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# Cytotoxicity screening of some Turkish plants against renal cancer cells

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**ABSTRACT**: In this contribution, we performed cytotoxicity screening of 51 extracts from 17 plant species, representing 14 families growing in Turkey. Cytotoxic activities of the dichloromethane, ethyl acetate, and methanol extracts of the plants were investigated against the renal (A498 and U031) cancer cell lines. 28 extracts exhibited more than 50% growth inhibition at a 25 ug/mL concentration on both renal cancer A498 and UO31 cell lines. The extracts showed higher activity against UO31 cells than A498 cells. The dichloromethane extract of the aerial parts of *Scabiosa columbaria* ssp. *columbaria* showed the highest cytotoxic activity with 64% inhibition at a 25 ug/mL concentration on the renal cancer UO31 cell line. The methanol extract of the aerial parts of *Epilobium minutiflorum* showed the highest cytotoxic activity with 63% inhibition at a 25 ug/mL concentration on the renal cancer A498 cell line.
**KEYWORDS**: Cytotoxic activity; renal cancer; Turkish plants; *Scabiosa columbaria* ssp. *columbaria*

## 1. INTRODUCTION

Cancer remains one of the leading causes of death worldwide [1]. Kidney cancer (Renal cell cancer-RCC) is the second most common cancer of the urinary system [2]. In 2020, there were approximately 431,000 new cases (2.2% of all new cases) and 179,000 deaths due to renal cancer (1.8% of all death cases) [3]. The discovery of new compounds from natural sources to fight cancer has become a matter of great interest among researchers [4,5]. Plant extracts and plant-derived natural compounds such as glycosides, alkaloids, tannins, terpenes, coumarins, and flavonoids have been reported to inhibit the growth of cancer cells such as renal, lung, breast, and colorectal cancer cells [6-17]. The prognosis of patients with RCC remains poor, although advances in therapeutic approaches. Since therapeutic opportunities are limited, approximately less than 40% of patients survive five years or more after diagnosis. Besides, since RCC is resistant to chemotherapy and radiotherapy, RCC recurrence principally owes to a lack of routine adjuvant therapy in the clinic. So, it is required to detect new compounds and develop novel targeted therapies for RCC [7].

The present study aimed to evaluate the cytotoxic activities of 17 plant species, representing 14 families growing in Turkey, against the renal (A498 and U031) cancer cell lines. This paper also introduces new plant sources whose cytotoxic activities have not been reported previously.

## 2. RESULTS AND DISCUSSION

This study is the first cytotoxicity screening performed on these 17 plant species against the renal (A498 and U031) cancer cell lines.

Cytotoxic activities of 51 extracts obtained from 17 plant species tested against A498 and U031 renalcancer cell lines are shown in Table 1.

Among 51 extracts tested, 28 extracts (54.9%) exhibited growth inhibition of more than 50% at a 25 ug/mL concentration in both renal cancer (A498 and UO31) cell lines. Extracts showed higher activity against UO31 cells than A498 cells. The most potent activity was found for the $CH_2Cl_2$ extract of *Scabiosa columbaria* ssp. *columbaria* with 64% inhibition at a 25 ug/mL concentration against UO31 cell line. The $CH_2Cl_2$ extracts of *Campanula alliarifolia, Corylus avellana* and *Geranium divaricatum*; the EtOAc extracts of *Campanula lactiflora* and *Corylus avellana*; the MeOH extracts of *Campanula alliarifolia, Campanula*

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*lactiflora,Epilobium minutiflorum* and *Epilobium angustifolium* were also active with the inhibition of more than 60% at a 25 ug/mL concentration against UO31 cell line.The most active extracts against A498 cell line were the MeOH extract of *Epilobium minutiflorum* and the EtOAc extract of *Melampyrum arvense* with 60% inhibition at a 25 ug/mL concentration. Of all 17$CH_2Cl_2$ extracts, 15 showed more than 50% growth inhibition at a 25 ug/mL concentration in both renal cancer cell lines. 10 MeOH extracts and 3EtOAc extracts inhibited the growth of both renal cancer cell lines by more than 50% at a 25 ug/mL concentration. All three extracts of *Echium vulgare* and *Melampyrum arvense* exhibited more than 50% growth inhibition at a 25 ug/mL concentration in both renal cancer cell lines.

