Isolation of extracts of wormwood - effective natural insecticides of the terpenoid group

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Abstract. Extracts of wormwood (Artemísia absínthium) grown in the greenhouse complex of the Institute of Phytopathology were obtained. The resulting extracts were isolated from the dried ground parts of this plant (leaves and flowering tops) by subsequent extractions with a mixture of dichloroethane, acetone and petroleum ether, as well as a mixture of methanol, chloroform and water with the addition of water and chloroform to separate the chloroform layer and purification on activated carbon. By further separation of the obtained extracts by preparative column chromatography, biologically active fractions with different contents of natural insecticides from the group of terpenoids were isolated. The content of biologically active components in various fractions was determined by the methods of chromatomass-spectrometry. It was found that a mixture of low-polarity chloroform with a minimum amount of polar methanol most fully extracts chrysanthenone and chrysanthenyl acetate, cineole and borneol, and the minimum extraction of terpenoids is observed with an increase in the content of polar methanol in the eluent containing chloroform. The insecticidal effect of the isolated fractions on the large cereal aphid (Sitobion avenae) was studied. It was found that the maximum insecticidal effect (100% death of insects) was exhibited by the fraction of terpenoids isolated during extraction with a low-polarity eluent containing chloroform with a minimum amount of methanol. It was found that the fraction of terpenoids containing a high amount of chamazulene exhibited a moderate insecticidal effect (31% of insect mortality), while fractions with a low content of this compound exhibited low insecticidal activity. It was shown that extracts of wormwood have an insecticidal effect against the pest of cereal crops, large cereal aphid and can be used as environmentally friendly natural insecticidal additives to commercial preparations.

1 Introduction

It is known that essential oils have been used for many thousands of years for conservation, preparation of drugs and pharmaceuticals [1–5], exhibiting bactericidal [6–9], antifungal, antioxidant and insecticidal properties [10–14]. The biological activity of essential oils

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depends on their chemical composition and is determined by the plant species, as well as its geographic location, environmental factors and agronomic cultivation conditions [15, 16].

Plants belonging to the genus Artemisia, which is widespread throughout the world and is one of the largest in the Asteraceae family, appear to be promising producers of essential oils [17]. Representatives of this genus are producers of a large list of biologically active substances - sesquiterpene lactones, glycosides, alcohols (absintine, anabsintin), flavonoids, coumarins, phytoncides, etc., including essential oils, exhibiting extremely strong physiological and pharmacological properties with a wide range of biological actions. The essential oil obtained from plants of the wormwood genus contains hundreds of compounds responsible for its biological activity [18]. Among such compounds, first of all, it should be noted the components of the terpene series, which are unsaturated carbons with high chemical and biological activity. So, chrysanthene (isomer of verbenone) and its derivative chrysanthone - bicyclic monoterpene ketone and alcohol, respectively, have a disinfecting effect. Chrysanthone acetate is an ester of chrysanthone and acetic acid, has antiallergic, sedative and antispasmodic effect [19, 20]. 1,8-Cineol is a monocyclic terpene, used as an antiseptic and expectorant. Borneol and the camphor obtained during its oxidation - bicyclic monoterpene alcohol and ketone, respectively, are used as analeptic, sedative, antiseptic and analgesic agents. In addition to the indicated characteristics of camphor, it should be noted that camphor oil has been used for a long time to control moths as an insecticidal agent. Terpineols - monoterpene alcohols, existing in the form of 5 isomers, are used as antimicrobial agents and for the preparation of perfume compositions. Caryophyllene is a bicyclic terpene hydrocarbon, used to compose perfume compositions, fragrances for soap, and antiseptics. The presence of even only these noted compounds formed in wormwood plants determine the prospects for the use of representatives of this genus in various areas of the national economy.

As follows from the data presented, the literature data on the chemical composition of certain species of the genus wormwood concern, first of all, biologically active components used in medicine, the pharmaceutical industry and the perfumery industry. At the same time, data on the insecticidal effect of wormwood are insufficiently covered in the literature, and studies of the chemical components responsible for such types of biological activity are represented by a small number of works.

