Artemisia jacutica Drob. as the source of terpenoids

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Abstract. The object of research is Artemisia jacutica Drob. from Absinthium section, Asteraceae family. The aerial part of A. jacutica was collected in Buryatia in 2017-2018. In this research we identified three types of terpenoid-containing structures: nonspecialized parenchyma cells with the inclusion of essential oils’ droplets, schizogenous intercellular space and essential oil glands. Also, we isolated the essential oil from the aerial part, and identified its component composition using GC-MS method. The dominant components were γ-eudesmol, chamazulene, neryl-3-methylbutanoate, neryl-isobutanoate and geranyl-3-methylbutanoate. Using IR-spectroscopy we made the screening of the sum of extractives for the presence of γ-lactones in water, ethanol and chloroform extracts. Then, the quantitative content of the total sum of sesquiterpene lactones from samples collected in 2017 and 2018 was 1.13±0.04 % and 1.05±0.07 %, respectively. More than that, we identified arglabin in CO2- and chloroform (1:10) extracts using HPLC-UV method. The extract yield was 0.79% and 2.39%, respectively. The yield of arglabin in these extracts was 0.001% and 1.15%, respectively.

1. Introduction
It is well known that there are three sections of wormwoods in Siberian flora: Artemisia, Abrotanum and Absinthium. Mostly Absinthium DC is represented by subshrubs: Artemisia rutifolia, Artemisia rupestris, only Frigidae type, that includes A. absinthium, A. sieversiana, A. macrocephala, A. jacutica, is represented by herbaceous species. The diagnostic feature of this section is a large number of trichomes on leaves, stems, phyllary and flower disc [1].

The object of our study is Artemisia jacutica Drob. It is an annual or biennial plant with 40 cm high, that has thick, wrinkled trichomes. A. jacutica is a xerohalophyte. The geographic range of this species is presented by some distance away to northeast from the main distribution of A. sieversiana closely related species: Arctic and Eastern Siberia. It is widely spread in steppes, gravels along the banks of rivers, along roads, garbage places, salt earth, along the banks of salt lakes, on sand, in thicket [1].

The specific attention of scientists is aimed at the studying of essential oil of this species, as it is a promising source of sesquiterpenoid - chamazulene (up to 40%). Thus, the essential oil of A. jacutica has a wound-healing effect [2]. According to literature data the composition of essential oils isolated from samples collected in native habitat and introduced on experimental sections of Botanical garden of Institute of Biology of YB SB AS USSR was studied [3], also there was made an analysis of the essential oil’s composition of A. jacutica from flora of Buryatia in comparison with closely related species [4]. More than that, A. jacutica is a rich source of sesquiterpene lactones. So, the aim of this work is to study the terpenoids of A. jacutica of flora of Buryatia.
2. Models and Methods

2.1. Plant material
The object of the research is *Artemisiae jacuticae herba* collected on the territory of the Republic of Buryatia (Eravninsky region), Russia, in 2017-2018 in a flowering stage.

2.2. Microscopic analysis
The microscopic analysis of the aerial part of *A. jacutica* was conducted using standard method [5]. The microphotographs were made on optical microscopes with a MicroVizor imaging system (Lomo).

2.3. Isolation and analysis of essential oil
We extracted 30 g of powdered plant material with hydrodistillation for 3 h in a Clevenger-type collector apparatus. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out to determine the composition of the essential oil. GC-MS analysis was performed using an Agilent 6890 Gas Chromatograph (Agilent Technologies) equipped with an HP 5973 quadrupole Mass Selective Detector (Hewlett-Packard, CA, USA) and an HP-5MS capillary column (30 m × 0.25 mm × 0.2 μm; Hewlett-Packard).

