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Study of Chemical Composition of *Asparagus racemosus* Roots

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Abstract—The chemical composition of *Asparagus racemosus* roots is studied by the methods of gas chromatography and high-performance liquid chromatography. Marker substances characteristic of this type of raw materials are revealed. The dioscin content in the roots of *A. racemosus* is quantitatively determined.

Keywords: Asparagus racemosus, gas chromatography, high-performance liquid chromatography, protodioscin, dioscin

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Asparagus racemosus is a medicinal plant widely used in oriental medicine. The plant contains steroid saponins (protodioscin, dioscin, etc.) that have an effect on the human body similar to natural hormones, normalize the female reproductive system by facilitating the acceleration of the transformation of estradiol to estrol, and affect the synthesis of progesterone. In addition, isoflavones, alkaloids, polysaccharides, and other groups of biologically active substances are constituents of *Asparagus racemous* [1]. Formulations based on the roots of *A. racemosus* normalize the female hormonal system and have antioxidant and immunostimulating effects.

The raw material of *A. racemosus* is included in the Ayurvedic Pharmacopoeia [2]. This species is not included in edition XIII of the State Pharmacopoeia of the Russian Federation; however, medicinal plants' raw materials and preparations based on them are supplied to the Russian pharmaceutical market.

The purpose of our work is to qualitatively and quantitatively study the roots of *A. racemosus*, as well as determine marker substances characteristic of this type of raw material.

EXPERIMENTAL

Sample preparation. The object of the study is the dried roots of *A. racemosus* (Mumbai, India) and extracts from them.

The component composition of the volatile fraction of chloroform and acetonitrile extracts was determined by gas chromatography with a mass-selective detector (GC–MS). A chloroform extract from the raw material was obtained by the method of circulation extraction. To do this, 35 g of dried and ground *A. racemosus* roots in a gauze pouch were loaded into a Soxhlet extractor and exhaustively extracted with 230 mL of a mixture of chloroform and 95% ethyl alcohol (10 : 1) for 4 h in a water bath. The resulting extract was concentrated to a volume of 100 mL and used for further studies [3, 4]. An acetonitrile extract was obtained by infusing the *A. racemosus* root powder in acetonitrile (1 : 1). During the sample preparation, vials with the obtained extracts were placed in a Sapfir ultrasonic bath-stirrer (without heating) for 10 min. Then, 10 mL of the extract was placed in a plastic flask and centrifuged on an Ohaus Split 16000-rpm centrifuge at 16000 rpm for 2 min. After that, 1 mL of the extract was taken with a microdoser from the surface to prevent the entry of raw material particles and placed in the injector of a GC–MS instrument [5].

Chromatography conditions. The work was carried out on an Agilent Technologies 6850 Series II gas chromatograph equipped with an Agilent Technolo-

Table 1. Parameters of gradient mode of chromatography

Time, min	Mobile phase A, %	Mobile phase B, %
0	70	30
20	50	50
30	30	70
35	30	70
36	70	30
42	70	30

Mobile phase A, deionized water acidified with trifluoroacetic acid to pH 3.0; mobile phase B, acetonitrile.

Table 2. Composition of chloroform extract from *Asparagus racemosus* roots

Compound	Retention time, min	Relative content, %
2,6,10-Trimethyltetradecane	20.176	1.33
Tert-Hexadecanethiol	20.28	1.31
2-Hexyl-1-decanol	21.204	2.76
Dibutyl phthalate	21.795	4.91
Eicosane	22.184	4.36
Androst-5,7-dien-3-ol-17-one	22.393	1.16
Pentacosane	23.123	5.61
	24.019	6.06
	24.874	6.91
N-Phenyl-2-naphthylamine	24.304	20.66
Tetracosane	25.694	7.6
Hetriacontan	26.521	7.14
	27.501	7.07
Bis(2-ethylhexyl)phthalate	26.945	5.74

gies 5973 Network mass-selective detector, a HP-5MS column (30 m × 0.25 mm); the column temperature was 30–240°C. The temperature rose at a rate of 5°/min; the final isothermal section lasted 10 min. Evaporator temperature 200°C; injector temperature 30°C; and carrier gas (helium) speed of 1 mL/min.

