In vitro anticancer and cytotoxic activities of some plant extracts on HeLa and Vero cell lines

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Summary

Purpose: The aim of our study was to evaluate the effect of in vitro anticancer and cytotoxic activity of the methanolic extracts of 14 medicinal plants, 8 of which are endemic species in Anatolia, against the human HeLa cervical cancer cell line and to compare to the normal African green monkey kidney epithelial cell line (Vero) using the MTT colorimetric assay.

Methods: Values for cytotoxicity measured by MTT assay were expressed as the concentration that causes 50% decrease in cell viability ($IC_{50}$, μg/mL). The degree of selectivity of the compounds can be expressed by its selectivity index (SI) value. High SI value (>2) of a compound gives the selective toxicity against cancer cells (SI = $IC_{50}$ normal cell/$IC_{50}$ cancer cell).

Results: Dose-dependent studies revealed $IC_{50}$ of 293 mg/mL and >1000 mg/mL for Cotinus coggygria Scop., $IC_{50}$ of 265 μg/mL and >1000 mg/mL for Rosa damascena Miller, $IC_{50}$ of 2 μg/mL and 454 mg/mL for Colchicum sanguicolle K.M. Perss., $IC_{50}$ of 427 μg/mL and >1000 μg/mL for Centaurea antiochia Boiss. var. praelata (Boiss & Bal) Wagenitz on the HeLa cells and the Vero cells, respectively. Four plants showed significant SI values which were 227 for Colchicum sanguicolle K.M. Perss (endemic species), >3.8 for Rosa damascena Miller, >3.4 for Cotinus coggygria Scop. and >2.3 for Centaurea antiochia Boiss. var. praelata (Boiss & Bal) Wagenitz (endemic species).

Conclusion: According to our study, 4 methanolic extracts of 14 tested plants exhibit greater activity on the HeLa cell line and little activity on the Vero cell line, meaning that these plants can be evaluated for potential promising anticancer activity.

Key words: anticancer activity, crude extracts, cytotoxic activity, HeLa cell line, MTT assay, Vero cell line

Introduction

Natural products have historically and continually been investigated for promising new leads in pharmaceutical development [1]. Many of the plant substances are used in traditional medicine because they are readily available in rural areas and cheaper compared to modern therapeutic agents [2]. The World Health Organization (WHO) has reported that about 80% of the world population depends on traditional medicine for their primary health care [3]. Out of total 250,000 plant species existing on earth approximately one thousand have anticancer activities [4].

Cancer is one of the most dangerous diseases in humans and presently there is a considerable amount of new anticancer agents from natural products [5]. According to WHO, cancer is one of the leading causes of death worldwide, which accounted for 7.6 million deaths (around 13%) of the world’s population in 2008. Furthermore, WHO estimated that the worldwide deaths are likely to rise to over 11 million in 2030 [6]. The potential of using natural products as anticancer drugs was...
Anticancer plant extracts on HeLa and Vero cell lines

recognized in 1950’s by U.S National Cancer Institute (NCI). Since 1950 major contributions have been made for the discovery of naturally occurring anticancer drugs [5]. In a recent report [3], about 60% of the currently used anticancer drugs have been isolated from natural products, mostly of plant origin. The use of medicinal plants is believed to contain a wide spectrum of polyphenolics, flavonoids, alkaloids, terpenoids and saponins compounds, which might have therapeutic properties and hinder cancer formation [5].

Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents [7]. Herbal medicine remains one of the common forms of therapy available to much of the world’s population. According to the World Health Organization, about three quarters of the world’s population currently uses herbs and other forms of traditional medicine to treat diseases. Even in USA, use of plants and phytomedicine has increased dramatically in the last two decades. A National Centre for Complementary and Alternative Medicine has been established in USA. The herbal products have been classified under ‘Dietary Supplements’ and are included with vitamins, minerals, amino acids and other products intended to supplement the diet. However, the scientific basis for the bioactivity and the underlying molecular mechanisms for most of these products is presently unknown or incomplete. Many naturally occurring substances present in the human diet have been identified as potential chemopreventive agents, and consuming relatively large amounts of vegetables and fruits can prevent the development of cancer [8]. Plant molecules and their semi-synthetic and synthetic derivatives are important sources of antitumor drugs. Over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them [9]. Several chemotherapeutic drugs derived from plants, such as vinca alkaloids, paclitaxel, camptothecins and podophyllotoxins are used in cancer therapy. Using modern analytical and chemical techniques, novel natural compounds from herbs can be isolated by fractionation and isolation. It has been estimated that only 5-15% of 250,000 species of higher plants have been screened systematically for natural bioactive compounds. To study new therapeutic approaches, cell lines are used to investigate in vivo antiproliferative activities of novel compounds [10] while in vitro cytotoxicity of plant extracts is commonly the first step of research for anticancer compounds from natural sources [11].

For this study, we selected 14 medicinal plants, 8 of which are endemic species in Anatolia, to evaluate their in vitro anticancer activity against the HeLa cell line in comparison with normal African green monkey kidney epithelial (Vero) cell line.

Methods

Plant selection and collection

Fourteen medicinal plants were chosen for in vitro anticancer activity and cytotoxicity testing by considering previous literature reports and ethnobotanical information. The selected plants, some of which are endemic, belong to different family groups and were collected from different districts of Turkey (Table 1).

Preparation of extracts

The dried plant material (Crataegus microphylla, Teucrium sandrascum, Centaurea nerifoliae, Olea europaea, Salvia hypargeria, Cotynus coggyria, Hypericum kotshyanum, Nepeta ilatia, Stachys cretica subsp. vacillans, Scorzonera tomentosa, Origanum sipleum, Rosa damascena, Centaurea antiochia var. praektal) were percolated with methanol (95%) at room temperature. The methanolic extracts (ME) were evaporated to dryness under pressure and controlled temperature (40 to 50 °C) in a rotary evaporator. The dried cormus of Colchicum sanguicole was extracted with methanol (95%) in a Soxhlet apparatus. The ME was evaporated to dryness under pressure and controlled temperature (40 to 50 °C) in a rotary evaporator. All the extracts were kept at -20 °C and were then lyophilized. In this way, crude methanolic extracts were obtained. The ME were dissolved in distilled water, and diluted in Eagle’s minimum essential medium (EMEM) (Sigma, USA).

Cell cultures

The HeLa cell line and the Vero cell line were grown and maintained in EMEM with Earle’s saline, supplemented with an antibiotic-antimycotic mixture (penicillin 100 U/mL, streptomycin 100 mg/mL, amphotericin B (0.25 mg/mL), and 10% fetal bovine serum (Sigma, USA). Cells were maintained in a humidified atmosphere containing 5% CO₂ at 57°C.

In vitro cytotoxicity assay

The cytotoxicity assays were performed according to the microculture MTT method [12,13]. The cells were harvested (2.0–2.8x10⁵ cells/well) and inoculated
Anticancer plant extracts on HeLa and Vero cell lines

