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Research Article

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Compositional study of phenolic compounds of *Cistanche salsa (C. A. Mey) G. Beck*, growing in the Republic of Kazakhstan

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ABSTRACT

By high performance liquid chromatography with mass detection the component composition of phenolic compounds of a new kind of medicinal plants - stolons of Cistanche salsa (C.A. Mey) G. Beck, growing in the territory of the Republic of Kazakhstan, has been studied. 10 substances of phenolic nature, with echinacoside (1097,9 mg/100g), acteoside (943,5 mg / 100g) and tubuloside B (794 mg / 100g) dominating, were identified. These compounds can be considered as a standard substance in the development of standardization parameters of stolons of Cistanche salsa.

Keywords: Cistanche salsa, HPLC / MS, acteoside, tubuloside, echinacoside standardization.

INTRODUCTION

Cistanche salsa *(C.A. Mey) G. Beck* is a parasite plant family Orobanchaceae, which grows in the territory of the Republic of Kazakhstan and has industrial feedstock [1-2].

In literature provides information about the general direction of the pharmacological action of cistanche. For example, studies *Shimoda H., Tanaka J.* and all. [3] showed hypocholesterolemic effect, and the results *Feng-rui Yang, Du-su Wen* and all. [4] prove hepatoprotective activity of cistanche stolons. *Ze-dong Nan, Ke-wu Zeng* and all. [5] reported about anti-inflammatory activity. Polysaccharide complex cistanche salsa in experiment demonstrated immunomodulatory effects [6]. The scientific values of Herba Cistanche lie in the treatment of kidney deficiency, impotence, female infertility, morbid leucorrhea, profuse metrorrhagia and senile constipation [7-8]. Chinese scientists have studied in detail the chemical composition of cistanche stolons. Obtained phenolic glycosides: tubuloside, acteoside, echinacoside and other. It is reported about the contents of iridoids and lignans [9-10, 12-16]. A complex of polysaccharide is studied in detail [11].

However, despite considerable raw stocks of the Cistanche in territory of Kazakhstan, domestic medicines are absent. In connection with the problem of import substitution and, as a consequence, an increase of the domestic products share in the range of pharmacies there is a need of the expansion of primary sources of herbal medicines. Therefore, the study and the subsequent standardization of new types of vegetable raw materials with a view to their introduction in pharmaceutical manufacturing are actual.

Objective is to study the component composition of phenolic compounds of stolons of Cistanche salsa using HPLC method.

EXPERIMENTAL SECTION

Materials: Stolons of Cistanche salsa were harvested in the desert of Moinkum, Almaty region, the village of Bakanas in June-July 2014.

Methods: Analysis was performed by high performance liquid chromatography / mass spectrometry using the system of a liquid chromatograph HP 1100 Series model (company Agilent Technologies, Inc., CA, USA), equipped with a flowing vacuum degasser, a four-channel low pressure gradient pump, an automatic injector, column oven, UV and mass spectrometry detectors.

Chromatographic separation of the phenolic compounds was performed using a column «ZORBAX Eclipse XDB-C18», the size of 2,1 × 50 mm, which is filled with octadecylsilyl silica gelpolymer having a particle size of 1,8 microns. Chromatography was carried out in the following regime: the mobile phase velocity of 0,2 ml / min; eluent operating pressure of 175-200 kPa; column oven temperature of 30 ° C; 2 mkl sample volume; gradient eluent feed mode (eluent A – methanol, eluent B – 0,2% formic acid solution): 0-36 min 10% A - 90% B; 36 min - 100% B.

Investigations were carried out under the following detection parameters: the scale of measurement of 1,0; scan time of 0,5 s; spectrum removing options of 200-550 nm (for each peak); the wave lengths of 254, 334, 350, 410, 450, 550 nm.

Chromatography of solutions was performed at least three times till the requirements for compliance with the chromatographic system were met.

Obtaining an extract and preparation of the sample: Sample preparation was carried out in two stages: 1) obtaining an extract from Cistanche raw materials; 2) proper sample preparation. The extract was prepared as follows: 2 g of initially crushed dry stolon Cistanche salsa was inundated in the hermetic vessel with 80% methyl alcohol until mirror formed and was left for the material to soften. After that 80% methyl alcohol was added to achieve ratio feed – extractant 1:10; then microwave extraction was carried out according to the following program: start - 5 minutes, extraction - 10 min, cooling - 10 min.

