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ORIGINAL ARTICLE

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. praealta (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. praealta (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

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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 saponin compounds, which might have therapeutic properties and hinder cancer formation [3].

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 novel compounds and their effects on tumor cells. Cell lines are used as cancer models 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 sandrasicum, Centaurea nerimaniae, Olea europaea, Salvia hypargeia, Cotynus coggygria, Hypericum kotschcyanum, Nepeta italica, Stachys cretica* subsp. *vacillans, Scorzonera tomentosa, Origanum sipyleum, Rosa damascena, Centaurea antiochia* var. *praealta*) 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 sanguicolle* 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 % CO_2 at 37°C.

In vitro cytotoxicity assay

The cytotoxicity assays were performed according to the microculture MTT method [12,13]. The cells were harvested (2.0– 2.8x10^5 cells/well) and inoculated

Table 1. List of plants used in the current study

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

in 24-well plates. The cells were washed with phosphate buffered saline (PBS) and were 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 dimetyl 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).

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]. Each SI value was calculated using the formula:

$$SI = IC_{50} \text{ normal cell}/IC_{50} \text{ cancer cell}$$

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 sanguicolle* 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 IC_{50} 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.*,

Table 2. Cytotoxic activity expressed as IC_{50} (µg/ml) of plant extracts

Plant extracts	*HeLa IC_{50} µg/mL±SD*	*Vero IC_{50} µg/mL±SD*	*SI*
Crataegus microphylla	576±3.06	>1000	>1.7
Teucrium sandrasicum	513±1.53	593±3.12	1.2
Centaurea nerimaniae	253±0.764	194±1.04	0.8
Olea europaea	>1000	>1000	≥1
Salvia hypargeia	>1000	>1000	≥1
Cotinus coggygria	293±1.00	>1000	>3.4
Hypericum kotshcyanum	507±1.53	367±1.15	0.7
Nepeta italica	980±3.01	>1000	>1
Stachys cretica	759±1.53	426±1.76	0.6
Scorzonera tomentosa	987±1.73	195±2.08	0.2
Origanum sipyleum	>1000	>1000	≥1
Rosa damascena	265±1.00	>1000	>3.8
Colchicum sanguicolle	2±0.02	454±3.06	227
Centaurea antiochia	427±3.06	>1000	>2.3

Data are the means of triple experiments. Selectivity Index (SI) = IC_{50} Vero cell/ IC50 HeLa cell. SI value > 2 indicating high selectivity [15,16]

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 IC_{50} 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 IC_{50} 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-

ties. A methanol extract of the leaves and flowers 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 methanolic 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. *praealta*, according to the SI values.

The medicinal importance of the genus Colchicum is attributed to the presence of tropolonic alkaloids, mainly colchicines. Colchicine has been found to possess antitumor and antiinflammatory properties and still keeps its importance in the treatment of many diseases [23]. The most attractive result in our research comes from the methanolic 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 anticancer agent, was 13.5. Therefore, the methanolic extract of *Colchicum sanguicolle* could be evaluated as a very strong anticancer agent.