Table 1. List of plants used in the current study

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Specimen number (ISTE)</th>
<th>Plant part used</th>
<th>Districts of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crataegus microphylla C. Koch</td>
<td>Rosaceae</td>
<td>76223</td>
<td>Leaves</td>
<td>Golcuk-Bolu</td>
</tr>
<tr>
<td>Teucrium sandrascicum O. Schwarz (Endemic)</td>
<td>Lamiaceae</td>
<td>87526</td>
<td>Aerial parts</td>
<td>Koycegiz-Mugla</td>
</tr>
<tr>
<td>Centaurea nerimaniae Ş. Kültür (Endemic)</td>
<td>Asteraceae</td>
<td>98163</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Olea europea L.</td>
<td>Oleaceae</td>
<td>106286</td>
<td>Leaves</td>
<td>Sarkoy-Tekirdag</td>
</tr>
<tr>
<td>Salvia hypargeia Fisch.&amp; Mey. (Endemic)</td>
<td>Lamiaceae</td>
<td>98205</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Cotinus coggygria Scop.</td>
<td>Anacardiaceae</td>
<td>80926</td>
<td>Leaves</td>
<td>Kırklareli</td>
</tr>
<tr>
<td>Hypericium kotshyanum Boiss. (Endemic)</td>
<td>Hypericaceae</td>
<td>98173</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Nepeta italica L.</td>
<td>Lamiaceae</td>
<td>98192</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Stachys cretica L. subsp. vacillans Rech. Fil</td>
<td>Lamiaceae</td>
<td>98166</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Scorzonera tomentosa L. (Endemic)</td>
<td>Asteraceae</td>
<td>98954</td>
<td>Aerial parts</td>
<td>Malatya</td>
</tr>
<tr>
<td>Origanum sipyleum L. (Endemic)</td>
<td>Lamiaceae</td>
<td>86060</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
<tr>
<td>Rosa damascena Miller</td>
<td>Rosaceae</td>
<td>106285</td>
<td>Flowers</td>
<td>Isparta</td>
</tr>
<tr>
<td>Colchicum sanguicollie K.M. Perss (Endemic)</td>
<td>Colchicaceae</td>
<td>48868</td>
<td>Cormus</td>
<td>Antalya</td>
</tr>
<tr>
<td>Centaurea antiochia Boiss. var. praealta (Boiss. &amp; Bal) Wagenitz (Endemic)</td>
<td>Asteraceae</td>
<td>98247</td>
<td>Aerial parts</td>
<td>Mersin</td>
</tr>
</tbody>
</table>

In 24-well plates. The cells were washed with phosphate buffered saline (PBS) and then inoculated with and without the extract (final extract concentrations range 5-1000 μg/mL). After 72-hr incubation, the medium was aspirated. 150 mL of MTT solution (5 μg/mL in PBS, pH 7.2) were added to each well and the plates were incubated for 4 hrs at 37 °C. After incubation, 750 mL of dimethyl sulfoxide were added to each well, followed by gentle shaking for 15 min to solubilize the formazan dye. Absorbance was read at 540 nm using a photometer (μQuant, Bio-Tek Instruments Inc, USA) and the surviving fraction was calculated according to the following formula:

% viability = (absorbance of extracts treated cells/absorbance of control cells) x100.

All experiments were performed in triplicate and mean values were used for calculation. Spectrophotometric determinations were performed using Quant Universal Microplate Spectrophotometer (Bio-Tek, Instruments Inc, USA) and the surviving fraction was calculated according to the following formula:

\[ SI = \frac{IC_{50} \text{ normal cell}}{IC_{50} \text{ cancer cell}} \]

Selectivity index (SI)

The degree of selectivity of the compounds was expressed by its SI value as suggested by Badisa et al. [14]. High SI value (>2) of a compound suggests selective toxicity against cancer cells, while a compound with SI value <2 is considered to give general toxicity which can also cause cytotoxicity in normal cells [15].

Statistics

Values from all experimental groups were acquired by using GraphPad Prism Software (GraphPad Prism version 5.0, GraphPad Software, San Diego California USA, Anonim-c) and analyzed using one-way ANOVA test. The significance between control and experimental groups was determined by Dunnett’s test and a p value <0.05 was considered as statistically significant.

Results

Plant species and the parts of plants used for extract preparation and the specimen number are shown in Table 1. Results on cytotoxicity of extracts on Vero and HeLa cervical cancer cell lines are shown in Table 2. The final concentration of methanol was <0.1%. Six of the 14 tested extracts on the Vero cells and 11 of the 14 tested extracts on the HeLa cells showed cytotoxic activity. Colchicum sanguicollie K.M. Perss methanolic extract showed the highest cytotoxic activity against HeLa cancer cell line. Centaurea nerimaniae Ş. Kültür methanolic extract showed the highest cytotoxic activity against Vero normal cell line. Olea europaea L., Salvia hypargeia Fisch.& Mey. and Origanum sipyleum L. exhibited no cytotoxic activity against the two types of cell lines.

