Study on Major Biologically Active Compounds in The Field Dodder

A. I. Jusupova
Department of Genetics and Molecular Biology
Al-Farabi-Kazakh National University, Almaty, Kazakhstan

ABSTRACT

Qualitative and quantitative properties of different types of biologically active compounds (amino acids, fatty acids, carbohydrates, flavonoids, and microelements) were determined both for original plant material and isolated substance. Presence and significance of the compounds as well as their quantitative alterations are discussed.

Key words: field dodder, substance, biologically active compound.

INTRODUCTION

According to the data of the Kazakh Ministry of Agriculture various dodder species are spread now on the territory of more than 168 000 ha (Review, 2002). The dodders belong to stem weeds referred to as damaging major crop sowings, especially under irrigated conditions. Frequent heat abundance and watering of irrigated areas may hinder intensive crop growth since dodder seeds germination period may last annually over 7-8 months. These species are supplied with special stem suckers (known as *haustorium*) which can 'pump out' virtually all useful nutrients circulating in host plant.Thus, affected plants firstly slow down their growth, then get yellow and finally perish. The field dodder (*Cuscuta campestris* Juncker) is ascribed to be the most widely spread and harmful dodder species registered for South-Eastern Kazakhstan. It is known to affect different plant families, more than 200 species in total, including crops. In particular, contamination with parts of the field dodder may lead to decreasing alfalfa harvests assessed to reach almost 50% annually (Zharasov Sh. U., Abdraimov N.S., 2002).

Interestingly, but due to its high adaptive features the field dodder may be useful against different plant, animal and human pathogens and pathologies. The fact was earlier established for other dodder species, namely *Cuscuta chinensis* Lam. and *Cuscuta europaea* L. These species were used in alternative medicine as the sources of certain agents revealing a broad range of healing properties, such as anti-inflammatory, tonic, haemostatic, expectorative, diuretic, cholagogue, laxative, antiseptic, and anti-neoplastic activities (Teegaurden R., 1998).

The task of present investigation was to isolate extractable compounds of the field dodder to further determine chemical compositions which may potentially be effective for medicinal aims.

MATERIALS AND METHODS

Dried samples (containing stems, flowers and seeds) of the field dodder collected in the alfalfa fields in 2005 (Baiserke-Agro Co., Talgar District of Almaty region) were ground to final size of approximately 1-3 mm.

Biologically active compounds were determined using specific developers (NH_3 and $AlCl_3$ for the flavonoids, NaOH for the anthraquinones, lactone assay for the coumarins, extensive foaming test for the saponins, $FeCl_3$ and iron ammonium alums for the phenolics and related acids, *o*-toluidine and urea for carbohydrates (aldoses and ketoses, respectively), ninhydrin for amino acids, Dragendorf's reagent and picric acid for alkaloids (Pashinina L.T., 1979, Grinkevich N.I., Sophronich L.M., 1983).

Quality of plant material was examined visually as by such characteristics as extractability, humidity, ash content, ash HCl-insoluble compounds by standard methods implied to conventional pharmacopeian samples (State Pharmacopeia of USSR, 1990).

Free amino acid and fatty acid contents were determined by gas chromatography (Adams P. 1974, Kuksis A., 1986).

Quantitative analysis of flavonoids was carried out using spectrophotometric absorption at 430 nm (Grinkevich N.I., Sophronich L.M., 1983). Total quantity of flavonoids was calculated in reference to quercetin and 100% dry material (X, %) in consent to the following formula:

$$X = \frac{D \times 525 \times 100 \times 100 \times 100}{764.6 \times m \times 2 \times (100 - W)},$$

where:

D – optic density of examined solution;

764.6 – specific index of the quercetin-$AlCl_3$ complex absorbing at 430 nm;

m – mass of the material (g);

W – weight loss (%) in result of drying.

Qualitative and quantitative parameters for different microelements were confirmed by atomic absorption based on corresponding spectra (Havezov I., Calev D., 1983).

RESULTS AND DISCUSSION

Quality of plant material and optimal extraction solvents were determined. Humidity and total ash of plant material comprised 2.57 and 5.4 %, respectively. HCl-insoluble compounds level (19.4%) indicates high degree of mineralization of plant source under this study. Optimal extraction agents for the set of biologically active compounds were indicated to occur 50% ethanol and 50% acetone as shown in Table 1. The solvents appeared to be dual, of hydrophobic and hydrophilic nature. These solvents are able to extract complete amounts of respective substances from plant material.