In order to establish the components responsible for the insecticidal effect of plants of the genus wormwood, we isolated various fractions of extracts of wormwood (Artemisia absinthium), carried out a chemical analysis of their composition, and studied the effectiveness of the action of the obtained fractions on wheat infected with a large cereal aphid (Sitobion avenae).

2 Methods

2.1 Conditions for growing bitter wormwood

The research was carried out in the greenhouse complex of the Institute of Phytopathology. Bitter wormwood was grown in a greenhouse complex, in the amount of 96 plants, on podzolic soil with a loamy texture, pH 6.0, with a humus content of 2.0–2.1%, exchangeable sodium 5.7–5.8%, exchangeable potassium 12.0–12.3 mg, calcium 1.3 mg, available phosphorus 2.4–2.6 mg per 100 g of soil. Sowing of wormwood was carried out at a soil temperature of 10 °C and an air temperature in a greenhouse box of 15–16 °C at the beginning of March 2018. General cultivation practices were followed during the growing season. The ground part of the plants (leaves and flowering leafy tops) was collected in two steps in June-July 2019: the basal leaves were plucked without petioles, before flowering, at
the stage of budding; the tops were cut to a length of 20–25 cm at the beginning of flowering at optimum maturity. The harvested plant mass was spread in a 5 cm layer on wooden pallets and dried for three days in the open air at a temperature of 25–27 °C and then in drying ovens at a temperature of 50 °C. Dried plants containing 80–85% dry matter were crushed by grinding in a mechanical mill to a fine powder, stored in tight bags in a dark place at room temperature.

2.2 Extraction of wormwood plants

Extraction of crushed wormwood plants was carried out by extracting the obtained dry powder three times in portions of 1 g with a mixture of dichloroethane, acetone and petroleum ether (10 : 7 : 1) with a volume of 100 ml, organic extracts were combined, filtered, the solvent was distilled off on a rotary evaporator to obtain 15–18% concentrate, which was passed through activated carbon three times. The resulting concentrate was extracted three times with a mixture of methanol, chloroform and water (10 : 20 : 8) with a volume of 50 ml, each time adding 30 ml of water and 30 ml of chloroform each time, the chloroform layer was separated, the extracts were combined and the resulting extract was purified again with activated carbon, the solvent was distilled off on a rotary evaporator.

2.3 Preparative extract chromatography

The final product in the form of a dark green oil was separated on a preparative chromatographic column (Silica gel 60 sorbent 0.04-0.063 nm, 230-400 mesh), successively eluting with hexane / chloroform (1 : 7, fraction I), methanol / chloroform mixtures (1 : 20, fraction II), methanol / chloroform (1 : 10, fraction III), methanol / chloroform (3 : 10, fraction IV), followed by distillation of eluents. The completeness of the separation of the components was monitored by TLC methods on Sorbfil plates PTSKh-P-A-UV, PTSKh-AF-A-UV (silica gel, UF 254/365).

2.4 Chromatmass spectrometric analysis

Fractions I – IV were separated on a Shimadzu GCMS-QP 2010 Ultra chromatmass spectrometer with a mass selective detector at an ionization energy of 70 eV using a column 25 m long, 1 mm inner diameter, stationary phase thickness 0.33 μm, helium carrier gas. Data analysis was performed in the ITDS system, comparing with known reference search library samples.

2.5 Biotesting of fractions

The insecticidal effect of end products I – IV (diluted at the rate of 0.1 g of the product per 99 ml of a mixture of methanol and water 30/70) was tested by spraying wheat infected with a large cereal aphid (Sitobion avenae).

