SHORT COMMUNICATION

Phytoecdysteroids-containing extract from *Stachys hissarica* plant and its wound-healing activity

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**ABSTRACT**

A number of phytoecdysteroid compounds, such as ecdysterone, polipodin V, 2-deoxy-20-hydroxyecdysone, integristeron A and 2-deoxydizon were isolated from *Stachys hissarica* plant and their structures were confirmed by NMR, mass and IR spectroscopy. In addition, the biological activity of the *S. hissarica* plant’s extract was tested on rats for wound healing activity. It was shown that the extract at repeated oral (per os) administration at a dose of 10 mg/kg speeds up the healing process of linear skin wounds in rats. The wound-healing activity of *S. hissarica* extract is confirmed to be effective and exceeds known drug methyluracil (2,4-dioxo-6-methyl-1,2,3,4-tetrahydropyrimidine), especially in case of alloxan induced diabetic animals.

**1. Introduction**

Phytoecdysteroids are natural compounds with various biological activities. It is well-known, these compounds possess a pronounced protein anabolic activity (Bathori et al. 2008). Thus,
based on the same class of compounds (ecdysterone), within our Institute of the Chemistry of Plant substances (ICPS) was developed a drug – ecdysten that has strong stimulating effect on regenerative processes in organism, and which was isolated from plant *Rhaponticum carthamoides* (Syrov 1994, 2000, 2009).

However, taking into account that *R. carthamoides* is endemic plant and doesn’t grow in Uzbekistan, it was important to find an alternative source for phytoecdysteroids that available in local plants. As the result of wide search for ecdysteroid-containing plants within the local flora, a few plants of *Stachys* family (Shishkin 1963; Radulović et al. 2007; Mohamed & Mohamed 2014; Farooq et al. 2015; Leporini et al. 2015) are been found that have a significant ecdysten-like effect. Thus, *Stachys hissarica* (Lamiaceae family) is frequently found on a territory of Central Asia, particularly in Tashkent and Fergana regions of Uzbekistan (Mamadalieva et al. 2014).

The current report for the first time presents data on the availability, composition and quantitative content of phytoecdysteroids in *S. hissarica*. In addition, we present here the results of the effect of total ecdysteroid-containing preparation (TEP) of this plant on the process of skin wounds healing.

2. Results and discussion

A preliminary investigation of *S. hissarica* plant has confirmed the presence of phytoecdysteroids in it and allowed to isolate and identify its main ecdysteroids (Figure 1), such as 20-hydroxyecdysone (1) (0.33% of dry plant’s weight), polipodin B (2) (0.22%), 2-deoxy-20-hydroxyecdysone (3) (0.2%), integristeron A (4) (0.2%) and 2-desoxyecdyster (5) (0.01%) (Galbraith & Horn 1969; Ramazanov 2007; Ramazanov, Bobayev, et al. 2007; Ramazanov, Saatov, et al. 2007; Ramazanov et al. 2007).

The isolated individual ecdysteroids have been identified on the basis of the IR spectroscopy, $^1$H NMR spectroscopy and mass spectroscopy, and as well as by comparison with reference compounds (see Supplementary Information). Table 1 provides the physicochemical data for the individual substances and ecdysteroids’ yield from *S. hissarica* plant. The NMR $^1$H and $^{13}$C spectra are recorded by VN MRS-400 (Varian) NMR spectrometer with an operating frequency of 400 MHz.
As the next step, the plant’s extract was investigated for biological activity. The TEP from *S. hissarica* was used in a number of biological experiments where the wound healing activity of summary extract, TEP, of investigated compounds was tested. The performed experiments have shown that in case of TEP from *S. hissarica* the healing process for rats with incisional linear wounds was significantly faster than for control. Thus, after administration of the TEP, on the day 7, there was a complete separating of the scab which was formed within 1–2 days after wounding. A well-formed scar was noticed under the separated scab. In case of control animals, the scab was completely separated only on 12th day and the formed scar was less firm in comparison to the group, which treated with TEP from *S. hissarica*. Table 2 provides mechanical properties data on formed scars for control and experimental groups of rats. As shown in the table, the necessary tensile strength (weight that required to rupture a scar) is 382.4 g for control group. At the same time, for rats treated with TEP from *S. hissarica* the tensile strength is 48.7% higher. As it can be seen from Table 2 the wound-healing activity of TEP from *S. hissarica* is higher than activity of well-known control compound methyluracil, a stimulator of regenerative processes.

