Chromato-mass-spectrometric study of alcohol extracts Plantago L.

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Abstract. The authors conducted a study of alcohol extracts of Plantago lanceolata L. and Plantago major L. using chromatography-mass spectrometry. Fitol, squalene, vitamin E, sitosterol, and sugars were found and identified in all studied types of plantains, among which isosorbide was identified on the chromatograms. The difference of the studied species in the composition of lipophilic substances was also revealed. Squalene and Methyl 7-methycyclopenta [c] pyran-4-carboxylate are found in extracts from Plantago major raw materials. In the Plantago lanceolata samples there are compounds: norlean-17-en-3-on and 3-[3-Bromophenyl]-7-chloro-3.4-dihydro-10-hydroxy-1.9 (2H, 10H) -acridinedione.

1. Introduction
The search for new sources of natural biologically active substances (BAS) is a pressing issue of medical and biological science. An important source of BAS are wild and cultivated medicinal plants. In modern Russia, herbal remedies are increasingly used for the treatment and prevention of various diseases. The strategy of drug provision of the population of the Russian Federation for the period up to 2025 requires the development of import-substituting drugs, which also implies the optimization of the use of domestic medicinal plant materials [1]. Qualitative changes in the study of the chemical composition of LSR have contributed to the solution of this problem, since the introduction of modern spectral and physicochemical re-search methods (gas-liquid chromatography, high-performance liquid chromatography, NMR spectroscopy, chromatography-mass spectrometry) has opened new opportunities for researchers.

This fact prompted us to study the natural sources of biologically active substances using modern methods. Exploring the flora of the Urals and the Volga region during expeditions (1999-2017), we paid attention to the plants of the family Plantaginaceae, which have a vast range in Eurasia and have been widely used in herbal medicine since ancient times.
The aim of our study was the study of biologically active substances *Plantago maxima*, *Plantago major* and *Plantago lanceolata* by gas chromatography-mass spectrometry. Previously, there was no study of Plantago LSR extracts using the GCMS method.

2. Materials and methods

The objects of our study were selected species of the genus *Plantago* L.:
- *Plantago maxima* Juss.ex Jacq.;
- *Plantago lanceolata* L.;
- *Plantago major* L.

*Plantago maxima* - herbaceous perennial plant with a powerful taproot, truncated stalk and collected in the outlet thick and broad petiolate leaves elliptical or egg-shaped, growing in steppe zone grows on solonetsous, bottomland steppized meadows, along rivers and lakes. In the Orenburg region, *Plantago maxima* grows over floodplain, sometimes saline, meadows in the floodplain of the Ural and Sakmara rivers. It blooms from June to August.

*Plantago lanceolata* is a herbaceous stem perennial plant with a wide growing area in Russia - the European part, the Caucasus, the middle and southern Urals, Western and East-ern Siberia, and the Far East. It grows in meadows, forest glades, along roads, on garbage places, gardens, vegetable gardens. On the territory of the Urals and the Volga region, Plan-tago lanceolata is commonly found on calcareous and sandy soils.

*Plantago major* is a perennial herbaceous plant distributed almost throughout the entire territory of Russia, with the exception of the Extreme North. It grows on roadsides, paths, wastelands, near housing, in vegetable gardens, gardens, crops. *Plantago major* - one of the oldest medicinal plants. Avicenna also used Plantago leaves as a wound healing and hemo-static agent. Plantago seedpads contain about 10-12% (up to 16%) of mucous substances. The highest content of mucus was found in Plantago oval, which, however, has less ability to swell than the major and lanceolate.

The current literature describes the use of species of the genus *Plantago* - *Plantago major* and *Plantago lanceolata*. Numerous pharmacological studies of drugs from the leaves of *Plantago major* (*Plantago lanceolata*) prove their versatile action in the treatment and prevention of many diseases [2].

Water infusions of *Plantago major* enhance the secretion of bronchial mucus, thereby showing an expectorant effect. Therefore, infusions, decoctions and syrups from *Plantago major* leaves are indicated for bronchitis, tuberculosis, other diseases of the respiratory organs, as well as diseases of the gastrointestinal tract, including gastric ulcer and duodenal ulcer. *Plantago major* drugs are used externally as a bacteriostatic and wound-healing agent for inflammatory diseases of the oral cavity and nasopharynx, bruises, wounds, cuts, chronic ulcers, burns, phlegmons, boils, purulent wounds. In obstetrics and gynecology, plantain is used for endometritis, myometritis, parametritis, adiexitis, as well as for infertility due to anovulation, with metrorrhagia [2, 3]. Extracts from the leaves of plantain leaves have a sedative and hypotensive effect [3].

