $[\alpha]_D$ + 94.2 ± 2° (*c*0.50, MeOH). ^{13}C NMR spectrum (100 MHz, DMSO-d6, δ, ppm): 33.359 (C-1), 68.405 (C-2), 68.757 (C-3), 34.904 (C-4), 78.500 (C-5),199.768 (C-6), 119.354 (C-7), 165.037 (C-8), 36.965 (Cc-9), 43.832 (C-10), 21.100 (C-11), 30.949 (C-12), 46.866 (C-13), 82.873(C-14), 30.312 (C-15), 20.249 (C-16), 48.619 (C-17), 17.210 (C-18), 16.512 (C-19), 76.232 (C-20), 21.034 (C-21), 75.709(C-22), 26.123 (C-23), 41.445 (C-24), 66.423 (C-25), 29.028 (C-26), 30.090 (C-27).

20-Hydroxyecdysone (3). White crystals, mp 240–241°C (Me_2CO), $[\alpha]_D$+63.2 ± 2° (*c* 6.30, MeOH). UV spectrum (C_2H_5OH, λmax, nm) (log ε): 245 (4.01). IR spectrum (KBr, v, cm^{-1}): 3435 (OH), 1665 (7-en-6-keto group).

Compound **3** was identified by direct comparison of its TLC with that of an authentic sample of 20-hydroxyecdysone [29].

Comment [a8]: Full form of TLC?

Ecdysterone-20,22-acetalisovaleric aldehyde(4). UV spectrum (C_2H_5OH, λmax, nm) (log ε): 242 (4.01). IR spectrum (KBr, v, cm^{-1}): 3440 (OH), 1670 (7-en-6-keto group).

Compound **4** was identified by direct comparison of its TLC with that of an authentic sample of 20-hydroxyecdysone [30].

Integristeron A (5). White powder, mp 246–248°C, $[\alpha]_D$ + 36.1 ± 2° (*c* 0.43; metanol). UVspectrum 245nm (Igε 4.00). in IR spectrum 3400 and 1660cm^{-1}.

Cyasterone(6). White powder, mp 157–158°C, $[\alpha]_D$ + 60.0 ± 2° (*c*1.0, Py). ^{13}C NMR spectrum (100 MHz, C_5D_5N, δ, ppm): 32.959 (C-1), 68.556 (C-2), 68.622 (C-3), 34.987 (C-4), 51.903 (C-5), 203.961 (C-6), 122.359 (C-7), 166.343 (C-8), 38.480 (C-9), 42.962 (C-10), 21.868 (C-11), 32.568 (C-12), 48.704 (C-13), 84.669 (C-14), 32.417 (C-15), 21.498 (C-16), 49.210 (C-17), 18.427 (C-18), 16.432 (C-19), 77.305 (C-20), 21.620 (C-21), 74.513 (C-22), 50.533 (C-23), 39.223 (C-24), 40.423 (C-25), 179.716 (C-26), 24.962 (C-27), 80.359 (C-28), 19.860 (C-29)

Ecdysterone-20,22-acetalisovalerian (7). UV spectrum (C_2H_5OH, λmax, nm) (log ε): 242 (4.01). IR spectrum (KBr, v, cm^{-1}): 3440 (OH), 1670 (7-en-6-keto group).

Compound **7** was identified by direct comparison of its TLC with that of an authentic sample of 20-hydroxyecdysone [30].

Compound **7** had the same molecular mass as 4 and presented the same NMR features except a triplet at (δ 5.05; t: 5.3; 1H) in place of the triplet signal a-H (δ 5.28; t: 5.2; 1H) for **4**. This triplet could be assigned to the signal observed for an epimer of **4** at the asymmetric carbon of the acetal function.

2-Deoxy-α-ecdysone(8). White powder, mp 240–241°C, $[\alpha]_D$ + 92.1 ± 2° (*c* 0.55; metanol). ^{13}C NMR spectrum (100 MHz, C_5D_5N -d6, δ, ppm): 29.49 (C-1), 28.07 (C-2), 59.86 (C-3), 33.12 (C-4), 51.65 (C-5), 202.42 (C-6), 121.40 (C-7), 166.33 (C-8), 34.48 (C-9), 37.01 (C-10), 21.01 (C-11), 31.72 (C-12), 48.01 (C-13), 84.04 (C-14), 31.73 (C-15), 24.87 (C-16), 48.22 (C-17), 16.00 (C-18), 24.44 (C-19), 43.00 (C-20), 14.72 (C-21), 74.10 (C-22), 26.80 (C-23), 42.48 (C-24), 70.02 (C-25), 29.92 (C-26), 30.45 (C-27).

