IN VITRO CYTOTOXIC PROPERTIES OF SIX ARTEMISIA L. SPECIES

Şüra BAYKAN EREL¹*, Serdar GÖKHAN ŞENOL², Fadime AYDIN KÖSE³, Petek BALLAR³

¹Ege University, Faculty of Pharmacy, Department of Biochemistry, 35100 Bornova, İzmir, TURKEY
²Ege University, Faculty of Science, Department of Botany, 35100 Bornova, İzmir, TURKEY
³Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 35100 Bornova, İzmir, TURKEY

Abstract

Methanolic extracts of Artemisia L. taxons (Artemisia absinthium L., Artemisia arborescens L., Artemisia campestris L., Artemisia scoparia Waldst&Kit., Artemisia santonicum L., Artemisia vulgaris L. and Artemisia arborescens L.) were investigated for their cytotoxic activities against three human cancer cell lines, MCF7, A549, HeLa, and two human normal cell lines, A7R5 and 293T. The cytotoxic activities were analyzed by real-time cell analysis system measuring electrical impedance. Artemisia scoparia Waldst&Kit. showed significant effect on MCF7 (IC⁵⁰: 34 μg/mL) and HeLa (IC⁵⁰: 90 μg/mL). A. absinthium exhibited selective cytotoxic activity on MCF7 (IC⁵⁰: 270 μg/mL).

Keywords: Artemisia, Cytotoxic, MCF7, A549, HeLa, A7R5, 293T.

Altı Artemisia L. Türünün in vitro Sitotoksik Özellikleri

Bu çalışmada Artemisia L. (Artemisia absinthium L., Artemisia arborescens L., Artemisia campestris L., Artemisia scoparia Waldst&Kit., Artemisia santonicum L., Artemisia vulgaris L. ve Artemisia arborescens L.) türlerine ait metanollü ekstrelerin üç insan kanserli hücre hattında (MCF7, A549, HeLa) ve iki normal insan hücre hattında (A7R5 ve 293T) sitotoksik aktiviteleri araştırıldı. Sitotoksik aktiviteler elektriksel impedans ölçümü ile gerçek zamanlı hücre analiz sistemi ile ölçüldü. Sonuçta Artemisia scoparia Waldst&Kit ekstresinin MCF7 (IC⁵⁰: 34 μg/mL) ve HeLa (IC⁵⁰: 90 μg/mL) üzerinde oldukça yüksek aktivite göstergi saptandı. Ayrıca A.absinthium’un da MCF7 (IC⁵⁰: 270 μg/mL) üzerinde seçici etkisi olduğu gözlandı.

Anahtar kelimeler: Artemisia, Sitotoksik, MCF7, A549, HeLa, A7R5, 293T.

*Correspondence: E-mail: sura.bayikan@ege.edu.tr
Tel:+ 90 232 311 10 10-3963; Fax: +90 232 388 52 58
INTRODUCTION

Cancer is the failure of the mechanisms that regulate normal cell growth, proliferation and cell death. It is generally used as a collective name for more than 100 diseases characterized by uncontrolled growth of abnormal cells including malignant tumors of different sites (breast, lung, cervix etc.). Cancer is currently the cause of %12 of all deaths worldwide and in 20 years time it is expected to increase from 6 million to 10 million (1).

Despite all the studies and advances on cancer research, it is still urgent to identify new anticancer agents with low toxicity against non-tumor cells. Natural products have a significant role in the treatment of cancer in worldwide. According to Newmann and Cragg more than 3000 plant species have been listed as used in cancer treatment. Over %60 of currently used anticancer agents are derived from natural sources. Also the molecules derived from natural sources play a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most of human diseases (2).

The genus *Artemisia* is one of the largest and most widely distributed genera of Asteraceae. Generally aromatic herbs and shrubs are distributed mainly in the Northern hemisphere and temperate zones of Europe, Asia and America (3,4). Most of the members of the genus have characteristic scent and taste. The genus is having been interested due to the diversified biology and chemistry of the constituents, the frequent application in traditional medical practice and the rich source of the plant material (5). Phytochemical reports on *Artemisia* species deal mainly with terpenoids, flavonoids, coumarines, steroids and polyacetylenes (3).

There are 23 species of *Artemisia* genus in the Flora of Turkey and several species have been used traditionally as stomachic, antimalarial, antidiabetic and antihelmintic in Anatolia since ancient times (6-8). A. absinthium L., A. arborescens L., A. campestris L., A. scoparia Waldst&Kit., A. santonicum L., and A. vulgaris L. are mainly distributed in West Anatolia. In this study in vitro cytotoxic activities of these plants were evaluated against three cancer cell lines, MCF7 (human breast cancer cell line), A549 (human lung cancer cell line), HeLa (human cervical cancer cell line) and two non-cancer cell lines, 293 HEK (human embryonic kidney cell line) and A7R5 (rat vascular smooth muscle cells).

EXPERIMENTAL

Plant material

Plants were collected from West Anatolia, taxonomically identified and voucher specimens have been deposited in the herbarium of Ege University (IZEF), Faculty of Pharmacy, Izmir, Turkey. Collection sites and dates are presented in Table 1.

Preparation of extracts

Aerial parts of the plants were dried under shade and powdered. Crude materials (50 g) were extracted successively with 1 L methanol each, using a Soxhlet extractor for 6 h. Solvent evaporated to dryness under reduced pressure on rotary evaporator. Dried extracts were stored at 4 °C until studied. Extract yields are presented in Table 1.

Cell lines and culture medium

MCF7, A7R5, 293 HEK, A549 and HeLa cell lines were obtained from ATCC. All cells were cultivated in a humidified incubator at 37°C with 5% CO₂. Cells were cultured in DMEM
supplemented with L-glutamine (2mmol/L) and 10% fetal bovine serum. All the tissue culture reagents were purchased from Biological Industries (Israel).

