

Cytotoxic activity of the fruit extracts of *Heptaptera cilicica* (Boiss. & Balansa) Tutin

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ABSTRACT

The fruit extracts of *Heptaptera cilicica* (Boiss. & Balansa) Tutin were screened for cytotoxic activities on the colon (COLO205 and KM12) and kidney (UO31 and A498) cancer cell lines. The highest cytotoxic activities were observed in the dichloromethane extract of the fruits with IC₅₀ values of 12.1 mg/mL and 12.2 mg/mL on the COLO205 and KM12 cell lines, respectively. In contrast, the ethyl acetate extract of the fruits exhibited moderate cytotoxic activity with IC₅₀ values of 33.6 mg/mL and 37.2 mg/mL against the COLO205 and KM12 cell lines. Dichloromethane and ethyl acetate extracts of the fruits exhibited weak cytotoxic activity against the UO31 and A498 cancer cell lines with IC₅₀ values greater than 50 mg/mL. Aqueous-methanol extract of the fruits showed cytotoxic activity against all cancer cell lines with IC₅₀ value greater than 50 mg/mL.

Keywords: cytotoxic activity, *Heptaptera cilicica*, Apiaceae

INTRODUCTION

Cancer, a significant public health problem, is the second leading cause of death worldwide. Colorectal cancers are the third estimated new cancer cases and deaths among adult Americans in 2022, and kidney cancer (Renal cell can-

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cer-RCC) is the second most common cancer of the urinary system¹. In 2023, there were approximately 81,800 new cases and 14,8900 deaths due to kidney and renal pelvis cancer, 153,020 new patients, and 52,550 deaths due to colon and rectum cancer². Natural products are important sources for the discovery of new anticancer drugs³. This study was designed to evaluate the cytotoxic activities of *Heptaptera cilicica* fruits on colon and kidney cancer cells during our ongoing studies on the genus *Heptaptera* (Apiaceae). There are 11 species of *Heptaptera* Marg. & Reuter found in the world four of them: *H. anatolica* (Boiss.) Tutin, *H. anisoptera* (DC.) Tutin *H. cilicica* (Boiss. & Balansa) Tutin and *H. triquetra* (Vent.) Tutin are growing in Turkey^{4,5}. *H. cilicica* is an endemic species distributed in the Mersin province of the Mediterranean region of Turkey⁵. *Heptaptera* species have been reported to contain sesquiterpene coumarin derivatives⁶⁻¹². Coumarins are well-known plant secondary metabolites for their cytotoxic, anti-cancer, anti-inflammatory, antibacterial, antidiabetic, antileishmanial, antiviral, and anti-cholinesterase effects¹¹⁻¹⁸.

In this study, the fruit extracts of *Heptaptera cilicica* were investigated for their cytotoxic activities against the colon (COLO205 and KM12) and kidney (UO31 and A498) cancer cell lines.

METHODOLOGY

Plant material

The fruits of *Heptaptera cilicica* were collected in Mersin province between Tarsus and Karakütük village in June 2013 and identified by Prof. A. Duran. A voucher specimen (A. Duran 9591) was deposited in the Herbarium of Selçuk University, Faculty of Sciences, Department of Biology (KONYA).

Extraction

Pulverized fruits (50 g) of the plant were sequentially extracted by maceration with dichloromethane (CH₂Cl₂) and methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to yield crude extracts. Dichloromethane and methanol extracts of the fruits were 0.72 g (1.44%) and 2.45 g (4.90%), respectively. The methanol extract was redissolved in a mixture of methanol/water (10:90) and then partitioned with ethyl acetate (EtOAc); the resulting extracts were separately concentrated in vacuo to dryness. The yields of ethyl acetate and aqueous-methanol extracts of the fruits were 0.92 g (1.84%) and 1.44 g (2.88%), respectively.

Cytotoxicity assay on cancer cells

The assay used for this study was a two-day, two-cell line XTT bioassay¹⁹, an *in vitro* antitumor colorimetric assay developed by the MTL Assay Development and Screening Section. Cancer cell lines used were colon (COLO205 and KM12) and kidney (UO31 and A498). Sanguinarine was used as a positive control. The assay was performed as described previously¹².

RESULTS and DISCUSSION

In this study, the cytotoxic activity of the fruits of *H. cilicica* was reported for the first time. In our research, the dichloromethane extract of the fruits had a strong inhibitory activity on the colon cancer COLO205 and KM12 cell lines. The ethyl acetate extract of the fruits showed moderate inhibitory activity on the COLO205 and KM12 cell lines. Aqueous-methanol extract of the fruits showed cytotoxic activity against the COLO205 and KM12 cell lines with IC₅₀ values greater than 50 µg/mL. All the extracts had cytotoxic activity on UO31 and A498 renal cell lines greater than 50 µg/mL concentrations. The cytotoxic activities detected in these extracts are shown in Table 1.

Table 1. Cytotoxic activities of the extracts

Extracts	IC ₅₀ values (µg/mL)			
	UO31	A498	COLO205	KM12
1	> 50	> 50	12,1	12,2
2	> 50	> 50	33,6	37,2
3	> 50	> 50	> 50	> 50

1: CH₂Cl₂ extract; 2: EtOAc extract; 3: Aqueous-methanol extract

The highest cytotoxic activities were observed in the dichloromethane extract of the fruits with IC₅₀ values of 12.1 mg/mL and 12.2 mg/mL on the COLO205 and KM12 cell lines, respectively. The ethyl acetate extract of the fruits exhibited moderate cytotoxic activity with IC₅₀ values of 33.6 µg/mL and 37.2 µg/mL on the COLO205 and KM12 cell lines. Dichloromethane and ethyl acetate extracts of the fruits exhibited weak cytotoxic activity against the UO31 and A498 cancer cell lines with IC₅₀ values greater than 50 mg/mL. Aqueous-methanol extract of the fruits showed cytotoxic activity against all cancer cell lines with IC₅₀ value greater than 50 mg/mL. Previously, Tosun and Miski reported the

cytotoxic activities of the fruit extracts of *Heptaptera anisoptera*²⁰, *H. anatolica*²¹, and *H. triquetra*²² on the COLO205 and KM12 cell lines. The cytotoxic activities of the dichloromethane, ethyl acetate, and aqueous-methanol extract of the *H. cilicica* fruits on the COLO205 and KM12 cell lines were similar to those of the fruit dichloromethane, ethyl acetate, and aqueous-methanol extracts of *H. anatolica*. Among these four *Heptaptera* species, the dichloromethane and ethyl acetate extracts of the fruits of *H. anisoptera* showed the best cytotoxic activities. The ethyl acetate extracts of the *H. anatolica*, *H. triquetra*, and *H. cilicica* fruits showed moderate to weak cytotoxic activities. Aqueous-methanol extracts of the fruits of these four *Heptaptera* species showed cytotoxic activity against the COLO205 and KM12 cell lines with IC₅₀ values greater than 50 mg/mL. Since some sesquiterpene coumarin derivatives were isolated from the chloroform extracts of the fruits of *Heptaptera anatolica*⁹, *H. anisoptera*⁹ and *H. cilicica*²³, and the cytotoxic activities of sesquiterpene coumarins were reported earlier¹¹⁻¹³, it can be concluded that the secondary metabolite(s) responsible for the cytotoxic activity of the fruits of *H. cilicica* may be sesquiterpene coumarin(s). The dichloromethane extract of the fruits of *H. cilicica* will be subjected to bioactivity-guided fractionation.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors made equal contributions to the study.

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