4.2 Biological part

As the next step, the plant extracts containingphytoecdysteroids sum were investigated for biological activity.

In control mice hanging by the neck fold for a long time, a rather characteristic picture of the stress reaction developed [24], [25]. Their adrenal gland mass increased in relation to the adrenal gland mass of intact animals by 42.6%, they showed a significant decrease in the content of ascorbic acid and

cholesterol by 56.5 and 49.1%, respectively. The mass of thymus gland decreased by 39.5, the spleen - by 48, and the liver by 24.4%. A significant decrease in glycogen content (by 30.1%) and a noticeable activation of lipid peroxidation processes were noted in the liver, as indicated by an increase of 69.2% in the content of MDA in the organ. Although a few, but distinct ulcerations were observed in the stomach (see Table 2).

The introduction (before starting of the stressful effect) of phytoecdysteroid sum to mice in many respects impeded the development of the noted negative changes in the animal organism. First of all, the phytoecdysteroid sum substantially inhibited the adrenal hypertrophy and the decrease of ascorbic acid and cholesterolin their content. As a result, the adrenal mass was only 9.8% higher in this group of animals than in intact animals at p> 0.05. The content of ascorbic acid and cholesterol at the same time had a clear tendency to normalize (only 8.4 and 15.4% lower than intact values).

The phytoecdysteroid sum also protected the thymus and spleen from involution. Their mass in this case was only 8.9 and 11.6 lower than that of intact animals. The phytoecdysteroid sum significantly prevented trophic disturbances in the gastric mucosa, and significantly reduced the number of bleedingulcerations in it. Table 2 shows that the sum of phytoecdysteroids was not inferior to the known Eleutherococcus extract with the same action in their ability to increase the adaptive potential of the body to a stressful effect [25], and in some cases had an even more pronounced effect.

A high ability ofphytoecdysteroids sum to increase the general non-specific resistance of the body to complex or adverse environmental conditions was also revealed in a series of other experiments. Themice after swimming to complete fatigue (rather severe stress associated with the fact that water is not their characteristic habitat) were removed from the bath with water and at the same time, injected the amount of phytoecdysteroids (similarly to the reference drug), then had one hour rest, and again forced to swim.

Table 2. The effect the sum of phytoecdysteroid on some manifestations of the stress response in mice under conditions of stress hanging in comparison with the extract of eleutherococcus (M± m, n=6)

Experiment conditions	Intact animals	STRESS		
		Control	Sum of phytoecdysteroids	Extract of eleutherococcus
Mass of organs, mg				
Adrenal glands	6.1 ±0.27	8.7 ±0.67[1]	6.7 ±0.33[2]	70±0.36[2]
Thymus	483± 2,1	29.2 ± 1,9[1]	44.0± 1,9[2,3]	342±0.94[1,2]
Spleen	1776± 7.6	92.0 ±7,2[1]	157,0± 6.2[2,3]	1224±4.8[1,2]
Liver	3106± 192	2349 ±170[1]	2957± 163[2]	2678±159
Content, mg %				
Ascorbicacidinadrenalglands	299± 10,6	130 ± 7.5[1]	274± 9.9[2,3]	169±7.0[1,2]
Cholesterolinadrenalglands	2153+113	1097±52.7[1]	1871± 108[2,3]	1407±32.6[1,2]
Glycogen in liver	1861+67.4	1301±86.6[1]	1748± 42.2[2]	1590±57.4[1,2]
MDA in liver, nmol/mg of protein	0.500± 0.04	0,846±0.03[1]	0,516± 0.03[2,3]	0,619±0.02[1,2]
Stomach ulcers				
Total	-	1.51± 0.43	0.3± 0.21[2]	1.0±0.36[3]

***Note:** * - Reliably in relation to the corresponding indicators of intact animals, 2 - to the control, 3 - reliably between groups of animals receiving the sum of phytoecdysteroids and eleutherococcus extract (p <0.05)*

