Cytotoxic Activity of the Root and Fruit Extracts of *Heptaptera Anisoptera* (DC.) Tutin

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ABSTRACT

Cytotoxic activities of the root and fruit extracts of *Heptaptera anisoptera* (DC.) Tutin were investigated on the colon cancer COL0205 and KM12 cell lines. The dichloromethane extract of the roots of *H. anisoptera* showed cytotoxic activity with IC₅₀ values of 3.1 and 3.9 μg/mL on the COL0205 and KM12 cell lines, respectively. Cytotoxic activity of the dichloromethane extract of the fruits were similar to those of root extract with IC₅₀ values of 5.5 and 4.8 μg/mL on the COL0205 and KM12 cell lines, respectively.

Keywords: Cytotoxic activity, *Heptaptera anisoptera*, Apiaceae

INTRODUCTION

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 Turkey¹². *Heptaptera* species are known to contain sesquiterpene coumarin derivatives³⁴, these compounds have various biological activities such as; cytotoxicity, P-glycoprotein inhibitory, cancer chemoprevention, anti-inflammatory, antibacterial, antileishmanial, antiviral, antidiabetic, etc.⁵⁹.
Extraction

Coarsely powdered roots (150 g) and fruits (50 g) of the plant were separately and sequentially extracted at room temperature with dichloromethane (CH₂Cl₂) and methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to dryness. Dichloromethane and methanol extracts of the roots were 3.89 g, 2.59% and 9.59 g, 6.39%, respectively. Dichloromethane and methanol extracts of the fruits were 5.73 g, 11.46% and 1.97 g, 3.94 %, respectively. 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 roots were 1.18 g, 0.79% and 8.41 g, 5.6%, respectively. Ethyl acetate and aqueous-methanol extracts of the fruits were 0.25 g, 0.5% and 1.72 g, 3.44%, respectively.

Cytotoxicity Assay on Colon Cancer Cells

The assay used for this study was a two-day, two cell line XTT bioassay⁶⁰, 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 roots and fruits of *H. anisoptera*. The dichloromethane extracts of the roots and fruits exhibited strong inhibitory activity on the colon cancer COLO205 and KM12 cell lines. The ethyl acetate extract of the fruits exhibited strong inhibitory activity on the COLO205 cell lines with IC₅₀ value of 4.5 ug/mL but only a moderate inhibitor activity on KM12 cell lines. The cytotoxic activities observed with these extracts are shown in Table 1.

Table 1. Cytotoxic activities of extracts

| Extracts | COLO205 | KM12 |
|----------|---------|------|
| 1: CH₂Cl₂ extract of the roots; 2: EtOAc extract of the roots; 3: aqueous-methanol extract of the roots | 3.1 | 3.9 |
| 2 | > 50 | 20.5 |
| 3 | > 50 | > 50 |
| 4: CH₂Cl₂ extract of the fruits; 5: EtOAc extract of the fruits; 6: aqueous-methanol extract of the fruits | 5.5 | 4.8 |
| 5 | 4.5 | 15.2 |
| 6 | > 50 | > 50 |

The dichloromethane extract of the roots of *H. anisoptera* showed cytotoxic activity with IC₅₀ values of 3.1 and 3.9 ug/mL on the COLO205 and KM12 cell lines, respectively. The cytotoxic activities of dichloromethane extract of the fruits were similar to those of root extract with IC₅₀ values of 5.5 and 4.8 ug/mL on the COLO205 and KM12 cell lines, respectively. The ethyl acetate extract of the fruits showed cytotoxic activity with IC₅₀ values of 4.5 and 15.2 ug/mL on the COLO205 and KM12 cell lines, respectively. Whereas, the ethyl acetate extract of the roots showed a moderate cytotoxic activ-
ity with IC\textsubscript{50} values of 20.5 \textmu{g}/mL on the KM12 cell lines but no inhibitor activity up to 50 \textmu{g}/mL on the COLO205 cell lines. Previously, Appendino \textit{et al.} reported umbelliprenin, badrakemin, badrakemone, colladonin (major compound), colladin, 14-acetoxybadrakemin, 14-acetoxybadrakemone, 14-hydroxycolladonin from the chloroform extract of the roots and more polar compounds samarcandin, samarcandone, conferol, conferone, feselol, 9,10,11-trihydroxyumbelliprenin, 9,10,11-5'-tetrahydroxyumbelliprenin, 9, 10, 5'-triacetoxy-11-hydroxyumbellipreni, 10, 11, 5'-trihydroxyumbelliprenin from the chloroform extract of the fruits of \textit{H. anisoptera} collected from Diyarbakır in June 1991\textsuperscript{3,11,12}. Cytotoxic activity of certain sesquiterpenic coumarins were described earlier\textsuperscript{4,5}, thus, the cytotoxic compound(s) of the roots and fruits of \textit{H. anisoptera} may be this type of compound(s). Bioactivity guided fractionation of the dichloromethane extracts of the roots and fruits of \textit{H. anisoptera} is planned to isolate and identify their cytotoxic principles.

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