Acta Pharm. Sci. Vol 60:(2), 2022
DOI: 10.23893/1307-2080.APS.6009

Cytotoxic activity of the fruit extracts of *Heptaptera anatolica* (Boiss.) Tutin

Fatma TOSUN[1*], Mahmut MISKI[2]

1 Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey
2 Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey

ABSTRACT

Cytotoxic activities of the fruit extracts of *Heptaptera anatolica* (Boiss.) Tutin were investigated on the colon cancer COLO205 and KM12 cell lines. The dichloromethane extract of the fruits of *H. anatolica* showed the best cytotoxic activities with IC_{50} values of 17.9 and 15.1 ug/mL on the COLO205 and KM12 cell lines, respectively. Whereas, the ethyl acetate extract of the fruits showed moderate cytotoxic activity with IC_{50} values of 23.4 ug/mL against the KM12 cell lines.

Keywords: Cytotoxic activity, *Heptaptera anatolica*, Apiaceae

INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death[1]. Natural sources have a great potential for the discovery of new anticancer drugs[2]. As part of our continuing studies on the genus *Heptaptera* (Apiaceae), we report here the cytotoxic activity of *Heptaptera anatolica* fruits.

The genus *Heptaptera* Marg. & Reuter (Apiaceae) is represented by 10 species worldwide, four of them; *H. cilicica* (Boiss. & Bal.) Tutin, *H. anisoptera* (DC.) Tutin, *H. anatolica* (Boiss.) Tutin and *H. triquetra* (Vent.) Tutin are growing in Turkey[3,4]. *Heptaptera* species are known to contain sesquiterpene coumarin derivatives[5-9], these compounds have various biological activities such as; cytotoxicity, P-glycoprotein inhibitory, cancer chemopreventive, anti-inflammatory, antibacterial, antileishmanial, antiviral, antidiabetic, etc.[8-14].

*Corresponding author:
E-mail: ftosun@medipol.edu.tr,
Phone: +905334798035
ORCIDs:
Fatma Tosun: 0000-0003-2533-5141
Mahmut Miski: 0000-0003-2653-0563
(Received 13 Sept 2021, Accepted 13 Dec 2021)

METHODOLOGY

Plant Material

The fruits of *Heptaptera anatolica* were collected in the vicinity of Izmir in June 2013 and identified by Prof. A. Duran. A voucher specimen (A. Duran 9703) was deposited in the Herbarium of Selçuk University, Faculty of Sciences, Department of Biology (KONYA).

Extraction

Coarsely powdered fruits (50 g) of the plant were sequentially extracted at room temperature with dichloromethane (CH_2Cl_2) and methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to dryness. Dichloromethane and methanol extracts of the fruits were 8.31 g, 16.62% and 8.67 g, 17.34 %, 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. Ethyl acetate and aqueous-methanol extracts of the fruits were 1.33 g, 2.66% and 7.14 g, 14.28%, respectively.

Cytotoxicity Assay on Colon Cancer Cells

The assay used for this study was a two-day, two cell line XTT bioassay[15], an in vitro antitumor colorimetric assay developed by the MTL Assay Development and Screening Section. Colon cancer cell lines used were COLO205 and KM12. Cells were maintained and passed weekly in RPMI-1640 medium with phenol red (Gibco, Carlsbad, CA, USA) and supplemented with 2 mM L-glutamine (Quality Biologicals, Inc., Gaithersburg, MD, USA) and 10% fetal bovine serum (Hyclone, Logan, UT, USA). Cells were placed in a humidified incubator with an atmosphere of 5% CO2 and 95% air and a temperature of 37° C. Cells were placed in a humidified incubator with an atmosphere of 5% CO2 and 95% air and a temperature of 37 °C. Cells used in the assay were harvested with RPMI-1640 medium, without phenol red (Gibco, Carlsbad, CA, USA) and supplemented with 2 mM L-glutamine (Quality Biologicals, Inc., Gaithersburg, MD, USA) and 10% fetal bovine serum without antibiotics. Harvested cells were counted using a Cellometer Auto T4 cell counter (Nexcelom Bioscience LLC, Lawrence, MA, USA) and plated in 384-well flat-bottom polystyrene microtiter plates (Nunc, Nunc A/S, Denmark), at a density of 5000 cells/well for COLO205 and 5000 cells/well for KM12. The cells were incubated in a 5% CO2/95% air and 37 °C incubator for 24 h. After incubation, test samples were added to plates using a Biomek FX robotic liquid handling workstation (Beckman/Coulter, Fullerton, CA, USA). The robot performed eight 2-fold serial dilutions of the sample and

then transferred the sample from the source plate to the assay plate. The plates used were Costar 384-well round-bottom plates (Corning Inc., Corning, NY, USA). Cells were further incubated with samples for 48 h, at which time the XTT reagent was added. Viable cells reduced the XTT to a colored formazan product, and after an additional 4 h incubation period the amount of formazan produced was quantified by absorption at 450 nm, using a 650 nm reference. Sanguinarine was used on each plate as a positive control.

