CYTOTOXIC COMPONENTS OF *Cuscuta*

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Glycoside-type antitumor compounds have been reported in the extract of Chinese dodder (*Cuscuta chinensis*) [1]. The structures of the observed compounds have not been established. Individual components were identified in the hydrolysate of the active fraction. Thus, the hydrolysate contained a trisaccharide consisting of two rhamnoses and one glucose and cuscutic and other acids specific to dodder [1].

Herein we communicate the isolation of a highly active cytotoxic compound from dodder using murine cancer cells as the test culture. The work used dodder extracts from three common species in Uzbekistan, *C. europea*, *C. lupuliformis*, and *C. attenuate*, which parasitize meadow grass, willow runners, and bushy fruits, respectively. Dodder samples were collected in June during flowering.

The cytotoxicity of fractions was tested using KML cells (murine melanoma) [2]. Cells were dispersed (40,000/mL) in RPMI-1640 nutrient medium (3 mL). Samples (dry compound, 100 μg/mL) were added after 24 h. Cells were contacted with the compounds for 24 h. ^{3}H-Thymidine (10 μCi) was added to the tubes after 1 h. The cytotoxic activity was determined from the suppression of ^{3}H-thymidine incorporation into the acid-insoluble material. The degree of suppression of radioactive material incorporation was determined in percent of the control using the formula

$$100 - [(\text{tracer inclusion in sample/inclusion in control}) \times 100\%].$$

The positive control was the antitumor compound platin—carboplatin at a dose suppressing DNA synthesis by 50%.

Comparison of the cytotoxicity of the aqueous alcohol extracts of the three dodder species showed that the extract of *C. europea* inhibited DNA synthesis by 81.2%; extracts of *C. attenuate* and *C. lupuliformis*, by 49.7 and 21.6%, respectively. Therefore, further experiments were carried out using fractions of *C. europea*. The cytotoxicity of the extract of this dodder speices collected at various sites was practically the same.

Table 1 shows that the cytotoxic components were extracted better by polar solvents. Chromatographic separation of the active components gave good results using the hydrophobic stationary phase phenyl-sepharose with subsequent separation by HPLC on a RP C_{18}-Ultrasphere column and a Beckman chromatograph (USA). Components obtained by preparative HPLC (HPLC-1 to HPLC-3) were highly cytotoxic. Yields of these fractions from dodder raw material were 0.04, 0.12, and 0.18%, respectively.

The physical chemical properties of the resulting fractions, which were homogeneous under rechromatography conditions, showed that fractions HPLC-1 to HPLC-3 contained components with molecular weights 304, 469, and 645 (MALDI-TOF), respectively. The IR spectrum was very similar to that of rutin-like glycosides. Fraction HPLC-3 with the highest inhibiting activity in cancer cell culture was conjugated to rabbit immunoglobulin using the known carbodiimide method [3]. The cytotoxicity of the conjugate obtained with a immunoglobulin:ligand ratio 1:50 was at least 70% retained relative to the activity of the initial fraction. This property of the HPLC-3 fraction indicates that it can be used to formulate highly specific antitumor agents via conjugation to the appropriate antibodies against cancer cells.

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TABLE 1. Inhibition of DNA Synthesis by Fractions of *C. europea* Extract in Cancer Cell Culture

Fraction	Dose, μg/mL	^{3}H-Thymidine incorporation, counts/min	Inhibition of DNA synthesis, %
$CHCl_3$ extract	100	30500 +/- 2000	9.8
EtOH extract	100	28500 +/- 1500	15.7
Aqueous-ethanol extract (1:1)	100	19700 +/- 1200	41.7
Aqueous extract	100	16800 +/- 1200	50.2
Phenyl-sepharose eluate	100	4500 +/- 800	86.7
HPLC-1	100	14300 +/- 1000	57.7
HPLC-2	100	4050 +/- 800	88.0
HPLC-3	100	3450 +/- 600	89.8
Carboplatin (positive control)	30	17100 +/- 1000	49.4
Control (without test compound)	0	33800 +/- 2500	0

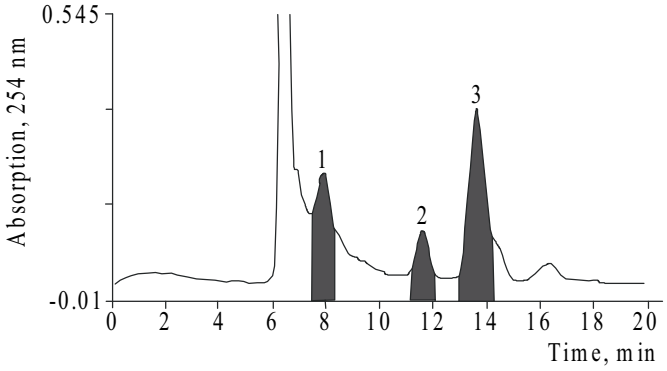

Fig. 1. HPLC of aqueous extract of dodder. Peaks of cytotoxic fractions are filled. Numbers over peaks correspond to HPLC-1, -2, and -3 in Table 1.

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