Qualitative and quantitative determination of steroid saponins in the powder of *A. racemosus* roots was carried out by high-performance liquid chromatography (HPLC). For sample preparation, an accurate weighed portion (0.566 g) of ground raw material of *A. racemosus* was placed in a 50-mL extraction flask, and 20 mL of 70% methanol was added, followed by extraction for 15 min in an ultrasonic bath [6]. The analysis was carried out on an Agilent 1290 liquid chromatograph with spectrophotometric and mass spectrometric (6460) detectors. Zorbax XDB-C18 column (150 × 4.6 mm, 5 μm; Agilent, United States).

Chromatography conditions. Security Guard C18 (4 × 3 mm; Phenomenex, United States) precolumn, column temperature 25°C, flow rate 1 mL/min, injection volume 20 μL, gradient modes for protodioscin and dioscin determination are shown in Table 1.

The conditions for UV detection were selected based on the spectrophotometric analysis data (wavelength 205 nm with spectral bandwidth 4 nm). Mass spectrometric detection conditions: ion scanning mode for positive ions, mass range of scanned ions from 400 to 1500 *m/z*; nebulizer pressure 70 psi; drying gas flow rate and temperature were 12 L/min and 350°C, respectively.

RESULTS AND DISCUSSION

Using the GC–MS method, we identified 11 substances in a chloroform extract from the *A. racemosus* roots (Table 2, Fig. 1).

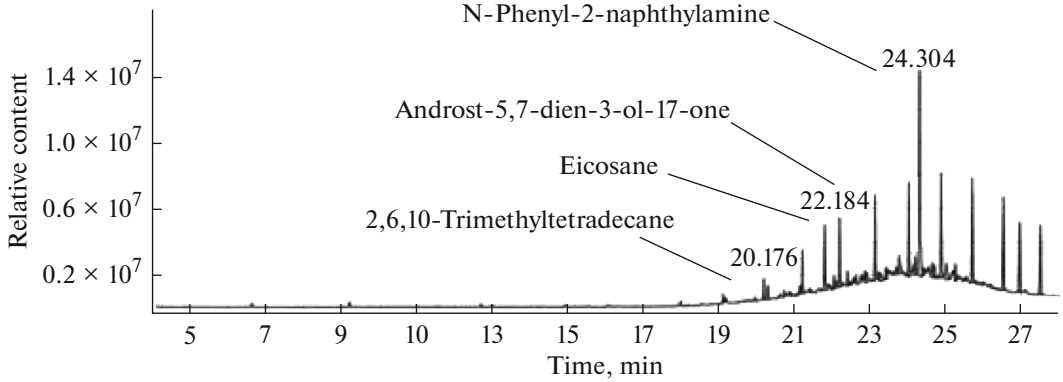

Fig. 1. GC–MS chromatogram of chloroform extract from *A. racemosus* raw material. For chromatographic conditions, see text.

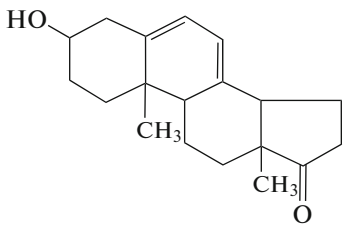

Fig. 2. Structural formula of androst-5,7-dien-3-ol-17-one.