Table 1. Optimal extraction agents for the field dodder.

Solvents	H_2O	**50% ethanol**	96% ethanol	acetone	**50% acetone**	benzene	ethyl-acetate
Extraction extent, %	4,12	**22,41**	19,03	20,32	**23,72**	2,86	1,73

Taking into account ecological and economical objectives, 50% ethanol was chosen as the optimal extraction agent. Amino acids were determined by specific developer ninhydrin using paper and gas chromatography. The field dodder contained all essential amino acids comprising 29.65% of total amino acids, or 2793 mg per 100 g of plant material. Contents of essential amino acids were determined to be, in mg: Val – 255, Leu – 588, Iso – 367, Thr – 410, Met – 202, Phe – 407, Lys – 336, Trp – 228. Presence of all essential amino acids in the field dodder seeds may affect better seed viability in the course of weed's adaptation to host plant. Besides, the evidence may witness to growing biological value of plant source (Lukmanova K.A. et al., 2000).

Fatty acids were determined by hydrolysis followed by the methylation with diazomethane improving volatile properties. Saturated fatty acids made up 13.84%, whereas unsaturated fatty acids comprised 86.16% (51.31% of which were monoenic and 34.85% polyenic acids). Contents of unsaturated fatty acids were established to be, in %: palmitoleic ($C_{16:1}$) – 0.91, oleic ($C_{18:1}$) – 49.50, linoleic ($C_{18:2}$) - 31.30, linolenic ($C_{18:3}$) – 0.27, eicosenoic ($C_{20:1}$) – 0.90, eicosadienoic ($C_{20:2}$) – 3.28. Hence, concentrations of unsaturated fatty acids substantially almost 6 times surpass those for saturated. This may evidence in favor of considerable biological activity of the field dodder and high viability of the species. Significant iodine number (104.95) was detected by the degree of

fatty acids desaturation. Linoleic and linolenic acids are regarded to be essential, whereas polyenic fatty acids may serve as precursors or structural analogues of a number of biologically active compounds or integrated components of cell membranes (Kemertelidze E.R, Dalakishili S.M., 1996).

Flavonols determination was carried out with ammonia (revealing of C-groups) and $AlCl_3$ (determination of *o*-phenol-linked hydroxyl groups), as shown in Table 2.

Table 2. Flavonols identification.

№	Reference standards	R_f	day light	UV light	NH_3+$AlCl_3$
1	Quercetin	0.69	yellow	yellow	dark yellow
2	Myricetin	0.90	light yellow	light yellow	yellow
3	Rutin	0.55	yellow	yellow	dark yellow
4	Plant material	0.68	yellow	yellow	dark yellow
		0.55	yellow	yellow	dark yellow

As indicated in the table above, R_f values and spot colours of tested plant material and corresponding reference standards were almost similar (see the data referred to quercetin and rutin). This indicates presence of quercetin and rutin in the source. To determine phenolics and phenolic acids, phenol-containing or phenolic acid references of known structure were used in the same way. R_f values and spot colours of corresponding reference standards resembled to similar features of the source. Thereby, pyrocatechine and coffeic acid were detected in the field dodder. Quantitatively, the amount of flavonoids did not exceed 4.23%. Identification of carbohydrates was carried out by comparing mobility values with the references in butanol-acetic acid-water mixture (40:12.5:29) to be specifically developed afterwards. Results are given in Table 3.

Table 3. Carbohydrates of the field dodder.

№	Reference carbohydrate	R_f	day light	UV light	*o* - toluidine
1	Xylose	0,38	-	-	violet
2	Saccharose	0,33	-	-	green-gray
3	Glucose	0,39	-	-	green
4	Arabinose	0,36	-	-	violet
5	Lactose	0,30	-	-	brown
6	Rhamnose	0,45	-	-	brown
7	Maltose	0,27	-	-	brown-green
8	Fructose	0,42	-	-	pale brown
9	Galactose	0,37	-	-	brown-green
10	Mannose	0,44	-	-	green
11	Plant material	0,33	-	-	green-gray
		0,42	-	-	pale brown

As seen from the table, R_f values and spot colors of corresponding standards revealed the presence of saccharose and fructose in plant material. Qualitative and quantitative contents of microelements were assayed by atomic absorption of the spectra. The data are briefly summarized in Table 4.

Table 4. Identification of microelements.