3 Results and Discussion

The results of experimental studies on the chromatographic extraction of components from extracts of wormwood and their content in various fractions are presented in the table1.
Table 1. Pyrethrine content in fractions I – IV (%).

| Compound                  | Fraction I | Fraction II | Fraction III | Fraction IV |
|---------------------------|------------|-------------|--------------|-------------|
| Chrysanthenone            | 16.44      | 34.92       | 12.51        | 5.37        |
| Cineol                    | 4.72       | 8.39        | 5.06         | 3.94        |
| Camphor                   | 5.80       | 4.93        | 6.48         | 8.01        |
| Borneol                   | 5.59       | 5.98        | 5.46         | 4.82        |
| Chrysanthenyl acetate     | 13.03      | 14.93       | 12.05        | 8.96        |
| Hamazulen                 | 8.63       | 6.63        | 3.01         | 2.06        |
| Germacren                 | 3.09       | 1.59        | 2.39         | 5.04        |
| Terpinenol                | 2.02       | 2.36        | 1.83         | 1.04        |
| Spatulenol                | 1.73       | 1.83        | 1.89         | 2.02        |
| Dihydrochamazulene        | 2.59       | 3.18        | 3.89         | 2.23        |
| Evdesmol                  | 2.07       | 1.05        | 1.68         | 2.45        |
| Caryophyllene             | 1.69       | 1.72        | 1.28         | 1.22        |
| Bornyl acetate            | 0.61       | 0.69        | 0.51         | 0.42        |
| Citronellol               | 0.53       | 0.60        | 0.42         | 0.36        |
| Caryophyllene oxide       | 0.43       | 0.48        | 0.49         | 0.53        |

Experimental data show that the most complete recovery of biologically active components (up to 90%) was observed for fraction II containing such compounds as chrysanthenone (> 34%), cineole (> 8%), chrysanthenyl acetate (> 14%), chamazulene (> 6%). For fraction I, the extraction of active components was somewhat lower (up to 65%). The smallest recovery was observed for fractions III (up to 60%) and IV (up to 50%). Thus, the best result was achieved when using a mixture of low-polarity chloroform with a minimum amount of polar methanol as an eluent. The active components were recovered slightly worse when using an almost non-polar mixture of chloroform with a small amount of hexane. An increase in the content of polar methanol in the eluent led to a decrease in the recovery of biologically active compounds. When processing wheat infected with a large cereal aphid (Sitobion avenae), it was found that the maximum insecticidal effect was shown by fraction II (100% of insect death), containing the maximum amount of chrysanthenone and chrysanthenyl acetate - bicyclic analogs of monocyclic ketones (pyrethron, cinerone, jasmine) or monocyclimon keto alcohols (pyrethronolone, cinerolone, jasmolone), which are included as an alcohol component in the natural insecticides of pyrethrins. Fractions I and III, containing rather close values of chrysanthenone (16.44% and 12.51%) and chrysanthenyl acetate (13.03% and 12.05%), significantly differed in their insecticidal effect. Fraction I showed a moderate insecticidal effect (31% of insect mortality), while fraction III showed a significantly lower activity (12% insect mortality), with a similar content of the noted compounds. Biologically active components such as cineole, camphor, and borneol also had similar values in fractions I and III. These data indicated that the difference in the insecticidal effect of fractions I and III is determined by the content of another compound. Such a compound is probably chamazulene (an isomer of naphthalene exhibiting insecticidal activity), the content of which in fraction I is more than twice the content in fraction III. Thus, the experimental data indicate that fraction II, containing the maximum amount of chrysanthenone and chrysanthenyl acetate, has the strongest effect on large cereal aphids. The content of cineole, camphor, and borneol in fractions I and III is comparable, which indicates an insignificant role of these compounds in the insecticidal action on large grass aphids, while the content of chamazulene in fraction I significantly exceeds its content in fraction III, which probably determines the difference insecticidal activity of these fractions.

4 Conclusions
It is shown that as a result of the research, new methods have been developed for the isolation of the components of essential oils of wormwood, which are necessary for the production of environmentally safe insecticidal preparations.

It has been shown that extracts of wormwood have a high insecticidal effect against such a pest of cereal crops as the great cereal aphid and can be successfully used as effective biologically active additives to commercial insecticidal preparations.

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