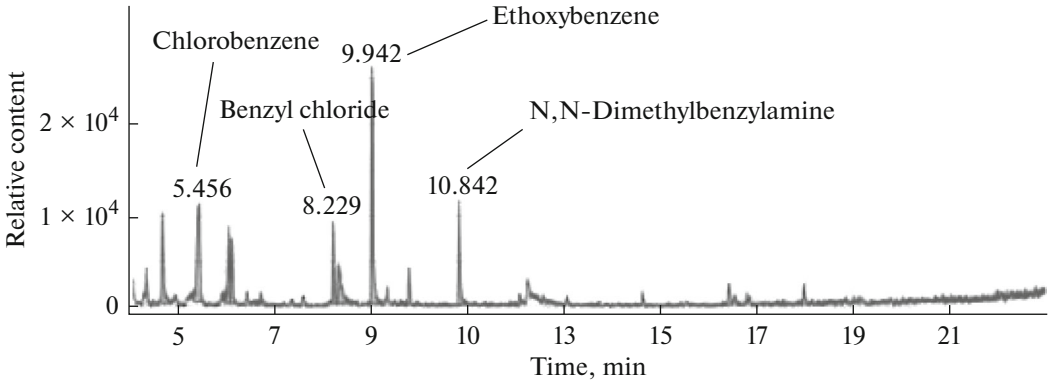

Fig. 3. GC–MS chromatogram of acetonitrile extract from the *A. racemosus* raw material. For chromatographic conditions, see text.

Androst-5,7-dien-3-ol-17-one (1.16%) from the group of phytosteroids, which was found in the chloroform extract of *A. racemosus* (Fig. 2), can be considered as one of the marker substances with a greater degree of reliability; however, it was not detected in the composition of the acetonitrile extract. This was probably due to the difference in the polarity indices of the solvents.

The component composition and chromatogram of the GC–MS analysis of the volatile fraction of the acetonitrile extract are presented in Table 3 and Fig. 3. It was found that N-phenyl-2-naphthylamine (20.66%) predominates among the substances of the volatile fraction of the chloroform extract; ethoxybenzene (24.39%) and chlorobenzene (20.32%), in the acetonitrile extract.

In the HPLC–MS analysis of the *A. racemosus* roots, we detected a chromatographic peak (Fig. 4) corresponding to the retention time close to that for the protodioscin reference (the protodioscin retention time is 22.22 ± 0.05 min); and in the mass spectrum, there was the most intense ion $m/z = 1033$ (by 2 units of molecular weight greater than for the standard sample). Most likely, this is due to the fact that this compound is an analog of protodioscin with a reduced double bond. It can be assumed that the reduced protodioscin is contained in the studied extract from the raw materials of medicinal plants. The peak with a retention time of 20.8 min corresponds to dioscin (Fig. 5), since it coincides with the retention time and spectra of the standard. The content of dioscin in the sample was at

Table 3. Composition of acetonitrile extract from *Asparagus racemosus* roots

Compound	Retention time, min	Relative content, %
Neopentyl glycol	4.699	10.68
Chlorobenzene	5.456	20.32
Ethylbenzene	6.075	7.72
	6.137	6.8
Benzyl chloride	8.229	7.33
2-(Formyloxy)-1-phenylethanone	8.347	9.36
Ethoxybenzene	9.042	24.39
N,N-Dimethylbenzylamine	10.842	9.07