RESULTS and DISCUSSION

This is the first report on the cytotoxic activity of the fruits of *H. anatolica*. The dichloromethane extracts of the fruits exhibited strong inhibitory activity on the colon cancer COLO205 and KM12 cell lines. The ethyl acetate extract of the fruits exhibited moderate inhibitory activity on the KM12 cell lines. The cytotoxic activities observed with these extracts are shown in Table 1.

Table 1. Cytotoxic activities of the extracts

Extracts	Cytotoxic activity (IC50 values in ug/mL)	
	COLO205	KM12
1	17.9	15.1
2	> 50	23.4
3	> 50	> 50

1: CH_2Cl_2 extract of the fruits; 2: EtOAc extract of the fruits; 3: aqueous-methanol extract of the fruits

The dichloromethane extract of the fruits of *H. anatolica* showed the best cytotoxic activities with IC_{50} values of 17.9 and 15.1 ug/mL on the COLO205 and KM12 cell lines, respectively. The ethyl acetate extract of the fruits showed moderate cytotoxic activity with IC_{50} values 23.4 ug/mL on the KM12 cell lines and a weak cytotoxic activity against the COLO205 cell line with IC_{50} value greater than 50 ug/mL. Previously, Appendino *et al.* reported samarcandin, samarcandone, conferone, feselol and more polar compounds 9,10,11-trihydroxyumbelliprenin and 9,10,11-5'-tetrahydroxyumbelliprenin from the chloroform extract of the fruits of *H. anatolica* collected from Mardin in June 1991[6]. Cytotoxic activity of certain sesquiterpene coumarins were described earlier[8-10], thus, the cytotoxic compound(s) of the fruits of *H. anatolica* may be this type of compound(s). Bioactivity guided fractionation of the dichloromethane extracts of the fruits of *H. anatolica* is planned to isolate and identify their cytotoxic principles.

ACKNOWLEDGMENT

We thank Dr. John Beutler, Molecular Targets Laboratory, CCR, NCI, Frederick, MD, U.S.A. for the cytotoxic activity testing.

We thank Prof. A. Duran for the collection and identification of plant material.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics. CA Cancer J Clin, 2021; 71: 7–33.

2. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/ 1981 to 09/2019. J Nat Prod, 2020; 83: 770–803.

3. The Plant List, A Working List of All Plant Species, Accessed July 30, 2019 at http://www.theplantlist.org/tpl1.1/search?q=Heptaptera

4. Herrnstadt I, Heyn CC, Heptaptera Marg. & Reuter. In Flora of Turkey and the East Aegean Islands, Davis, P., Ed.; Edinburgh University Press, Edinburgh, 1972; Vol. 4, pp 388-390.

5. Appendino G, Özen HÇ, Tagliapietra S, Cisero M. Coumarins from Heptaptera anisoptera. Phytochemistry, 1992; 31: 3211-3213.

6. Appendino G, Özen HÇ, Nano GA, Cisero M. Sesquiterpene coumarin ethers from the genus Heptaptera. Phytochemistry, 1992; 31: 4223-4226.

7. Appendino G, Özen HÇ, Jakupovic J. A sesquiterpene coumarin ether and a coniferyl ester from Heptaptera anisoptera. Fitoterapia, 1993; 64: 505-506.

8. Tosun F, Beutler JA, Ransom TT, Miski M. Anatolicin a Highly Potent and Selective Cytotoxic Sesquiterpene Coumarin from the Root Extract of Heptaptera anatolica. Molecules, 2019; 24: 1153-1160.

9. Tosun F, Aytar EC, Beutler JA, Wilson JA, Miski M. Cytotoxic Sesquiterpene Coumarins from the Roots of Heptaptera cilicica. Rec Nat Prod, 2021;15(6): 529-536.

10. Nazari ZE, Iranshahi M. Biologically active sesquiterpene coumarins from Ferula species. Phytother Res, 2011; 25: 315-323.

11. Gliszczynska A, Brodelius PE. Sesquiterpene coumarins. Phytochem Rev, 2012; 11: 77–96

12. Dastan D, Salehi P, Aliahmadi A, Gohari AR, Maroofi H, Ardalan A. New coumarin derivatives from Ferula pseudalliacea with antibacterial activity. Nat Prod Res, 2016; 30(24):2747-2753.

13. Amin A, Tuenter E, Cos P, Maes L, Exarchou V, Apers S, Pieters L. Antiprotozoal and antiglycation activities of sesquiterpene coumarins from Ferula narthex exudate. Molecules, 2016; 21: 1287-1296.

14. Iranshahi M, Rezaee R, Najafi MN, Haghbin A, Kasaian J. Cytotoxic activity of the genus Ferula (Apiaceae) and its bioactive constituents. Avicenna J Phytomed, 2018; 8 (4): 296-312.

15. Devkota KP, Covell D, Ransom T, McMahon JB, Beutler JA. Growth inhibition of human colon carcinoma cells by sesquiterpenoids and tetralones of Zygogynum calothyrsum. J Nat Prod, 2013; 76: 710-714.