Previously six of these 17 plants were tested on different cancer cell lines. For example, the extracts of *Clinopodium vulgare* were evaluated for cytotoxic activities against the Caco-2, HepG2, and MCF-7 cancer cell lines [18] and A2058 (human metastatic melanoma), HEp-2 (epidermoid carcinoma, larynx, human) and L5178Y (mouse lymphoma) cell lines [19] and CaOV (human testis cystadenocarcinoma), HeLa (human cervical adenocarcinoma), HT-29 (human colorectal adenocarcinoma) [20]. Previous studies reported that the leaf and stem extracts of *Corylus avellana* significantly reduced the viability of the HeLa (cervical cancer), HepG2 (liver hepatocarcinoma), and MCF-7 (breast cancer) cell lines and contained over 100 compounds as organic acids, triacylglycerols, phytosterols, phenolic acids, diarylheptanoids, flavonoids, tannins, isoflavones, lignans, terpenes, and taxanes. (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxy- phenyl) heptane 3-O-ß-D-glucopyranoside and quercitrin were identified as contributors to the strong cell viability-reducing activity of *C. avellana* leaf extracts [21]. *Rhododendron ponticum* were tested against human prostate carcinoma (DU145) and human prostate adenocarcinoma (PC3) cell lines [22]; *R. luteum* extracts on A549 breast, colon, prostate, liver and lung cancer cell lines [23, 24]; *Sedum spurium* extracts against the Hep-2 human larynx epidermoid carcinoma cells [25]. *Epilobium angustifolium* extracts have been shown to exhibit potent antiproliferative activity on the LNCap prostate carcinoma cell lines [26] and has been reported to contain ellagitannins, flavonoids, and phenolic acids [27,28] and Urolithin C, metabolites of *Epilobium* ellagitannins, has been explained to responsible for the activity [27]. In this study, the MeOH extract of *Epilobium angustifolium* and EtOAc extract of *Corylus avellana* exhibited more than 60% growth inhibition at a 25 ug/mL concentration against UO31 cell line. The remaining extracts of these six plants showed between 41% and 60% growth inhibition at a 25 ug/mL concentration in both renal cancer (A498 and UO31) cell lines.

No reports in the literature deal with the cytotoxic activities of *E. vulgare,S. columbaria* ssp. *columbaria*, *C.alliarifolia, C. lactiflora, G. divaricatum,L. pratensis,E. minutiflorum, M. arvense,G. leiocarpum, T. minus*and *U. minor*.

## 3. CONCLUSION

This study is the first cytotoxicity screening performed on these 17 plant species against the renal (A498 and U031) cancer cell lines. Of all 17 plant species, 11 were investigated for the first time for the cytotoxic activities in this study.

Previous studies on the among the most active plants (*S. columbaria* ssp. *columbaria*, *C. alliarifolia, C. lactiflora, G. divaricatum, C. avellana* and *M. arvense*) *Scabiosa columbaria* ssp. *columbaria* has been reported to contain six phenolic compounds; chlorogenic acid was the major compound and exhibited antioxidant activity [29]. *Campanula* species have been reported to contain polyphenols, steroids, phenylpropanoid derivates, triterpenes, and polyacetylenes and show antioxidant activity [30,31]. Previous phytochemical studies on the *Geranium* genus have shown the presence of phenolic compounds such as gallic, ellagic, quinic acid, quercetin, geraniin, and corilagin [32-33]. *Melampyrum arvense* has been reported to exhibit antimicrobial and antioxidant activity and contain phenolic compounds [34]. There are no reports in the literature dealing with the cytotoxic activities of the five plants (*S. columbaria* ssp. *columbaria*, *C. alliarifolia, C. lactiflora, G. divaricatum*and *M. arvense*) of the most active six plants.

In conclusion, bioactivity-guided fractionation of the *S. columbaria* ssp. *columbaria*, *C. alliarifolia, C. lactiflora, G. divaricatum, C. avellana* and *M. arvense* extracts, which exhibited growth inhibition of more than 60% at a 25 ug/mL concentration, are planned to isolate and identify their cytotoxic principles.

## 4. MATERIALS AND METHODS

### 4.1. Plant materials

Collection sites and voucher numbers of the plants are listed in Table 1. Voucher specimens are deposited in the Herbarium of Faculty of Pharmacy of Istanbul Medipol University, Istanbul, Turkey (IMEF). Used plant part is leaves for *Corylus avellana* and *Ulmus minor*. Aerial parts are used for the remaining 15 plants.