Extraction temperature regime: on the right - 360°, on the left - 110-120 ° C. Purification of the obtained extract from ballast material was achieved through centrifugation at 9000 rev / min for 5 min. The purified extract was decanted and evaporated to ointment like state. Sample preparation was performed as follows: the obtained extract was dissolved in 20% dimethyl sulfoxide solution in methanol (9,6mg extract per 1 ml of solvent) and filtered through a Teflon membrane filter having a pore size of 0,45mkl into the vial for analysis.

Management of chromatographic system, obtaining of chromatograms and calculating of results were performed using the Agilent software. Identification of the mixture components was carried out by comparing their retention parameters, the data of the UV spectra and mass spectra with those of standard substances or using literature data.

Statistical analysis: The data were presented as mean ±SD of five experiments. The results were processed by methods of variation statistics using a standard statistical software package «Statistica 6,0»

RESULTS

Cistanche salsa extract chromatograms are shown in Figure 1. Results of determination of phenolic compounds and their retention parameters are given in the Table 1.

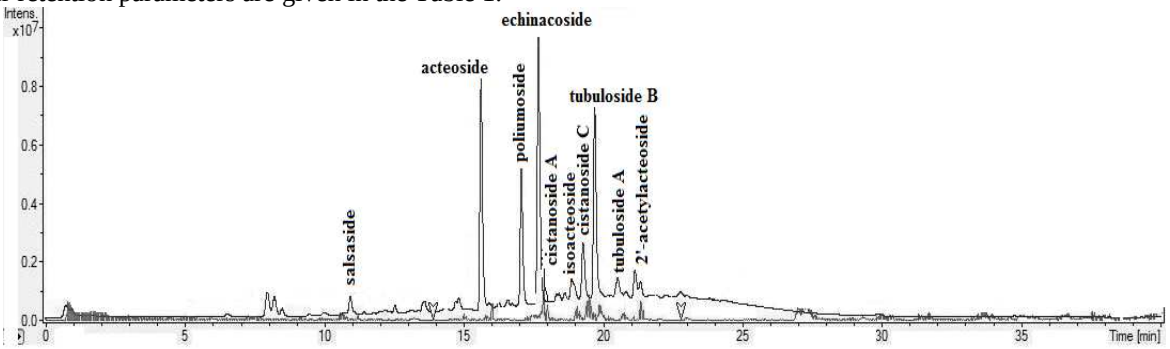

Fig. 1 Chromatogram of the extract Cistanche salsa

Table 1 The results of the study of phenolic compounds in stolons Cistanche salsa

#	Retentiontime, min	Content,mg 100 g	Compound
1.	10,9	64,6±0,01	salsaside
2.	15,6	943,5±0,01	acteoside
3.	17,1	568,3±0,01	poliumoside
4.	17,7	1097,9±0,01	echinacoside
5.	17,9	62,7±0,01	cistanoside A
6.	18,9	82,1±0,01	isoacteoside
7.	19,3	228,7±0,01	cistanoside C
8.	19,7	794,1±0,01	tubuloside B
9.	20,5	72,6±0,01	tubuloside A
10.	21,1	110,5±0,01	2'-acetylacteoside

DISCUSSION

In stolons of Cistanche salsa by HPLC / MS analysis 10 substances of phenolic nature were identified. Among the identified compounds echinacoside (1097,9 mg / 100g), acteoside (943,5 mg / 100g) and tubuloside B (794 mg / 100g) were dominating. The obtained data are correlated with the given in literature results of the study of individual species Cistanche growing in different regions of Asia [13-16]. Echinacoside and acteoside are the main components which are the basis for standardization of officinal species Cistanche deserticola and Cistanche tubulosa, listed in the Chinese Pharmacopoeia [17].

CONCLUSION

In stolons of Cistanche salsa by HPLC / MS analysis 10 substances of phenolic nature were identified. Taking into consideration that on the territory of Kazakhstan genus Cistanche is represented mainly by species C. Salsa, which has a sufficient resource base, we can recommend harvesting and the standardization of this raw material using echinacoside and acteoside as standard substances.

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