The similar pattern is observed for animals with alloxan-induced diabetes, though in this case the healing process and formation of wound scar was much slower. The slow wound healing in alloxan-induced diabetes is caused by metabolism deterioration at this kind of pathology in rats, including the skin (Kashkin 1968; Blakytny & Jude 2006). As it is known, phytoecdysteroids are optimising the metabolic processes, and first of all, optimising a carbohydrate metabolism that demonstrated by hypoglycemic effect, by increasing of oxidation–reduction potential of cellular system, by considerable increase in production of macroergic phosphorus compounds (Syrov et al. 2012). Consequently, phytoecdysteroids are noticeably activating all these processes of skin wounds’ healing at these conditions. From Table 2 can be seen that on the 10th day of observation of rats with alloxan-induced

### Table 1. Physical and chemical properties of phytoecdysteroids isolated from *Stachys hissarica*.

| Compound No | Compound name       | Composition     | M, °C | [α], (CH₃OH) | Yield, % of plant’s mass |
|-------------|---------------------|----------------|-------|-------------|--------------------------|
| 1           | 20-Hydroxyecdysone  | C₂₇H₄₄O₇        | 241–242 | +63.2 ± 2   | 0.35                     |
| 2           | Polipodin B         | C₂₇H₄₄O₈        | 252–254 | +94.2 ± 2   | 0.25                     |
| 3           | 2-Desoxy-20-hydrindoxyecdysone | C₂₇H₄₄O₆      | 235–237 | +82.2 ± 2   | 0.20                     |
| 4           | Integristeron A     | C₂₇H₄₄O₆        | 246–248 | +36.1 ± 2   | 0.20                     |
| 5           | 2-Desoxyecdysone    | C₂₇H₄₄O₅        | 234–235 | +93.2 ± 2   | 0.01                     |

### Table 2. The data on tensile strength of wound scars formed after incisional linear wounds on rats’ skins (on a 10th day of administered total ecdysteroid-containing preparation from *S. hissarica* and methyluracil), M ± m, n = 6.

| Experimental conditions | Tensile strength of post-operative scar (g) | Difference in tensile strength of a scar in comparing to control (%) | P₁ – to the test I | P₂ – to the test II |
|-------------------------|---------------------------------------------|--------------------------------------------------------------------|-------------------|---------------------|
| Test I (normal animals) | 382.4 ± 12.6                                | –                                                                  | –                 | –                   |
| TEP from *Stachys hissarica* | 568.8 ± 18.4                              | 48.7                                                               | P₁ < 0.001        | –                   |
| Methyluracil            | 522.2 ± 16.8                               | 36.5                                                               | P₁ < 0.001        | P₂ < 0.001          |
| Test II (diabetes)      | 286.4 ± 23.4                               | –                                                                  | P₁ < 0.01         | –                   |
| Diabetes + TEP from *Stachys hissarica* | 520.6 ± 32.6                              | 81.8                                                               | P₁ < 0.01         | P₂ < 0.001          |
| Diabetes + methyluracil | 413.2 ± 29.4                               | 44.3                                                               | P₁ < 0.50         | P₂ < 0.01           |
diabetes, the applied force to rupture the scar is 286.4 g, while in case of TEP-treated rats the applied force is 520.6 g. Thus, in current study the wound-healing effect of TEP from *S. hissarica* was much better in comparing to known methyluracil, and it was even more pronounced than for normal animals (Table 2).

Thus, the TEP, from *S. hissarica*, have a potential to be a highly effective wound-healing medication.

### 3. Conclusion

In conclusion, the present study experimentally demonstrates the effective wound healing activity of ecdysteroid-containing preparation from *S. hissarica*. Furthermore, comprehensive chemical and spectroscopic research is conducted to reveal the main active components of the plant extract that provide synergistic wound-healing effect. The main revealed active components are 20-hydroxyecdysone, Polipodin B, 2-deoxy-20-hydroxyecdysone, Integristeron A and 2-deoxy-α-ecdysone. It was shown that the extract of the whole plant at repeated oral (per os) administration at a dose of 10 mg/kg speeds up the process of healing of linear skin wounds in rats. The wound-healing activity of *S. hissarica* extract exceeds known drug methyluracil (2,4-dioxo-6-methyl-1,2,3,4-tetrahydropyrimidine), especially in case of alloxan-induced diabetic animals.

### Disclosure Statement

No potential conflict of interests was reported by the authors.

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