

Screening some of plants from *Silene* Genus for 20-Hydroxyecdysone

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Abstract: Eight *Silene* species include *S. brachuica*, *S. guntensis*, *S. linicola*, *S. oreina*, *S. praemixta*, *S. pseudotites*, *S. viridiflora* and *S. wallichiana* were screened for the main ecdysteroid 20-hydroxyecdysone by HPLC using the UV spectroscopy. HPLC analyses have shown all *Silene* plants except *S. oreina* contain 20-hydroxyecdysone, but in different concentration. Studies have shown that *S. praemixta* and *S. viridiflora* are rich phytoecdysteroids containing plants and the yields of total ecdysteroids are 2.0% and 1.6%, respectively. The results of investigation species of *Silene*: *S. brachuica*, *S. praemixta*, *S. viridiflora*, *S. guntensis*, *S. linicola*, *S. pseudotites* and *S. wallichiana* showed that the yields of 20E of these plants are 0.03, 0.27, 0.35, 0.082, 0.367, 0.071 and 0.08% respectively. The plants of *S. linicola*, *S. praemixta* and *S. viridiflora* suggested to be used for production of ecdysteroids containing preparations.

Key words: Phytoecdysteroids, Caryophyllaceae, *Silene*, 20-hydroxyecdysone,

1. Introduction

Phytoecdysteroids are contentedly widespread in the plant world. They are isolated from the main types of higher plants - ferns, gymnosperms and angiosperms, but their function in plants are yet studied insufficiently. The application of phytoecdysteroids is a promising alternative to the use of anabolic-androgenic steroids because of the apparent lack of adverse effects. Toxicity is very low, at an LD₅₀ for 20-hydroxyecdysone (20E) of 6.4 g/kg (per os) and 9.0 g/kg (pre oral) [1]. 20E may be extended to treatments of pathological conditions where anabolic steroids are routinely applied. 20E does not bind to the cytosolic steroid receptors, but rather is likely to influence signal transduction pathways, just as the anabolic steroids, possibly via membrane bound receptors. One of the most cited effects of phytoecdysteroid application is the increase of muscle size.

The tonic and anabolic preparations are produced from the plants *Rhaponticum carthamoides* (Asteraceae), *Pfaffia irisinodes* (Amaranthaceae), *Ajuga turkestanica*

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(Lamiaceae) and *Serratula coronata* (Asteraceae). The limited availability of the natural resources and relatively low content of 20E in these plants leads to a high cost of these preparations. Regional variations in the concentration levels of 20E range from 2.10-5–3% of dry weight with the average being between 0.001-0.01%.

More than 170 *Silene* species (family Caryophyllaceae) have been analyzed for their phytoecdysteroid content, and 140 of them were found to be positive and 93 different ecdysteroids have been detected from these plants. Some of them contain a high concentration of 20-hydroxyecdysone, such as *Silene otites* (almost 1%) and *Silene multiflora* (1.9%) [2]. It is established that the promising 20E containing species are plants of genus *Silene* L. and it is necessary to find novel plants among this genus. In this study we screened content of 20E from aerial parts 8 *Silene* species plants: *S. brachuica* Boiss., *S. guntensis* B. Fedtsch., *S. linicola* L., *S. oreina* Schischk, *S. praemixta* M. Pop., *S. pseudotites*, *S. viridiflora* L. and *S. wallichiana* Klotzsch.

2. Materials and Methods

2.1. Plant Material

Aerial parts of the *Silene* species such as *S. brachuica* Boiss., *S. guntensis* B. Fedtsch., *S. linicola* L., *S. oreina* Schischk, *S. praemixta* M. Pop., *S. pseudotites*, *S. viridiflora* L. and *S. wallichiana* Klotzsch. were collected in the Surkhan-Darya, Samarkand, Tashkent, Namangan regions and Botanical Garden of Uzbekistan in the summer time during 2011-2013. Aerial parts were collected when the plants were at the flowering stage. The plants were identified at the Department of Herbal Plants, Institute of the Chemistry of Plant Substances, Uzbekistan, by Dr. O.A. Nigmatullaev and voucher specimens were deposited at this Department.

2.2. Extraction

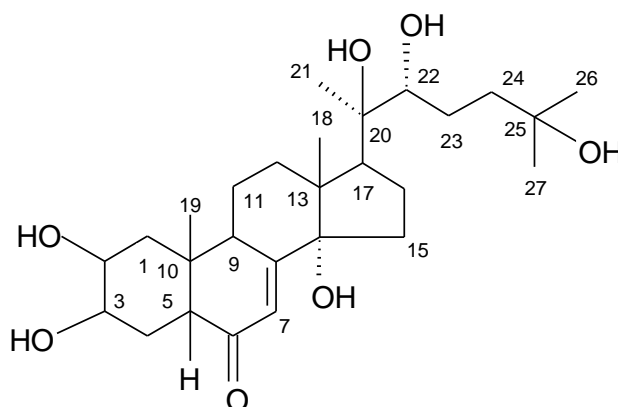
The collected plant material was air-dried away from direct sunlight at room temperature and then ground to a fine powder in a Waring blender. After grinding 100 g unit of the powdered plant material was extracted for 24 h in 500 ml of methanol. The extraction solvent were subsequently filtered to remove plant debris and then evaporated to dryness using a rotary vacuum at 40°C. Solvents methanol, acetonitrile and water were purchased from Sigma (Italy).