Microelements	Pb	Cu	Zn	Ni	Co	Mn	Fe	Cd
Quantity (in % per 1 g)	$7.35 \cdot 10^{-3}$	$1.71\ 10^{-2}$	$4.88 \cdot 10^{-2}$	$2.71\ 10^{-3}$	$1.28\ 10^{-3}$	$1.33\ 10^{-2}$	$2.55\ 10^{-1}$	$4.65\ 10^{-4}$

The results reflect the presence of listed microelements in the field dodder as well as the host and the soil

Various conditions to be optimal as a scheme of isolating combination of biologically active compounds were proposed: 50% ethanol extraction (to be added to plant material in ratio of 1:8, v/v), at 23-25^{0}C, for 18 hours at 2 times process cycling. As seen from Table 5, time of extraction extending over 18 hours may cause the drop of effectiveness.

Table 5. Optimal time of extraction.

№	time, hours	mass, g	Volume of the solvent, ml	Substance release, %
1	4	50	300	21.12
2	5	50	300	21.37
3	6	50	300	21.83
4	7	50	300	21.91
5	8	50	300	21.52
6	10	50	300	21.96
7	14	50	300	22.24
8	18	50	300	22.37
9	20	50	300	21.2
10	40	50	300	21.35
11	48	50	300	21.76

Fixing each of the parameters is considered as the best way for determining optimal characters described above.

Table 6 summarizes the results of amino acid identification in pure substance isolated by the procedure suggested above.

Table 6. Amino acid contents.

Amino acids	Amount (mg) per 100 g of the substance
Alanine	1156
Glycine	386
Valine*	442
Leucine*	515
Isoleucine*	296
Threonine*	268
Serine	707
Proline	602
Methionine*	115
Asparagine	857
Cysteine	90
Oxiproline	32
Phenylalanine*	415
Glutamine	1826
Ornithine	16
Tyrosine	415
Histidine	162
Arginine	419
Lysine*	326
Tryptophane*	228
Total amount of essential amino acids	2605
Total amount of all amino acids	9273
Amount of essential amino acids of total amount of all amino acids, %	28,09

*essential amino acids

The result clearly showed that all essential amino acids can be found in the substance, what was determined also for original plant source. However, the quantity of essential amino acids in the substance referred to their total amount has reduced by 1.56% to make up 28.08%.

Table 7 depicts alterations in fatty acid quantities attributed to the substance isolated by a proposed scheme. Fatty acid composition was determined according to the data obtained for collected parts of plant material.

Table 7. Fatty acid quality spectrum and quantification in the substance.

Fatty acid	Symbol	content %
Lauric	$C_{12:0}$	1,2
Myristic	$C_{14:0}$	2,2
Palmitic	$C_{16:0}$	8,3
Palmitoleic*	$C_{16:1}$	0,8
Stearic	$C_{18:0}$	4,2
Oleic*	$C_{18:1}$	50,4
Linoleic*	$C_{18:2}$	22,7
Linolenic*	$C_{18:3}$	2,2
Arachidic	$C_{20:0}$	1,7
Eicosenoic*	$C_{20:1}$	2,4
Eicosadienoic*	$C_{20:2}$	1,5
Geneicosanic	$C_{21:0}$	1,4
Behenic	$C_{22:0}$	1,0
Total	100%	
Amount of unsaturated fatty acids of total amount of fatty acids, %	80	

*unsaturated fatty acids

As seen from the table, unsaturated fatty acids composed 80% of total amount (nearly 4 times more than saturated). Ration of mono- to polyenic acids was calculated as 6.7 : 3.3. Percentage of polyenic unsaturated fatty acids (26.4%) revealed in the substance was higher by 8.45 %, than corresponding quantity established for plant material. Noticeable enrichment of monoenic acids may increase antioxidant activity of the substance (Kemertelidze E.R, Dalakishili S.M., 1996)

Information on qualitative and quantitative contents of other biologically active groups (e.g. flavonoids and carbohydrates) appeared to be less varied.

Data on microelement contents detected in the substance are presented in Table 8. It emphasizes that the content of heavy metals, and primarily Cd and Pb, does not exceed maximal rate, what allows to imply the substance in medicinal aims (Listov S.A., 1990).

Table 8. Identification of microelements in the substance.