The obtained results presented in Table 3. In intact (in this version of the experiment — control animals), the duration of repeated swimming in relation to the first was 45.2%. Under the influence of phytoecdysteroid sum and eleutherococcus extract, the recovery process was faster. In the first case, the duration of the repeated swimming in relation to the previous was 89.1%, and in the second - 80.0%, respectively. Table 4 shows that the sum of phytoecdysteroids, like the extract of eleutherococcus, exhibits a very distinct antitoxic effect to alcohol (attributed to adaptogenic remedies [28]. So, the introduction of 25% ethanol solution at a dose of 9.8 g/kg, all control animals died. The death rate of individuals in the group of mice receiving the sum of phytoecdysteroids was 45%, and in the group of mice receiving eleutherococcus, the antitoxic effect to alcohol is very pronounced (65%).

Table 3. The effect of phytoecdysteroid sum on the recovery process after "physical work" to complete fatigue in comparison with the extract of eleutherococcus (M±m, n=6)

Experiment conditions	First swimming duration	Repeat swimming duration	Duration of repeated swimming in relation to the first one, %
Intact animals	33.3 ±1.3	15.3 ±0.76[1]	45.9
Phytoecdysteroid sum	34.0± 1,5	30.3 ± 1.3[2]	89.1
Extract of eleutherococcus	33.5± 1.2	26.8 ±1.2[1,2]	80.0

Note: *1 –Reliably in respect to the duration of the first swimming control, 2 - reliably between the duration of repeated swimming of intact animals and those receiving the sum of phytoecdysteroids and eleutherococcus extract (p <0.05)*

Table 4. The effect of the phytoecdysteroids sum on the survival of mice after intraperitoneal administration of lethal dose of ethanol in comparison with the extract of eleutherococcus

Experiment conditions	Number of animals in the group	Number of dead animals			
		In1 hour		In 24 hours	
		Unit	%	Unit	%
Control	20	5	25	15	100
Phytoecdysteroids sum	20	3	15	9	45
Extract of eleutherococcus	20	4	13	13	65

A similar pattern observed in the tissue hypoxia model. Preliminary single administration of the phytoecdysteroidsum increased the life expectancy of mice with intraperitoneal administration of sodium nitroprusside by 74%. Eleutherococcus extract in this case was less effective (effect only 31%) (seeTable 5).

Table 5. The effect of the phytoecdysteroids sum on the life expectancy of mice with tissue hypoxia in comparison with the eleutherococcus extract (M±m, n=6)

Experiment conditions	Number of animals in the group	Life expectancy	Life expectancy increase (%)
Control	10	9.3 ±0.52	
Phytoecdysteroids sum	10	16.2 ± 1.5[*,**]	74
Extract of eleutherococcus	10	12.2 ±0.66[*]	31

Note: *1 –Reliably in respect to the control, 2 - reliably between the duration of intact animals and those receiving the sum of phytoecdysteroids and eleutherococcus extract (p <0.05)*

The ability of the sum of phytoecdysteroids to prevent negative changes in the animal organism under acute stress accompanied by disturbances in individual metabolic processes indicated the possibility of its use to prevent the development of the pathological changes symptom complex in response to various destabilizing factors affecting both individual organs and systems, and the organism as a whole.

5 Conclusion

Ourstudiesconclude that*Sileneclaviformis*contains 2-deoxyecdysterone **(1)**, polypodine B (**2**), 20-hydroxyecdysone (**3**), ecdysterone-20,22-acetalisovaleric aldehyde**(4)**,integristeron A (**5**),cyasterone**(6)**, ecdysterone-20,22-acetalisovalerian **(7)**, 2-deoxy-α-ecdysone**(8)**. The compounds 2 and 6 are reported for the first time from this genus.

The biological activity (stress-protective effect) of the mentioned phytoecdysteroidsstudied for the first time. The sum of phytoecdysteroids reduces negative changes in mice after immobilization stress. It prevents the involution of the thymus and spleen and increases adrenal glands mass (i.e., normalizes the content of ascorbic acid and cholesterol in the adrenal glands). In the liver of animals after stress, the sum of phytoecdysteroids prevents a sharp decrease of glycogen, eliminates the imbalance of lactic and pyruvic acids, supports homeostasis of macroergic phosphoric compounds, increases the activity of antioxidant enzymes, and inhibits lipid peroxidation. The stress-protective effect of phytoecdysteroidswas more pronounced in compare to the eleutherococcus extract.

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