**Determination of cell viability by Real-Time Cell Analyzer (RTCA)**

Cells were grown and expanded in 100 mm tissue culture dishes. After reaching 60-80% confluence, cells were washed with PBS and detached from the flasks by trypsin/EDTA treatment. meanwhile, 100 μL of cell culture media at room temperature was added into each well of E-plate 96 and background of E-plate was measured. To determine the optimum cell number, 5,000, 10,000, 20,000 and 40,000 cells/well were seeded for each cell line. After 30 min of incubation at room temperature, E-plates were placed into the Real-Time Cell Analyzer MP (RTCA) station. Cells were grown and impedance was measured every 30 min afterwhile as the cell index (CI) value. To determine the effect of test extracts, optimum number of cells (MCF7:7500, HeLa:7500, A545:10.000, A745:10.000 and 293T:20.000) for each cell line were seeded. 16-24 hrs later cells were exposed to test extracts at different concentrations (25, 100, 250, 500 and 1000 μg/mL). CI values were monitored every 2 min for 2 hrs to visualize the fast drug response and then every 30 min for the late drug response. The electrical impedance was measured by the RTCA software of the xCELLigence system as a dimensionless parameter termed CI. All the measurements were done at least in triplets and IC\(_{50}\) values were determined using RTCA software.

| Table 1. Collection sites, dates, voucher specimens and yields of *Artemisia* L. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Turkish name** | **Locality** | **Date** | **Voucher Specimen** | **Extract Yield (%)** | **Oil Yield (%)** |
| A. absinthium | Pelinotu, Akpelin | Antalya, Alanya, 1514m 37°47'10"N / 28°56'05"E | 29.07.2003 | 5657 | 17.78 | 1.1 |
| A. arborescens | - | Muğla, Bodrum, 0m | 14.04.2005 | 5802 | 24.76 | 1.2 |
| A. campestris | Kara yavşanotu | Denizli, Altindere, 850m 37°47'10"N / 28°56'05"E | 30.09.2003 | 5665 | 21.96 | 0.7 |
| A. scoparia | - | Manisa, Salihli, 112m 38°28'46"N / 28°03'56"E | 30.09.2003 | 5662 | 17.16 | 0.9 |
| A. santonicum | Deniz Yavşanı | Balikesir, Edremit, 0m 39°33'43"N / 26°57'02"E | 03.09.2003 | 5661 | 21.76 | 0.4 |
| A. vulgaris | Ayvadana | Denizli, Baskarci, 450m 37°45'42"N / 28°58'48"E | 30.09.2003 | 5663 | 14.92 | 0.4 |

**RESULTS AND DISCUSSION**

The results of the cytotoxic activity tests are shown in Table 2. Among the extracts, *A. scoparia* had a strong effect on MCF7 and HeLa cells with (IC\(_{50}\): 34 and 90 respectively) while it showed moderate effect on the A549 and normal cells. *A. absinthium* had a significant selective activity on MCF7 (IC\(_{50}\): 270) among all tested cell lines. *A. vulgaris* extract also exhibited inhibitory activities against all cell lines with significant IC\(_{50}\) values except A549...
cells. *A. santonicum* and *A. arborescens* extracts were not active on A549 but had moderate effects on the other cell lines. *A. campestris* showed the weakest cytotoxic activity.

In many studies, cytotoxic activities of various *Artemisia* species have been reported against several cell lines. However this is the first report for these *Artemisia* L. species against these cell lines mentioned above. In a previous report Nibret and Wink was evaluated cytotoxic activities of ethanol and dichloromethane extracts of four *Artemisia* species against HL-60. *Artemisia absinthium* methanolic extract was shown to have strong inhibitory activity (IC$_{50}$: 57.90 μg/mL) (9). Consistent with our results, *A. campestris* was reported as the weakest plant among 36 medicinal plant species in a cytotoxic screening study against K562 human leukemia cells (10).

Most of the reports dealing with the cytotoxic activity studies of *Artemisia* species have showed that the sesquiterpenes are the main responsible compounds for the activities (11-13). Strong activities of *A. scoparia* and *A. absinthium* needs to be explained by further phytochemical investigations on these plants.

### Table 2. Cytotoxic activity results of *Artemisia* species on different cell lines.

<table>
<thead>
<tr>
<th>Species</th>
<th>MCF7</th>
<th>A549</th>
<th>HeLa</th>
<th>A7R5</th>
<th>293T</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>270</td>
<td>NA</td>
<td>NA</td>
<td>894</td>
<td>NA</td>
</tr>
<tr>
<td><em>A. arborescens</em></td>
<td>547</td>
<td>NA</td>
<td>542</td>
<td>541</td>
<td>647</td>
</tr>
<tr>
<td><em>A. campestris</em></td>
<td>790</td>
<td>&gt;1000</td>
<td>NA</td>
<td>899</td>
<td>500</td>
</tr>
<tr>
<td><em>A. santonicum</em></td>
<td>530</td>
<td>NA</td>
<td>&gt;1000</td>
<td>336</td>
<td>570</td>
</tr>
<tr>
<td><em>A. scoparia</em></td>
<td>34</td>
<td>370</td>
<td>90</td>
<td>495</td>
<td>485</td>
</tr>
<tr>
<td><em>A. vulgaris</em></td>
<td>190</td>
<td>778</td>
<td>284</td>
<td>382</td>
<td>317</td>
</tr>
</tbody>
</table>

NA: Not Active, *: Values are mean of triplicate analysis

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### REFERENCES


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