Micro-elements	Fe	Ni	Co	Mn	Cu	Zn	Cd	Pb	Mg	Na	K
Quantity (mcg per g)	76,24	6,21	2,83	144,4	11,42	28,38	0,67	19,87	543,61	4544,5	2878,4

As it is clear from Table 8, the substance contains considerable amounts of almost all necessary microelements, namely high quantities of Na, K and Mg. These elements are essentially significant, as they play important role in mostly all living organisms. Na is one of the main extracellular cations, taking part in stabilizing cell osmotic pressure. If it is deficient, this may cause dysfunction of nerve pathways, damage in blood circulation, functioning of smooth muscles, and etc. K is also ubiquitous cellular and intercellular cation directly involved in excitation and conductivity of cardiac muscles and different plant cells. Mg is important as the co-factor of enzymes modifying phosphorylated substrates (in co-ordinated joint action with phosphatases, phosphokinases, and desoxyribo-nucleases). Mg is also required for DNA and RNA accumulation by means of the complex formation with (deoxy)ribo-NTPs and enzymes involved in DNA and RNA biosynthesis (Avcin A.R., 1991).

Results of changes in qualitative and quantitative properties of other biologically active compounds (e.g., flavonoids and carbohydrates) were also clarified.

SUMMARY

Optimal scheme for extraction of the substance from the field dodder was proposed. It included the treatment of plant material with 50% ethanol (1:8, v/v) at 23-25^{0}C for 18 hours with 2 times process cycling. Qualitative and quantitative properties of different types of biologically active compounds (amino acids, fatty acids, carbohydrates, flavonoids, and microelements) were determined both for original plant material and isolated substance. Presence and significance of the compounds as well as their quantitative alterations are discussed.

REFERENCES

1. **Review on quarantine objects distribution**. Astana, 2002. – P. 33.
2. **Zharasov Sh. U., Abdraimov N.S., 2002**. Measures of field dodder control on the south-east of Kazakhstan//*Actual problems of plant protection in Kazakhstan*, Almaty- p. 166-181.
3. **Teegaurden R., 1998.** The Ancient Wisdom of the Chinese Tonic Herbs. New York: Warner Books- p. 35-36.
4. **Pashinina L.T. , 1979.** Methodical directions to quanlitative and quantitative analysis of natural polyphenols and carbohydrates. – Almaty: KazGU- P. 47.
5. **Grinkevich N.I., Sophronich L.M., 1983.** Chemical analysis of medicinal plants. Moscow . – P. 175.
6. **State Pharmacopeia of USSR – Moscow:** Medicine, XI edition 42, 1990- P. 399.
7. **Adams P., 1974.** Determination of amino acids profiles biological samples by gas chromatography// *Chromatography*. - Vol. 95, №2. – p. 188-212.
8. **Kuksis A., 1986.** Lipids in book "Chromatography. Practical application of the method».- Moscow: Mir. – vol.1. - p. 130-134, 286-293.
9. **Havezov I., Calev D., 1983.** Atomic-absorbtion analysis. – Leningrad: Chemistry. - p.112-114.
10. **Lukmanova K.A. et al. Lukmanova K.A., Ryabchuk V.A., Salikhanova N.H., 2000**. Amino acid and mineral content of phytopreparation alfalfa // *Pharmacy*. - №1. – p. 25-27.
11. **Kemertelidze E.P, Dalakishili C.M. , 1996.** Biologically active lipids of some plants, growing in Georgia. – Tbilisi: Mecniereba- p. 3-21.
12. **Listov S.A., Petrov N.V., Arzamascev A.P., 1990.** About content of heavy metals in medicinal plant material // *Pharmacy*. - № 2. - p. 19-25.
13. **Avcin A.R., Zhivoronkov A.A., Remi M.A., Strochkova L.S. , 1991.** Microelements of human. Moscow: Medicine- P. 496.

الملخص العربى

دراسة علي المكونات الرئيسية البيولوجية النشطه في حشيشة الدودير

جوسوبوفا .أ .أ

قسم الوراثة والبيولوجيا الجزيئيه-جامعة الفارابي كازاخ الدوليه-الماتي-كازاخستان

تم خلال هذا البحث دراسة خصائص الصفات الكمية والوصفية لبعض المكونات البيولوجية المختلفة مثل الاحماض الامينية والاحماض الدهنية والكربوهيدرات والفينولات والعناصر الغذائية الصغري على حشيشة الدودير فى كازاخسنان ووجد انها كانت موجوده ومعنويه في مكوناتها تماما وكميتها وتم مناقشتها.