2.3. HPLC analysis

The contents and quantity of the 20E in the extracts of *Silene* species were investigated by HPLC. Chromatographic profiles of the extracts were generated using a high performance liquid chromatograph LC-10ATvp connected to a UV-VIS detector SPD-10Avp (Shimadzu Co, Kyoto, Japan). Extracts were diluted to 1 mg/ml, filtered through 0.22 µm and 20 µl were injected. For separation of these extracts, a Nucleosil 100-5 C18 column with a size 250 mm × 4 mm (Macherey-Nagel GmbH & Co, KG) was used. Elution was carried out by a mobile phase consisted of A (water) and solvent B (acetonitrile) and the gradient profile was as follows: from 0% B to 5% B in 8 min, from 5% B to 85% B at 8-30 min, from 95% B to 100% B% at 30-35 min and at 100% B% until 40 min. Flow rate was 1 ml/min and detection was at 242 nm and 200 nm. The quantifications of 20-hydroxyecdysone in the extracts were carried out using a calibration curve of corresponding standards at different concentrations. Authentic phytoecdysteroid 20E was obtained from the Institute of the Chemistry of Plant Substance, Tashkent, Uzbekistan. The purity of the tested compound was >95 %, as determined by HPLC. Standard of 20E was dissolved in MeOH and stored in dark at 4 °C.

3. Results and Discussion

Plants comprise rich sources of ecdysteroids in high concentration and with broad structural diversity. Zibareva et al. (2000) indicate that some sections and groups of the genus *Silene* probably include only ecdysteroid-containing species (e.g., the sections Siphonomorpha, Dipterospermae, *Silene*, *Otites*), whereas others probably comprise only ecdysteroid-negative species (e.g., *Auriculatae*, *Conomorpha*, *Eudianthe*, *Heliospermae*, *Inflatae*). The highest content of ecdysteroids both in annual and perennial species was associated with the reproductive organs. Ecdysteroid content was also high in leaves and lowest in stems. In annual species, the highest concentrations were found during budding or flowering stages. In *S. linicola* and *S. viridiflora*, ecdysteroid concentrations followed the same pattern: reproductive organs > leaves > stems > roots and the highest concentrations of 20E were found in flowers [3, 4].



Scheme 1. 20-Hydroxyecdysone (**20E**)

Eight *Silene* species include *S. brachuica*, *S. guntensis*, *S. linicola*, *S. oreina*, *S. praemixta*, *S. pseudotites*, *S. viridiflora* and *S. wallichiana* were screened for the main ecdysteroid 20E by HPLC using the UV spectroscopy. HPLC analyses have shown all *Silene* plants except *S. oreina* contain 20E, but in different concentration. Studies have shown that *S. praemixta* and *S. viridiflora* are rich phytoecdysteroids containing plants and the yields of total ecdysteroids are 2.0% and 1.6% respectively (from weight of air dried aerial parts). The results of investigation species of *Silene*: *S. brachuica*, *S. praemixta*, *S. viridiflora*, *S. guntensis*, *S. linicola*, *S. pseudotites* and *S. wallichiana* showed that the yields of 20E of these plants are 0.03, 0.27, 0.35, 0.082, 0.367, 0.071 and 0.08% respectively. The chemical analysis of the present study confirms already reported [5-7]. Determination of structures and content of isolated 20E was established on basis of data of physical-chemical constants and HPLC. The absorption maximum is due to the presence of a conjugated ketone occurs near 242 nm.

20-Hydroxyecdysterone (20E), $C_{27}H_{44}O_7$, mp 241-242°C (acetone), $[a]_D^{20} +58.9 \pm 2^\circ$ (c 0.3, CH_3OH). **IR** spectrum (KBr, n, cm^{-1}): 3435 (OH), 1665 (7-en-6-on). **UV** λ_{max}^{MeOH} nm: 242 (log ϵ 3.98). **ESI-MS**, m/z (%): 480 $[M]^+$ (0.03), 462 (1), 446 (14), 444 (2), 411 (4), 408 (10), 393 (5), 363 (10), 345 (33), 327 (20), 301 (16), 300 (12), 161 (5), 143 (10), 125 (8), 107 (6), 99 (100), 81 (34), 69 (21). **HR-MS** m/z : for $C_{27}H_{47}O_7$ $[M+H]^+$ calcd. 481.3165, found 481.3233 [8].

4. Conclusion

Determination of content of 20E in *Silene* species were analyzed by UV–vis and mass spectral characteristics to standard. Based on validation results, the developed method proved useful for 20E analysis under the specified conditions. The conducted screening results confirm the content of 20E in those *Silene* plants of Uzbekistan. The plants of *S. linicola*, *S. praemixta* and *S. viridiflora* can be used for production of the tonic and anabolic preparation “Ecdisten”.

5. References

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