Juice (from fresh leaves) *P. major* is effective in the treatment of corneal wounds and has a pronounced antibacterial effect: it inhibits the growth of pathogenic staphylococcus at a dilution of 1: 2, the blue-pus pate disease at a dilution of 1: 4 and retards the growth of hemolytic streptococcus at a dilution of 1: 2.

Folk medicine in Russia recommends *Plantago major* leaf infusion for allergies, fever, diarrhea, hemorrhoids, inflammation of the bladder, cancer of the stomach and lungs. Fresh leaves are applied to wounds, abrasions, cuts, ulcers, and boils. Ointment with dried leaf powder is used as an effective treatment for pustular skin diseases. Plantain juice is prescribed for the treatment of corneal wounds, atopic dermatitis and acne [2].

In Chinese traditional medicine *Plantago major* drugs used as diuretic, antipyretic, respiratory infections, urinary tract diseases, conjunctivitis, toothache, as wound healing [2].

Plantaglucid, which contains a mixture of polysaccharides, is produced on the basis of *Plantago major* leaves, has an antispasmodic and anti-inflammatory effect, and is used in case of hypoeacid gastritis, gastric ulcer during the period of exacerbation and to prevent relapse.
The plant material (sheet) of Plantago was collected by us in the vicinity of the village of Kamenno-Ozernoe, 30 km south-east of the city of Orenburg on July 26, 2014. *Plantago lanceolata* is gathered on a dry meadow in a fescue-grass association. The leaf of *Plantago major* is on the edge of the riparian forest.

For the manufacture of extracts, a portion of the crushed raw material (d = 1.0 mm) was poured with 70% ethyl alcohol in a ratio of 1:10, shaken for 1 h and infused for 24 h. Next, we conducted a study of extracts by gas chromatography with mass-selective detection using an Agilent 7890A gas chromatograph with an Agilent 5975C mass spectrometer. Chromatography was carried out under the following conditions: non-polar HP-5ms column; ionization was carried out by electron impact; flow rate of the carrier gas (He) 1 ml / min; without dividing the flow; evaporator temperature 280 ° C; programmable column temperature: 5 min 70 ° C, then with a speed of 18 ° C / min it rose to 310 ° C and was kept for 10 min; registration of mass spectra was carried out on the total ion current; mass spectra obtained were compared with library mass spectra; used libraries: NIST08; tuning of the mass spectrometric detector was performed according to the standard Autotune setup program.

3. The chromato-mass-spectrometric study of alcohol extracts

In the studied species of plantain we found phytol, vitamin E, sieve-sterol and sugars, among which isosorbide were identified on the chromatograms.

Chromatograms of alcoholic extracts of plantain raw materials showed the difference in composition of the objects (Table 1, Fig. 1, 2). *Plantago major* raw materials contain squalene and Methyl 7-methylcyclopenta [c] pyran-4-carboxylate. There is a substance in *Plantago lanceolata*: 3-[3-Bromophenyl] -7-chloro-3,4-dihydro-10-hydroxy-1,9 (2H, 10H) -acridinedione. Method GCMS could not detect substances of polyphenolic natureTo solve this problem, it is necessary to continue the study using other methods.

In the studied *Plantago* species, unsaturated monoatomic alcohol fi-tol was detected (Fig. 3). In plants, phytol is part of the chlorophyll molecule. Chlorophyll is an ester of dicarboxylic acid chlorophyllin, in which one carboxyl group is esterified with a residue of methyl alcohol, and the second with a residue of phytol (C20-terpenoid). The pharmacological action of phytolan today has not been studied. Leading on the content of fitol samples Plantago maxima.

The content of tocopherol is somewhat higher in the raw materials of plantain-alcanthus (Fig. 4). Vitamin E (*α*-tocopherol) is a highly effective fat-soluble antioxidant, exhibiting a membrane-stabilizing, cytoprotective effect. When hypovitaminosis E in the human body de-velops degenerative changes in the muscular, parenchymal, connective and nervous tissues [2].

Methyl 7-methylcyclopenta [c] pyran-4-carboxylate found in the extracts of Plantago major belongs to the group of terpenoids and is noted in the literature as a component of complex hypolipemic and choleretic agents (Fig. 5) [4, 5].