This study has shown that Centaurea antiochia Boiss. var. praealta (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 development 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 activity of a sub-fraction of dichloromethane extract of Strobilanthes crispus on human breast and prostate cancer cells in vitro. BMC Complement Altern Med 2010;10:42-55.
2. Moglad EH, Abdalla OM, Koko WS, Saadabi AM. In vitro Anticancer Activity and Cytotoxicity of Solanum nigrum on Cancer and Normal Cell Lines. Int J Cancer 2014;10:74-80.
3. Latif A, Amer HM, Homad ME, Alarifi SA, Almajhdi FN. Medicinal plants from Saudi Arabia and Indonesia: In vitro cytotoxicity evaluation on Vero and Hep-2 cells. J Med Plants Res 2014;8:1065-1073.
4. Bibi Y, Nisa S, Zia, M, Waheed A, Ahmed S, Chaudhary MF. In vitro cytotoxic activity of Aesculus indica against breast adenocarcinoma cell line (MCF-7) and phytochemical analysis. Pharm Sci 2012;25:183-187.
5. Sharma J, Pitchaiah G, Satyavati D, Rao JV, Kumar-Vikram HS. In Vitro Anticancer Activity of Methanolic Extract of Roots of Glochidion zeylanicum (Gaertn.). Int J Res Pharmaceut Biomed Sci 2011;2:760-764.
6. Berrington D, Lall N. Anticancer Activity of Certain Herbs and Spices on the Cervical Epithelial Carcinoma (HeLa) Cell Line. eCAM 2012; Article ID 564927.
7. Kanimozhi D. In-Vitro Anticancer Activity of Ethanolic Extract of Cynodon dactylon Against HEP-2, HELA and MCF-7 Cell Lines. Int J Sci Res Rev 2012;1:10-23.
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 cancer cell lines. Curr Sci 2004;87:1585-1588.
9. Mesquita ML, Paula JE, Pessoa C et al. Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. J Ethnopharmacol 2009;123:439-445.
10. Svejda B, Moser VA, Sturm S et al. Anticancer Activity of Novel Plant Extracts from Trailliaedoxa gracilis (W. W. Smith & Forrest) in Human Carcinoid KRJ-I Cells. Anticancer Res 2010;30:5-64.
11. Erel SB, Demir S, Nalbantsoy A et al. Bioactivity screening of five Centaurea species and in vivo anti-inflammatory activity of C. athoa. Pharm Biol 2014;52:775-781.
12. Mosmann T. Rapid colorimetric assay for cellular grow and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 1983;65:55-63.
13. Karagöz A, Turgut-Kara N, Çakır Ö, Demirgan R, Arı Ş. Cytotoxic activity of crude extracts from Astragalus chrysochlorus (Leguminosae). Biotechnol Equip

2007;21:220-222.

14. Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM. Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF7 cells. Anticancer Res 2009;29:2993-2996.
15. Awang N, Aziz ZA, Kamaludin NF, Chan KM. Cytotoxicity and mode of cell death induced by Triphenyltin (IV) compounds in vitro. J Biol Sci 2014;14:84-93.
16. Machana S, Weerapreeyakul N, Barusrux S, Nonpunya A, Sripanidkulchai B, Thitimetharoch T. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. Chin Med 2011;6:1-8.
17. Erel SB, Demirci B, Demir S, Karaalp C, Baser KHC. Composition of the essential oils of Centaurea aphrodisea, C. polyclada, C. athoa, C. hyalolepis and C. iberica. J Essent Oil Res 2013;25:79-84.
18. Kılıç Ö. Essential oil composition of two Centaurea L. (Asteraceae) species from Turkey. Düzce Univers J Science Technol 2015;3:28-33.
19. Marcetić M, Bozić D, Milenković M, Malesević N, Radulović S, Kovacević N. Antimicrobial, antioxidant and anti-inflammatory activity of young shoots of the smoke tree Cotinus coggygria Scop . Phytother Res 2013;27:1658-1663.
20. Tavirani MR, Fayazfar S, Keshel SHMB, Rezaee MZR, Tavirani M, Khodarahmi R. Effect of essential oil of Rosa damascena on human colon cancer cell line SW742. Gastroenterol Hepatol Bed Bench 2013;6:25-31.
21. Akhlaghi AZ, Rakhshandeh H, Najaran ZT, Mousavi SH Study of cytotoxic properties of Rosa damascena extract in human cervixcarcinoma cell line. Avicenna J Phytomed 2011;1:74-77.
22. Zu Y, Yu H, Liang L et al. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules 2010;15:3200-3210.
23. Pırıldar S, Sütlüpınar NB, Atasever SE, Papouskova B, Šimánek V. Chemical constituents of the different parts of Colchicum baytopiorum and their cytotoxic activities on K562 and HL60 cell lines,. Pharm Biol 2010;48:32-39.