Four of the 14 tested extracts exhibited a substantial antiproliferative effect on HeLa cells. Dose-dependent studies showed IC₅₀ of 293 μg/mL and >1000 μg/mL, 265 μg/mL and >1000 μg/mL, 2 μg/mL and 454 μg/mL, 427 μg/mL and >1000 μg/mL and SI of >3.4, >3.8, 227 and >2.3 on the HeLa cells and the Vero cells for Cotinus coggygria Scop,
Anticancer plant extracts on HeLa and Vero cell lines

Rosa damascena Miller, Colchicum sanguicolle K.M. Perss and Centaurea antiochia Boiss. var. praealta (Boiss. & Bal) Wagenitz, respectively. Colchicum sanguicolle K.M. Perss methanolic extract showed the highest SI value.

Discussion

The main aims of analyzing crude plant extracts are either to isolate bioactive agents for direct use as drugs or to identify bioactive compounds that can be used as lead substances in the preparation of semi synthetic drugs [10]. A large number of novel anticancer drugs have been discovered from natural products in the past and new ones are continually being developed. These cytotoxic natural products may be able to play a significant role in treating selected cancers by working in concert with conventional chemotherapeutic drugs, thereby improving their efficacy or reducing their toxicity [1]. The results of our study show that Cotynus coggygria Scop., Rosa damascena Miller, Colchicum sanguicolle K.M. Perss and Centaurea antiochia Boiss. var. praealta (Boiss. & Bal) Wagenitz have promising anticancer activities in vitro.

The IC₅₀ values were used to determine the SI of each extract which represents the overall activity.

The extract from Colchicum sanguicolle K.M. Perss (SI=227), Rosa damascena Miller (SI ≥3.8), Cotinus coggygria Scop. (SI ≥3.4) and Centaurea antiochia Boiss. var. praealta (Boiss. & Bal) Wagenitz (SI ≥2.3) showed the most promising and selective cytotoxic activity against the HeLa cell line. In a previous study, actinomycin D, an anticancer agent, had an IC₅₀ values of 0.002 ± 0.0000395 μg/mL for the HeLa cell line and 0.027 ± 0.00021 μg/mL for the Vero cell line and its SI value was 13.5 [6]. In our study, especially the extract of Colchicum sanguicolle, an endemic species, exhibited highest cytotoxic effect on the HeLa cell line and low cytotoxicity on the Vero cell line. Therefore, Colchicum sanguicolle could be considered as a promising anticancer agent due to its high SI value.

The genus Centaurea L. is distributed in various regions of Turkey, represented by 192 taxa, 114 of which are endemic. High endemism ratio shows that Turkey is one of the gene centers of this genus [17]. In recent years, the Centaurea genus has attracted great interest among researchers from this area due to its widespread distribution and chemical properties [11]. The aerial parts of some Centaurea species are used in traditional medicine of many countries as antiprotozoal, antimicrobial, cytotoxic and antiinflammatory agents [18]. According to SI values found in the present study, it was shown that the methanolic extract of an endemic species Centaurea antiochia Boiss. var. praealta possesses an effective anticancer potential against the HeLa cancer cell line.

In traditional medicine of different countries, Cotinus coggygria has been used for its antiseptic, antiinflammatory and antihaemorrhagic proper-