Sitosterol, found in significant quantities in the test samples (Fig. 6), has a hypocoles-terolemic effect, since due to the similarity in the structure with cholesterol, it is competitively associated with micelles, inhibiting cholesterol absorption from the gastrointestinal tract. Sitosterol is used to treat benign prostatic hypertrophy, prostate carcinoma, and breast cancer, although the positive effect of treatment has not yet been proven [6]. Externally, sitosterol preparations have a protective, moisturizing, anti-inflammatory and wound-healing effect. Sitosterol - natural sunscreens that have the ability to absorb ultraviolet rays that inhibit mel-anogenesis. Sitosterol preparations are used to prevent the appearance of unwanted pigmentation and senile spots, to even out and refresh the complexion, to strengthen the dermal structure by inhibiting the enzymatic breakdown of proteoglycans and fiber structures of the dermis [6, 7].

Table 1. The results of the study of BAS Plantago method GHMS
| Chemical Name | Time holding | Plantago maxima | Plantago major | Plantago lanceolata |
|---------------|--------------|----------------|---------------|-------------------|
| Fitol         | 14.94        | ++++           | +++           | +++               |
| Vitamin E     | 19.38        | +              | +++           | ++++              |
| Sitosterol    | 20.70        | +++            | +++           | ++                |
| Squalene      | 18.03        | +++            | +             | -                 |
| Sahara        | -            | +              | +             | +                 |
| Isosorbide    | 12.23        | +              | +             | -                 |
| 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-acridinedione | 18.26 | -             | -             | +                 |
| Methyl 7-methylcyclopenta[c]pyran-4-carboxylate | 12.23 | -            | -             | +                 |
| Norlean-17-en-3-on | 20.82 | -            | +             | +                 |

* Designation *
- - not found
- +, ++, ++++, ++++ – degree of intensity of the peak substance on the chromatogram

**Figure 1.** Chromatogram of alcohol extraction *Plantago Maxima*

**Figure 2.** Chromatogram of alcohol extraction *Plantago major*
The following substances were found in the extract of *Plantago lanceolata*: 2-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9 (2H,10H)-acridinedione and Norlean-17-en-3-on (Fig. 7-8). The biological functions and pharmacological action of these substances to date has not been studied.

Squalene was detected in *Plantago major* samples (Fig. 9). Squalene is a fat-soluble hydrocarbon of the amtriterpene series. In human liver, squalene is produced as a precursor of cholesterol. Squalene is important for the formation of a lipid film that protects the body from the external environment, and is also found in the composition of low and very low density lipoproteins. Currently, a number of works prove the anti-cancer effect of squalene preparations, its antioxidant, anti-toxic, immunomodulatory and photoprotective effect [8, 9, 10]. Currently, the food and cosmetic industries produce a number of preparations containing squalene and exhibiting a wide range of functional effects on the body (lipid-lowering, hepatoprotective, antioxidant, anticarcinogenic, anti-inflammatory) [11, 12].
Figure 5. Mass spectrum of vitamin E (peak with retention time of 19.38 minutes)

Methyl 7-methylcyclopenta[c]pyran-4-carboxylate

Figure 6. The mass spectrum of the substance Methyl 7-methylcyclopenta [c] pyran-4-carboxylate with a retention time of 12.23

Figure 7. Mass spectrum of sitosterol (peak with retention time 20.70 min).
Figure 8. The mass spectrum of the peak with a retention time of 18.26 minutes and the formula of the compound 2-[3-Bromophenyl]-7-chloro-3.4-dihydro-10-hydroxy-1.9 (2H,10H) –acridinedione

Figure 9. Mass spectrum with retention time of 20.82 min, library mass spectrum and formula of norlean-17-en-3-on compound

4. Conclusion
Thus, on the basis of the studies performed, it can be argued that in the alcohol extracts of Plantago maxima Juss. ex Jacq., Plantago lanceolata L. and Plantago major L., phytol, squalene, vitamin E, sitosterol and sugar were detected and identified isosorbide. Chromatograms of alcoholic extracts showed differences in the composition of lipophilic substances 7-methylcyclopentane [c] pyran-4-carboxylate was found in extracts from Plantago major raw materials. In the Plantago lanceolata samples, norlean-17-en-3-on and 3-[3-Bromophenyl]-7-chloro-3.4-dihydro-10-hydroxy-1.9 (2H, 10H) -acridinedione are present, but not Squalene is found. Lead on the content of fitol, sitosterol and squalene Plantago maxima samples. The highest content of tocopherol is characterized by the raw material Plantago lanceolata.
Figure 10. The mass spectrum of squalene (peak with a retention time of 18.03 minutes)

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