### 4.2. Preparation of extracts

Air-dried and coarsely powdered leaves/aerial parts of the plants were sequentially extracted at room temperature with dichloromethane, ethyl acetate, and methanol. The extracts were separately concentrated in a rotary evaporator under reduced pressure to dryness. All extracts were stored at −20 ◦C prior to screening.

**Table 1**. Growth inhibition of the plant extracts at 25 ug/mL concentration on the renal cancer cell lines

| | | | Inhibition % | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A498 | | | UO31 | | |
| Plants | Voucher No | Collection sites | 1 | 2 | 3 | 1 | 2 | 3 |
| **Betulaceae** | | | | | | | | |
| *Corylusavellana* L. | IMEF1175 | Giresun-Şebinkarahisar | **53** | 47 | **50** | **60** | **61** | **58** |
| **Boraginaceae** | | | | | | | | |
| *Echium vulgare* L. | IMEF1161 | Giresun-Şebinkarahisar | **56** | **52** | **51** | **59** | **54** | **58** |
| **Campanulaceae** | | | | | | | | |
| *Campanulaalliarifolia*Willd. | IMEF1162 | Giresun-Şebinkarahisar | **53** | 47 | **52** | **60** | **52** | **61** |
| *Campanulalactiflora*M.Bieb. | IMEF1168 | Giresun-Şebinkarahisar | **53** | 46 | **54** | **56** | **60** | **62** |
| **Caprifoliaceae** | | | | | | | | |
| *Scabiosacolumbaria*ssp. *columbaria* L. | IMEF1141 | Trabzon-Zigana pass | **51** | 48 | **55** | **64** | **56** | **55** |
| **Crassulacaeae** | | | | | | | | |
| *Sedumspurium*M.Bieb. | IMEF1142 | Trabzon-Hamsiköy | **56** | 47 | 37 | **57** | **55** | 43 |
| **Ericaeae** | | | | | | | | |
| *Rhododendronluteum*Sweet | IMEF1150 | Trabzon-Altındere | **56** | 44 | 36 | **58** | **55** | 41 |
| *Rhododendronponticum* L. | IMEF1152 | Trabzon-Tonya | **53** | 48 | **53** | **55** | **54** | 45 |
| **Geraniaceae** | | | | | | | | |
| *Geraniumdivaricatum*Ehrh. | IMEF1131 | Trabzon-Zigana pass | **53** | 42 | **53** | **60** | 46 | **55** |
| **Lamiaceae** | | | | | | | | |
| *Clinopodiumvulgare* L. | IMEF1137 | Trabzon Zigana pass | 41 | 47 | **50** | 40 | **54** | **57** |
| **Leguminosae** | | | | | | | | |
| *Lathyruspratensis* L. | IMEF1167 | Giresun-Şebinkarahisar | **52** | 48 | 43 | **56** | **50** | 47 |
| **Onagraceae** | | | | | | | | |
| *Epilobiumangustifolium* L. | IMEF1147 | Trabzon-Tonya | **53** | 47 | **53** | **59** | 49 | **61** |
| *Epilobiumminutiflorum*Hausskn. | IMEF1171 | Giresun-Şebinkarahisar | **58** | 47 | **63** | **56** | 49 | **63** |
| **Orobanchaceae** | | | | | | | | |
| *Melampyrumarvense* L. | IMEF1055 | Trabzon-Tonya | **50** | **61** | **52** | **53** | **54** | **54** |
| **Papaveraceae** | | | | | | | | |
| *Glauciumleiocarpum*Boiss. | IMEF1135 | Ankara-Çankaya | 39 | **50** | 46 | 42 | **57** | **55** |
| **Ranunculaceae** | | | | | | | | |
| *Thalictrumminus* L. | IMEF1172 | Giresun-Şebinkarahisar | **50** | 48 | **50** | **57** | **52** | 47 |
| **Ulmaceae** | | | | | | | | |
| *Ulmusminor*Mill. | IMEF1166 | Giresun-Şebinkarahisar | **55** | 47 | 46 | **58** | 45 | **58** |

1. $CH_2Cl_2$ extract; 2. EtOAc extract; 3. MeOH extract

### 4.3. Cytotoxic activity assay

The assay used for this study was a two-day, two cell line XTT bioassay [17], an *in vitro* antitumor colorimetric assay developed by the MTL Assay Development and Screening Section. Renal cancer cell lines used were A498 and U031. Sanguinarine was used as a positive control. The assay was performed as described previously [16]. Cytotoxic activities of extracts are shown in Table 1.

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