Table 2. Cytotoxic activity expressed as IC₅₀ (μg/ml) of plant extracts

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>HeLa IC₅₀ μg/mL±SD</th>
<th>Vero IC₅₀ μg/mL±SD</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crataegus microphylla</td>
<td>576±5.06</td>
<td>&gt;1000</td>
<td>&gt;1.7</td>
</tr>
<tr>
<td>Teucrium sandrasicum</td>
<td>513±1.53</td>
<td>593±3.12</td>
<td>1.2</td>
</tr>
<tr>
<td>Centaurea nerimaniae</td>
<td>253±0.764</td>
<td>194±1.04</td>
<td>0.8</td>
</tr>
<tr>
<td>Olea europaea</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>≥1</td>
</tr>
<tr>
<td>Salvia hypargea</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>≥1</td>
</tr>
<tr>
<td>Cotinus coggygria</td>
<td>295±1.00</td>
<td>&gt;1000</td>
<td>&gt;5.4</td>
</tr>
<tr>
<td>Hypericum kotshcyanum</td>
<td>507±1.53</td>
<td>367±1.15</td>
<td>0.7</td>
</tr>
<tr>
<td>Nepeta italica</td>
<td>980±3.01</td>
<td>&gt;1000</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Stachys cretica</td>
<td>759±1.53</td>
<td>426±1.76</td>
<td>0.6</td>
</tr>
<tr>
<td>Scorzonera tomentosa</td>
<td>987±1.73</td>
<td>195±2.08</td>
<td>0.2</td>
</tr>
<tr>
<td>Origanum sipyleum</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>≥1</td>
</tr>
<tr>
<td>Rosa damascena</td>
<td>265±1.00</td>
<td>&gt;1000</td>
<td>&gt;3.8</td>
</tr>
<tr>
<td>Colchicum sanguicolle</td>
<td>2±0.02</td>
<td>454±5.06</td>
<td>227</td>
</tr>
<tr>
<td>Centaurea antiochia</td>
<td>427±±3.06</td>
<td>≥1000</td>
<td>&gt;2.3</td>
</tr>
</tbody>
</table>

Data are the means of triple experiments. Selectivity Index (SI) = IC₅₀ Vero cell/ IC₅₀ HeLa cell. SI value > 2 indicating high selectivity [15,16]
ties. A methanol extract of the leaves and flow-
eres exhibited antioxidant and cytotoxic properties 
[19]. Rosa damascena is well known as medicinal herb [20], it has significant antioxidant activity and shows strong oxidative prevention of DNA damage [21]. Cytotoxic effect of Rosa damascena on cancer cell lines has been reported on human lung (A549) and breast cancer (MCF-7) cell lines [22]. The present study has shown that the meth-
anolic extracts of Cotinus coggygria (SI>3.4) and 
Rosa damascena (SI>3.8) had stronger anticancer potential than the extract of Centaurea antiochia Boiss. var. paealta, according to the SI values.

The medicinal importance of the genus Col-
chicum is attributed to the presence of tropolonic alkaloids, mainly colchicines. Colchicine has been found to possess antitumor and antiinflammato-
ry properties and still keeps its importance in the 
treatment of many diseases [23]. The most attrac-
tive result in our research comes from the meth-
anolic extract of Colchicum sanguicolle. We have 
found that the SI value of the methanolic extract 
of Colchicum sanguicolle, an endemic species, was 
227, whereas the SI of actinomycin D, an antican-
cer agent, was 13.5. Therefore, the methanolic ex-
tract of Colchicum sanguicolle could be evaluated 
as a very strong anticancer agent.

This study has shown that Centaurea anti-
ochia Boiss. var. paealta (Boiss. & Bal) Wagenitz, 
Rosa damascena Miller, Cotynus coggygria Scop. 
and especially, Colchicum sanguicolle K.M. Perss have potent anticancer activities in vitro and 
consequently, could potentially be a source for a 
pharmacologically active products suitable for de-
velopment of novel anticancer agents. These plant 
extracts should be searched in detail to find the 
compounds responsible for the anticancer activity 
by isolation and purification studies in the future.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Yaacob N, Hamzah N, Kamal NN et al. Anticancer ac-
tivity of a sub-fraction of dichloromethane extract of 
Strobilanthes crispus on human breast and prostate 


6. Erel SB, Demir S, Nalbantsoy A et al. Bioactivity 
screening of five Centaurea species and in vivo an-

7. Kanimozi D. In-Vitro Anticancer Activity of Ethanol-
ic Extract of Cynodon dactylon Against HEP-2, HEIa 

8. Rao KVK, Shwartz SA Nair HK et al. Plant derived 
products as a source of cellular growth inhibitory 
pytochemicals on PC3M, DU-145 and LNC a prostate 

9. Mosmann T. Rapid colorimetric assay for cellular 
growth and survival: application to proliferation and 


11. Erel SB, Demir S, Nahtansoy A et al. Bioactivity 
screening of five Centaurea species and in vivo an-

12. Mosmann T. Rapid colorimetric assay for cellular 
growth and survival: application to proliferation and 

Anticancer plant extracts on HeLa and Vero cell lines