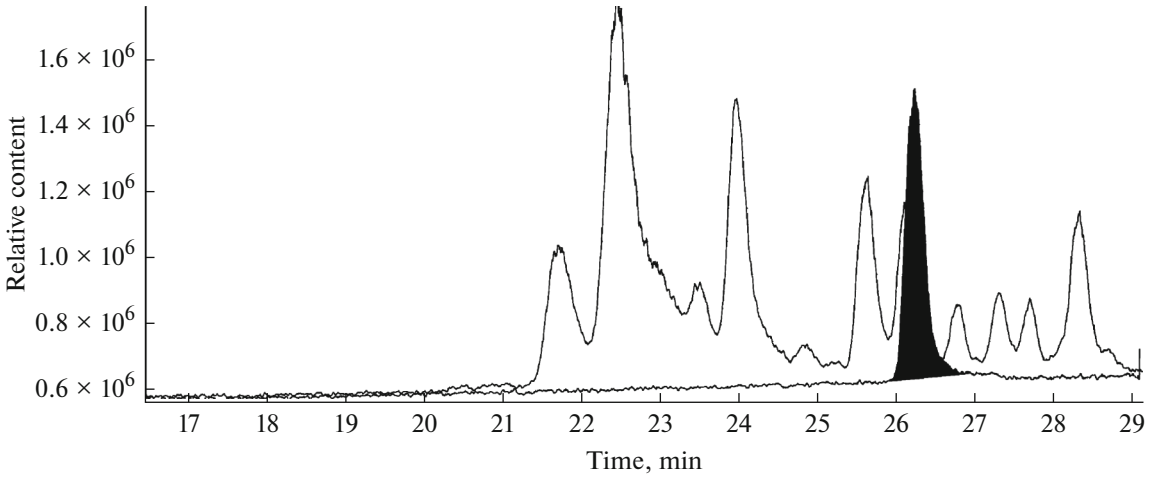

Fig. 4. Overlay of chromatograms of methanol extract from the *A. racemosus* roots and standard protodioscin solution. For chromatographic conditions, see text.

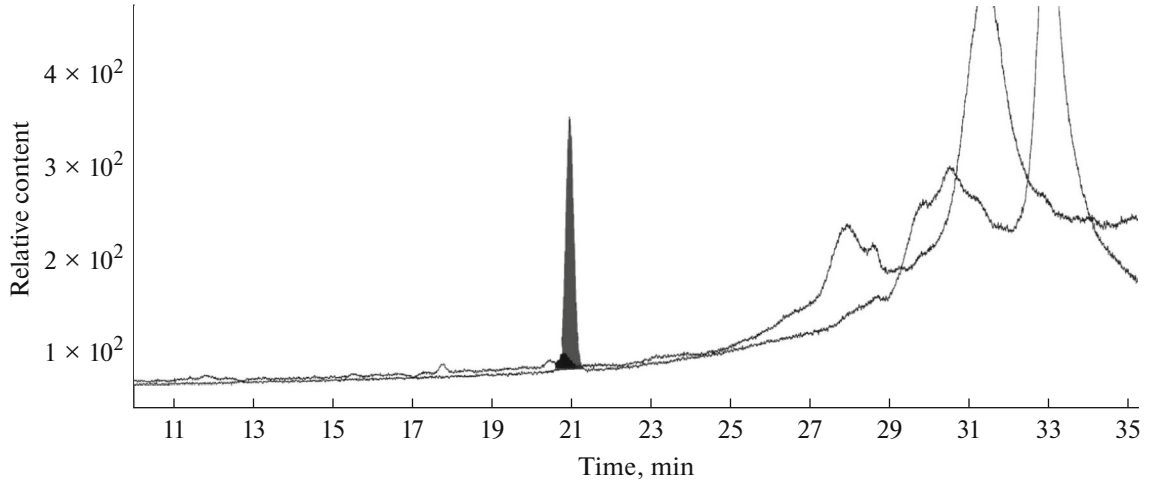

Fig. 5. Overlay of chromatograms of methanol extract from the *A. racemosus* roots and standard dioscin solution. For chromatographic conditions, see text.

the detection limit, which, in terms of the amount of extractant and weight of the sample, was 13.2 mg/kg.

Thus, we studied the component composition of the extracts from the *A. racemosus* roots. Eleven substances were identified in the composition of the chloroform extract by the GC–MS method; and 7 substances, in the composition of the acetonitrile extract. A compound from the phytosteroid group, androst-5,7-dien-3-ol-17-one can be proposed as a marker substance. A steroid saponin, reduced protodioscin, was found in the *A. racemosus* raw material by the HPLC–MS method. The quantitative content of dioscin (13.2 mg/kg) that can also be a marker substance for this type of raw material was determined.

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