2.4. Identification and analysis of sesquiterpene γ-lactones
The screening of the sum of extractive compounds for identifying γ-lactones was carried out using IR-spectroscopy method. The spectra were taken on an Agilent Cary 630 FTIR spectrometer after the preparation of a potassium bromide (KBr) disk (in the range from 3800 to 600 cm⁻¹). Then, quantitative determination of the total sum of sesquiterpene lactones in terms of arglabin were conducted using titrimetric method – acidimetry. And the quantitative analysis of arglabin in chloroform and CO₂ - extracts were conducted on an Agilent 1200 (Agilent Technologies, USA) high performance liquid chromatograph with a diode array detector. Separation was achieved using Zorbax Eclipse XDB-C18 column (4.6*150mm, 5 μm). The mobile phase consisted of aqueous solvent A: water and organic phase solvent B: acetonitrile; a flow rate was 1.0 mL/min; duration – 20 min in an isocratic mode.

3. Results and Discussion
According to the microscopic analysis there were revealed three types of terpenoid-containing structural elements in the aerial part of *A. jacutica*: nonspecialized parenchyma cells with the inclusion of essential oils’ droplets, schizogenous intercellular space and essential oil glands (Figure 1).

Using GC-MS method we determined the qualitative composition and quantitative content of the components of the essential oil of *A. jacutica* of flora of Buryatia. The essential oil was with dark blue colour, specific (wormwood) odour. The volume was 0.66% in terms of air-dry weight. The dominant components of the essential oil were γ-eudesmol (43.4%), chamazulene (22.1%), neryl-3-methylbutanoate (11.1%), neryl-isobutanoate (10.3%) and geranyl-3-methylbutanoate (3.2%). A comparative analysis of samples from Yakutia and Buryatia revealed the presence of such dominant components as 1,8-cineol (2.1-2.7%), neryl-2-methylbutanoate (2.8-3.4%), neryl-3-methylbutanoate (6.4-6.7%), chamazulene (45.8-53.6%). Also, α-bisabolol (15.2%) was dominant component that was found in samples from Yakutia, while γ-eudesmol (23.9%) - in sample from Buryatia. In general, the qualitative compositions of the dominant components of samples from Yakutia and Buryatia florases were similar, however, there were changes in the quantitative content of these components.
Figure 1. Types of terpenoid-containing structures in the flowers of *Artemisia jacutica* (zoom X150): 1 – essential oil glands on ovary; 2 – schizogenous intercellulae space in the style; 3 – essential oil glands on the flower-crown (dorsal and side views).

According to the literature data *A. jacutica* contains sesquiterpene γ-lactones that have high pharmacological activity and can be used in medicine. For example, arborescin (Figure 2) has a high cytotoxic activity [6], dihydroargolide (oxopelenolide B) (Figure 3) is an activator of the immune system (due to TCR-activated cells) [7]. According to the article [9] *A. jacutica* contains sesquiterpene lactone with antitumor activity — arglabin (Figure 4), that was found in CO2-extract of the aerial part [8].

![Figure 2. Arborescin.](image)
![Figure 3. Dihydroargolide.](image)
![Figure 4. Arglabin.](image)

Using IR-spectroscopy method we made the screening of the sum of extractives for the presence of γ-lactones. The presence of absorption bands in the range of 1740-1800 cm$^{-1}$ (characteristic of C = O γ-lactone) indicated the presence of sesquiterpene γ-lactones in the aerial part of *A. jacutica*: in water extract - 1746 cm$^{-1}$, ethanol and chloroform extracts - 1751 cm$^{-1}$.

Also, we determined the quantitative content of the total sum of sesquiterpene lactones in terms of arglabin in *A. jacutica*. For the sample collected in 2017 the total sum was 1.13 ± 0.04%, and for 2018 sample – 1.05 ± 0.07%.

More than that, we identified arglabin in CO2- and chloroform (1:10) extracts using HPLC-UV method with the absorption maximum of 204 nm. The extract yield was 0.79% and 2.39%, respectively. And the yield of arglabin in these extracts was 0.001% (chloroform extract) and 1.15% (CO2-extract).

4. Conclusion

According to the research *Artemisia jacutica* Drob. is a promising source of terpenoids that can be used in medicine. It has three types of terpenoid-containing structural elements in the aerial part. The essential oil is a rich source of chamazulene that shows wound-healing effect. Also, *A. jacutica* is a promising source of sesquiterpene lactones, the total sum is about 1.05-1.13%, including arglabin.
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